ANTI-DIARRHOEA ACTIVITY OF *Azanza garckeana* FRUIT AQUEOUS EXTRACT USING SWISS MICE

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CERTIFICATION

This is to certify that this undergraduate project work on the effect of *Azanza garckeana* on diarrhea was carried out by Ifechukwude Nonye Grace with matriculation number LSC1706042 in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this work to the God who sustained me all through the period of this work.

ACKNOWLEDGEMENTS

I will like to express my dearest thanks to my project supervisor, Dr. B.O. GABRIEL of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City for giving me this topic, guiding and supporting me throughout the beginning of this work to the very end.

To my H.O.D and amazing lecturers, this journey will not be complete without their endless effort, I honorably appreciate them.

I appreciate my parents, Mr. and Mrs. AUGUSTINE IFECHUKWUDE and my siblings who stood by me and provided all the moral, financial, spiritual and physical support I needed to make this a reality.

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ABSTRACT

Traditional medicine encompasses manual therapies, exercises, and spiritual therapies in addition to medical therapies, theories, and practices that involve medications derived from plants, animals, and minerals. This investigation aims to assess the ability of Azanza garckeana in treating diarrhea in Swiss mice. Castor oil-induced diarrhea and gastrointestinal transit models in mice were used to assess the antidiarrheal effects of various dosages of the plant extract (25, 50, and 100 mg/kg). According to the study's findings, the extract significantly ($p < 0.05$) reduced both the frequency of wet feces being defecated and the amount of feces produced overall when compared to the control group. In comparison to the untreated control, the extract triggered a substantial ($p < 0.05$) antimotility activity at higher doses. In conclusion, this study validated the ethnomedicinal report of Azanza garckeana as an efficacious anti-diarrhoea property, thereby required further study for compound elucidation and evaluation.

CHAPTER 1

INTRODUDUCTION

1.1 Background of Study

Traditional medicine encompasses manual therapies, exercises, and spiritual therapies in addition to medical therapies, theories, and practices that involve medications derived from plants, animals, and minerals. These methods may be used separately or in combination to treat, diagnose, prevent, or maintain health (Wyk *et al.,* 1999). Traditional medicine is described by the World Health Organization (WHO) as "the body of knowledge, skills, and practices based on theories, beliefs, and experiences that are indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness," whether these are based on explanations or not (World Health Organization, 2008).

Prior to the entrance of the Europeans, millions of Africans relied mostly on traditional medicine. The introduction of evidence-based medicine marked a significant turning point in the development of this tradition and culture (Abdullahi, 2011). In general, there is inadequate research and regulation around herbal medicines in Africa (Mills *et al.,* 2005). Traditional knowledge is currently incompletely documented and is typically passed down verbally. Misidentification or abuse of therapeutic plants can have serious negative repercussions (Helwig, 2005). Natural components are used in traditional therapies, which are highly significant. Traditional Korean medicine, traditional Chinese medicine, Ayurveda, Kampo, traditional

Korean medicine, and Unani are a few examples of medical systems that use natural treatments that have been practiced for hundreds or even thousands of years all over the world. These systems have grown into well-managed, organized healthcare systems. They may have defects in their many forms, but they nonetheless serve as a priceless archive of human knowledge (Fabricant and Farnsworth, 2001; Alves and Rosa, 2003).

In traditional medicine, just one herb or therapy may contain a number of phytochemical elements, such as alkaloids, terpenoids, flavonoids, etc. According to Parasuraman *et al.* (2014), these compounds typically work independently or in combination to provide the desired pharmacological effect. It is noteworthy that many of the plant-based medications used in modern clinical practice have their origins in traditional medicine (Li-Weber, 2009). Additionally, it has been established that traditional medicine is where many useful medications derived from plants were first discovered (Fabricant and Farnsworth, 2001). It is significant to highlight that while the explanation that follows gives a general overview of traditional medicine, it does not analyze every element and tradition connected to it in detail.

When viewed historically, traditional medicine has a long history that predates that of contemporary scientific medicine (Mark and Lyons, 2014). Traditional medicine has historical roots in prehistoric civilizations that used numerous natural cures and healing methods, including those in China, India, Egypt, and Mesopotamia Indigenous civilizations all around the world have created their own distinctive systems of healing based on their knowledge of the human body and how it interacts with the natural world. Systems of traditional medicine are often governed by a number of fundamental principles. Depending on the particular tradition, these guidelines may differ, however some recurring motifs can be found (WHO, 2005). The importance of a holistic approach to health, which recognizes the connection of the body, mind,

and spirit, is commonly emphasized in traditional medicine. Rather than concentrating only on treating symptoms or disorders, it emphasizes promoting general well-being. It also depends on organic compounds that come from minerals, plants, and animals to have therapeutic effects. For instance, herbal remedies make use of the therapeutic qualities of plant parts such leaves, stems, roots, and flowers (Bent, 2008). Traditional medicine frequently customizes therapies to the patient's particular needs, taking into account the patient's distinctive constitution, symptoms, and general state of health. The more uniform treatments that are frequently used in modern medicine contrast with this individualized approach (Tu *et al.,* 2002)

It has been practiced for many years and has proven beneficial in improving wellbeing and treating a variety of illnesses. There is evidence to support the therapeutic efficacy of traditional medicinal herbs, according to numerous studies that have looked into this topic. For instance, according to estimates from the World Health Organization (WHO), up to 80% of individuals in underdeveloped nations depend on traditional medicine for their primary healthcare (WHO, 2004). It is crucial to remember that traditional medicine's support is frequently based on historical and personal accounts rather than thorough scientific research. While some traditional treatments have received scientific approval, others might not have enough proof or may only have a basic grasp of how they work. The terms complementary/alternative/nonconventional medicine are used interchangeably with traditional medicine in some countries (WHO, 2000; Abdullahi, 2011)

1.2 AIM OF STUDY

The aim of studying the antidiarrheal activity of Azanza garckeana, also known as Gorontula is to determine if this plant or its extracts possess properties that can effectively treat or alleviate diarrhea.

1.3 OBJECTIVES OF STUDY

To study the antidiarrheal activity of Azanza garckeana includes:

Determininig if *Azanza garckeana* or its extracts can effectively reduce the frequency, duration, and severity of diarrhea.

Investigate how *Azanza garckeana* exerts its antidiarrheal effects. This involve studying its impact on intestinal motility.

Assess the safety profile of *Azanza garckeana*, including any potential side effects or toxicities.

Compare the antidiarrheal activity of *Azanza garckeana* with existing conventional treatments to evaluate its effectiveness relative to standard therapies.

Validate the established use of *Azanza garckeana* in folk medicine for treating diarrhea through scientific research.

These objectives aim to provide an in-depth knowledge of the potential advantages and mechanisms of Azanza garckeana in the treatment of diarrhea and contribute to evidence-based healthcare practices.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Azanza garckeana* (F. Hoffm.) Exell and Hillc

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 (Mojeremane and tshwenyane, 2004; Ochokwu *et al.,* 2014).

2.2 TAXONOMY OF *Azanza garckeana*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: Azanza

Specie: *Azanza garckeana*

Figure 2.1: Different parts of *Azanza garckeana.*

2.3 BOTANICAL DESCRIPTION

Azanza garckeana is a deciduous shrub that can reach heights of 3 to 15 meters, depending on the climate, and has a stem diameter of up to 25 centimeters at breast height. The tree has several stems that can be straight, crooked, or occasionally fork from the base (Orwa *et al.,* 2009). The bark is fibrous with longitudinal fissures and is tough, greyish-black or brown. The branches feature woody hairs, and the twigs are initially hairy before becoming smooth with age. The leaves are 8 by 12 cm and have distinctly rounded edges. They are always roundish, basic, and alternating. The leaves contain longitudinal fissures in the midrib and 3 to 5 lobes, each of which

is covered with brown star-shaped hairs (Orwa *et al.,* 2009). The leaf's tip is typically bluntly pointed or rounded. Five to seven nerves can be seen in the leaf's heart-shaped base. Dark colors characterize the young, velvety leaves. Large, solitary flowers that can reach a length of 6 cm bloom in the axils of the uppermost leaves. The capsules, which can be up to 4 cm long and 3 cm thick, have globular petals. According to Orwa et al. (2009), the hemispherical seeds are up to 10 mm long, 7 mm thick, and coated in 18 woolly floss that is brown in color. The globose fruit includes woody capsules that can be up to 3 or 4 cm in diameter. It is split into five sections, each of which has a seed.

2.4 ETHNOMEDICINAL PROPERTIES

Different parts of *A. garckeana* plant such as the roots, fruits, leaf and stem are used in the treatment of certain types of illnesses. 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 Msheila *et al.,* 2016). 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 (Morris, 1996; Soladoye and Oyesiku, 2008; Hedberg and Staugard, 1989; 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 and Maundu *et al.,* 1999). Leaf decoction of *A. garckeana* when taken orally is medicinally used for treating diabetes, edema and epilepsy (Maroyi, 2013; 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 (Gelfand *et al.,* 1985), it can also be used to

treat earache by applying drops of the root infusion in the affected ear (Gelfand *et al.,* 1985; Maroyi, 2011; 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 (Morris, 1996; Augustine *et al.,* 2011; Gelfand *et al.,* 1985; Esther *et al.,* 2017; Soladoye and Oyesiku, 2008). It's root and stem bark when taken orally is used in the treatment of sexually transmitted disease and gonorrhea (Morris, 1996; Nkafamiya *et al.,* 2015; Ndubani and Höjer, 1999). The root decoction mixed with Sterospermum kunthianum Cham is used to treat Asthma (Morris, 1996).

2.5 PHYTOCHEMICAL PROPERTIES

Tannin (0.22%), saponins (1.72%), alkaloids (3.70%), flavonoids (1.0%), phenols (2.60%), cyanogenic glucosides (0.33ug/g), and carotenoids (3.40%) are the phytochemicals found in *A. garckeana* seeds. Due to their diverse roles in medicine, nutrition, and genetics, these anti-nutrients are extremely important (Saka and Msousthi, 1994). According to Enzo (2007), tanins, phenols, saponins, alkaloids, and flavonoids may have a role in antibacterial and antiviral activities. Tannins and flavonoids boost colonic water and electrolyte absorption, while other phytochemicals work by limiting intestinal motility and some constituents have been demonstrated to inhibit specific enteropathogens, according to studies on the mode of action (Enzo, 2007). Similar to saponins, alkaloids are essential in *A. garckeana* seed due to their toxicity. *A. garckeana* seeds contain 3.70 mg/100 g of alkaloids and 1.72 mg/100 g of saponins, respectively, of these harmful byproducts. Saponins have a number of properties, including the capacity to bind cholesterol, the ability to hemolyze substances in aqueous solutions, and bitterness. Because of their innate ability to fight off germs, saponins are great candidates for

treating fungal and yeast infections. These substances acted as natural antibiotics, aiding the body's defenses against microbial invasion and illnesses (Sodipo *et al.,* 2000). Because *A. garckeana* contains saponins, it can be used medicinally to assist people fight fungus, bacteria, and viruses as well as increase the efficiency of killing specific types of tumor cells, particularly lung and blood cancers (Barakat, 1993).

Flavonoids are a class of widely dispersed polyphenolic chemicals that have a benzopyrone ring structure and have been shown to have antioxidant effects in a variety of biological systems. In addition to their antioxidant qualities, flavonoids have biological activities that include defense against cancers, free radicals, bacteria, ulcers, hepatotoxins, inflammation, and allergies (Barakat, 1993; Okwu, 2005). *A. garckeana* seed's ability to prevent cancer is attributed to the presence of flavonoids. For instance, flavonoids block estrogen synthetase, an enzyme that binds estrogen to receptors in several organs (Okwu, 2005; Ajayi, 2014). The presence of phenolic compounds helps to act as antimicrobial agent which makes the *A. garckeana* to be an antimicrobial agent (Ofokansi *et al.,* 2005). Tannins, which have a characteristically bitter taste, may be the cause of its mild bitter flavor. *A. garckeana* can therefore serve as a deterrent to rottenness.

2.6 PHARMACOLOGICAL ACTIVITIES

The plant is used as herbal remedy for diseases like cough, chest pains, infertility, menstruation abnormalities, sexually transmitted infections and hepatic impairments (Alfred, 2017; and Glew *et al.,* 2005). Azanza garckeana has been linked to a range of pharmacological actions that support some of its ethnomedicinal uses, according to published research.

2.6.1 Antimicrobial activity

Azanza garckeana extracts have shown antibacterial action against a variety of microorganisms, such as bacteria and fungi. According to Dikko (2016), Mutindi (2014), and Masila (2015), these characteristics point to its potential application as a natural antibacterial agent.

2.6.2 Antihyperglycemic

Azanza garckeana extracts may offer anti-diabetic activities, according to research (Amuri *et al.,* 2017). In experiments on animals, they were found to reduce blood glucose levels and enhance glucose tolerance. To determine their effectiveness and safety in treating diabetes in people, more research is necessary. Guinea pigs (*Cavia porcellus*) were given 500 mg kg of aqueous *A. garckeana* leaf extract to assess its hypoglycemic and antihyperglycemic effects, both under glucose baseline circumstances and in an oral glucose tolerance test with a 210-minute follow-up. According to Amuri *et al.* (2017), *A. garckeana* was active in the oral glucose tolerance test, inhibiting glycemia increase by 36.9% as opposed to glibenclamide's 50% hyperglycemic inhibition rate. This data supports the traditional use of *A. garckeana* leaf decoction as herbal medicine for diabetes in DRC (Amuri *et al.,* 2017).

2.6.3 Antimalarial activity

Antimalarial activities of aqueous and organic fractions of *A. garckeana* against Plasmodium falciparum was evaluated by Connelly *et al.* (1996). *A. garckeana* showed weak antimalarial activity with median inhibitory concentration which was >3 µg/mL (Mutindi, 2014). Antimalarial evaluations carried out by Connelly *et al.* (1996) demonstrated weak activities but such findings may imply that *A. garckeana* has bioactive constituents with potential in controlling mosquito vectors.

2.6.4 Antioxidant

Evaluated antioxidant potential of petroleum ether, ethyl acetate, acetone, methanol and water stem bark extracts of *A. garckeana* using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity (Mshelia *et al.,* 2016). The methanol stem bark extracts exhibited antioxidant activity with IC50 value of less than 100 µg mLG1 while acetone extracts exhibited activity with IC50 value of 160 µg mLG1 against the standard ascorbic acid activity with IC50 value of 220µg mLG1 (Mshelia *et al.,* 2016) These antioxidants activities of stem bark are probably due to the presence of flavonoids and phenolics (Ndhlala *et al.,* 2006). There is now a global trend towards the use of natural phenolics as antioxidants and functional ingredients due to their perceived safety and prevalence in wild edible fruits (Wu *et al.,* 2013).

2.6.5 Iron absorption

Iron absorption capability of aqueous extract of *Azanza garckeana* fruits *in vivo* by using everted gut sacs of Wistar albino rats evaluated by Ahmed *et al.* (2016) by administering 2 g/kg of *Azanza garckeana* aqueous extract to iron deficient rats for 3 weeks in a nutritional anemia experimental model. Administration of A. garckeana extracts caused slight alterations on hematological parameters of the nutritionally iron deficient rats except on red blood cells counts of these animals (Ahmed *et al.,* 2016). As a result, an in vitro model of iron absorption using *Azanza garckeana* extract revealed that it has characteristics that stimulated iron absorption. This effect may justify its use for treatment of iron deficiency anemia in Sudan (Ahmed *et al.,* 2016) as this plant contributes to enhancement of iron deficiency rather than providing the body with rich iron source. Therefore, this impact of *A. garckeana* extract may be related to its saponin concentration, which increases red blood cell synthesis and, consequently, their quantity.

2.7 DIARRHOEA

To meet the body's physiological requirements, the digestive system regulates the uptake and elimination of electrolytes and water. The 10 liters of fluid that enter the adult intestines each day are reabsorbed in a rate of more than 98 percent (Keusch, 2001). Normal feces often range in consistency from dry, firm pellets to softened, voluminous stools depending on the amount of leftover stool water, which is mostly connected to the indigestible fiber load. Individuals, days, and stools all have different levels of consistency. A standard definition of diarrhea is the passing of 3 or more stools in a day that are sufficiently liquid to take the shape of the container in which they are placed. This variance makes the definition more complicated. It is not diarrhoea when produced stool is passed frequently (Black and Lanata, 2002). Diarrhoea is characterized by loose, watery feces that occur more frequently than usual. The World Health Organization defines it as passing three or more loose or watery stools per day (or more frequently than is customary for the individual).

There are causes of diarrhoea outside underlying diseases. A liquid diet, food intolerance, stress, anxiety, and laxative use are a few examples. It is frequently an indicator of an intestinal infection, which may be caused by a variety of bacterial, viral, or parasite 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 diarrhoea bouts are self-limited (lasting a certain period of time and progressing at a constant rate of severity), diarrhoea 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 diarrhoea (WHO, 2017). Along with excrement, diarrhoea leads to the loss of electrolytes and water. To regain the lost fluids, you

must consume enough of liquids as dehydration can be life-threatening if it does not improve, worsens, or is not properly treated

2.8 CAUSES OF DIARRHOEAL

A number of diseases and conditions can cause diarrhoea, including:

2.8.1 Viruses

Among the viruses that can cause diarrhea include the norwalk virus, also known as the norovirus, enteric adenoviruses, astronucleoviruses, cytomegaloviruses, and viral hepatitis. The rotavirus is the most frequent cause of severe childhood diarrhea. The virus that causes coronavirus disease 2019 (COVID-19) has also been related to constipation, nausea, vomiting, and diarrhea.

2.8.2 Bacteria and parasites

Diarrhea is caused by ingesting contaminated food or drink that contains parasites or harmful microbes like E. coli. Traveler's diarrhea is a common name for diarrhea caused by germs and parasites in developing countries. Clostridioides difficile is another type of bacterium that can cause diarrhea. It may occur before, during, or following an antibiotic course.

2.8.3 Medications

A wide variety of medications, including antibiotics, can cause diarrhea. When used to treat illnesses, antibiotics eradicate both good and bad microorganisms. This can lead to diarrhea or another sickness like Clostridioides difficile by upsetting the usual bacterial balance in your intestines. Other drugs that cause diarrhea include antacids that include magnesium and anti-cancer drugs.

2.8.4 Intolerance to lactose

A sugar called lactose is present in milk and other dairy products. Those who have trouble processing lactose experience diarrhea after consuming dairy products. Because levels of the enzyme that aids in the digestion of lactose decline as you age, lactose intolerance may worsen.

2.8.5 Synthetic sweeteners

Some otherwise healthy people may experience nausea from artificial sweeteners such sorbitol, erythritol, and mannitol, which are non-absorbable sugars used in chewing gum and other sugar-free products.

2.8.6 Surgery

A surgical procedure to remove the gallbladder or a portion of the intestine can occasionally cause diarrhea. Additional gastrointestinal issues: There are various additional digestive disorders that can result in persistent diarrhea, in addition to IBS, Crohn's disease, ulcerative colitis, celiac disease, microscopic colitis, and small intestinal bacterial overgrowth (SIBO).

2.9 TYPES OF DIARRHOEAL

There are three distinct clinical kinds of diarrhea: acute watery diarrhoea, which can include cholera and last for a few hours or days; acute bloody diarrhoea, commonly known as dysentery; and persistent diarrhoea, which can linger for at least 14 days.

2.10 TREATMENT

Maintaining electrolyte and fluid balance is essential to controlling diarrhea (Gauchan and Malla, 2015). Pedialyte, Gatorade, or diluted fruit juice should be offered to patients. When diarrhea is more severe, IV fluid rehydration may be necessary (Santos, 1986). Foods with less fiber may contribute to firmer stools. Bananas, toast, oatmeal, white rice, apple sauce, and soup/broth make comprise the bland "BRAT" diet that Delate *et al.* (2013) found to be well tolerated and may aid with symptoms. Anti-diarrheal medication, such as anti-secretory or anti-motility medicines,

may be used to reduce stool frequency. However, they should be avoided by adults who have bloody diarrhea or a high temperature since they may worsen serious intestinal illnesses. Probiotic medication has been shown to decrease the intensity and duration of symptoms in patients with severe diarrhoeal disease. Chronic diarrhea is managed differently depending on the etiology (Schiller, 2017). To begin with, diarrhea must be categorized as either watery, fatty, or inflammatory. After categorization, the next level of management can be selected using an algorithm. Most often, additional fecal testing, lab work, or imaging are required. More invasive procedures like colonoscopies or higher endoscopies might be required (Delate *et al.,* 2013).

The Centers for Disease Control (CDC) released recommendations in 2003 for the management of acute diarrhoeal in children on an outpatient and inpatient basis, including referral criteria (King *et al.,* 2003). Among the medications used to treat diarrhoeal include bismuth subsalicylate and loperamide (brand name Imodium). Antibiotics may be recommended if a bacterial infection is the cause of the diarrhoeal. For bacterial diarrhoeal, common drugs include ciprofloxacin (Cipro), azithromycin (Zithromax), and metronidazole (Flagyl). Probiotics are given for some specific types of diarrhoeal, and oral rehydration solution, which has a precise balance of fluids and electrolytes, can help restore lost fluids and minerals brought on by diarrhoeal (King *et al.,* 2003).

2.11 PLANTS USED IN THE TREATMENT OF DIARRHOEAL

Both traditionally and pharmaceutically, the use of plants for disease treatment has grown considerably in popularity. Intriguingly, medicinal plants have played important roles throughout human history. It has been determined that ethnobotany, the scientific study of the traditional knowledge of using plants for traditional, medical, or religious purposes, is the best method for

finding amazing medicines (Heinrich *et al.,* 2004). Drinking calming herbal teas like catnip, chamomile, fennel, lemon, orange, rosemary, and peppermint tea is strongly advised during the healing process. Additionally, affected individuals should try to eat smaller meals more frequently. The use of specific potent plant species for treating and halting diarrhea was studied by researchers like Laloo and Hemalatha, (2011). The following are a few of these plants: *Cinnamomum tamala*, *Bauhinia Acuminata, Aegle marmelos*, Guava (*Psidium guajava*), Yarrow (*Achilea millefolium*), Soursop (*Annona muricata*), Carob tree (*Ceratonia siliqua*), Agrimony (*Agrimonia eupatoria*), Astragalus (*Astragalus membranaceus*), *Picrorhiza Kurroa*, Goldenseal (*Hydrasis canadensis*), Barberry (*Berberis vulgaris*), Psyllium (*Plantago ovata*), Chinese bayberry (*Myrica rubra),* Peppermint (*Mentha piperita*), *Moringa oleifera, Pongamia pinnata* and *Acorus calamus* (Laloo and Hemalatha, 2011).

Tannin is a naturally occurring polyphenol biomolecule that can be found in plants, fruit skins, seeds, leaves, bark, and wood. It is a potent compound present in these strong plants. Proteins and numerous other organic substances, including amino acids and alkaloids, bind to and precipitate out as tannin (tannoid), an astringent. When it comes to tightening and contracting human tissue, tannin's presence in these potent plants is a major factor. This leads to fluid retention and abrupt stoppage to diarrhoea (Laloo and Hemalatha, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 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.

3.2 Plant Preparation

Azanza garckeana fruit that has been freshly prepared was cleaned with distilled water, and shade-dried in a spotless setting that is kept at room temperature. Before being ground up using a British mechanical grinder, the plant materials were further dried using a controlled oven at 40 ^{0}C for 24 hours. Using the maceration procedure, 3,000 ml of distilled water was used to extract four kilograms (1500 g) of the fruit that had been ground up. The extract was subsequently condensed into a semi-solid state at a controlled standard temperature (45 ^{0}C) using a Search Tech Instruments HH-S Water Bath. The formula (% Yield = extract weight / powder sample weight x 100/1) was used to determine percentage yield.

3.3 Evaluation of antidiarrheal Activity

For the experiment, twenty-five (25) adult Swiss mice weighing 28–32 g were suitable. Five animals per cage were housed in wooden cages, and they were given unlimited access to food and water. The temperature was maintained at a constant(25 ± 1°C) with a relative humidity of 45–55%. Each technique involving the use of animals was approved by the institutional animal ethical committee, and the experiment was carried out in accordance with CPCSEA guidelines.

3.4.1 Castor oil-induced diarrhoeal in mice

Twenty-five (25) mice were split into five groups at random (n=5) after fasting for 18 hours. The treatment groups were given oral doses of the fruit extract (25, 50, and 100 mg/kg). 0.2 ml/kg of distilled water was given to the control group whereas 3 mg of loperamide was given to the reference group. The entire animals were then subjected to 0.5 ml/rat of castor oil administered orally 1 hour later. They were kept in a separate transparent plastic container with plain filter paper at the base (Awouters *et al.,* 1987; Okoh *et al.,* 2016). It took four hours to assess when the diarrhea started and how bad it was. In comparison to the control group, the total number of feces—both diarrheal and non-diarrheal—expelled was counted. The control group's overall diarrhoeal feces score was measured as 100%. The percentage of diarrhoeal inhibition was used to display the results.

3.4.2 Gastrointestinal motility test

Swiss mice were randomly splitted into five groups, with five mice in each group, fasted for 18hrs prior to the study and were given free access to water. The treatment groups were administered oral doses of the fruit extract at 25, 50, and 100 mg/kg body weight, respectively, while the control group was given distilled water (0.2 ml/kg body weight). The reference group

received standard drug (5 mg/kg body weight of atropine sulphate) administered intraperitoneally. An hour thereafter, 1 ml of castor oil was administered to each animal. An hour later, a charcoal meal (5% gum acacia, 10% activated charcoal) was administered orally in a dose of 1 ml. 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 (Pazhani *et al.,* 2001).

3.5 Data analysis

Graph Pad Prism version 6 was used to examine the results. Data were displayed as Mean S.E.M., and statistical significance was determined using One-way ANOVA, followed by Dunnett's test, where $p<0.05$ was determined as statistically significant.

CHAPTER FOUR

RESULTS

When compared to the untreated control, which displayed a significant decrease in the number of diarrheal, the findings of this study revealed that *Azanza garckeana* fruit aqueous extract elicited an inhibitory effect on castor oil induced diarrhoea across graded doses of the extract as expressed in Table 3.1

Table 3.1: Antidiarrhoeal effect of *Azanza garckeana* fruit aqueous extract in castor oil induced diarrhoeal in mice

Treatment	Dose mg/kg	Onset of stool (sec)	Total number of stools	Number of diarrhoea	Weight of stool (g)
Control	DW	18.67±0.95[a]	7.67±0.23[a]	7.33±0.76[a]	0.80±0.11[a]
Loperamide	3	72.67±5.71[c]	3.00±0.15[b]	3.00±0.55[b]	0.57±0.03[b]
AGFAE	25	31.33±1.62[b]	7.00±0.58[a]	5.67±0.33[b]	0.53±0.03[b]
AGFAE	50	48.00±2.70[b]	7.00±0.55[a]	5.00±0.58[b]	0.70±0.10[b]
AGFAE	100	66.67±3.85[c]	4.67±0.67[b]	3.33±0.33[b]	0.67±0.16[b]

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

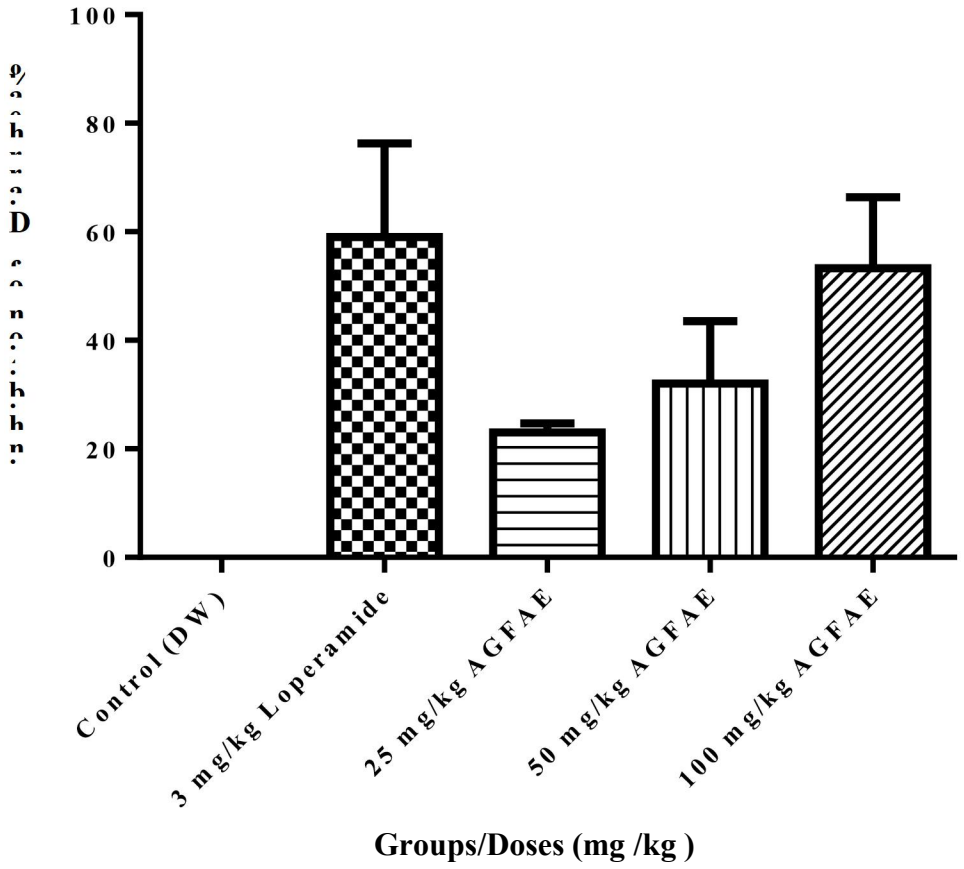

Figure 3.1: Percentage inhibition of *Azanza garckeana* fruit aqueous extract in castor oil induced diarrhoeal in mice

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.

Table 3.2 Antidiarrhoea effect of *Azanza garckeana* fruit *aqueous* extract using charcoal meal in Swiss mice induced diarrhoea in mice.

Treatment	Dose 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.

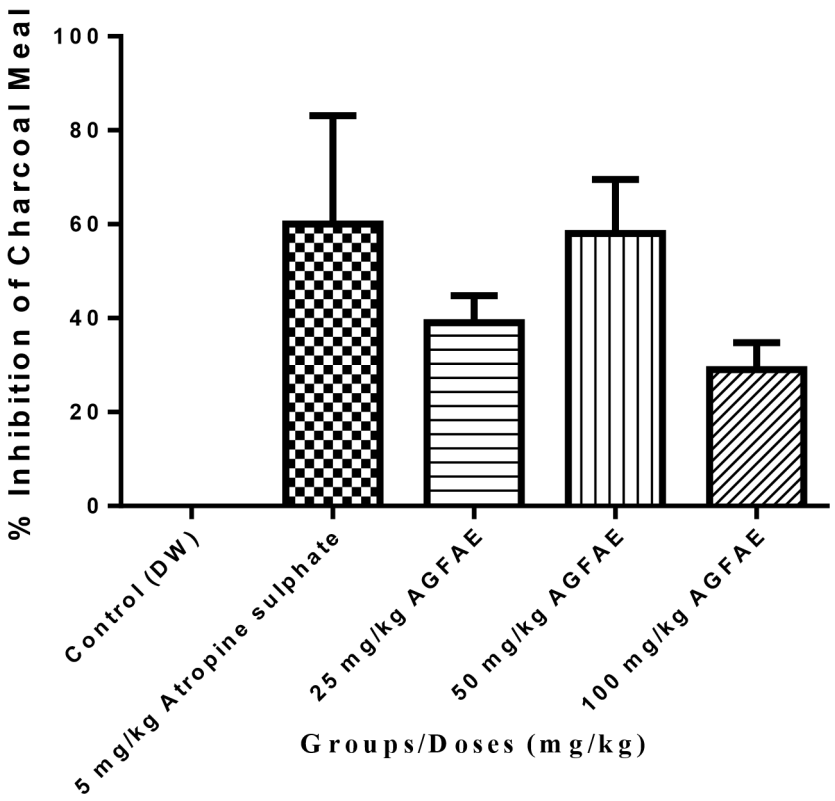

Figure 3.2: Percentage inhibition of *Azanza garckeana* fruit aqueous extract in charcoal meal induced-diarrhoea in mice

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.

CHAPTER FIVE

5.0 Discussion

This study utilized various experimental models of diarrhea in mice to assess the antidiarrheal activity of the aqueous extract of *Azanza garckeana* fruit. In every model, Castor oil was administered to each mouse to cause diarrhoea. 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 (Kulkami and Pandit, 2005). 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 (Racusen and Binder, 1979). Furthermore, this metabolite also alters the motility of GI smooth muscles (Matias *et al.,* 1978). 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 to 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 Abel *et al.* (2013) on aqueous extract of *Phoenix dactylifera* and Getnet *et al.* (2015) 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 (Begum *et al.,* 2013). Ricinoleic acid, the active metabolite of castor oil, causes intestinal mucosal irritation and inflammation, which triggers the generation of prostaglandin. The prostaglandins thus released stimulate secretion by preventing the reabsorption of sodium chloride and water (Pierce *et al.,* 1987). As a result, it's probable that the extract greatly reduces gastrointestinal hypersecretion and entero-pooling by enhancing reabsorption of water and electrolytes or by preventing artificially-induced accumulation of fluid in the intestines.

The research demonstrated that the aqueous extract of *A. garckeana* significantly inhibited castor oil-induced diarrhea in experimental mice at a dose of 100 mg/kg, with a similar effect to that of Loperamide (standard drug). The extract also significantly inhibited the gastro-intestinal motility test using charcoal at a specific dose of 50 mg/kg. The antidiarrheal activity that has been discovered may be due to tannins, alkaloids, saponins, sterols, and terpenoids that are found in plants.

This was in agreement to the findings of Getnet *et al.* (2015) on the effects of *Lantana camara linn's* aqueous and methanol fractions on diarrhoea in animal models.

CONCLUSION

According to the study's findings, *A. garckeana* had a suppressive effect on both frequency of defecation and moist faecal excretion. Additionally, it inhibits intestinal secretion and gastrointestinal propulsion brought on by castor oil. The presence of certain phytochemicals may

be a factor in the extract's antidiarrheal properties. These findings offer evidence in favor of the traditional usage of *A. garckeana* stem as a diarrhea treatment.

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