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Anti-Diarrhoeal Activity of *Azanza garckeana* Fruit Aqueous Extract Using Swiss Mice

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Abstract

Traditional medicine encompasses medical therapies, methods, theories, and practices that employ products derived from plants, animals, and minerals, as well as spiritual and manual therapies and exercises. This study aimed to evaluate the antidiarrheal potential of *Azanza garckeana* in Swiss mice. Different doses of the plant extract (25, 50, and 100 mg/kg) were assessed using castor oil–induced diarrhea and gastrointestinal transit models. The results showed that the extract produced a significant ($p < 0.05$) reduction in the frequency of defecation of wet feces and in total fecal output compared with the control group. At higher doses, the extract also elicited significant ($p < 0.05$) antimotility effects relative to untreated controls. These findings validate the ethnomedicinal use of *Azanza garckeana* as an effective antidiarrheal agent and indicate the need for further studies on compound isolation, characterization, and pharmacological evaluation.

Keywords: Antidiarrheal Activity; *Azanza garckeana*; Aqueous Extract; Castor Oil–Induced Diarrhea; Swiss Mice.

Introduction

It is often referred to as Goron Tula (kola of Tula) in Hausa, is a member of the Malvaceae family. It is solely grown in the Gombe State hamlet of Tula in Nigeria. It is a versatile fruit native to tropical Africa. It is a significant food and medicinal plant that is frequently used as herbal medicine in Northern Nigeria (Ahmed *et al.,* 2016). More than 20 human diseases and afflictions have reportedly been treated with *Azanza garckeana* in traditional medicine. According to Alfred (2017) and Glew *et al.* (2005), the plant is utilized as a herbal remedy for conditions like cough, chest pains, infertility, irregular menstruation, STDs, and hepatic impairments. Amino acids, alkaloids, ascorbic acid, carotenoids, flavonoids, glucosides, phenols, lipids, tannins, and saponins are only a few of the different kinds of bioactive metabolites that have been isolated from *A. garckeana* (Akinnifesi *et al.,* 2004).

English (common names): snot apple, azanza, and tree hibiscus. Local names include "goron tula" in Nigeria (Hausa), "morojwa" in Botswana, and "*Thespesia garckeana*" in South Africa (Jigan *et al*., 2017 Ochokwu *et al.,* 2014).

The ripe fruits are used in the treatment of anaemia (Ahmed *et al.,* 2016), when eaten raw or cooked to eat it's relish, it can be used to treat malaria, this was practiced in Zambia (Chinsembu, 2016). Application of the fruit poultice can be used to treat Abseccess (Ochokwu *et al.,* 2015 and Kumar *et al.,* 2015). The ripe fruits when taken orally is used for Aphrodisiac, this has been evaluated in Nigeria (Dikko *et al.,* 2016). Also the ripe fruits or root decoction when taken orally is used to cure Infertility (Soladoye and Oyesiku, 2008; Dikko *et al.,* 2016). The stem and leaf decoction when taken orally has shown to have effect in the treatment of liver problems (Ochokwu *et al.,* 2015). Leaf decoction of *A. garckeana* when taken orally is medicinally used for treating diabetes, edema and epilepsy (Amuri *et al.,* 2017). It's root infusion when administered orally has shown to have Antiemetic properties, to treat chest pain, cough and aids in retained placenta, it can also be used to treat earache by applying drops of the root infusion in the affected ear (Maroyi, 2013). The root decoction of *A. garckeana* when taken orally can be used to treat Fever, mental illness, membrane rupture, Syphilis and also used to induce labor (Esther *et al.,* 2017). It's root and stem bark when taken orally is used in the treatment of sexually transmitted disease and gonorrhea (Nkafamiya *et al.,* 2015). The root decoction mixed with Sterospermum kunthianum Cham is used to treat Asthma..

Diarrhoeal is typically defined as the passing of three or more stools in a 24-hour period that are sufficiently liquid to assume the shape of the container in which they are placed. There are causes of diarrhoeal outside underlying diseases. A liquid diet, food intolerance, stress, anxiety, and laxative use are a few examples. It is frequently a sign of an intestinal infection, which may be brought on by a number of bacterial, viral, or parasitic species. Poor hygiene can cause an infection to spread from person to person or through tainted food or drinking water (WHO, 2017). Although the majority of diarrhoeall bouts are self-limited (lasting a certain period of time and progressing at a constant rate of severity), diarrhoeall can occasionally cause life-threatening complications. Dehydration (when your body loses a lot of water), electrolyte imbalance (loss of sodium, potassium, and magnesium), and renal failure (not enough blood or fluid is delivered to the kidneys) are all effects of diarrhoeall (WHO, 2017).

Materials and Methods

Plant Collection and Authentication

Fresh fruit of Azanza garckeana was purchased from Tula village in Gombe state. The plant was identified by Dr. O. Timothy in the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria. The plant was authenticated by Dr. H. A. Akinnibosun in the Herbarium Unit of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria, with voucher specimen number UBH-A371.

Plant Preparation

Freshly prepared fruit of *Azanza garckeana* washed in distilled water and shade dried in a clean and organized environment maintained at room temperature. The plant materials were further dried using regulated oven at 40 ^{0}C for 24 hours before being pulverized using British mechanical grinder. Four thousand grams (1500 g), the pulverized fruit was extracted using 3,000 ml of distilled water using maceration technique. The extract was then concentrated into semi-solid (HH-S Water Bath; Search Tech Instruments) regulated at standard temperature (45 ^{0}C). Percentage yield were calculated via the formula (% Yield = extract weight / powder sample weight x 100/1).

Evaluation of antidiarrheal Activity

Twenty-five (25) adult Swiss mice weighing 28-32 g were applicable for the experiment. Animals were housed in wooden cages (five per cage) and maintained under controlled room temperature (25 ± 1°C) with relative humidity of 45-55% under 12: 12 hr light and dark cycle for one week with free access to food and water ad libitum. Every procedure with the use of animals obtained approval from the Institutional Animal Ethical Committee, and the experiment being carried out in conformity with Guidelines for CPCSEA, with ethical number issued (LS22016)

Castor oil-induced diarrhoeal in mice

Twenty-five (25) mice were fasted for 18 hours and randomly divided into five groups (n=5). The fruit extract (25, 50, and 100 mg/kg) were given orally to treated groups. The control group received 0.2 ml/kg body weight of distilled water and reference group received 3 mg/kg body weight loperamide. An hour later, the whole animals were predisposed to 0.5 ml/rat of castor oil orally via gavage. They were kept in a separate transparent plastic container with plain filter paper at the base (Masila *et al.,* 2015). The onset and severity of diarrhoeal was evaluated for 4 hours. Total number of feces (diarrhoeal and non-diarrhoeal) expelled were compared with that of the control group. Total score of diarrhoeal feces for control group was measured as 100%. Results were presented as percentage inhibition of diarrhoeal.

Gastrointestinal motility test

Swiss mice were randomly divided into five groups (5 per groups) and fasted prior to the study for 18 hrs with freely access to water. Control group received distilled water orally (0.2 ml/kg body weight); treated groups were given the fruit extract at graded doses of 25, 50, and 100 mg/kg body weight orally. The reference group received standard drug (5 mg/kg body weight of atropine sulphate) i.p. An hour later, each animal was administered with 1 ml castor oil. Thereafter charcoal meal (10 % activated charcoal in 5 % gum acacia) at 1 ml via oral route was giving an hour thereafter. The entire animals were humanly sacrificed an hour thereafter and the distance travelled in the small intestine by charcoal meal, from pylorus to caecum were estimated and evaluated via distance moved in percentage (Lawal *et al.,* 2016).

Data analysis

Results were analyzed with Graph pad prism version 6. Data was presented as Mean ± S.E.M, and statistical significance were calculated using One way ANOVA, followed by Dunnett's test where $p < 0.05$ were considered statistically significant.

Results

The results obtained from this study showed that *Azanza garckeana* fruit aqueous extract elicited an inhibitory effect on castor oil induced diarrhoeal across graded doses of the extract when compared with the untreated control, which displayed a significant decrease in the number of diarrheal as shown in Table 1, 2.

Table 1: Antidiarrhoeal effect of *Azanza garckeana* fruit aqueous extract in castor oil induced diarrhoeal in mice

Treatment Dose	Doses (mg/kg)	Onset of stool	Total number of diarrhoeal	Total number of dry stool	Weight of stool	% inhibition
Control	DW	18.67±0.95^{a}	7.67±0.23^{a}	7.33±0.76^{a}	0.80±0.11^{a}	0.00
Loperamide	3	72.67±5.71^{c}	3.00±0.15^{b}	3.00±0.55^{b}	0.57±0.03^{b}	60.89
AGFAE	25	31.33±1.62^{b}	7.00±0.58^{a}	5.67±0.33^{b}	0.53±0.03^{b}	8.74
AGFAE	50	48.00±2.70^{b}	7.00±0.55^{a}	5.00±0.58^{b}	0.70±0.10^{b}	8.74
AGFAE	100	66.67±3.85^{c}	4.67±0.67^{b}	3.33±0.33^{b}	0.67±0.16^{b}	39.11

Values were expressed as Mean ± SEM and the level of significant as *p-value < 0.05, showed* the level, DW---- distilled water, AGFAE --- *Azanza garckeana* fruit *aqueous* extract

Table 2 Antidiarrhoeall effect of *Azanza garckeana* fruit *aqueous* extract using charcoal meal in Swiss mice induced diarrhoeall in mice.

Treatment	Doses (mg/kg)	Total length of Intestine (cm)	Length travel by charcoal meal (cm)	Weight of intestine (g)	Peristalsis index
Control	DW	33.33±6.68	33.00±4.04^{a}	1.50±0.29	99.00±6.07^{a}
Atropine	5	44.83±5.18	13.33±1.83^{c}	1.27±0.63	29.74±1.46^{c}
AGFAE	25	42.67±5.18	20.00±3.22^{a}	1.17±0.60	46.87±1.68^{b}
AGFAE	50	41.00±2.51	14.00±3.71^{b}	1.17±0.58	34.15±12.56^{c}
AGFAE	100	47.67±2.03	23.33±2.96^{c}	1.20±0.61	48.94±6.57^{b}

To calculate for % peristalsis index (PI) = LM/LSI LM----length of charcoal meal, LSI----length of small intestine. Values were expressed as Mean SEM and the level of

significant as *p-value < 0.05, showed* the level, DW---- distilled water, AGFAE --- *Azanza garckeana* fruit *aqueous* extract.

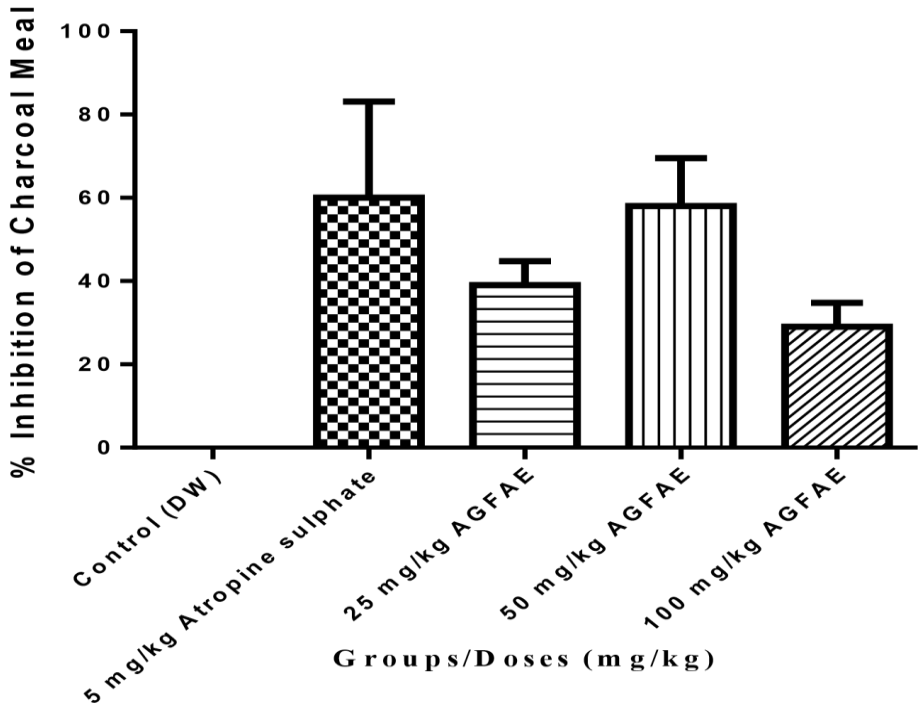

Figure 2: Percentage inhibition of *Azanza garckeana* fruit aqueous extract in charcoal meal induced-diarrhoeall in mice

Values were expressed as Mean SEM and the level of significant as *p-value < 0.05, showed* the level, DW---- distilled water, AGFAE --- *Azanza garckeana* fruit *aqueous* extract.

Discussion

This study was aimed to evaluate the antidiarrheal activity of the aqueous extract of *Azanza garckeana* fruit by using different experimental models of diarrhoeal in mice. In all models, diarrhoeal was induced by administering castor oil to each mouse. Castor oil produces diarrhoeal due to its active metabolite, a ricinoleic acid which is liberated by the action of lipases in the upper part of the small intestine (Lawal *et al*., 2019). It mediates its action by binding to EP3 prostanoid receptors on smooth muscle cells (Tunaru *et al.,* 2012) and facilitates the accumulation of fluid in the intestine by inhibiting absorption and enhancing secretion of fluid and electrolytes. Furthermore, this metabolite also alters the motility of GI smooth muscles. The secondary metabolites present in *Azanza garckeana* is implicated in its verse preventive, suppressive and curative measure, which is responsible for the many health benefit (Lawal *et al.,* 2015). The various phytochemicals included

alkaloids, tannins, flavonoids, saponins, sterols and terpenes, could be responsible for the anti-diarrheal property through promoting colonic water and electrolyte absorption (Zia-Ul-Haq *et al.,* 2012).

The results obtained from the graded doses of *Azanza garckeana* aqueous extract elicited a significant reduction in castor oil induced diarrhoeal when compared with the untreated control ($p < 0.05$). The inhibitory effect displayed by the extract indicated its effect to reduce diarrhea stool either by reducing the peristalsis movement leading to diarrhea by decreasing gastrointestinal tract movement, which the extract could possibly be involved by triggering or working through the path of sympathomimetic to cease wet stool. This finding agreed with the report of the study by Yilni *et al.* (2020) on aqueous extract of *Phoenix dactylifera* and Schiller (2017) specifically the aqueous and methanol fractions of *Lantana camara* linn. The maximal effect of the extract was similar to Loperamide, which is one of the most widely employed drugs against diarrhoeal disorder; as shown in present study Loperamide effectively antagonized diarrhoeal induced by castor oil (Umar *et al.,* 2019). The active metabolite of castor oil, ricinoleic acid, induces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins. The prostaglandins thus released stimulate secretion by preventing the reabsorption of sodium chloride and water. Thus, it is possible that the extract significantly inhibits gastrointestinal hypersecretion and entero-pooling by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid.

The study showed that the aqueous extracts of *A. garckeana* at a dose at 100 mg/kg exhibited a significant inhibition of castor oil-induced diarrhea in experimental mice, with a similar effect to that of Loperamide (standard drug), elicited a significant inhibition at a specific dose of 50 mg/kg of the extract in the gastro-intestinal motility test using charcoal. Tannins, alkaloids, saponins, sterols and terpenoids found in plants could be responsible for observed antidiarrhoeal activity. This concurred with the work of Schiller (2017) who specifically reported on the aqueous and methanol fractions of *Lantana camara* linn on diarrhoea using animal models

Conclusion

The results of this study revealed that *A. garckeana* displayed an inhibitory effect in the frequency of defecation and wet faecal excretion. Moreover, it also produces an

inhibitory effect on castor oil induced intestinal secretion and gastrointestinal propulsion. These antidiarrheal activities of the extract may be implicated due to the presence of certain phytochemicals. These findings provide a scientific support for a traditional use of the stem of *A. garckeana* as diarrhoeal remedy.

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