ANTI-DIARRHOEIC ACTIVITY OF THE AQUEOUS EXTRACT OF ORANGE (CITRUS SINENSIS) FRUIT PEELS IN CATOR OIL-INDUCED DIARRHOEA MODEL IN MICE, AND THE EFFECTS OF CITRUS SINENSIS EXTRACT ON RED BLOOD CELLS.

ANTIDIARRHEAL ACTIVITY

By

Davidson Chijioke NKWOCHA

LSC1705889

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY,

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

DECEMBER 2022

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, UNIVERSITY OF BENIN, BENIN CITY: IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.SC) HONOURS.

DECEMBER 2022

CERTIFICATION

We certify that this work was carried out by Davidson chijioke NKWOCHA of the department of Plant Biology and Biotechnology, Faculty of Life Sciences, University Of Benin, Benin City, Edo State, Nigeria.

Dr. O. Timothy ---------------------------

Project supervisor Signature/Date

Dr. Joseph Erhabor --------------------------

Project Coordinator Signature/Date

Prof. E. O. Akpaja --------------------------

Head of department Signature/Date

DEDICATION

This project work is dedicated to God Almighty for his indefinite mercies, love and goodness over me and for granting me the grace to accomplish it.

ACKNOWLEDGEMENT

I am grateful to almighty God for the gift of today, without his inspiration, and strength this would not be possible. I want to particularly thank Dr. O. Timothy for his fatherly guidance and support.

My sincere appreciation goes to the head of department, Prof . E. O. Akpaja, to the project Coordinator Dr. Joseph. Erabor and to the technical assistant at the phytomedicine research Department Dr Benjamin and Mr paul.

Also, I am grateful to my parents Mr & Mrs Sampson NKWOCHA. O. For their love and support throughout the course of this project, to my friends and colleagues that gave me their support I am grateful.

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ABSTRACT

Acute diarhoea is one of the major illness that cause death in children, despite clinical intervention and the use of oral rehydration therapy. Thus, there is need to discover other effective, affordable and accessible treatments for this disease. Therefore, this study was carried out to investigate the effects of *citrus sisnensis* peel on castor oil-induced diarhoea in mice.

CHAPTER ONE

INTRODUCTION

1.1 CITRUS SINENSIS

In addition to being consumed as a fruit, *citrus sinensis Osbeck,* often known as sweet orange, is utilized as medicine in many nations. Tropical and subtropical regions are home to the *Rutaceae* family, which is where it is thought to have first appeared. Oranges are presently the fruit that are grown most widely worldwide, with an annual production of approximately 110 million tons of citrus fruit (*Blauer,* 2016).

There are three different varieties of *citrus* in the subgenus *Citrus* (Swingle), family *Rutaceae*, and subfamily *Aurantioideae*: *Citrus, fortunella* (kumquat), and *Poncirus trifoliata*. There are three genera and 18 distinct species, but there are also several hybrids that are found all over the world as a result of other natural mutations (Guo and Deng., 2001). Citrus is commonly farmed in Nigeria and many other tropical and subtropical regions (Piccinelli *et al,* 2008).

In terms of production volume, citrus is the second-largest fruit crop in the world, behind bananas, with more than 108 million tons (FAO, 2006). The sweet orange (*Citrus sinensis*) is a member of this family and a significant source of vitamins, especially vitamin C, as well as calcium, potassium, thiamine, niacin, and magnesium (Angew, 2007). With an estimated 60 million metric tonnes produced globally in 2005, valued at a total of $9 billion, oranges are a significant fruit crop commercially. Half of the amount was given by Brazil and the United States of America (Goudeau *et al.,* 2008; Bernardi *et al.,* 2010).

The FAO reports that there were nine million hectares of citrus plants around the world in 2009, with an estimated production of 122.3 million tons, of all the fruit crops, sweet oranges are the most productive (Xu *et al.,* 2013). By 2500 BC, oranges were being grown in China and were known as "Chinese apples." Oranges most likely originated in South East Asia (Nicolosi *et al.,* 2008). It is currently virtually produced all over the world as a source of food for people because of its outstanding nutritional value, source of vitamins, and other uses. Problems including inadequate pollen production, self-incompatibility, and muscular embryos are connected to seed propagation (Mortton, 1987). Therefore, the typical method of ensuring the replication of known-quality cultures is through budding onto suitable rootstocks.

Arsingrin (2011) suggested tissue culture as a method for creating high-quality plantlets. But are prone to root infections, which restrict productivity (Katzer *et al.,* 1999), to distinguish it from closely related species like sour orange, C. aurantium, C. reticulata, and mandarin orange, sweet orange (*Citrus sinensis*) is a tiny, evergreen tree that can grow as tall as 15 m in some situations. It is now commercially grown around the world in tropical, semi-tropical, and some warm temperate countries, making it the most commonly planted fruit tree in the world.

It is believed to have originated in southern China, where it has been farmed for many years (Nicolosi *et al.,* 2000; Ehler, 2011). The leathery, evergreen leaves of the orange plant are 6.5–15 cm long, 2.5–9.5 cm wide, and have elliptical, oblong, and oval shapes. Occasionally, the petioles of the leaves develop thin wings. It bears fragrant, five-petalled white flowers with 20 to 25 yellow stamens, either arranged singly or in whorls of six, each whorl being approximately 5 cm wide. The tiny, fragrant, hermaphrodite flowers are either white or purple, and they provide nectar for pollinating insects. The 6.5 to 9.5 cm diameter fruit, which can range from globose to oval in shape, ripens to orange or yellow.

The two distinct anatomical parts of the fruit are the pericarp, often referred to as the peel, skin, or rind, and the endocarp, commonly referred to as the pulp and juice sacs (Fig. 1). The skin's epidermis is made of epicuticular wax, and it possesses a significant number of tiny aromatic oil glands that produce the smell. Variety, the environment, and growth rate all have an impact on wax production. a profusion of microflora, mostly composed of bacteria and fungi, that is frequently seen on the skin and is more common in humid situations. This supports the need for thorough fruit washing before consumption or juice and essential oil extraction. The outer flavedo, or epicarp, is what makes up the pericarp and is primarily formed of parenchymatous cells and cuticle. Embedded oil glands produce terpenoid aromatic compounds such as valencene, limonene, and alpha/beta sinsenal (Goudeau *et al.,* 2008). Under the epidermis is the flavedo, which has a unique yellow, green, or orange color. The oliferous vesicles that are found inside the fragile, ultrafine flavedo can be collected by scraping the flavedo layer.

The inner, typically colorless flavedo layer of mesophyll undergoes character and thickness alterations that impact fruit growth (Webber, 1989). Albedo, or mesocarp, which lies beneath the flavedo, is made up of tubular-shaped cells that have been linked together to form the tissue mass that has been squashed into the intercellular space. Albedo consists of several flavonoids, which gives juice its bitter taste when added. The fruit's flesh or pulp, which is often divided into 10 to 14 segments and comes in a variety of colors from yellow to orange to red, is typically juicy and sweet.

There are seedless varieties of the fruit. When ripe, the thick, many-seeded fruit, known as a hesperidium, resembles a berry in appearance. Nucleic acids and proteins are difficult to extract from fleshy juice sacs due to the buildup of sugars, organic acids, and a significant amount of water. The vesicles that contain juice that are located in the endocarp and carpels, and which, from the perspective of synthetic biology, should be seen as the liquid discharged by the cytoplasm and by the vacuoles in the vesicles' interior cells.

The majority of the fruit is made up of a spongy tissue comparable to the albedo. The Malay Archipelago, China, Southeast Asia, the Malay Archipelago, New Caledonia, and Australia all have ancient cousins of the citrus plant, yet it's probable that most citrus cultivars have an intriguing origin (Atta *et al.,* 2012). It is believed to have originated from the interspecific hybridization of a few early citrus species, while its genetic background is uncertain (Xu *et al.,* 2013). As a result, based on flavour and origin (Mediterranean and Spanish oranges), its numerous cultivars are grouped into four primary categories or groups. As a single mutation in a Brazilian convent in 1820, navel oranges received their name from the way they appear (the segmented skin resembles a human navel). 2007; Ehsani *et al.*

It is the source of all navel oranges because they do not produce seeds and are propagated through graft cuttings. The popularity of these oranges increased with time, which aided in their global success. The Valencia orange was named after Valencia, a Spanish city well known for its orange production, by agronomist William Wolfkill (Ehsani *et al.,* 2007). Blood oranges, which are distinguished by their scarlet flesh, can vary in sweetness or sourness depending on the variety. The three types of blood oranges: moro, tarocco, and sanguinello,each have a distinctive flavour, size, and country of origin. Blood oranges are used in beverages, sorbets, and preserves.

The Seville orange is a type of bitter orange that is mainly grown around the Mediterranean. There are many nations where the Seville orange originated. According to legend, this fruit has been discovered in Sicily, Guam, and ancient Fuji. It is unknown if Seville, Spain, is where it was initially grown. Spanish immigrants brought this orange to St. Augustine, Florida, where it was farmed and sent to England. It is used to make marmalade, wine, and liquor (Ehsani *et al.,* 2007). One of the key ingredients in many Italian wines, the Chinotto bitter orange, is mostly farmed in Italy. They have different leaves than other varieties since they originate from orange trees with myrtle leaves (Ehsani *et al.,* 2007). Peel quality plays a specific economic role in the production of oranges since factors including shape, colour, scent, texture, and ease of peeling have an impact on consumer demand. However, orange peels make up around 44% of the fruit body, generating a significant number of byproducts, according to (Li *et al.,*2006).

These orange peels are often discarded as waste, which poses a serious disposal problem and potential environmental risk. Given the significant amount of "waste" that is created in the food supply chain, orange peels have a great deal of potential to be used as a value-added product, including for the recovery of natural antioxidants, pectin, enzymes, or for cosmetic purposes.

There are numerous ways to achieve the vast value-added product potential of orange peels, including recovering natural antioxidants, pectin, enzymes, or creating ethanol, organic acids, essential oils, and prebiotic single cell proteins (Mamma & Christakopoulos, 2014). Along with fibre, phenolics, and flavonoids, the C. sinensis peel also contains considerable amounts of vitamin C and other minerals. Its two main divisions are the epicarp and mesocarp.

The cuticle and parenchymatous cells make up the majority of the coloured epicarpal surface. It has several tiny oil glands that emit different smells, as well as an epidermis comprised of epicuticular wax. The smooth, white mesocarp rests below the epicarp. It is made up of tubular-like cells that combine to produce the tissue mass compressed into the intercellular space, according to (Favela-Hernández *et al.,*2016). The C. sinensis peel has been used as a traditional medicine in several regions of the world to cure ringworm infections, stomachaches, skin inflammation, and to improve cardiovascular health (Li *et al.,*2006; Ghasemi & Ebrahimzadeh, 2009). Numerous potent antioxidants found in citrus peels have been found to have antioxidant actions, including metal chelation and free radical scavenging. Reactive oxygen species (ROS) play a crucial role in a number of diseases, including cancer, cardiovascular dysfunction, neurological diseases, and the ageing process, therefore exploring the active phytochemicals in the Citrus sinensis peel is encouraging (Rafiq *et al.,* 2016). Citrus sinensis has the highest concentration of GOFA among citrus peel extracts, according to a recent study on the finding of 40-geranyloxyferulic (GOFA), which has previously been shown to have neuroprotective and colon cancer-chemopreventive effects in rats (Genovese & Epifano, 2012; Genovese *et al.,* 2013). The phytochemicals in C. sinensis peels make them potential therapeutic agents or low-cost yet nutrient-dense food additives due to their widespread availability worldwide.

1.1.2 TAXONOMY CLASSIFICATION OF ORANGE

Kingdom	*Plantae*
Subkingdom	*Viridiplantae*
Division	*Tracheophyta*
Subdivision	*Spermatophytina*
Class	*Magnoliopsida*
Super order	*Rosanae*
Order	*Sapindales*
Family	*Rutaceae*
Genus	*Citrus*
Species	*Citrus sinensis Osbeck*

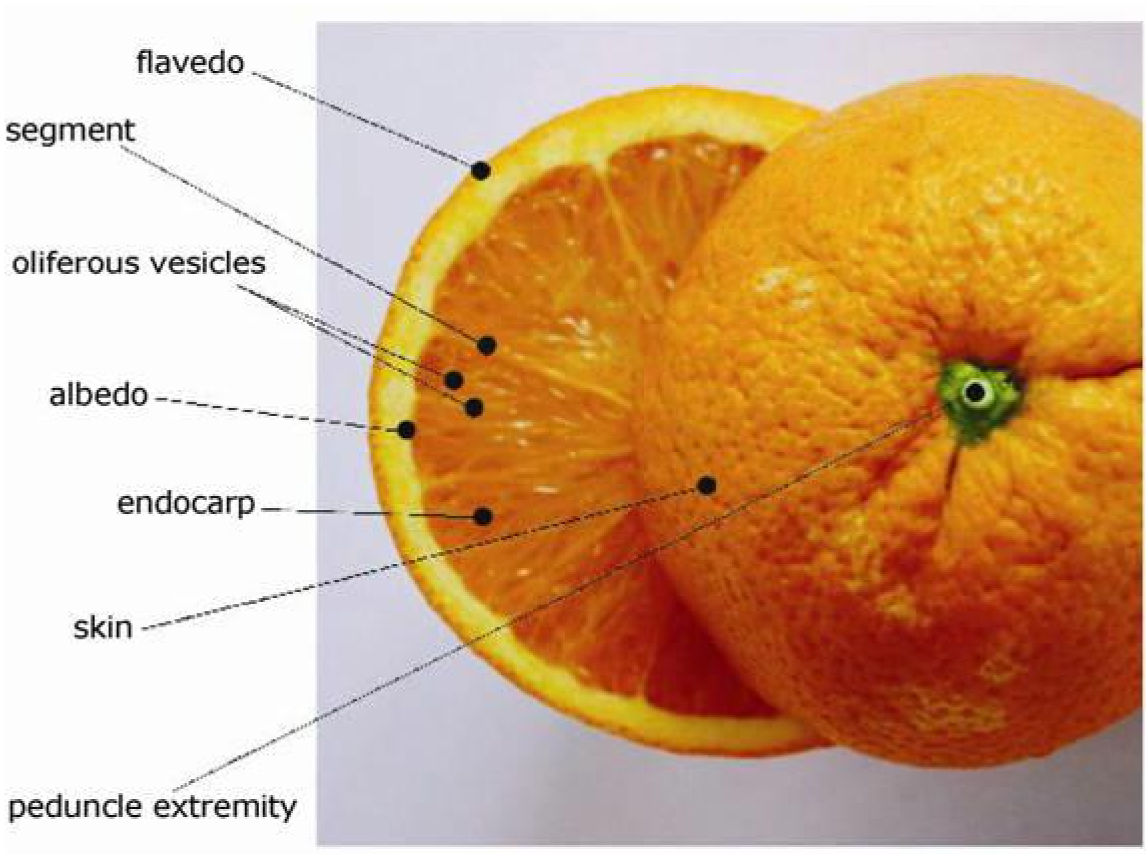

Fig 1: Structure of *Citrus* fruit.

1.1.3 DIARRHOEA

Diarrhoea is a condition in which a person defecates with a soft or in the form of watery stool and the frequency is more frequent than usual (three or more times) in one day. The fecal water content which forms a liquid or semi-liquid consistency has more than 200 grams or 200 ml / 24 hours. Based on the duration, diarrhoea can be classified into acute diarrhea that past within 2 weeks, persistent diarrhoea that last more than 2 weeks or more, and chronic diarrhoea that last more than 30 days or years.

Diarrhoea can cause mild to severe dehydration as well as malnutrition which causes Severe Acute Malnutrition (SAM) in children under five years.3,4 Severity of diarrhoea is categorized according to the degree of dehydration.

Diarrhoea is still one of the biggest health problems in the world. According to research conducted by The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), there were around 4.4 billion episodes of diarrhea cases in all age groups in 2016. Furthermore, in children under five, episodes of diarrhoea is 1.1 billion cases. According to GBD, cases of diarrhoea in individuals under five have a mortality rate of 70.6 deaths per 100,000 individuals.6 Based on Indonesia Health Profile 2017; There were at least 21 cases of diarrhoea with a Case Fatality Rate (CFR) of 1.97% or 1.725 deaths.7 In 2018, the coverage rate of diarrhoea cases in children under five in Central Java is 40.91%. This is higher than the national average of 37.88%.

Diarrhoea occurs due to impaired absorption, abnormalities in intestinal motility, and osmotic abnormalities in the intestinal tract. The clinical manifestations of diarrhoea are well known, but diarrhoea case management is still inadequate.9 The management diarrhoea and dehydration which is currently used is fluid management by administering Oral Rehydration Solution (ORS) with zinc supplementation parallel to the clinical criteria. Other managements of diarrhoea are antibiotics, peristalsis, astrigents, absorbents, and selective antisecretions.

Astringent is an antidiarrheal which has a mechanism of action in the process of mucosal shrinkage and controls intestinal irritation resulting in the decrease of fecal water content. One of the example of astringent is tannins.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Peels of *Citrus sinensis*, was obtained from an urban area around Ring Road, Benin City, Edo State, in January 20222. 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.

Preparation and extraction of plant material

Citrus sinensis peels were separated, bathed in running tap water, and shade dried in a clean environment away from sunlight. After being further dried in a controlled oven at 40°C for 10 minutes, the peels were ground in a British mechanical grinder.

Maceration was used to extract 2.5L of distilled water from 1.61 kg of ground leaves. After that, extracts were put into semi-solid using a water bath (model: HHS Search tech instruments) containers and cooled to a consistent temperature (40°C). The yield of extract was determined according to the formula (% Yield = extract weight / powder sample weight x 100/1) was used to determine percentage yield.

Plate1: REGULATED DRYING OVEN

Plate 2: PULVERIZED ORANGE PEELS **Plate 3:** SENSITIVE BEAM BALANCE

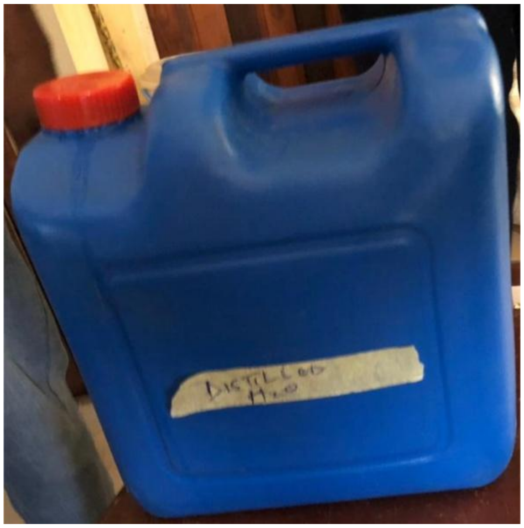

Plate 4: DISTILLED WATER **Plate 5a:** MACERATION PROCESS

Plate 5b: MACERATION CONT'D

Phytochemical analysis

Utilizing accepted practices; the plant's aqueous extract was subjected to qualitative chemical screening for the determination of the tannins, alkaloids, and flavonoids (Trease & Evans, 2001).

Test for tannins

Filtered combination of distilled water (10 mL) and the aqueous extract (1 mL), the ferric chloride reagent was added three drops to the filtrate. A blue-black or green precipitate, indicating the presence of gallic or catechol tannins, respectively.

Test for alkaloids

The aqueous extract (0.2 mL) was stirred with 5 mL of 1% aqueous hydrochloric acid on a steam bath. Then, 1 millilitre of the filtrate was added to 3 drops of Mayer's reagent, and the other millilitre was added with a matching amount of Dragendorff's reagent. Turbidity or precipitation was considered evidence of the presence of alkaloids when using these reagents.

Test for flavonoids

Concentrated hydrochloric acid (5 drops) and metallic magnesium were added after boiling the aqueous extract (2 mL). A red or orange colour suggested the presence of flavonoids.

Experimental Animal

30 Swiss albino mice weighed between 21 and 28 g each. Animals were house in clean wooden cage (6 per cages) and maintain at 25 ± 1°C via relative humidity of 45-55 % under 12: 12 hrs light and dark cycle for a week with free access water and feed ad libitum. The Institutional Animal Ethical Committee gave its ethical approval, and the experiment was carried out in accordance with CPCSEA Guidelines. One week before the trial, all mice were acclimated to the working (lab) environment.

Plate 6: ACCLIMATIZATION OF EXPERIMENTAL ANIMALS.

Experimental protocol

Swiss albino mice were selected into five groups of three mice each (n= 3), for each of the two antidiarrheal test models 15 mice were used. Group 5 (untreated control rats). In all both models, the negative control groups were treated with the vehicle (distilled water, 10ml/kg). The positive controls were treated with Loperamide 2mg/kg (in castor oil-induced diarrhea and enteropooling models) and atropine 1mg/kg (gastrointestinal motility model). The other groups (Groups 3, 4, and 5) in each model received 50, 100, and 200mg/kg doses of the crude extract respectively.

Castor Oil-Induced Diarrhea in Mice

This test was done based on the method used by (Awouters *et al.,* 1978). Fifteen mice were fasted for 18 hours and divided into five groups with three animals in each group. The first group received distilled water (10 ml/kg) and the second group received loperamide (2 mg/kg), serving as negative and positive controls, respectively. (Groups 3, 4 and 5) in each model received 50, 100, and 200mg/kg doses of the extract respectively. After one hour, all the animals received 0.5 ml of castor oil orally. The animals were kept in separate metabolic cages. The severity of diarrhea was assessed for hours. The mean total number of faeces (dry and wet diarrheal droppings) was determined and compared with the negative control group. The total score of diarrheal faeces for the negative control group was considered as 100%. The percent inhibition of total defecation and that of diarrhea were calculated using the following formulas:

% inhibition of defecation =

$$\frac{\text{Total number of faeces in the negative control} - \text{total number of faeces in the treated group}}{\text{Total number of faeces in the negative control}} \times 100$$

% inhibition of diarrhea =

$$\frac{\text{Total number of diarrhea faeces in the negative control} - \text{total number of diarrhea faeces in the treated group}}{\text{Total number of diarrhea faeces in the negative control}} \text{ X } 100$$

Statistical Analysis

All column graphs were stated in the tables as the mean ± SEM of the values. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by a post hoc Duncan's method for multiple comparisons. Differences in P-values < 0.05 were considered statistically significant. The results were analysed using Graph Pad Prism 9.0.

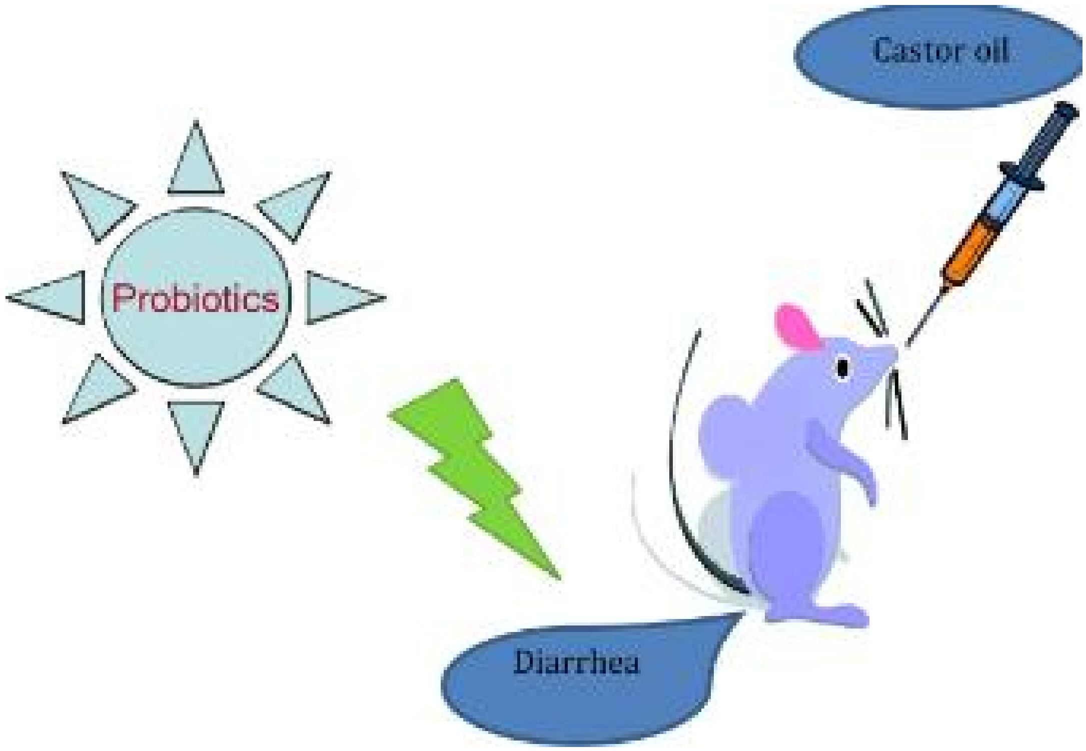

Plate 7: CASTOR OIL-INDUCED DIARRHEA

CHAPTER THREE

3.0 RESULTS

3.1 Red blood cell counts

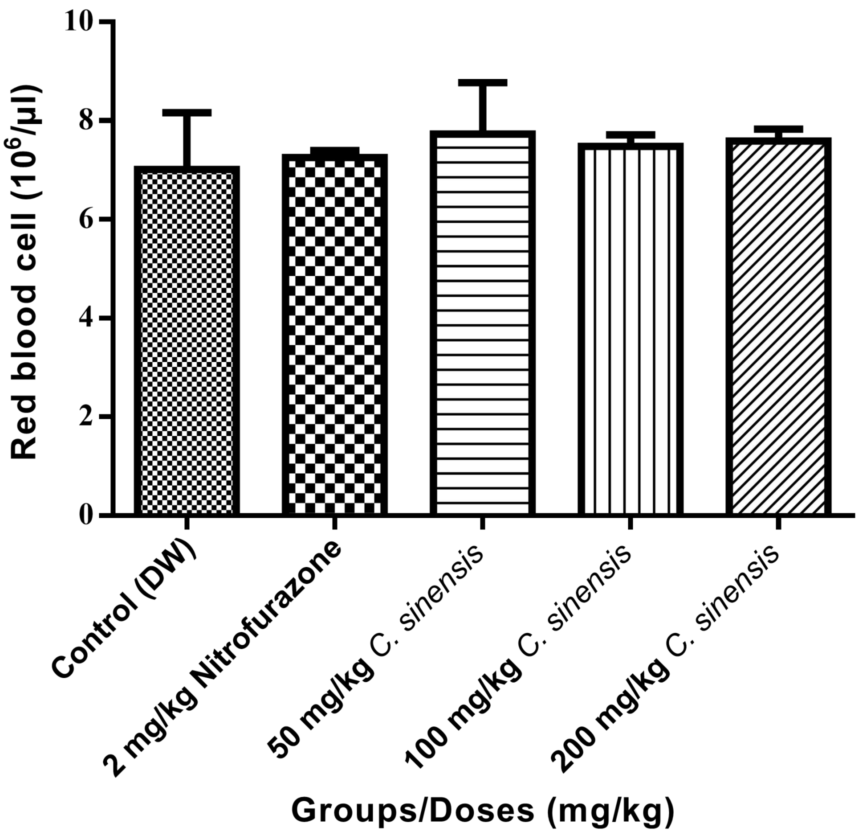

Figure 3.1: Effect of *Citrus sinensis* peel aqueous extract on the red blood cells

3.2 Castor oil induced diarrheal

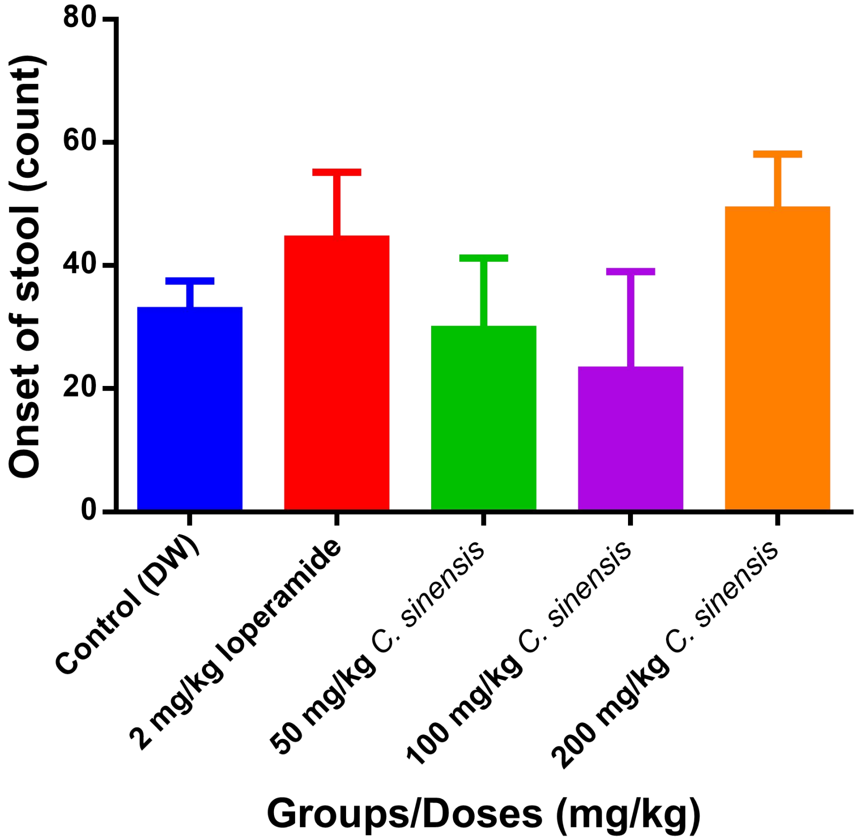

Figure 3.2: Effect of *Citrus sinensis* peel aqueous extract on the onset of stool

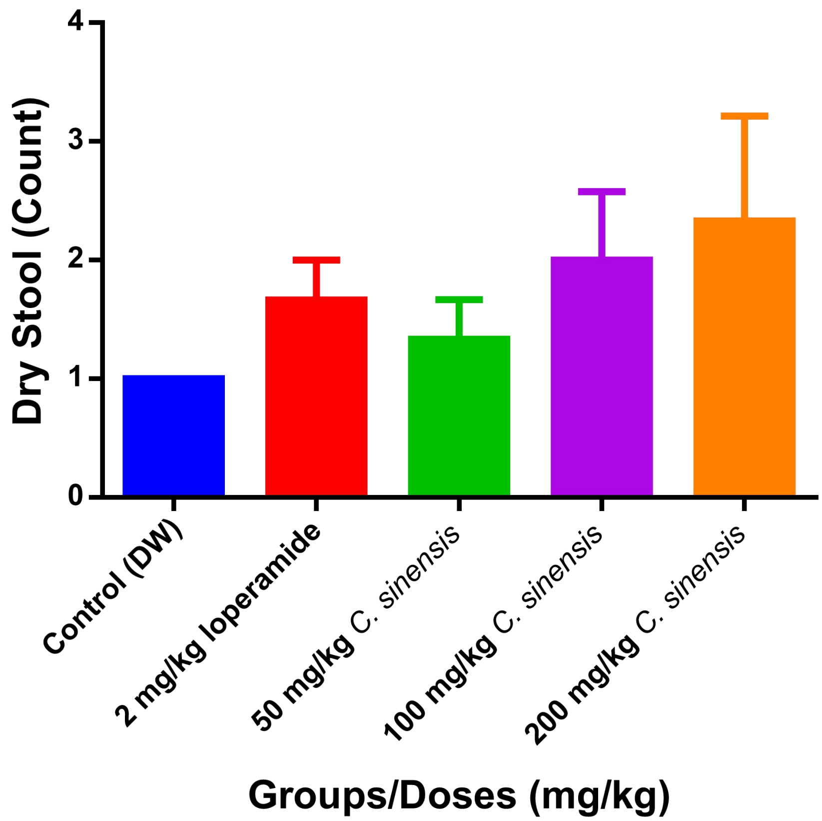

Figure 3.3: Effect of *Citrus sinensis* peel aqueous extract on the Dry stool

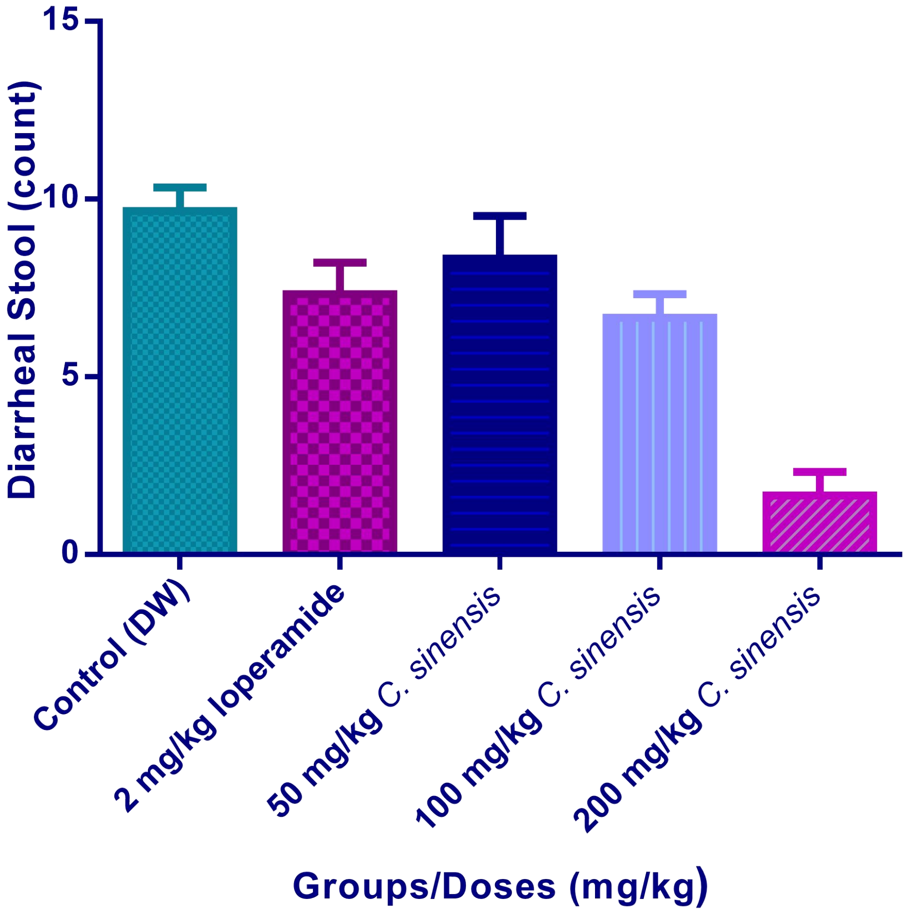

Figure 3.4: Effect of *Citrus sinensis* peel aqueous extract on the Diarrheal stool

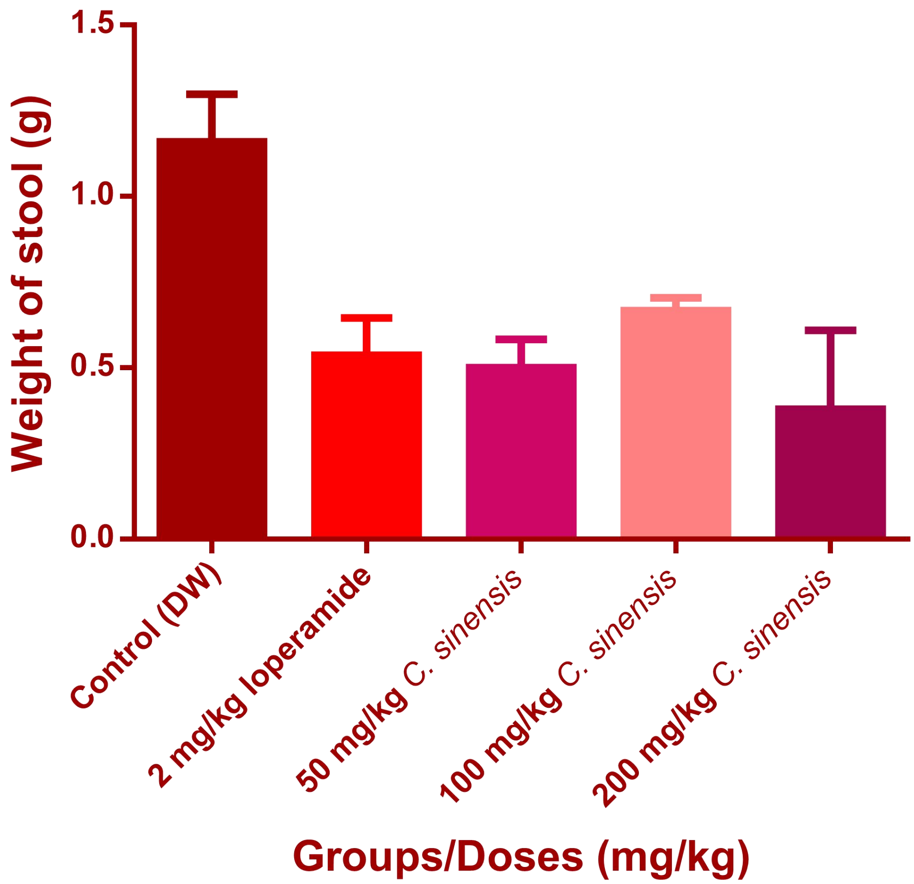

Figure 3.5: Effect of *Citrus sinensis* peel aqueous extract on the Weight stool

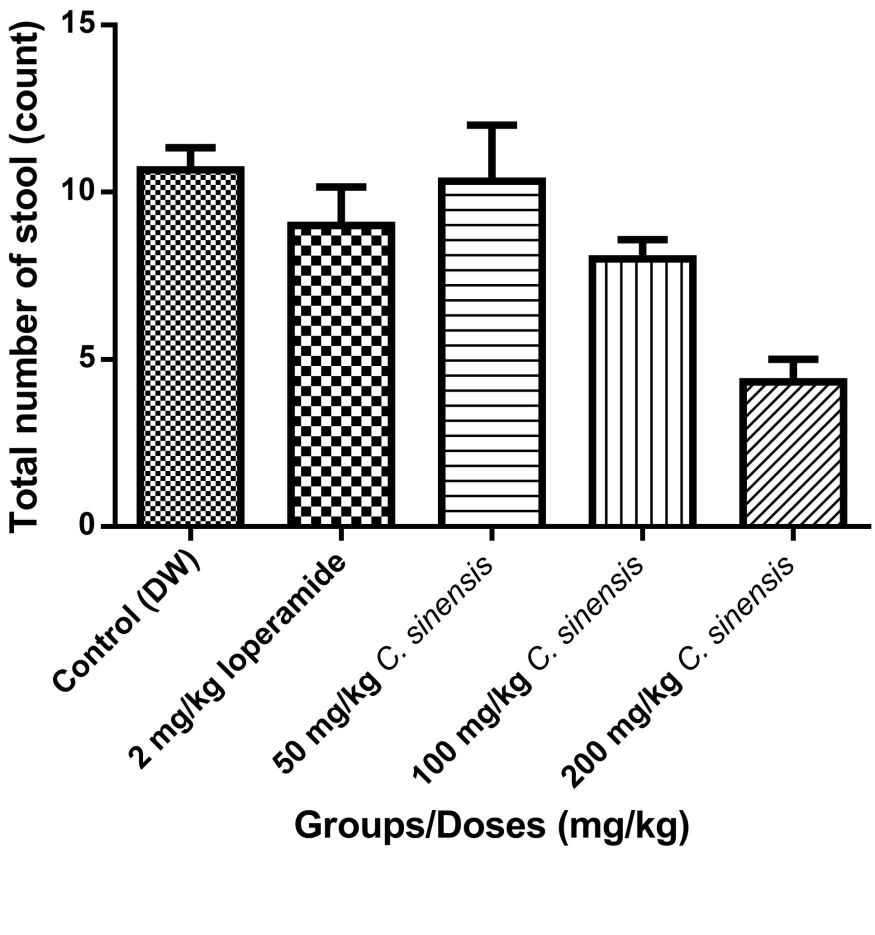
Total number of stool (count)
15
10
5
0
Control (DW)
2 mg/kg loperamide
50 mg/kg C. sinensis
100 mg/kg C. sinensis
200 mg/kg C. sinensis
Groups/Doses (mg/kg)

Figure 3.6: Effect of *Citrus sinensis* peel aqueous extract on total number of stool

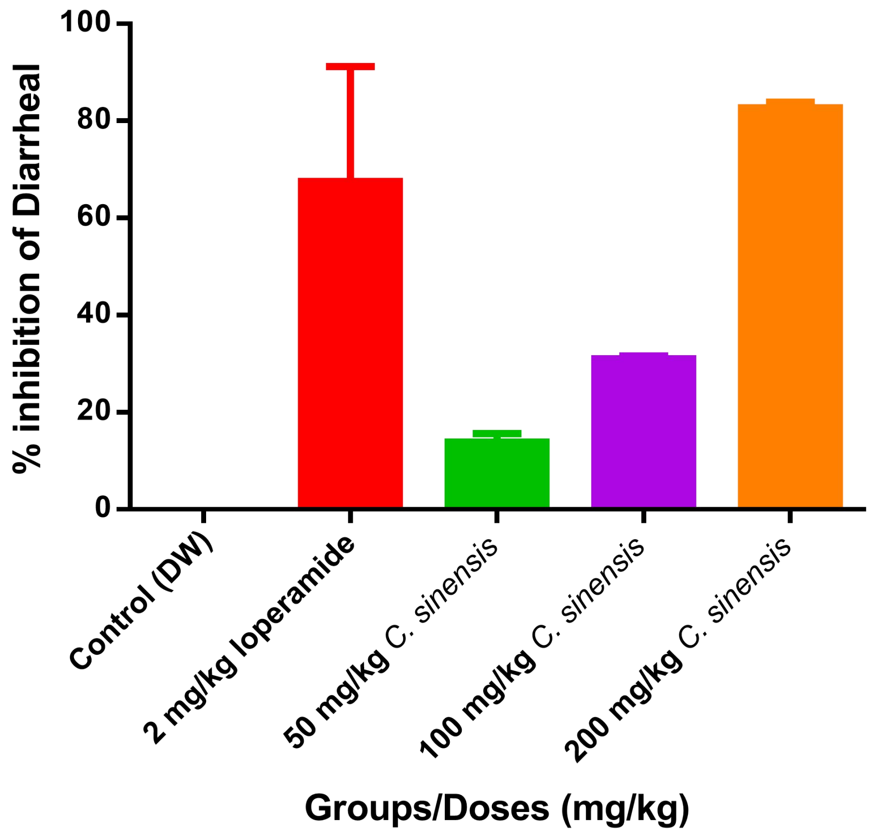

Figure 3.7: Effect of *Citrus sinensis* peel aqueous extract on the percentage inhibition of diarrheal

Preliminary Phytochemical Screening

The preliminary phytochemical screening test of aqueous peel extract of *Citrus sinensis* showed the presence of phenols, tannins, flavonoids, and alkaloids.

Effect of the Extract on Castor Oil-Induced Diarrhea

The aqueous extract of the peels of *Citrus sinensis* reduced the total quantity of faeces as well as the amount of diarrhoeic feces in the castor oil-induced diarrheal mice in a dose-dependent manner, and the results were statistically significant. At a dosage of 50 mg/kg, 100mg/kg and 200mg/kg respectively. The strongest antidiarrheal efficacy among the fractions was 100mg/kg and 200mg/kg. In test animals, both fractions decreased the overall quantity of faeces and the total number of moist faeces activity. In comparison to the control, both fractions were successful in lowering both the overall quantity of faeces and the amount of wet faeces.

CHAPTER FOUR

DISCUSSION

Therefore, this study was proposed and conducted to evaluate the anti-diarrhoea activity of the aqueous extract of orange (citrus sinensis) peels in cator oil-induced diarrhea model in mice, and the effects on citrus sinensis extract on the red blood cells.

The plant extract significantly ($p < 0.001$) reduced the total fecal output and diarrheal drops in castor oil-treated mice in the one-hour observation compared with the negative controls.

The three serial doses of the extract were found to produce a reduction in the frequency of defecation and diarrheal output to the same significant level that was produced by loperamide. Furthermore, at the highest dose 200 mg/kg, the plant extract significantly ($p < 0.001$) delayed the onset of diarrhea caused by castor oil when compared with the negative controls. The percent reduction in the frequency of defecation and diarrhea was increased with a corresponding increase in the dose of the plant extract.

The percent inhibition produced by the highest dose of the extract was closer to the inhibition produced by loperamide. This increasing pattern of percent inhibition in the total number of fecal output instances and diarrheal episodes with increasing dose of the extract plus significant delay in the onset of diarrhea by the highest dose implies that the plant extract inhibits diarrhea more effectively at relatively higher doses. In this study, the aqueous extract of *Citrus sinensis* has been shown to reduce castor oil-induced diarrheal episodes and intestinal secretion and motility. So, these results can be taken as scientific evidence showing that this plant has an antidiarrheal activity. But the exact mechanism of action of the plant extract for these effects was not determined. The possible mechanisms of action of the extract can be proposed based on the pathophysiologic processes of diarrhoea and the actions of castor oil to induce diarrhoea.

Diarrhoea is caused by four pathophysiologic processes: increased luminal osmolarity, electrolytes secretion, decreased electrolytes absorption, and abnormal intestinal motility causing reduction in intestinal transit time. In the intervention of diarrhea, antimotility and antisecretory agents remain as the main agents used to decrease such pathophysiologic changes.

Castor oil has been widely used for induction of diarrhea in antidiarrheal activity studies because it releases Ricinoleic acid, a metabolite that causes diarrhea, upon metabolism in the gut. Ricinoleic acid initiates diarrhea via mechanisms such as irritation of GI mucosa, leading to the release of prostaglandin which stimulates gastrointestinal motility and electrolyte secretion, reducing electrolyte absorption from the intestine and colon; these are similar to the pathophysiologic processes resulting in diarrhea.

Therefore, the antidiarrheal activity of the plant might be due to the activities that oppose the actions of castor oil for induction of diarrhea or pathophysiologic processes leading to diarrhea. The extract has been shown to decrease the intestinal fluid accumulation. This suggests that the plant extract may decrease water and electrolyte secretion to the intestinal lumen while promoting their absorption, which in turn could decrease intestinal overload and distension, leading to a decrease in intestinal motility giving a longer time for absorption and water contents of the fecal drops and hence overall reduction in the total number of defecation instances and diarrheal drops in treated groups. This is consistent with the mechanism of action of loperamide for its antidiar-rheal effect as presented in the literatures. In addition, the extract may have an anticholinergic activity and cause reduction in intestinal motility and secretion, which is in agreement with the action of loperamide on the intestine

The aqueous contains phenols, tannins, flavonoids, saponins, terpenoids, and glycosides, as asserted by preliminary phytochemical screening tests, and most of these secondary metabolites were reported to have an antidiarrheal activity. Reports in the literatures showed that tannins have an antispasmodic and muscle relaxant effect, flavonoids inhibit prostaglandin induced intestinal secretion, saponins inhibit histamine release, terpenoids inhibit the release of prostaglandins, and phenols reduce intestinal secretion and transit and have an astringent action. All these actions lead to the inhibition of diarrhoea by decreasing intestinal secretion and motility. Therefore, the antidiarrheal activity of the plant extract may be produced by these chemical constituents.

Conclusion

In conclusion, the aqueous extract of *Citrus sinensis* has an antidiarrheal activity as revealed by reductions in the total fecal output and diarrheal drops. Hence, this study supports the use of the plant in the treatment of diarrhea in the traditional settings.

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