ANTI-DIARRHOEAL ACTIVITY OF *Chrysobalanus icaco* (COCO PLUM) FRUIT METHANOL EXTRACT USING SWISS MICE

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2023.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY. IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECNIQUES)

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CERTIFICATION

This is to certify that the undergraduate research work on anti-diarrhoeal activity of *Chrysobalanus icaco* (coco plum) fruit methanol extract using Swiss mice was carried out by OLADIMEJI BOLANLE TEMITOPE with the matriculation number LSC1706116 in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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EXTERNAL SUPERVISOR **DATE**

DEDICATION

I dedicate solely this project to God Almighty for the privilege to grace throughout the research.

ACKNOWLEDGEMENTS

I hereby acknowledge God Almighty for giving me the grace, opportunity and strength necessary for the completion of my undergraduate research work.

My heartfelt gratitude goes to my selfless supervisor, DR. B. O. GABRIEL. Thank you so much for the patience, guidance and time you spared for me during the period of research. Your effort and sacrifices have ushered, into reality, this project. May God bless you richly.

Also my profound appreciation to the Head of Department, DR. E. O. OSHOMOH and the entire staff of the Department of Science Laboratory Technology.

To my late dad (my mirror), MR BAYODE OLADIMEJI, my ever-stunning mum (my golden heroine), MRS KEMISOLA OLADIMEJI, and all the great men and women who have impacted in my life. I have come this far because of your unflinching financial and moral support, constant motivation, and inspirational messages. Thank you very much for everything you have sacrificed for my sake towards my educational pursuit, and ultimately for believing in my dreams. God abundantly bless you.

Finally, I acknowledge all and sundry who in one way or the other contributed towards a successful completion of this research. God bless you all.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

ABSTRACT

Diarrhoea is the excretion or passage of watery stools at least twice or three times within 24-hour. Traditional medicines are largely underused as a source of health care. Insights and innovative approaches from traditional medicine can also directly impact the economy and public health. The aim of this study is to evaluate the anti-diarrhoeal activity of *Chrysobalanus icaco* (coco plum) fruit methanol extract in Swiss mice. Diarrhoea was induced in Swiss mice using castor oil model. Parameters such as consistency, frequency, and duration of stool were investigated and compared with the control groups. The results obtained from this study showed that *C. icaco* fruit methanol extract elicited an inhibitory effect in castor oil induced diarrhoea across graded doses of the extract when compared with the untreated control. Also, a significant decrease in the number of diarrhoea compared with the untreated group $p < 0.05$). In conclusion, the findings of this study demonstrated that *C. icaco* fruit methanol extract possessed a significant anti-diarrhoeal activity. However, further research is warranted to elucidate the underlying molecular mechanisms and to determine its long-term safety and efficacy in human subjects.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The World Health Organization (WHO) defines traditional medicine as the body of knowledge, abilities, and procedures derived from theories, beliefs, and experiences that are unique to particular cultures and used to maintain health as well as to prevent, identify, and treat physical and mental illnesses (Che *et al*., 2017). The method includes massage, spiritual treatments, and other locally and culturally specialized techniques in addition to medicines made from plants, animals, and minerals (Chali *et al*., 2021).

Traditional medicines are largely underused as a source of health care. Insights and innovative approaches from traditional medicine can also directly impact the economy and public health (Graz *et al*., 2011). In order to provide the primary healthcare needs of 65 to 80 percent of the world's population, traditional medicine is used (Pan *et al*., 2014). Additionally, they are crucial in providing communities with treatment. (Ozioma and Chinwe, 2019). Accessibility, cost, money, educational level, effectiveness, and lack of access to contemporary health services were the main justifications for utilizing TM. (Tizazu *et al*., 2020). The diversity of Ethiopian civilizations is reflected in the ancient medical methods used there. The Ethiopian population used traditional medicine to cure illnesses and promote overall health. Ethiopia's drug laws and health policies take traditional medicine into account (Kassaye *et al*., 2006). The oldest medical practice in existence, TM is used to cure and prevent both physical and mental diseases. A range of health- and life-threatening disorders were traditionally fought by different communities using a variety of practical treatment techniques. TM is also referred to as complementary and

alternative medicine, ethnic medicine, or other names, and it continues to be very important in many nations today (Abdullahi, 2011).

WHO estimated that a sizable portion of the global population still relies on TMs for medical care (Parasuraman *et al*., 2014), distinct nations have distinct laws governing TM at the moment. The entire market size of the TCM sector in 2012 was roughly equal to that of the Chinese pharmaceutical sector (Lu, 2013). According to research, 80% of people in Africa utilize TM, either alone or in conjunction with western treatment (Boakye *et al*., 2015). In contrast, due to the popularity of mainstream treatment, traditional Aboriginal medicine in Australia is in danger of disappearing (Oliver, 2013). In Israel, a country with a diverse ethnic population, modern medicine is dominating and traditional medicine is dwindling (Lev, 2006). Although the majority of people worldwide use these TM systems, many Western medical professionals believe that they lack reliability (Parasuraman *et al*., 2014). The value of TM cannot be undervalued in the study and creation of contemporary pharmaceuticals. Despite its cryptic nature, it has a wide range of applications in non-Western medical technology or practices. In Traditional Chinese Medicine, a single herb or formula may contain a variety of phytochemical components, including alkaloids, terpenoids, flavonoids, etc. Generally speaking, these substances work individually or in combination to provide the desired pharmacological effect (Parasuraman *et al*., 2014). It is noteworthy that TM (Li-Weber, 2009) served as the source for many of the plant-based medications used in clinical practice today. Additionally, it has been established that the numerous beneficial medications made from plants were found by their use in TM (Fabricant and Farnsworth 2001). As part of the WHO's TM Program, a thorough analysis of the pharmacopoeias of developed and developing countries as well as the related international scientific literature was carried out about 20 years ago. The purpose of that investigation was to ascertain whether modern drug discoveries had in fact been inspired by TM and whether there

was any connection between various chemicals' use today and their use in TM. The study proved that TM had in fact played a sizable part in the development of efficient new drugs by focusing on diverse components utilized in drugs produced from plants in various countries. In that study, 122 compounds were examined, and it was shown that 94 plant species were the source of these chemicals, 80% of which were linked to medicinal effects in folk medicine (Fabricant and Farnsworth 2001).

The acceptance, practicality, and accessibility of TMs have been and will continue to be beneficial for the study of novel drugs (Ngo *et al*., 2013). Modern medications based on TMs include artemisinin and other antimalarial medications, as was previously mentioned. Doctor Hong Ge (AD 284–384) wrote in his book Zhou Hou Bei Ji Fang about the effectiveness of *Artemisia annua* L. in treating malaria at the beginning of China's Jin Dynasty. This is the earliest instance of Artemisia annua L. being used to cure malaria that has ever been found, and it demonstrates how advanced Chinese physicians were in terms of medical treatment 1700 years ago (Zhao *et al*., 2012; Li and Zhou, 2010). The study of artemisinin, also known as qinghaosu in Chinese, has advanced significantly. New artemisinin analogs and derivatives have been created, and investigations into the biological activities and associated mechanisms have also been made. As a result, artemisinin and its potent derivatives are widely used as novel anti-malarial medications throughout the world (Li, 2012).

1.2 AIM OF THE STUDY

To evaluate the anti-diarrhoeal activity of *Chrysobalanus icaco* (coco plum) fruit methanol extract on laboratory swiss mice.

1.3 OBJECTIVES

- Induce experimental diarrhoea in laboratory swiss mice using a suitable method (castor oil-induced) to mimic the condition of interest.
- Evaluate the anti-diarrhoeal activity of *Chrysobalanus icaco* fruit methanol extract by measuring and comparing parameters such as stool consistency, frequency of defecation, and weight of feces between the control and treatment groups.
- Assess the effect of *Chrysobalanus icaco* fruit methanol extract on gastrointestinal motility using methods such as intestinal transit time or charcoal meal test.
- Examine the potential mechanisms of action involved in the anti-diarrhoeal activity of *Chrysobalanus icaco* fruit methanol extract, including its effect on intestinal fluid secretion, electrolyte balance, and gut microbial composition.
- Compare the anti-diarrhoeal efficacy of *Chrysobalanus icaco* fruit methanol extract with a standard anti-diarrhoeal drug to establish its relative effectiveness.
- Propose potential applications and future directions for the development of *Chrysobalanus icaco* fruit methanol extract as a natural anti-diarrhoeal agent based on the research findings.
- Summarize and present the study results in a comprehensive report, including statistical analysis and data interpretation, to contribute to the scientific knowledge on the anti-diarrhoeal properties of *Chrysobalanus icaco* fruit methanol extract.

CHAPTER 2

LITERATURE REVIEW

2.1 *Chrysobalanus icaco* (Coco Plum)

There are many names for *C. icaco*, including cocoplum, gbafilo, fat-pork, icaco, cacco, abajeru, grageru, zicaque, icacillo, and caramio, to mention a few. It is utilized all over the world for both medicinal and culinary purposes. (Araujo-Filho *et al.,* 2016; Prance, 1972; Davies and Zibokere, 2011). Due to its considerable plasticity to establish itself in different plant associations and soils with high salt concentrations, its range is fairly extensive. Tropical regions, low woods, beach vegetation, and mangroves with sandy soils are where it is most commonly found naturally. It appears as a little tree with leaves that are alternating, simple, leathery, orbicular in shape, bright green in color, and highly variable in size. Its height can range from 1.5 to 7 m, and its width can be between 5 and 20 cm. The fruit is a drupe that is 2 to 5 cm long. It exposes one or, less frequently, two seeds with flat-convex cotyledons. The pulp is white and cottony, connected to the seed, soft, and tasty (Espinosa-Osornio *et al*., 2002). Both immature and ripe fruits can be found in the same bunch, and it is typically accessible from spring through summer. The immature fruits are green, and as they ripen, they take on pink, purple, and white tones (Brown and Frank, 2018).

2.1.1 Taxonomy Hierarchy

Kingdom - Plantae

Subkingdom - Viridiplantae

Infrakingdom - Streptophyta

Superdivision - Embryophyta

Division - Tracheophyta-

Subdivision - Spermatophytina-

Class - Magnoliopsida

Superorder - Rosanae

Order - Malpighiales

Family - Chrysobalanaceae

Genus - Chrysobalanus

Species - *Chrysobalanus icaco* L.

2.1.2 Ethnobotanic use of *Chrysobalanus icaco*

It is a fruit with low levels of domestication, and it is primarily semi-cultivated in backyard gardens as an attractive plant, a shade tree, or as a living fence. Given that it is used to cure malaria, gastrointestinal disorders, fever, and hypertension, it has significant economic, nutritional, and medical importance in Africa. Natives of Arraial do Cabo, Ilha do Cardoso, Marudá, Pará State, Recife, Pernambuco, Rio de Janeiro, and other Brazilian cities use the leaves, stems, and roots as infusions. Even in conventional markets, *C. icaco* is offered for sale as a medicine, mostly to treat diabetes and high cholesterol. The fruits, roots, stem, or leaves are produced as an infusion for the treatment of inflammations, chronic diarrhoea, bleeding, abdominal pain, dysentery, and other conditions in Brazil. (Araújo-Filho *et al.,* 2016; Feitosa *et al.*, 2012). Fruit, leaf, root, and stem infusions are used to heal stomach disorders and bleeding in El Salvador and Trinidad (Castilho and Kaplan, 2011). The medical benefits of these fruits should be widely known because research might be done to enhance the lives of those who consume them and to capitalize on the transformation business, which would increase revenue for the area.

Figure 1: Immature green fruits and leaves of *Chrysobalanus icaco*

2.1.3 Phytochemicals from *Chrysobalanus icaco*

From the fruits, leaves, roots, seeds, and stems of *C. icaco*, many secondary metabolites have been extracted and identified. The majority of research has been on phenolic chemicals, particularly flavonoids like anthocyanins and flavonols. Myricetin and its glycosylated derivatives are among the most prevalent flavonoids and are regarded as chemotaxonomic indicators of *C. icaco* (Paracampo *et al*., 2017). According to Da Silva *et al*., (2013), *C. icaco* leaves contain gallic acid 0.45 mg/g, myricetin 0.78 mg/g, and quercetin 0.14 mg/g, as well as total flavonoids 6.64 ±1.08 mg CE/g and total phenolic compounds 51.30± 2.71 mg GAE/g. This distinguishes it from several aromatic and medicinal plants extensively utilized in international gastronomy and ethnopharmacology (Skerget *et al*., 2005; Katalinic *et al*., 2006). According to De Brito *et al*., (2007), the majority of anthocyanins in *C. icaco* fruits come from delphinidin, petunidin, and peonidin, with 100 mg/100 g DW, 367 mg/100 g DW, and 25 mg/100 g DW, respectively. Venancio *et al*., (2017) isolated glycosylated and acylated anthocyanins from *C. icaco* fruits, focusing on the delphinidin 3-(6″-acetoyl) galactoside or delphinidin 3-(6″-oxaloyl) arabinoside (1,396 g/mL), delphinidin-3-glucoside (1,162 g/mL), peonidin 3-(6″-acetoyl) glucoside or peonidin 3-(6″-oxaloyl) arabinoside (689 µg/mL), petunidin 3-(6″-acetoyl) galactoside or petunidin3-(6″-oxaloyl) arabinoside (611 µg/mL), cyanidin 3-glucoside (382 µg/mL), and petunidin 3-glucoside, peonidin 3-glucoside (345 µg/mL).

Barbosa *et al*., (2006) found through HPLC/MS glycosylated flavonols in the leaves of *C. icaco*, specifically derivatives of myricetin and rutin like myricetin 3-O-glucoronide, myricitrin, myricetin 3-O-rutinoside, and quercitrin. These compounds have demonstrated antioxidant, antiobesogenic, and hypoglycemic activity (White *et al*., 2016a). Castilho and Kaplan (2011) extracted the antibacterial compound 7-O-methylkaempferol from the leaves of *C. icaco*. This compound proved effective against S. aureus and S. pyogenes.

2.1.4 Chemical composition of *C. icaco*

2.1.4.1 *Mineral constituents*

Minerals have a critical role in maintaining the permeability of cell membranes, muscular contraction, heart function, blood clotting, and the production of protein and red blood cells (Aremu *et al*., 2005). The most prevalent minerals in *C. icaco* seeds are calcium, potassium, magnesium, and sodium, with values of 93.4, 340, 173, and 30 mg/g, respectively (Aguiar *et al*., 2011). Numerous physiological processes, including blood clotting, bone and tooth development, muscular contraction, and the activity of specific enzymes, all depend on calcium (Aremu *et al*., 2005). Prothrombin's transformation into thrombin is aided by it. Numerous other enzymes, including adenosine triphosphatase, succinate dehydrogenase, and lipase, are also activated by calcium (Soetan *et al*., 2010). While magnesium activates numerous enzyme systems and maintains the electrical potential in the nerves, sodium and potassium are needed to keep the osmotic balance of body fluids, the pH of the body, and the control of glucose homeostasis (Barbagallo *et al*., 2007; Palmer & Clegg, 2016). The seeds contain 2.9 mg/g of iron, which is necessary for the correct operation of the central nervous system (Riccardi & Brown, 2010) and promotes the oxidation of carbohydrates, proteins, and lipids (Aremu *et al*., 2005). In addition to being a cofactor for several enzymes involved in neurotransmitter packing, it also participates in their production (Ponka, 2000; Beard, 2001). Lactate dehydrogenase, DNA, and RNA polymerase are just a few of the enzymes that benefit from zinc's (0.8 mg/g) role as a cofactor and component. It has also been suggested that zinc-dependent enzymes play a role in the metabolism of macronutrients and cell division (Jurowski *et al*., 2014). Therefore, eating a diet high in cocoplums could be an affordable way to get these necessary elements.

2.1.4.2 Macronutrients constituents

The amount of amino acids in the seed is somewhat modest, with serine (0.18 mg/g) and aspartic acid (0.18 mg/g) being present in larger concentrations. They can, however, be employed as biomarkers to separate the seed from others (Aguiar *et al*., 2011). A 100 g serving of *C. icaco* seed contains 24.9 g of total carbohydrates, 1.58 g and 1.76 g of reducing and non-reducing sugars, and 19.8 g of insoluble fiber (Aguiar *et al*., 2011). As a result, the seed may act as a low-cost supply of fibre, improving satiety and improving laxative action (Dhingra *et al*., 2012; Marlett & Fischer, 2003). It could lessen the mutagenic and carcinogenic potential of undesirable compounds (Unnati *et al*., 2013) as well as cholesterol and glucose levels (Gunness and Gidley, 2010).

2.1.5 Pharmacological properties of *C. icaco*

Numerous studies have reported the different pharmacological properties of *C. icaco*.

2.1.5.1 Anticancer and antitumor properties

After being treated with 20 μg/mL for 48 hours, the anthocyanins isolated from *C. icaco* exhibit specific chemo-preventive effect against proliferative HT-29 cancer cells (Venancio *et al*., 2017). Furthermore, it was demonstrated that a triterpene (pomolic acid) extracted from *C. icaco* was efficient against the human erythroleukemia K562 cell line and inhibited the growth of Lucena 1, a vincristine-resistant derivative of the K562 cell line exhibiting various drug resistance traits (Fernandes *et al*., 2003). High-performance liquid chromatography-mass spectrometry analysis was utilized to further analyse the anthocyanin content of *C. icaco* fruit. The results showed that the most prevalent (Venancio *et al*., 2017). anthocyanin present was delphinidin 3-(6″-oxalyl) arabinoside, with 1396 μg/mL. Generally speaking, anthocyanins cause cancer cells to undergo apoptosis by increasing the generation of reactive oxygen species (ROS).

2.1.5.2 Anti-diabetic properties

A study by de Oliveira *et al*., (2013) found that giving *C. icaco* leaves as an aqueous extract lowered fasting blood glucose levels when compared to metformin at the same dosage (400 mg/kg). This was also consistent with another study that found that giving *C. icaco* leaves orally to alloxan-induced diabetic mice for 33 days significantly decreased their fasting blood glucose levels (White *et al*., 2016).

When *C. icaco* fruit extracts (2 mL/kg body weight) were given orally to streptozotocin-induced diabetic rats over a period of 18 days, the animals not only lost weight but also experienced a 19% drop in fasting blood glucose (Nayak *et al*., 2011). This plant may have antidiabetic properties because of its α-amylase inhibitory activity, which lessens the conversion of polysaccharides to glucose. It might possibly be because the plant has antioxidant properties that lower the quantity of free radicals produced when a person has diabetes (de-Oliveira *et al*., 2013).

2.1.5.3 Anti-obesity properties

Research indicated that through increasing muscle glucose uptake and fat consumption, the aqueous extracts of *C. icaco* might decrease weight gain and improve the lipid profile of diet-induced obesity in mice (Portela-de-Sá *et al*., 2020) and Wistar rats (White *et al*., 2016). This could be as a result of its increased ability to mobilize muscle and burn fat and glucose, which prevents further fat from being absorbed and deposited (White *et al*., 2016).

2.1.5.4 Antioxidant properties

According to a prior study, *C. icaco* leaves' aqueous extract had strong antioxidant activity and substantially lower half-maximal inhibitory concentration values than those of seven other plants (de-Silva Port's *et al*., 2012). Additionally, it has been demonstrated that the leaves reduce oxidative stress in kidney damage caused by doxorubicin (Venâncio *et al*., 2012). In addition to comet assay biomarkers, these were thought to be caused by the

inhibition of decreased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex activities and increased ROS-scavenging ability (de-Oliveira Barbosa *et al*., 2013). These also significantly reduce DNA fragmentation in bone marrow and erythrocytes.

Myricetin, a phytochemical commonly utilized as a chemotaxonomic marker in the Chrysobalanaceae family, has been identified by some research as the primary phytochemical in *C. icaco* leaves (de Oliveira *et al*., 2014). It's one of the substances that gives *C. icaco* its antioxidative qualities. Its significance in averting oxidation and acting as strong ion scavengers of free radicals has thus been established (Adonizio *et al*., 2006; Nayak *et al*., 2011). Thus, the presence of phytochemical components like flavonoids and terpenes may account for the antioxidant action shown by plant extracts. The flavonoid content of *C. icaco*'s leaves and fruits, such as rutin, quercitrin, and myricitrin, significantly increased the plant's antioxidant capacity (Araújo-Filho *et al*., 2016).

2.1.5.5 Anti-inflammatory properties

It has been established that the water-based extract of *C. icaco* stem bark has anti-inflammatory qualities (de-Oliveira *et al*., 2014). Mice's edema during the phases of the inflammatory response was significantly reduced when aqueous extracts of *C. icaco* were administered orally. This characteristic was linked to the suppression of several pro-inflammatory mediators, including nitric oxide, histamine, and prostaglandins (Araújo-Filho *et al*., 2016). A study conducted on rats treated with acetic acid also shown the anti-inflammatory properties of aqueous extracts of *C. icaco* leaves in the treatment of cramps and stomach pain (Morris, 2003; Posadas *et al.,* 2004). When mice were given an aqueous extract of *C. icaco* leaves at doses of 100–400 mg/kg body weight, their pain-response behaviours were significantly reduced, as compared to the control group, at all treatment levels (Guzzo *et al*., 2008).

The inhibition of pro-inflammatory enzymes like lipoxygenase, cyclooxygenase-2, and nitric oxide synthase may be the mechanism behind *C. icaco*'s potential anti-inflammatory effect, which would mitigate the inflammatory process (de-Oliveira *et al*., 2014).

2.2 DIARRHOEA

Diarrhoea is a type of gastrointestinal infection that can be transmitted from person to person due to poor hygiene or by a number of bacterial, viral, and parasite organisms in tainted food or water (Peter and Umar, 2018). Diarrhoea generally lasts several days if untreated. Diarrhoea has been defined as an increase in the volume, fluidity, and pace of faeces production with minor consistency alterations. The evaluation of stool frequency along with the measurement of stool fluid content serves as a diagnostic sign. The World Health Organization (WHO) defined diarrhoea as the excretion or passage of watery stools at least twice or three times in a 24-hour period. However, other factors, including stool frequency, consistency, and the ability of parents to recognize diarrhoea in their children, are crucial in determining whether or not diarrhoea has actually occurred (Peter and Umar, 2018).. Regardless of frequency or consistency, blood in the stool can frequently be used to diagnose acute diarrhoeal diseases or dysentery.

2.2.1 Main Categories

There are three main categories of diarrhoeal disorders: acute, chronic, and persistent. The most frequent type of diarrhoea disorder, acute diarrhoea frequently begins suddenly, is brought on by infections, and is treated or cleared within 14 days (Peter and Umar, 2018). Chronic diarrhoea typically results from birth abnormalities in the body's digestive and absorbing systems and lasts for at least 14 days. When problems like malnutrition are present, subsequent infections frequently cause persistent diarrhoea. Children under the age of five are thought to have 2.5 billion bouts of diarrhoea each year, and estimates indicate that total

incidence has been largely steady over the last two decades (Peter and Umar, 2018).. More than half of these instances are found in Africa and South Asia, regions where diarrhoeal illnesses are more likely to cause fatalities or other serious consequences. The age of a kid and the seasons have a significant impact on the prevalence of diarrhoeal illnesses. The most vulnerable kids are the little ones: The first two years of life are when the incidence is highest, and it decreases as a child becomes older. Diarrhoea continues to be the predominant factor in both morbidity and mortality among children in underdeveloped nations (Peter and Umar, 2018). Even though the condition is easily treatable with oral rehydration therapy (ORT), diarrhoea diseases are a major source of illness and death among young children and are the third highest cause of child mortality and infant fatalities in poor and middle-income nations (Peter and Umar, 2018).. Exposure to infections that cause diarrhoea is commonly linked to drinking tainted water, preparing food improperly, and improperly disposing of human waste. Diarrhoea and its treatment are a top issue for health services because to the high cause-specific mortality rate and the availability of a successful treatment (Peter and Umar, 2018).

2.2.2 THE MAIN CAUSATIVE AGENTS OF DIARRHOEA

The most common causes of diarrhoea are listed below:

2.2.2.1 Bacterial infections: Diarrhoea brought on by enteric bacterial infections is a major issue worldwide, particularly in tropical and developing nations, and affects both new-borns and young children as well as older children and adults. A very wide variety of germs, including *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *vibrios*, and *Clostridium difficile*, are responsible for the disease (Gracey, 1996).

2.2.2.2 Viral infections*:* One of the most frequent causes of severe diarrhoea is rotavirus.

Other viruses, such as enteric adenoviruses, caliciviruses, astroviruses, the Norwalk virus, and viruses similar to the Norwalk virus, may also play a significant role in the development of diarrhoea in humans (Gracey, 1996). Parasites can settle in the digestive

system after entering the body through food or drink. *Giardia lamblia, Entamoeba histolytica*, *Cyclospora cayetanensis*, and *Cryptosporidium* are some of the parasites that can cause diarrhoea. Some people find it difficult to digest certain food ingredients, such as the sugar lactose found in milk or the gluten present in wheat and barley. A few types of laxatives, antacids, and antibiotics (including clindamycin, cephalosporins, and sulfonamides). Intestinal diseases such as celiac illness or inflammatory bowel disease. Functional bowel disorders, for example, irritable bowel syndrome, where the intestines do not function appropriately (Gracey, 1996). .

2.2.3 TRANSMISSION ROUTES

Faecal-oral transmission, which includes consuming tainted food or water, direct contact with faeces, and person-to-person contact, is how infectious diarrhoea is spread. When indoor water storage facilities or/and water sources are contaminated (equivalent to domestic domain and public domain contamination), transmission patterns for water-borne diarrhoea occur (Jessen *et al*., 2004; Jessen *et al*., 2002). The residential environment is where diarrhoea is most frequently transmitted (Jessen *et al*., 2004).

There are four ways for the main infectious agents to spread to human hosts through the environment: from human to human, from human to human multiplying in the environment, from human to human via animal, and from animal to human via animal (Curtis *et al*., 2000). The majority of instances of endemic disease likely originate either through human-to-human transmission or from the human-to-human transmission of pathogenic agents that have multiplied in the environment in settings when faecal pollution of the household environment is significant.

2.2.4 TREATMENT STRATEGIES

The goal of any anti-diarrhoeal medication is to replace or minimize fluid and electrolyte loss, reduce stool frequency and any other symptoms such as stomach pain, reduce faecal losses,

and eventually reduce disease duration and severity (Collise and Nomalungelo, 2012). As a result, the use of oral rehydration solutions (ORS) to restore fluid and electrolyte loss in diarrhoeic patients is a must for effective treatment. There are several formulas of these solutions, but the fundamental constituents are water, electrolytes (such as salt), and glucose. The sodium/glucose co-transport proteins on the brush border cells of the intestinal lumen pull sodium and glucose from the gut into the cells, which is how they work (Collise and Nomalungelo, 2012). Water is reabsorbed from the gut into the body as cellular osmotic pressure rises. This action balances electrolytes and hydrates the patient. Rice starch and other concentrated carbohydrates are also being included in modern ORS formulations, with the benefit that a greater amount of cellular substrate will also drive active salt absorption, resulting in symptom alleviation. A combination of ORS and zinc has also been reported to relieve diarrhoeal symptoms and hasten recovery in many patients around the world. This medication is being promoted because it may be a method to reduce unnecessary antibiotic use, particularly in youngsters. Furthermore, a 10–14-day course of zinc during and after diarrhoea has been shown to reduce disease recurrence in the next 2-3 months (Collise and Nomalungelo, 2012). Despite the alleviation provided by ORS, one of the key challenges limiting their use in developing-world rural and semi-urban regions is a lack of parental understanding about their use. It is also challenging to successfully deliver the medication to patients whose purging episodes are accompanied by vomiting. In this instance, professional medical assistance may be required to provide intravenous fluid replacement. Such personnel are hard to come by in African rural villages (Collise and Nomalungelo, 2012).

2.2.4.1 Anti-Motility and Anti-Secretory Agents

Anti-motility drugs (for example, loperamide and diphenoxylate-atropine combos) work by increasing intestinal transit time and increasing the potential for fluid and electrolyte re-

absorption (Collise and Nomalungelo, 2012). However, due to the possibility of central nervous system side effects, these drug classes are normally not recommended for children and young babies. Bismuth salicylate's anti-secretory qualities have been proven to reduce the number of unformed stools by roughly 50% in patients with travellers' diarrhoea. Bismuth salicylate possesses antibacterial and anti-inflammatory characteristics in addition to its anti-secretory capabilities, making it an excellent choice for the treatment of diarrhoea. However, due to its large pill burden, delayed onset of action, and the existence of unpleasant side effects such as tinnitus and black tongue, this medicine is not a popular choice (Collise and Nomalungelo, 2012).

2.2.4.2 Antimicrobial Therapy

Antimicrobial therapy decreases the severity of accompanying symptoms such as fever and stomach pain while also shortening the duration of the illness and preventing complications. It also reduces secondary cases by preventing the transfer of diarrhoeic germs from person to person (Collise and Nomalungelo, 2012). However, due to the possible concerns of drug resistance, side effects, and treatment cost, the use of antibiotics in the treatment of diarrhoea is being treated with care. There is also concern that antibiotic medication may worsen patients' clinical conditions due to its effect on gut bacteria. Most antibiotics are only advised for the treatment of acute bloody diarrhoea in children. When prescribing antibiotics for diarrhoea, doctors must be aware of not only the most likely bacteria, but also their antimicrobial susceptibility patterns and safety profiles (Collise and Nomalungelo, 2012).

2.2.4.3 Treatment with Indigenous Herbal Medicines

Medicinal cures made from indigenous plants are virtually always the only readily available and economical treatments for diarrhoea control in many developing-world rural areas. Extracts, decoctions/concoctions, or ashes of various plant components (roots, rhizomes, tubers, aerial portions, stem barks, and leaves) are used as treatments for diarrhoea and other ailments in these tribes (Collise and Nomalungelo, 2012). Most of these plants' anti-diarrhoeal properties have been scientifically confirmed, with isolated active components, according to the literature. Many of the plants' anti-diarrhoeal effect has been linked to the presence of tannins, alkaloids, saponins, flavonoids, steroids, and/or terpenoids. However, only a few of these molecules have made it into pharmaceutical shelves as anti-diarrhoeal medications following years of development and clinical review (Collise and Nomalungelo, 2012).

These findings offer pharmacological support to the anecdotal, ethnomedical usage of medicinal herbs as diarrhoea treatments and highlight the need for additional research in this field. Only around 20-30% of the world's 350,000 plant species have been properly studied, and only about 5-10% are currently known to be utilized in traditional medicine. Plants are responsible for at least two out of every ten medicines prescribed in hospitals, with the majority of them discovered through the use of indigenous medicinal plants (Collise and Nomalungelo, 2012). It is also predicted that at least seven out of every ten cancer medications have been generated from medicinal plants. South Africa is home to 10% of the world's terrestrial plants, many of which are utilized as herbal medicines and remain relatively undiscovered. As a result, there is an urgent need for thorough research on South Africa's flora since it holds the promise of discovering novel treatments and making substantial contributions to the country's health care system (Collise and Nomalungelo, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Collection and Authentication

Fresh fruit of *Chrysobalanus icaco* was purchased from Uselu market Egor LGA, Benin city Edo 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-C201.

3.2 Plant Preparation

Freshly prepared fruit of *Chrysobalanus icaco* was rinsed 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 0C for 24 hours before being pulverized using British mechanical grinder. Four thousand grams (1800 g), the pulverized fruit was extracted using 4,000 ml of absolute methanol 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).

3.3 Evaluation of anti-diarrhoeal 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.

3.3.1 Castor oil-induced diarrhoea in mice

Twenty-five (25) Swiss 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 of 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 (Awouters *et al*., 1987; Okoh *et al*., 2016). The onset and severity of diarrhoea was evaluated for 4 hours. Total number of faeces (diarrhoeal and non-diarrhoeal) expelled were compared with that of the control group. Total score of diarrhoeal faeces for control group was measured as 100%. Results were presented as percentage inhibition of diarrhoea.

3.3.2 Gastrointestinal motility test

Twenty-five (25) Swiss mice were randomly divided into five groups (5 per groups) and fasted prior to the study for 18 hours with free 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. 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 afterwards 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).

3.4 Data Analysis

Results were analysed 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.

CHAPTER FOUR

RESULTS

The results obtained from this study showed that *Chrysobalanus icaco* fruit methanol extract elicited an inhibitory effect on castor oil induced diarrhoea across graded doses of the extract when compared with the untreated control, which displayed a significant decrease in the number of diarrhoea as shown in Table 4.1

This effect was found to be statistically significant ($p < 0.050.001$). In the present study, the potential anti-diarrhoealeffect of *Chrysobalanus icaco* fruit methanol extract was evaluated using a castor oil-induced diarrhoea mice model (Table 4.1).

Table 4.1 Anti-diarrhoeal effect of *Chrysobalanus icaco* fruit methanol extract in castor oil induced diarrhoea 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	7.67±0.23[a]	7.33±0.76[a]	0.80±0.11
Loperamide	3	72.67±5.71	3.00±0.15[c]	3.00±0.55[c]	0.57±0.03
CIFME	25	52.67±5.84	5.67±0.33[b]	4.33±0.67[b]	0.57±0.14
CIFME	50	57.00±3.00	4.33±0.88[b]	2.00±0.15[b]	0.37±0.02
CIFME	100	35.00±1.53	6.67±0.22[b]	5.00±1.00[b]	0.80±0.06

Values were expressed as Mean ± SEM and the level of significant as *p-value < 0.05,* showed the level, DW---- distilled water, CIFME --*Chrysobalanus icaco* fruit methanol extract

The bar chart illustrated the significant inhibitory effect of *Chrysobalanus icaco* fruit methanol extract on castor oil-induced diarrhoea in mice. The extract demonstrated a dose-dependent response, with varying concentrations of the extract. The highest dose of the extract exhibited the most substantial inhibitory effect, with a percentage inhibition. The results highlighted the potential of fruit methanol extract as an effective anti-diarrhoeal agent. The observed dose-dependent response suggests a direct relationship between extract concentration and anti-diarrhoeal activity. These findings are consistent with the traditional use of *C. icaco* in various medicinal practices for gastrointestinal disorders. The positive outcomes of this study provide a foundation for further investigations into the active compounds within the *C. icaco* fruit methanol extract responsible for its anti-diarrhoeal effects. Additionally, these results underscore the potential of *C. icaco* as a valuable natural source for the development of novel therapeutic interventions against diarrhoea.

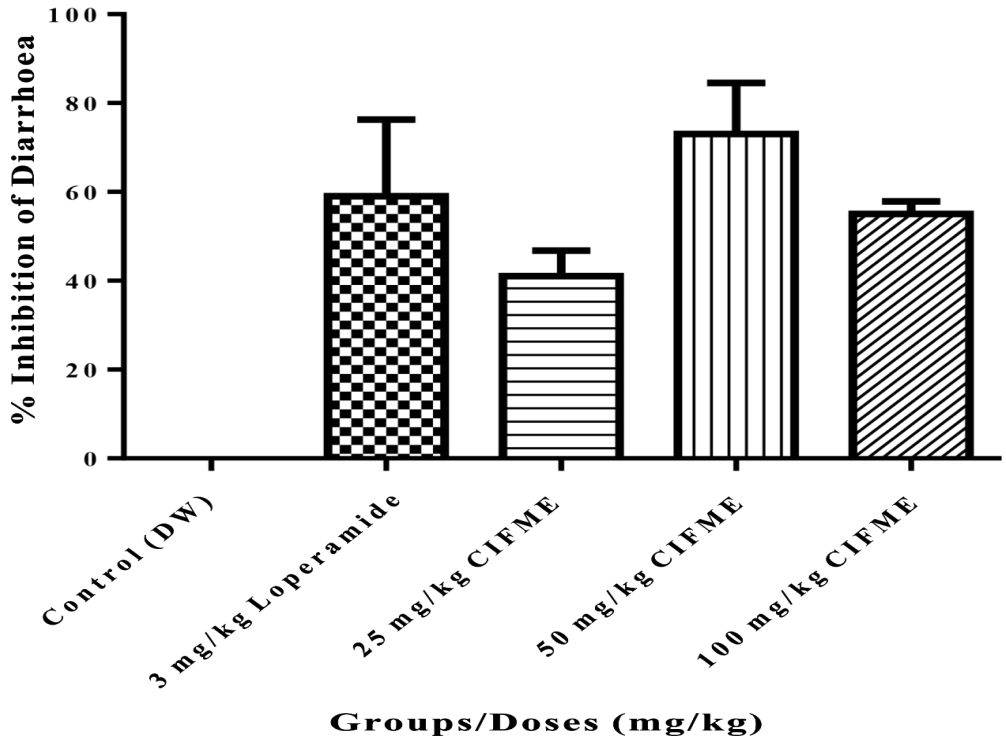

Figure 4.1: Percentage inhibition of *Chrysobalanus icaco* fruit methanol extract in castor oil induced diarrhoea in mice. Values were expressed as Mean ± SEM and the level of significant as *p-value* < *0.05,* showed the level, DW---- distilled water, CIFME --*Chrysobalanus icaco* fruit methanol extract.

The effects of *Chrysobalanus icaco* fruit methanol extract on key parameters associated with charcoal meal-induced diarrhoea in mice. The "Distance Travelled by Charcoal Meal" in the intestine, indicated the peristalsis motility of the gastrointestinal tract. The "Percentage inhibition of diarrhoea illustrated the extent of diarrhoea prevention achieved by the extract. The percentage inhibition of diarrhoea highlighted the extract's ability to mitigate diarrhoea induction at lowest dose (25 mg/kg), had a better inhibitory effect. The observed dose-dependent responses support the extract's efficacy in reducing diarrhoea -associated symptoms and suggest its potential therapeutic relevance in treating diarrhoeal disorders.

Table 4.2: Anti-diarrhoeal effect of *Chrysobalanus icaco* fruit methanol extract in charcoal meal induced-diarrhoeal 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]
CIFME	25	45.00±2.75	8.67±0.78[c]	1.47±0.75	19.27±4.28[c]
CIFME	50	39.00±2.52	17.17±1.59[c]	1.03±0.52	44.03±2.22[b]
CIFME	100	44.00±1.53	19.50±3.18[c]	1.27±0.64	44.32±1.62[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, CIFME --*Chrysobalanus icaco* fruit methanol extract

The effectiveness of *Chrysobalanus icaco* fruit methanol extract as an anti-diarrhoeal agent evaluated on charcoal meal-induced diarrhoea in mice. The results showed the effects of the extract in graded dose when compared with the controls. At highest dose (100 mg/kg), the extract exhibited a noticeable reduction in the distance travelled by the charcoal meal along the intestine, indicated an inhibitory effect in gastrointestinal tract motility. This reduction was pronounced at lower doses (25 and 50 mg/kg) of the extract, indicated a dose-dependent trend. At 25 mg/kg of the extract a significant delay in the onset of diarrhoea stool when compared with the untreated control.

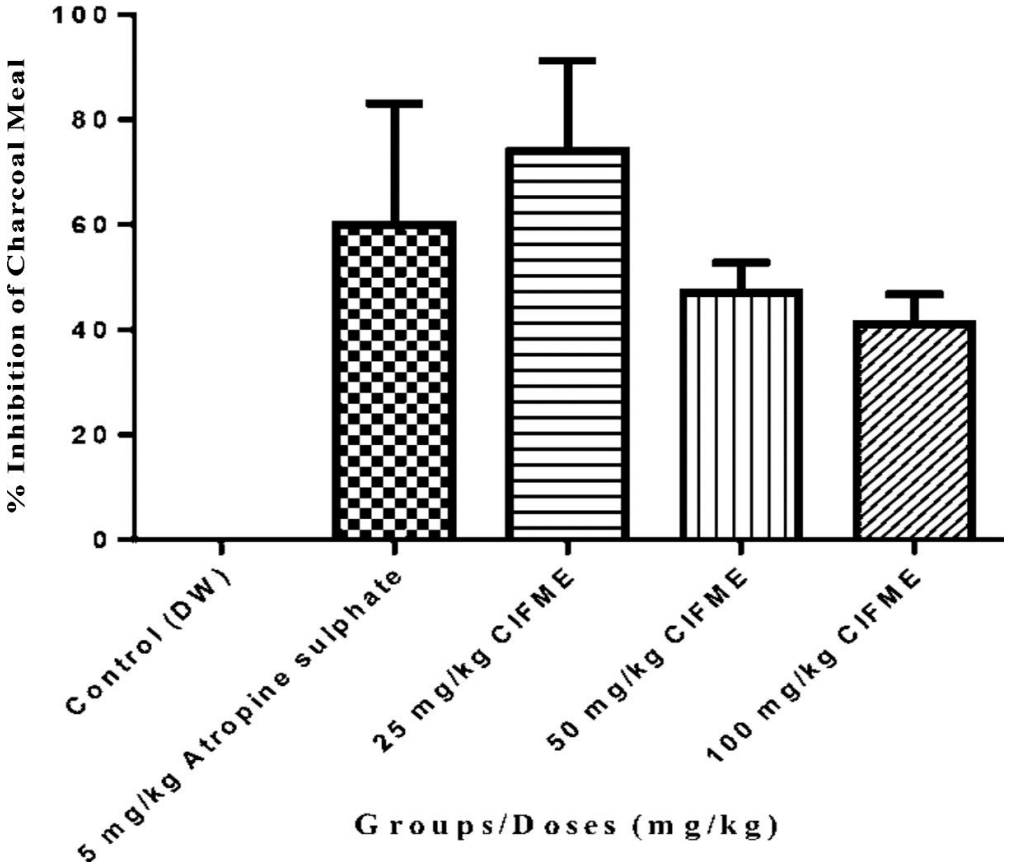

Figure 4.2: Percentage inhibition of *Chrysobalanus icaco* fruit methanol extract in charcoal meal induced-diarrhoeal in mice. Values were expressed as Mean ± SEM and the level of significant as *p-value* < *0.05,* showed the level, DW---- distilled water, CIFME --*Chrysobalanus icaco* fruit methanol extract

CHAPTER FIVE

DISCUSSION

Diarrhoea is a common gastrointestinal disorder that can lead to dehydration and electrolyte imbalances if left untreated. Natural products have long been of interest in the search for effective and safe anti-diarrhoeal agents. *Chrysobalanus icaco*, a plant known for its medicinal properties, was selected for this study due to its traditional use in treating diarrhoea in some folk medicine practices. The investigation of potential mechanisms of action revealed that the extract likely exerts its anti-diarrhoeal effects through multiple pathways. The castor oil induced diarrhoea model is frequently used to assess the anti-diarrhoeal properties of medications. Ricinoleic acid is the substance in the oil that is the most active. The intestinal mucosa becomes inflamed and irritated when exposed to ricinoleic acid. The irritation alters the intestinal mucosa's electrolytic permeability by enhancing the small intestine's peristaltic activity. Prostaglandins can be released as a result of this cascade events, which promoted motility, secretion and reduced the absorption of sodium and potassium ions (Dash *et al*., 2014). This present study, elicited that, the fruit methanol extract of *Chrysobalanus icaco* at graded doses (25, 50 and 100 mg/kg) inhibited the severity of castor oil and charcoal meal induced diarrhoea (Table 4.1, 4.2 and Figure 4.1, 4.2). Due to castor oil and the restriction of prostaglandin release, it could be implicated that the extract possibly had a preventive measure in lowering electrolyte permeability in the intestine. The extract's suppression on the intestinal fluid build-up may also indicate the reasons, for the gastrointestinal function inhibition (Dash *et al*., 2014). Previous studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants could be due to the presence of tannins, alkaloids, saponins, flavonoids, sterol and triterpenes (Dash *et al*., 2014). The anti-diarrhoeal properties of the *C. icaco* fruit methanol extract could be implicated owing to the

presence of flavonoids, which have shown to possess antioxidant properties (Araújo-Filho *et al*., 2016). The comparative analysis of graded doses of the extract with the standard anti-diarrhoeal drug (loperamide) showed that the extract's efficacy was comparable at 50 and 100 mg/kg CIFME indicated its potential effect as an alternative treatment for diarrhoea. The study also explored the potential involvement of oxidative stress and antioxidant activity in mediating the anti-diarrhoeal effect of the extract. Further investigations into the extract's antioxidant potential and its ability to scavenge reactive oxygen species could provide valuable insights into its mechanism of action.

CONCLUSION/RECOMMENDATION

In conclusion, the findings of this study demonstrate that *Chrysobalanus icaco* fruit methanol extract possesses a significant anti-diarrhoeal activity across the animal model used. The extract's ability to reduce stool frequency, improve stool consistency, and modulate gastrointestinal motility suggested its potential as a natural anti-diarrhoeal agent. However, further research is warranted to elucidate the underlying molecular mechanisms and to determined its long-term safety and efficacy in human subjects. The results of this study contribute to the growing body of knowledge on the medicinal properties of *Chrysobalanus icaco* and pave the way for the development of novel and effective anti-diarrhoeal treatments from natural sources.

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