



Research Article

Antidiarrhoeal Effects of Methanolic Stem Bark Extract of *Isoberlinia tomentosa* (Leguminosae) in Albino Rats

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ABSTRACT

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The research aimed to evaluate the antidiarrhoeal effect of the methanolic stem bark extract of *Isoberlinia tomentosa*. The stem bark of *Isoberlinia tomentosa* was collected air-dried, ground to powder, and extracted with methanol using the maceration method to give a percentage yield of 8.58% w/w. The antidiarrhoeal effect of the methanol stem bark extract on castor oil-induced diarrhoea, intestinal charcoal meal transit, and castor oil-induced enteropooling was determined. The stem bark extract of *Isoberlinia tomentosa* exhibits significant dose-dependent protection ($p < 0.05$) against castor oil-induced diarrhoea with the highest protection of 71.0% obtained at the highest dose tested (400mg/kg). The extract showed a significant difference in intestinal charcoal meal transit in the rat at 100, 200, and 400 mg/kg ($p < 0.05$) as it had 40.5%, 52.5%, and 62.5% inhibition respectively when compared to distilled water, the negative control (0% inhibition). Atropine however produced a significant increase ($p < 0.05$) in intestinal charcoal meal transit at 83.9% inhibition. There were no significant differences ($p > 0.05$) between the extract doses of 100, 200, and 400 mg/kg administered. The methanol extract at 100, 200, and 400 mg/kg showed 20.4%, 68.0% and 77.0% fluid accumulation respectively. The positive control Atropine (5 mg/kg) had 81.0% inhibition of intestinal content, when compared to the negative control, which had 0% inhibition. In conclusion, the results obtained from this study revealed that the stem bark extract of *Isoberlinia tomentosa* possesses an antidiarrhoeal effect.

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INTRODUCTION

Medicinal plants are those plants whose chemical contents have some physiological effect on the body's chemistry. From the earliest times, mankind has used plants in an attempt to cure disease and relieve physical suffering (Dawa *et al.*, 2018). The folkloric use of plants to treat ailments has triggered the interest of researchers in tapping the valuable and enormous treasure packed in plants' nature (Ahvazi *et al.*, 2012). The therapeutic value of medicinal plants is due to substances found in the plant tissue that produce a definite physiological action on the human body. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants (Ahvazi *et al.*, 2012). Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 7 billion people of the world live in less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs (Ahvazi *et al.*, 2012). Medicinal plants are the backbone of traditional medicine, which means more than 3.3 billion people in less developed countries utilize medicinal plants regularly. There are nearly 2000 ethnic groups in the world, and almost every group has its traditional medical knowledge and experiences (Dawa *et al.*, 2018). The use of medicinal plants in the treatment of diseases has generated renewed interest in recent times, as herbal preparations are increasingly being used in human healthcare systems. Diarrhoea is one of the common clinical signs of gastrointestinal disorders caused by both infectious and non-infectious agents. Diarrhoea in particular remains a major concern in Nigeria resulting in high mortality rates when left untreated (Dawa *et al.*, 2022) Traditional medicine remains the only health care available

in many rural areas in Nigeria like the rural community of Kangaruwa in Borno state, located in a very remote area in the Northeastern part of the country. With limited access to modern medicine, the local population uses medicinal plants to treat most diseases (Dawa *et al.*, 2022).

The plant *Detarium microcarpum* was used from the creation of the world as remedies to cure diseases. From ancient times people have knowledge of chemistry they used different methods of extraction for them to use the plant as herbal remedies (Dawa *et al.*, 2022). The beginning of the development of herbal medicines was concurrent with the development of chemistry and isolation, purification, and determination of plant compounds. In the past, the drug discovery of the biological compounds from plant materials and the process of identifying the structures of active compounds from the extracts were problematic depending on the complexity of the compounds and might take weeks, months, or even years (Jamshidikai *et al.*, 2018).

Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level and has maintained its popularity worldwide (Nedi *et al.*, 2004) About 60-85% of the population in every country in the developing world has to rely on traditional medicine. The practice of traditional Medicine is widespread in Nigeria China, India, Japan, Pakistan, Sri Lanka, Thailand, and Korea (WHO, 2012).

Medicinal plants are used for treatment because they have certain properties, including synergistic actions. The constituents of the plant may interact with each other, and this interaction can be beneficial for both or adverse to either of them or eliminate the harmful effects of both. Plant-derived compounds can dramatically

improve hard-to-treat illnesses, such as cancer (Nedi *et al.*, 2004). Plant components are also characterized by their ability to prevent the development of certain diseases (Nedi *et al.*, 2004). The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demand an increase in the number of herbal drug manufacturers as well as a reduction in the use of chemical drugs (Jamshidi-kai *et al.*, 2018).

Isobерlinia tomentosa is a hardwood tree native to African tropical savannas and Guinean forest-savanna mosaic dry forests (Gele-kakai and Sinsin, 2009). At one time this tree was disregarded as a useful resource but with the selective felling of such valuable species as *Khaya senegalensis* and *Azelia Africana*, it is now better appreciated that it contains antidiarrhoeal activities based on scientific findings and the claims of traditional healers in Amuda community. This study aimed to evaluate the antidiarrhoeal activities of *Isobерlinia tomentosa* stem bark extract.

Isobерlinia (*Leguminosae*, *Caesalpinioidea*) is an African tree genus growing mainly in woodlands and tree-savannas. Two species are found in Benin namely; *Isobерlinia doka* (grows on clay soil and well-drained soils) and *Isobерlinia tomentosa* (grow on stony and sloping soils) (Gele-kakai and Sinsin, 2009). The Wari-Marо Forest Reserve is partly composed of woodlands and tree-savannas with *Isobерlinia* natural stands. To prevent the decline of these stands, as was the case for *K. senegalensis* and *A. africana*, a sustainable management strategy should be applied, with special attention to *Isobерlinia spp.* This requires better knowledge of the structure of the stands (Gele-kakai and Sinsin, 2009).

Materials and Methods

Sample Collection and Identification

The fresh sample stem bark of *Isobерlinia tomentosa* was collected from Kulp Theological

Seminary Kwarhi Hong Local government Adamawa state, Nigeria, and authenticated by a Plant taxonomist with a voucher number UMC/DC/342. The sample was dried under shade rendered free of foreign material through manual picking and ground with a wooden mortar and pestle to a fine powder.

Plant Extraction

The pulverized dried stem bark of *Isobерlinia tomentosa* (3000 g) was macerated with absolute methanol at 25°C for (72 hours) in a round bottom flask with occasional shaking. The soaked sample was passed through a muslin cloth to remove the vegetative debris and the extract was then filtered through Whatman No 1 filter paper. The crude extract was concentrated to dryness at 25°C. The extract was weighed, labelled, and subjected to further analysis. 200g of the crude extract was subjected to defatting with n-hexane and then partitioning with chloroform, ethyl acetate, and n-butanol base on their polarities using the dry technique.

Animals

Ninety (90) Wistar albino rats weighing between 100-200g were obtained from Jos, Plateau State. They were kept in plastic rat cages and allowed to acclimatize to the laboratory environment for a minimum period of one week before the commencement of the experiments. They were fed with grower's mash (Vital Feed Nig Ltd, Jos, Nigeria) and water was provided *ad libitum*. The experiments were conducted in compliance with the international guiding principles for biochemical research involving animals (CIOMS and ICLAS, 2012).

Antidiarrhoeal Studies of *Isobерlinia tomentosa*

Effect of the Methanol Stem Bark Extract of *Isobерlinia tomentosa* on Castor oil-induced Diarrhoea

The method of Williamson, *et al.*, 1996. Was used to evaluate the effect of the methanol extract on castor oil-induced diarrhea. Thirty

(30) Wistar strain albino rats of both sexes weighing between 100-200 g were used for the experiment. The rats were denied food for 12 hours but were provided with water *ad libitum*. They were divided into five groups of six rats each. Groups B, C, and D were dosed orally with 100, 200, and 400mg/kg of methanol crude extract of *Isobertinia tomentosa* respectively. Group A was administered 2 ml of normal saline (Dona Nig. Ltd) orally. Group E was administered 3mg/kg loperamide (RPG Life

Science Ltd, Ankleshwar) intraperitoneally (i.p) as the standard drug. The rats were separated singly in cages lined with white blotting paper. After one hour, each rat was given 1ml castor oil (Bellson's and Co. Ltd, England), orally and observed for 6 hours for wet or watery faeces. The wet faeces of each rat were counted and recorded at the end of the experiment. The percentage protection was calculated using equation 1. (Dawa, *et al.* 2018)

$$\% \text{ protection} = \frac{A-B}{A} \times 100 \quad (\text{equation 1})$$

Effect of the Methanol Stem Bark Extract of *Isobertinia tomentosa* on Gastro Intestinal Transit of Charcoal Meal in Rats

The method of Chitme *et al.* (2003) was adopted to study the effect of the methanolic root extract of *Isobertinia tomentosa* on the gastrointestinal transit of charcoal meal in rats. Thirty (30) albino rats weighing between 100-200 g were used for this experiment. The rats were denied food for eighteen hours but were allowed access to water *ad libitum*. They were divided into five groups of six rats each. Group A served as the control and was given 2 ml of normal saline orally. Rats of groups B, C, and D were given 100, 200, and 400mg/kg of methanol crude

extract respectively. Group E rats were treated with 3mg/kg atropine (Fugisawa U.S.A Inc) intraperitoneally as the standard drug. After 10 minutes, 1 ml of a charcoal meal (5 % activated charcoal (Kochlight Laboratories Ltd. England) suspension in 10 % solution of acacia powder) was given orally to each rat. The rats were sacrificed after 30 minutes and the abdomen opened. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. The percentage intestinal transit of the charcoal meal was calculated using the formula;

$$\% \text{ Intestinal Transit} = \frac{X}{Y} \times 100 \quad (\text{equation 2})$$

A reduction in the gastrointestinal propulsion of the charcoal meal was an indication of a laxative effect.

Effect of the Methanol Stem Bark Extract of *Isobertinia tomentosa* on Castor Oil-Induced Enteropooling

The method of Robert *et al.* 1976 was used to evaluate the effect of the methanol stem bark extract *Isobertinia tomentosa* on the intraluminal fluid accumulation in rats. Thirty (30) albino rats weighing between 100-200g were used for this experiment. The rats were fasted overnight and

then divided into five groups of six rats each. Group A served as the control and was given 2 ml of normal saline per body weight orally. Groups B, C, and D were given 100mg/kg, 200 mg/kg, and 400mg/kg orally of the methanol crude extract respectively. Group E was treated with 3 mg/kg Atropine intraperitoneally (i.p) to serve as the control drug. After one hour, all the rats were sacrificed and the intestine was removed and weighed. The content of the intestine was collected by milking and the

weight of the empty intestine and the content was also measured.

Statistical Analysis

Data presented as mean \pm standard deviation (S.D), also, One-way analysis of variance (ANOVA) was used to test for significance and mean and $P < 0.05$ was considered significant.

Results

Effect of Methanolic Stem bark Extract of *Isoberlinia tomentosa* on castor oil-induced Diarrhoea

After one hour of administration of castor oil, the rat's diarrhoea was watery and clinically apparent in all the animals in the control group, for the next six hours. This was reduced by loperamide (3 mg/kg) (95.5 %). A similar reduction in the number of defecations after six hours was achieved with the stem bark extract

of *Isoberlinia tomentosa* at doses of 100, 200, and 400 mg/kg. The methanol stem bark crude extract of *Isoberlinia tomentosa* at 100, 200, and 400 mg/kg significantly inhibited defecation (46.3 %, 56.5 %, and 71.0 %). The effect of methanol stem bark extract of *Isoberlinia tomentosa* at (400 mg/kg) was comparable to that of loperamide, the standard antidiarrhoeal agent at a dose (95.5 %) 3 mg/kg concentration. There was no significant difference between the effect of the extract doses ($p > 0.05$), however, there was a significant difference between the effect of the positive control, 3 mg/kg of loperamide ($p < 0.05$) when compared to the extract doses of 100, 200, and 400 mg/kg on the total number of stools and wet stool as presented Table 1

Table 1: Effect of extract of *Isoberlinia tomentosa* on Castor oil-induced Diarrhoea

Dose mg/kg	Weight of Stools in 6 hours (g \pm SEM)	% Protection
100 mg/kg	7.40 \pm 0.98 ^{ab}	46.3
200 mg/kg	6.00 \pm 0.55 ^{ac}	56.5
400 mg/kg	4.00 \pm 1.10 ^{bcd}	71.0
Control		
+ Ve	0.60 \pm 0.24 ^d	95.6
Control -V	13.80 \pm 1.11	00

Key: + Ve Control: 3 mg/kg Loperamide, -Ve Control: 2ml Normal saline

Values of Mean with the same alphabetical super script across the column are not significantly different from each other ($p > 0.05$)

Effect of Methanolic Stem Bark Extract of *Isoberlinia tomentosa* on Gastrointestinal Transit of Charcoal Meal in Rats

The methanolic stem bark extract of *Isoberlinia tomentosa* 100 mg/kg, 200 mg/kg, and 400 mg/kg dose of extract produced 40.5 %, 52.5%, and 62.5 % gastrointestinal transit induced by

castor oil respectively. Atropine however produced a significant decrease ($p < 0.05$) in the percentage of intestinal transit (83.9 %). There was no significant difference ($p > 0.05$) between the methanol stem bark crude extract of *Isoberlinia tomentosa* doses 100, 200, and 400 mg/kg administered. However, there was a significant decrease ($p < 0.05$) between the effects of the methanol stem bark crude extract of *Isoberlinia tomentosa* when compared to the

negative control (rats pretreated with distal water) and positive control (rats treated with atropine) as presented in Table 2.

Table 2: Effect of Extract of *Isoberlinia tomentosa* on Gastrointestinal Transit of Charcoal Meal in Rats

Dose mg/kg	Total Length of Intestine (g±SEM) in cm	Movement of Charcoal (g±SEM) in cm	% Intestinal Transition	% Inhibition
100 mg/kg	77.00 ± 2.68 ^b	38.00 ± 2.98 ^a	49.3	40.5
200 mg/kg	83.40 ± 1.40 ^b	32.80 ± 1.16 ^a	39.3	52.5
400 mg/kg	80.80 ± 3.18 ^b	25.00 ± 1.38	30.9	62.5
Control +Ve mg/kg	74.80 ± 0.86 ^b	12.40 ± 0.51	13.3	83.9
Control -Ve mg/kg	79.60±0.98 ^b	66.00±1.30	82.9	00

Key: + Ve Control: 3 mg/kg Atropine, - Ve Control: 2 ml Normal saline

Values of Mean with the same alphabetical super script across the column are not significantly different ($p > 0.05$)

Effect of Stem Bark Extract of *Isoberlinia tomentosa* on Castor Oil-Induced Entropooling

Castor oil-induced entropooling is not influenced by atropine 3 mg/kg in rats. Methanolic extract of *Isoberlinia tomentosa* 100, 200, and 400 mg/kg produced a dose-dependent

reduction in intestinal weight and volume. Methanolic stem bark extract of *Isoberlinia tomentosa* 100, 200 and 400 mg/kg dose produced 30.4 %, 68.0 %, 77.0 % and 81.0 % inhibition of the volume of intestinal content respectively with significance ($p < 0.05$). The percentage of fluid accumulation of the intestinal content was also reduced to 37.0, 27.0, 14.9, and 12.8 significantly at all doses (Table 3).

Table 3: Effect of Extract of *Isoberlinia tomentosa* on Castor oil-induced Enteropossoling

Dose mg/kg	Weight of Intestine and Content (g±SEM)	Weight of Empty Intestine (g±SEM)	Weight of Content (g±SEM)	% Fluid Accumulation	% Inhibition
100	4.96±0.60	3.52±0.54 ^a	1.84±0.02	37.0	30.4
200 mg/kg	3.93±0.24	2.87±0.12 ^a	0.83±0.02 ^{ab}	27.0	68
400 mg/kg	3.97±0.23	3.02±0.32 ^a	0.59±0.16 ^{ac}	14.9	77
control +Ve	3.82±0.34 ^b	3.33±0.31 ^a	0.50±0.06 ^{bc}	12.8	81
Control -ve	5.56±0.42 ^a	2.95±0.41 ^a	2.63±0.12	47.1	00

Key: +Ve control: 3 mg/kg Atropine, -Ve control: 2 ml normal saline

Values of Mean with the same alphabetical super script across the column are not significantly different ($p > 0.05$)

Values of Mean with different superscripts across the column are significantly different from each other ($p < 0.05$)

DISCUSSION

Diarrhoea (loose motions) is the passage of 3 or more losses of liquid stools per day or more frequently than is normal for the individual. Diarrhoea is not itself a disease, but can be a symptom of several diseases and sometimes may be associated with abdominal pain, which may reduce after a stool is passed (Dawa *et al.*, 2018). Diarrhoea occurs due to the irritation within the lining of the small or large intestine, which leads to decreased water absorption and hence increases in water being passed with stools (Dawa *et al.*, 2022). Four clinical types of diarrhoea can be recognized, each reflecting the basic underlying pathology and altered physiology: acute watery diarrhoea (including cholera), which lasts several hours or days: the main danger is dehydration; weight loss also occurs if feeding is not continued; acute bloody diarrhoea, which is also called dysentery: the main dangers are damage of the intestinal mucosa, sepsis and malnutrition; other complications, including dehydration, may also occur (Dawa *et al.*, 2022).

Persistent diarrhoea, which lasts longer,: the main danger is malnutrition and serious non-intestinal infection; dehydration may also occur; diarrhoea with severe malnutrition (marasmus or kwashiorkor): the main dangers are severe systemic infection, dehydration, heart failure, and vitamin and mineral deficiency. The management of each type of diarrhoea should prevent or treat the main danger(s) that each present. Many factors such as food poisoning, infection (bacterial, viral, parasitic), food intolerance malnutrition, intestinal disease, and sometimes medication can contribute to diarrhoea. Castor oil has been reported to induce

diarrhoea by increasing the volume of intestinal contents and preventing the re-absorption of water (Dawa *et al.*, 2018). The plant extract of *Isoblerlinia tomentosa* at doses of 100, 200 and 400 mg/kg of methanol crude extract significantly decreased ($p < 0.05$) the total number of wet faeces produced upon administration of castor oil at 400 mg/kg was comparable to the control group. The effect of the highest dose of the methanolic crude extract of *Isoblerlinia tomentosa* was similar to that of the standard drug. Therefore, it may probably assume that the anti-diarrhoeal action of the extracts was mediated by an anti-secretory mechanism. These include castor oil decreasing fluid absorption, increasing secretion in the small intestine and colon, and effects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effects due to its active component of ricinoleic acid Mamza *et al.* (2014), Dawa *et al.* (2018). The propulsion of the charcoal meal through the gastrointestinal tract decreased significantly ($p < 0.05$) from 100, 200, and 400 mg/kg, of methanol crude extract of *Isoblerlinia tomentosa* compared to the control group. Similarly, the effect of the highest dose of the methanol crude extract of the graded dose was similar to that of the standard drug. These observations suggest that the methanol crude extracts reduced diarrhoea by inhibiting peristalsis, gastrointestinal motility, and castor oil-induced enteropooling. It may be equally effective in the prevention and curing of diarrhoea. The significant inhibition of the castor oil-induced enteropooling in rats suggests that the extract of *Isoblerlinia tomentosa* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enter pooling effect Dawa *et al.* (2018), Mascolo *et al.* (1999).

CONCLUSION

The methanolic stem bark extract of *Isoblerlinia tomentosa* has been shown to possess some antidiarrhoeal activity. Bio-guided assay of the isolated bioactive compound should be further

studied. Likewise, characterization using a spectroscopic technique such as ¹HNMR, ¹³CNMR UV-visible, and IR-Spectroscopy should be carried out to confirm the chemical structures of the bioactive constituents responsible for the antidiarrhoeal activity. Even though there was no work done on *isoberlinia tomentosa* that shows the antidiarrhoeal activities it seems that *isoberlinia tomentosa* is based on traditional medicine and the research prove that it can treat diarrhoea.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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