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Antidiarrhoeal effects of methanol leaf extract of *Carissa edulis* Vahl. (Apocynaceae) in albino rats

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Abstract
The research aims at evaluating the chemical contents of methanolic leaf extract of *Carissa edulis*. The leaf of *Carissa edulis* was collected air-dried, ground to powder and extracted with methanol by cold extraction method to give a percentage yield of 28.03% $^{w}/_{w}$. The antidiarrhoeal effect of the methanol leaf extract on castor oil-induced diarrhoea, intestinal charcoal meal transit and castor oil-induced enteropooling were determined. The leaf extract of *Carissa edulis* produced a significant dose dependent protection ($p<0.05$) against the castor oil-induced diarrhoea with the highest protection of 53.50% obtained at the highest dose tested (800mg/kg). The extract showed a significant intestinal charcoal meal transit ($p<0.05$) as it had 15.80%, 41.90%, and 50.30% 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 76.50% inhibition. There were no significant differences ($p>0.05$) between the extract doses 200, 400, 800 mg/kg administered. The methanol extract at 200, 400, and 800 mg/kg showed 27.00%, 40.80% and 51.40% fluid accumulation respectively. The positive control loperamide (5 mg/kg) had 79.50% inhibition of intestinal content when compared to the negative control (distilled water treated rats) which had 0% inhibition. The results obtained from this study revealed that the leaf of *Carissa edulis* possesses antidiarrhoeal effect.

Keywords: Diarrhoea, Antidiarrhoea, *Carsisa edulis*, Phytochemicals

Introduction

Medicinal plants are those plants whose chemical contents have some physiological effect on the body chemistry. From the earliest times, mankind has used plants in an attempt to cure diseases and relieve physical suffering [1]. The folkloric use of plants to treat ailments has triggered interest of researchers into tapping the valuable and enormous treasure packed in plants by nature. The therapeutic value of medicinal plants is due to substances found in the plant tissues 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. 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 5.2 billion people of the world live in the 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. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences [1].

Carissa edulis Vahl is widespread in many parts of Africa. It belongs to the family Apocynaceae and grows at forest edges, in forests and woodlands where Euphorbia, Acacia, and Croton commonly occur, especially on rocky hillsides, clay soils, black cotton soils, in dry and moist low- and midlands of altitude 1500-2500 m [2]. The plant is a well-known African medicinal plant widely used in traditional treatment of headaches, chest pains, rheumatism, gonorrhoea, syphilis and rabies. The plant roots have been used in Africa for a variety of medicinal purposes. The vapour from a hot aqueous root bark infusion is inhaled as treatment for chest congestion and the root powder is applied to toothache to relieve pain. The roots are also used to treat gastric ulcers and the decoction is used to treat malaria [3, 4]. Phytochemically, seven sesquiterpenes, benzonoids lignans, propanoids, cumarins phenolic [5, 6, 7], steroids, terpenes, tannins, flavonoids and cardiac glycosides [8] have been isolated from *Carissa edulis*.

In pharmacological studies, root wood extracts induces diuretic effect in rats [2]. The extract of the leaves of *Carissa edulis* reduces blood glucose level in streptozotocin diabetic rats, indicating the presence of compounds with hypoglycemic activity [9]. Aqueous extracts of roots of *Carissa edulis* have exhibited significant activity against the *Herpes simplex* virus (HSV) *in vitro* and *in vivo* for both wild type and resistant strains of HSV [10].

Carissa edulis is used traditionally for the treatment of headaches, chest complaints, rheumatism, gonorrhoea, syphilis, rabies and as a diuretic [2]. Folkloric uses of this species include the treatment of fever, sickle cell anaemia and hernia.

Inspite of the use of *Carissa edulis* in traditional medicine it is pertinent therefore to test the methanol extract on diarrhoea given the fact that micro organisms are becoming increasingly resistant to synthetic agents

The increase in search for the phytotherapeutic chemical constituents in plant materials has been remarkably

astonishing most especially in recent times. Scientific justification of the folkloric use of *Carissa edulis* as anti-diarrhoea would help to provide information for the basis of the use of this plant for medicinal purposes.

Materials and Methods

Equipments used for pharmacological studies were: rodents' plastic cages, surgical gloves, face mask, 1 and 2 ml syringes, beakers, surgical blades. Instruments used for pharmacological studies were: analytical weighing balance.

Chemicals used for pharmacological studies were distilled water (Dona Nig. Ltd), castor oil (Bellsons and Co. Ltd, England), 5 % activated charcoal (Kochlight Laboratories Ltd. England), loperamide (RPG Life Science Ltd, Anleshwar), atropine (Fugisawa U.S.A Inc).

Sample Collection and Identification

Fresh sample of leaf of *Carissa edulis* was collected from Hebarium of Faculty of Pharmacy, University of Maiduguri Borno State, Nigeria and authenticated by a plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Nigeria. The sample was air dried under shade rendered free of foreign material through manual picking, labeled (663C) and ground with a wooden mortar and pestle to a powder.

Plant Extraction

Five hundred gram (1000 g) of the pulverized dried leaves of *Carissa edulis* was extracted by cold extraction method (maceration) with 2.5 L of absolute methanol at room temperature for seventy two hours (72 hr) in a round bottom flask with occasional shaking. The soaked sample was passed through a muslin cloth to remove the vegetative debris and the liquid was filtered through Watman No. 1 filter paper. The crude extract was concentrated to dryness. The extract was weighed, labelled and subjected to further analysis.

Animals

Seventy five (75) Wistar albino rats weighing between 100-200 g were brought from Jos, Plateau State. They were kept in plastic cages and allowed to acclimatize to the laboratory environment for two weeks 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 [11].

Antidiarrhoeal Studies of *Carissa edulis*

Effect of the Methanol Leaf Extract of *Carissa edulis* on Castor Oil-Induced Diarrhoea

The method of Williamson *et al.* [12] was used to evaluate the effect of the methanol leaf extract on castor oil induced-diarrhoea. Twenty five (25) Wistar strain albino rats of both sexes weighing between 100-200 g were used for the experiment. The rats was denied food for 12 hr but were provided water *ad libitum*. They were divided into five groups of five rats each. Groups B, C and D were dosed orally with 200,400 and 800 mg/kg of the extract respectively. Group A was given 2 ml distilled water orally. Group E was given 5 mg/kg loperamide intraperitoneally as the standard drug. The rats were separated singly in cages lined with white blotting paper. After one hour, each rat was given 1 ml castor oil, orally and observed for 6 hr 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 the formula below [13, 14].

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

Effect of the Methanol Leaf Extract of *Carissa edulis* on Gastro Intestinal Transit of Charcoal Meal in Rats

The method of [12, 15] was used to study the effect of the methanol extract of *Carissa edulis* on gastrointestinal transit of charcoal meal in rats. Twenty five (25) 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 five rats each. Group A served as the control and was given 2 ml distilled water orally. Rats of groups B, C and D were given 200, 400 and 800 mg/kg of the methanol extract orally 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 charcoal meal [5 % activated charcoal] 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;

$$\% \text{ Intestinal Transit} = \frac{\text{Movement of Charcoal (cm)}}{\text{Total Length of Intestine (cm)}} \times 100$$

A reduction in the gastrointestinal propulsion of the charcoal meal is an indication of an antidiarrhoeal effect [13] while an increase in the gastrointestinal propulsion indicates a laxative effect.

Effect of the Methanol Leaf Extract of *Carissa edulis* on Castor Oil-Induced Enteropooling

The method of Robert *et al.* [16] was used to evaluate the effect of the methanol leaf extract on the intraluminal fluid accumulation in rats. Twenty five (25) albino rats weighing between 100-200 g were used for this experiment. The rats were fasted overnight and then divided into five groups of five rats each. Group A served as the control and was given 2 ml distilled water orally. Groups B, C and D were given 200, 400 and 800 mg/kg orally of the extract respectively. Group E was treated with 5 mg/kg loperamide intraperitioneally to serve as the control drug. After one hour, all the rats were sacrificed and the intestine removed and weighed. The content of the intestine was collected by milking and the weight of the empty intestine and the content was also taken.

Statistical Analysis

Data where applicable were presented as mean ± standard deviation (S.D). One way analysis of variance (ANOVA) was

used to test for significance and mean and P<0.05 was considered significant.

Antidiarrhoeal Activity of the Leaf Extract of *Carissa edulis*

Effect of Methanol Leaf Extract of *Carissa edulis* on Castor Oil-induced Diarrhoea

After 1 hour of administration of castor oil to the rats, the diarrhoea was clinically apparent in all the animals in the control group, for the next 6 hours. This was reduced by Loperamide (5 mg/kg) (98.5 %). A similar reduction in the number of defecations after six hours was achieved with the leaf extract of *Carissa edulis* at the doses of 200, 400 and 800 mg/kg. The methanol leaf crude extract of *Carissa edulis* at 200, 400 and 800 significantly inhibited the defecation (36.60 %, 39.40 % and 53.50 %). The effect of methanol leaf extract of *Carissa edulis* at (800 mg/kg) was comparable to that of loperamide, the standard antidiarrhoeal agent at a dose (98.50 %) 5 mg/kg concentration. There was no significant difference between the effect of the extract doses (p>0.05), however, there was a significant difference (p<0.05) between the effect of the positive control, 5mg/kg loperamide when compared to the extract doses of 200 mg/ml, 400 mg/ml and 800 mg/ml on the total number of stools and wet stool as presented in Table 1.

Table 1: Effect of Extract on Castor Oil-Induced Diarrhoea

S/N	Group	Dose (mg/kg)	mean±SEM total no. of wet stools	% inhibition
1	A	–ve control (saline)	14.20 ± 0.86^{a}	0
2	B	200	9.00 ± 0.84^{ab}	36.6
3	C	400	8.60 ± 0.51^{ac}	39.4
4	D	800	6.60 ± 0.93^{ad}	53.5
5	E	5 (loperamide)	0.20 ± 0.20^{abcd}	98.5

Means with different letters are significantly different from each other (p<0.05).

Effect of Methanol Leaf Extract of *Carissa edulis* on Intestinal Transit in Rats

The methanol leaf extract of *Carissa edulis* 200 mg/ml, 400 mg/ml and 800 mg/kg dose of extract produced 71.00 %, 49.00 % and 41.90 % intestinal transit induced by castor oil respectively. Atropine however produced a significant decrease (p<0.05) in percentage intestinal transit (19.80%). There was no significant difference (p>0.05) between the methanol crude exract of *C. edulis* leaf doses 200, 400 and 800 mg/kg administered. However there was a significant difference (p<0.05) between the effects of the methanol crude extracts of *C. edulis* leaf when compared to the negative control (rats pretreated with distle water) and positive control (rats treated with atropine) as presented in Table 2.

Effect of Methanol Leaf Extract of *Carissa edulis* on Castor oil-Induced Entropooling

Castor oil-Induced enteropooling is not influenced by atropine 3 mg/ml in rats. Methanol extract of *Carissa edulis* 200, 400 and 800 mg/ml produced a dose dependent reduction in intestinal weight and volume. Methanol extract of *Carissa edulis* 200, 400 and 800 mg/kg dose produced 27.00 %, 40.80 %, 51.40 % and 79.50 % inhibition of the volume of intestinal content respectively with significance (p<0.05). The weight of intestinal content was also reduced significantly at all the doses (Table 3).

Table 2: Effect of Extract on Intestinal Transit in Rats

S/N	Group	Dose (mg/kg)	Mean ± SEM Total length of Intestine (cm)	Mean ± SEM Movement of charcoal (cm)	% Intestinal Transition	% Inhibition
1	A	–ve control (saline)	93.40 ± 3.14^{a}	79.20 ± 5.13^{a}	84.40	00
2	B	200	93.40 ± 3.14^{a}	66.60 ± 10.73^{bc}	71.00	15.80
3	C	400	99.80 ± 1.99^{a}	49.00 ± 3.07^{ad}	49.00	41.90
4	D	800	85.80 ± 2.69^{a}	36.80 ± 0.97^{ab}	41.90	50.30
5	E	5 (loperamide)	88.00 ± 6.63^{a}	15.00 ± 2.89^{acd}	19.80	76.50

Mean with same letter no significant different (p>0.05).

Mean with different letters are significant different from each other (p<0.05).

Table 3: Effect of Extract on Castor oil-Induced Entropooling

S/N	Group	Dose (mg/kg)	Mean ± SEM Weight of intestine + Content (g)	Mean ± SEM Weight of empty intestine (g)	Mean ± SEM Weight of Content	% Inhibition
1	A	–ve control (saline)	6.82 ± 0.16^{a}	3.86 ± 0.27^{a}	2.86 ± 0.19^{a}	0.00
2	B	200	6.48 ± 0.45^{b}	4.52 ± 0.28^{b}	2.06 ± 0.16^{ab}	27.00
3	C	400	5.66 ± 0.86	4.02 ± 0.59^{c}	1.68 ± 0.07^{ac}	40.80
4	D	800	4.50 ± 0.51^{a}	3.02 ± 0.52	1.38 ± 0.09^{abd}	51.40
5	E	5 (loperamide)	3.54 ± 0.17^{ab}	2.16 ± 0.10^{abc}	0.58 ± 0.21^{abcd}	79.50

Mean of different letters significantly different from each other (p<0.05).

Discussion

Diarrhoea (loose motions) is the passage of 3 or more loss 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. Diarrhoea occurs due to the irritation within the lining of the small or large intestine, which leads decrease water absorption hence increase in water being passed with stools. Many factors such as food poisoning, infection (bacterial, viral, parasitic), food intolerance malnutrition, intestinal disease and some time 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 [17]. The plant extract at doses of MCE 200, 400 and 800 mg/kg significantly decreased ($p < 0.05$) the total number of wet faeces produced upon administration of castor oil at 800 mg/kg was comparable to the control group. The effect of the highest dose of the MCE extracts 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 decrease fluid absorption, increases secreation in the small intestine and colon and effects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinolic acid [18].

The propulsion of the charcoal meal through the gastrointestinal tract decreased significantly ($p < 0.05$) from MCE at 200, 400 and 800 mg/kg, compared to control group. Similarly, the effect of the highest dose of the MCE extracts of graded dose was similar to that of the standard drug. These observations suggest that the 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 *C. edulis* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effect [18, 19].

Conclusion

The methanol leaf extract of *Carissa edulis* has been shown to possess some antidiarrhoeal activity. Bio-guided assay of the isolated bioactive compound should be further studied. Likewise, characterization using physical technique such as ^{1}HNMR, ^{13}CNMR UV-visible and IR-Spectroscopy should be carried out in order to confirm the chemical structures of the bioactive constituents responsible for the antidiarrhoeal activity.

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