**Antidiarrhea activity of ethyl acetate fraction of *Ocimum gratissimum* in castor oil-induced diarrhea in rats**

**ABSTRACT**

Diarrhea is a common gastrointestinal disorder that is one of the causes of morbidity and mortality in both children and adults. *Ocimum gratissimum* has been used in traditional medicine as a household remedy for various diseases, including diabetes, biliary diseases, cough, wound healing, and diarrhea. This study investigated the effect of ethyl acetate fraction of *Ocimum gratissimum* leaves in castor oil-induced diarrheal rats. The acute toxicity study was done using Lorke’s method. The antidiarrheal properties of the ethyl acetate fraction of *O. gratissimum* leaves were investigated using castor oil-induced diarrhea, intestinal motility, and enteropooling models in Wistar rats. The diarrhea untreated (negative control) group received water orally, the positive control group was given 3 mg/kg loperamide orally, the test groups were administered three dose levels (200, 400 and 800 mg/kg) of the fractions. The results of LD50 showed that the plant is safe for therapeutic purposes. It was observed that the fractions showed no acute toxicity at a dose of 1.6 g/kg. The graded fractions delayed the onset of diarrhea and reduced fecal parameters significantly as compared with the negative control in a dose-dependent manner in the castor oil-induced diarrheal model. The charcoal meal test revealed that the fraction produced a significant anti-motility effect at all tested doses as compared with negative control. The result of the enteropooling test revealed that the graded fractions produced a significant decrease in the volume and weight of intestinal contents. The percentage inhibition of diarrhea in the fraction increased in a dose-dependent manner. The anti-diarrheal activity could be associated with the minerals, vitamins, and phytochemicals present in this fraction. The results suggest that ethyl acetate fraction of *O. gratissimum* has antidiarrhea activity. The findings provide scientific support and validate the use of *O. gratissimum* leaves for the treatment of diarrhea.

**Keywords:** Diarrhea, *Ocimum gratissimum*, Castor oil, Anti-motility, Enteropooling, Intestinal motility

1. **INTRODUCTION**

“The use of plants as therapies for various ailments has formed the basis of our modern medicinal sciences conforming to the World Health Organization” (Yin, 2024), “about 80% of Asia and Africa’s population use traditional medicine as a form of healthcare for treatment of diseases including gastro-intestinal disorder. Diarrhea is a popular gastrointestinal disorder characterized by increased frequency of abdominal pain, wet stool and bowel movement. In developing countries like Nigeria, it is a common cause of morbidity and mortality among the young and the old age. About 90% of diarrheal deaths worldwide are attributed to unsafe water, inadequate sanitation and poor hygiene. In Nigeria, diarrhea prevalence rate is 18.8% and is one of the worst in sub-Sahara Africa and above the average of 16%. Annually, it accounts for over 16% of child deaths and an estimated 150,000 deaths mainly amongst children under five years” (UNICEF, 2014).

“Diarrhea Infection is spread through contaminated food or drinking water, or from person to person as a result of poor personal hygiene and also be caused by food intolerance, food poisoning or as a side effect of certain medications” (Nansunga *et al.,* 2014). “Excessive loss of fluid in diarrhea is associated with an imbalance between the absorptive and secretory mechanisms of water and electrolytes in the intestinal tract, accompanied by hypermotility” (Ezeja *et al*., 2012). “Globally in 2019, diarrhea is the 8th leading cause of death among all ages, which is responsible for 1.6 million deaths” (Abuzerr *et al.,* 2019) and “second cause of death next to respiratory infections among under five years of age children throughout the world is diarrhea” (Mihrete *et al*., 2014). “The incidence of diarrhea is still high (about 7.1 million per year), despite the efforts of international organizations to control this disease” (Budyanra, 2017). “Diarrhea is one of the most prominent diseases treated by traditional medicines” (Maroyi *et al*., 2016).

“The use of plant materials for medical purposes dates back to the history of mankind” (Onochie *et al*., 2020; Enemchukwu *et al*., 2022; Ezeigwe *et al*., 2025). “Medicinal plants exhibited their therapeutic properties due to important phytochemicals, minerals and vitamins found in them” (Ezeigwe *et al*., 2020; Ani *et al*., 2024; Achara *et al*., 2025). “*Ocimum gratissimum* (*O. gratissimum*) is a herbaceous plant commonly found in tropical Asia and in the coastal areas of Nigeria, where it is used for the treatment of ailments such as diarrhea, urinary infections, fever, and dysentery” (Busari *et al*., 2021). “The plant is an erect small plumb with many barnacles usually not more than 1m high” (Gulati, 2023). “It is a plant belonging to Lamiceae family known in Nigeria as Nchuanwu (Igbo), Efinron (Yoruba) and Dan doya tugida (Hausa). In Eastern Nigeria, it serves as a source of stimulation and condiment in soup due to its minty aromatic flavor. The flowers and the leaves of this plant are rich in essential oil, so it is used in the preparation of teas and infusions” (Rabelo *et al*., 2003)

“The tribes of Nigeria use the leaves extract in the treatment of diarrhea, while the cold leave infusions are used for the relief of stomach upset and haemorrhoids” (Kouitcheu *et al*., 2006). “The presence of saponins, terpenoids, glycosides, and alkaloids in the aqueous extract of O. gratissimum may further contribute to its anti-inflammatory and anti-oxidative activities” (Oyem et al., 2021). “Investigated the anti-diarrhea effects of an aqueous extract of O. gratissimum leaves. They were able to demonstrate the anti-diarrhea efficacy of the extract on castor oil-induced diarrhea in rats, as evidenced by a substantial decrease in the amount of wet faeces in rats treated with the extract” (Offiah and Chikwendu, 1999). Ugbogu *et al.* (2021) reviewed that “various in vivo and in vitro studies have shown that O. gratissimum and its bioactive constituents possess pharmacological properties such as antioxidant, anti-inflammatory, anticancer, hepatoprotective, antidiabetic, antihypertensive, anti-diarrhea, and antimicrobial properties which indicate that O. gratissimum has a strong preventive and therapeutic effect against several diseases” (Ugbogu *et al*., 2021).

“Furthermore, the ethanol extract of *Ocimum gratissimum* possesses coagulant activity and can serve as a lead to production of drug remedies to blood bleeding” (Osuala *et al*., 2021). Although there are studies on the antidiarrheal property of *O. gratissimum*, there is yet no investigation on the antidiarrheal property of the ethyl acetate fraction of *O. gratissimum* leaves. Hence, the Purposed of this study is to evaluate the antidiarrheal activities of ethyl acetate fraction of *Ocimum gratissimum* on castor oil-induced diarrhea.

1. **MATERIALS AND METHODS**

**2.1 Sample collection and identification**

The leaves of *O. gratissimum* were collected from Mgbakwu, Awka North Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. A voucher specimen (NAU 35A) was deposited in the herbarium of the department.

**2.2 Preparation of ethanol leaf extract of *O. gratissimum***

The leaves were properly washed and air dried at room temperature for two weeks. The dried leaves were pulverized into powder using corona manual grinding machine. Exactly 2 kg of the pulverized leaf powder was soaked in 10 litres of 70% ethanol for 24 hrs for ethanol extraction. The ethanol mixture was sieved using muslin cloth and filtered using Whatman no 1 filter paper. The filtrate was concentrated using water bath at 500C. The biological yield of the extract after extraction was 113.5g. The ethanol extract was stoppered in universal bottles and preserved in the refrigerator for use.

**2.3 Fractionation of leaf extract of *O. gtatissimum***

The crude ethanol leaf extract (113.5g) was fractionated by the method of Wu *et al.* (2005). The method involved successive extraction by increasing polarity with n-hexane, chloroform, ethyl acetate, n-butanol and water. Twenty grams (20g) of the ethanol extract was dissolved in 250ml of Methanol/Water (MeOH/H2O) (1:1) mixture and shaken with n-hexane (3 x 200ml). Combined extract was left to dry on the bench to yield n-hexane fraction. Methanol (MeOH) was further fractionated by successive solvent extraction with chloroform (2 x 200ml), ethyl acetate (1 x 200ml) and n-butanol (2 x 200ml). Each fraction was left to evaporate to dryness on the bench to yield n-hexane fraction (2.21g), chloroform fraction (3.21g), ethyl acetate fraction (8.32g), n-butanol fraction (2.34g) and water fraction (3.53g).

**2.4 Experimental animals**

Eighty-seven (87) male Wistar albino rats weighing between 130–150 g were obtained from Chris Experimental Research Farm, Mgbakwu, Awka North Local Government Area, Anambra State. They were sorted and housed in standard cages with a 12:12 light:dark cycle. They were fed with Top grower’s mash pellets and water *ad libitum*. The weights of the experimental animals were checked before the commencement of the experiment. All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Nnamdi Azikiwe Animal Care Ethics Committee.

**2.5 Acute toxicity (LD50) evaluation**

The median lethal dose (LD50) for the ethylacetate fraction was determined using Lorke’s method (Lorke, 1983). Twelve (12) rats were used for the LD50 study. The acute toxicity study was carried out in two phases. The first phase involved the administration of 10, 100 and 1000 mg/kg bodyweight of the fraction to three rats in each group respectively and monitoring the rats for a period of 24 hours and then 14 days for signs and symptoms of toxicity while the second phase involved the administration of higher doses of 1600, 2900 and 5000 mg/kg bodyweight of the fraction to one rats each in a group. The monitoring for both phases was extended to 14 days to have a longer period of observation as a modified Lorke’s method.

After the experiment, the LD50 can be estimated in the following manner.

LD50 is calculated mathematically as

**LD50** = √HNLD x LLD

Where HNLD = Highest non-lethal dose

 LLD = Least Lethal dose

**2.6 Animal grouping for diarrhea studies**

Seventy-five (75) male Wistar rats were randomized into three groups of twenty-five rats each for the three diarrhea models tested. The twenty-five (25) rats in each group was further randomized into five (5) groups of five rats each and used for the study. The groupings are as follows:

Group A: Diarrhea untreated (Negative Control)

Group B: Diarrhea + 3 mg/kg loperamide (Positive Control)

Group C: Diarrhea + 200 mg/kg ethylacetate fraction of *O. gratissimum* leaves

Group D: Diarrhea + 400 mg/kg ethylacetate fraction of *O. gratissimum* leaves.

Group E: Diarrhea + 800 mg/kg ethylacetate fraction of *O. gratissimum* leaves

**2.7 Induction of Diarrhea and Determination of Antidiarrheal Property**

**2.7.1 *Castor Oil-Induced Diarrhea Model***

The model and formular described by Nwodo and Alumanah (1991) were used in this study. Twenty-five healthy male Wistar albino rats were fasted for 24 hour, with free access to water and treated exactly when their fasting reached 24 hours. One hour later after treatment/administration all the rats except group A (Negative control) received 0.5 ml of castor oil by oral gavage, and then, they were individually placed on the floor of individual cages, which were covered with non-wetting transparent paper.

During the observation period of 4 to 5 hours, the time of onset of diarrhea, the number and weight of wet stools, the time when they started defecating, and the total number and weight of fecal output (both diarrheal and non-diarrheal) excreted by the rats were recorded and compared with the control group.

Finally, the percentage of diarrheal inhibition as well as the weight of wet and total fecal output were determined according to the formular below:

$$\% Inhibition of diarrhea=\frac{Mean of wet defecation(negative control-test)}{Mean number of wet defecation in negative control }X\frac{100}{1}$$

$$\% Of wet fecal output=\frac{Mean weight of wet feces of each treatment group}{Mean weight of wet feces of control }X\frac{100}{1}$$

$$\% Of total fecal output=\frac{Mean fecal weight of each treatment group}{Mean fecal weight of control }X\frac{100}{1}$$

**2.7.2 *Castor Oil-Induced Gastrointestinal Motility***

A gastrointestinal motility test was investigated according to the method and formular described by Tan et al, (1989). All rats were fasted for 24 hours with free access to water and were randomly assigned to 5 groups and treated as described in the study design. An hour later, 0.5ml of castor oil was administered. Sixty (60) minutes after the administration of castor oil, each rats received 1ml of 5% charcoal suspension in distilled water. Thirty (30) minutes later, each rat was then sacrificed by cervical dislocation, the abdomen was opened, and the small intestine was immediately dissected out from pylorus to caecum and placed lengthwise on a white paper. Therefore, the distance travelled by the marker from the pylorus and the total length of the intestine were measured with a calibrated ruler. The peristaltic index (PI) expressed as a percentage of the distance travelled by the charcoal meal relative to the total length of the small intestine as well as the percentage of movement as a function of the control was calculated.

$$Peristaltic Index (PI)=\frac{Mean distance travelled by charcoal meal }{Mean lenght of small intestine }X\frac{100}{1}$$

$$\% Inhibition of motility=\frac{Mean \% of distance travelled by the charcoal (negative control-test)}{Mean \% of distance travelled by the charcoal meal of negative control }X\frac{100}{1}$$

**2.7.3 *Castor Oil-Induced Enteropooling***

Intestinal fluid accumulation was evaluated according to the method described by OECD/OCDE (2008). Twenty-five (25) rats were fasted for 24 hours and treated as described in the grouping, just one hour before oral administration of 0.5ml castor oil. After 1 hour, the rats were sacrificed, the pyloric and caecum ends of the small intestine were tied, and the intestine was removed. The dissected small intestine was weighed, and intestinal contents were expelled into a measuring cylinder and then the volume was measured. The weight of the intestine after milking was taken and the difference between full and empty intestines was calculated.

Finally, the percentage of inhibition of intestinal secretion was calculated according to Suleman and Alemu, (2011) and Nwodo and Alumanah, (1991).

$$\%Inhibition using MVIC=\frac{\begin{array}{c}Mean volume of intestinal fluid\\(negative control-test)\end{array}}{Mean volume of intestinal fluid negative control }X\frac{100}{1}$$

Where; MVIC is mean volume of the intestinal content.

$$\%Inhibition using MVIC=\frac{\begin{array}{c}Mean weight of the intestinal fluid\\(negative control-test)\end{array}}{Mean weight of the intestinal fluid negative control }X\frac{100}{1}$$

Where; MWIC is mean weight of the intestinal content.

**2.7.4 *In vivo Antidiarrheal Index***

The *in vivo* antidiarrheal index (ADI) for the plant fraction and standard drug was determined by combining three parameters taken from the aforementioned models., it was then expressed according to the formula developed by Nwodo and Alumanah, 1991.

$$In vivo antidiarrheal index \left(ADI\right) 3\sqrt{D frequency X Gmeg X Pfrequency }$$

Where;

D frequency = the delay in defecation time or diarrheal onset obtained from castor oil- induced test (as % of control).$$DFreq (\%)=\frac{Mean onset of Diarrhea in min (Test-Negative Control)}{Mean onset of Diarrhea in mins of Negative Control }X\frac{100}{1}$$

G meg = is the gut meal travel reduction (as % of control).

$$Gmeq (\%)=\frac{Mean \% charcoal meal transit/Peristaltic index (Negative Control-Test) }{Mean \% charcoal meal transit/Peristaltic index of Negative Control }X\frac{100}{1}$$

P frequency = is the purging frequency as reduction in the number of wet stools (as % of control) from castor oil diarrhea model.

$$PFreq (\%)=\frac{Mean number of wet defecation (Negative Control-Test) }{Mean number of wet defecation of Negative Control }X\frac{100}{1}$$

**2.8 Statistical Analysis**

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results obtained were performed by using ANOVA and POS-HOC Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at *p*<0.05, *p*<0.01 and *p*<0.001.

1. **RESULTS**

**3.1 Result of Acute Oral (LD50) Toxicity Test**

Administration with 10, 100 and 1000 mg/kg bodyweight of ethyl acetate fraction of *O. gratisimum* showed no signs or symptoms of toxicity in the rats (table 1). The rats were observed to be normal within and after 24 hours of the administration. The extract was well tolerated at doses up to 1000mg/kg bodyweight in rat.

The rat that were administered 1600 mg/kg bodyweight of ethyl acetate fraction of *O. gratisimum* was slightly weak while the rat administered 2900mg/kg bodyweight was weak and the rat administered 5000 mg/kg bodyweight was very weak and died within three hours of the administration (table 2). The LD50 value is 3,807.89 mg/kg.

**Table 1:** Shows the result of the administration of low doses of the ethyl acetate fraction of *O. gratissimum* to the rats.

|  |  |  |
| --- | --- | --- |
| **Fraction/Dose (mg/kg body weight)** | **Observations** | **Mortality** |
| 10 | They were normal | 0/3 |
| 100 | They were normal | 0/3 |
| 1000 | They were normal | 0/3 |

Number of deaths per group = 0, Number of rats per group = 3

**Table 2:** Shows the result of the administration of high doses of the ethyl acetate fraction of *O gratissimum* to the rats.

|  |  |  |
| --- | --- | --- |
| **Fraction/Dose (mg/kg body weight)** | **Observation** | **Mortality** |
| 1600 | It was slightly weak | 0/1 |
| 2900 | It was weak | 0/1 |
| 5000 | It was very weak and died within 3 hours of administration | 1/1 |

Number of deaths per group = 0,0,1, Number of rats per group = 1

**3.2 Result of Antidiarrheal Studies in rat models**

**3.2.1 *Effect on Castor Oil-Induced Diarrheal Model***

The ethyl acetate fraction of *O. gratissimum* leaves was found to be effective against castor oil-induced diarrhea in terms of delaying the onset of diarrhea at 200 mg/kg, 400 mg/kg, 800 mg/kg tested doses as well as reduced the fecal parameters (number and weight of wet and total stools) compared to the negative control (table 3). Besides, the data revealed that the percentage of diarrheal inhibitions were 36.36% (*p*<0.05, *p*<0.01, *p*<0.001), 50.00% (*p*<0.05, *p*<0.01, *p*<0.001) and 68.18% (*p*<0.05, *p*<0.01, *p*<0.001) at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg, respectively. The number of wet feces was markedly reduced with ethyl acetate fraction of *O. gratissimum* at a dose of 800 mg/kg when compared with negative control. Loperamide 3 mg/kg (positive control) has also shown highest reduction of wet defecations with percentage inhibition of 100% (*p*< 0.05, *p*<0.01, *p*<0.001).

The observations made from table 3 shows that the antidiarrheal property of the ethyl acetate fraction of *O. gratissimum* was dose-dependent. The higher the dose, the longer the delay in the onset of diarrhea. Among the treatment groups that only received ethyl acetate fraction of *O. gratissimum*, Group that is administered with 200mg/kg extract had a delay of 68.40 minutes for diarrhea onset, followed by the group that was administered with 400mg/kg at 105.40 minutes while that of 800mg/kg fraction showed the longest delay of diarrhea onset at 126.40 minutes compared to all treatment groups except the group that was treated with the conventional drug which received 3 mg/kg loperamide and had a delay of 263.00 minutes in its onset of diarrhea.

**Table 3:** Antidiarrheal Effects of ethyl acetate fraction of *O. gratissimum* leaves on Castor oil-induced diarrheal model in rats.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dose Administered** | **Onset of Diarrhea (Minutes)** | **Number of Wet Faeces** | **Total Number of Fecaes** | **Average Weight of Wet Faeces (g)** | **Average Weight of Total Faeces (g)** | **% Inhibition/****Reduction** | **% Wet Fecal Outputs** | **% Total Fecal Outputs** |
| Diarrhea untreated (negative Control) | 45.20 ± 0.20 | 4.40 ± 0.40 | 5.18 ± 0.40 | 1.15 ± 0.14 | 1.24 ± 0.17 | - | - | - |
| 3mg/kg loperamide (Positive Control) | 263.00 ± 15.13a123 | 0.00 ± 0.00a123 | 0.70 ± 0.20a123 | 0.00 ± 0.00a123 | 0.03 ± 0.02a123 | 100.00 | 0 | 2.42 |
| 200mg/kg extract | 68.40 ± 6.74 | 2.80 ± 0.37a1 | 3.28 ± 0.40a1 | 0.46 ± 0.12a123 | 0.56 ± 0.12a123 | 36.36 | 40 | 45.16 |
| 400mg/kg extract | 105.40 ± 7.15a123 | 2.20 ± 0.37a123 | 2.38 ± 0.42a123 | 0.25 ± 0.03a123 | 0.32 ± 0.03a123 | 50.00 | 21.74 | 25.81 |
| 800mg/kg extract | 126.40 ± 2.71a123 | 1.40 ± 0.24a1 | 1.26 ± 0.35a123 | 0.04 ± 0.02a123 | 0.11 ± 0.03a123 | 68.18 | 3.48 | 8.87 |

Data are expressed as mean ± SEM (n=5); aCompared with negative control; 1*p*< 0.05, 2*p*< 0.01, 3*p*< 0.001.

**3.2.2 *Effect on Castor Oil-Induced Gastrointestinal Motility***

As presented in Table 4, the graded doses of the ethyl acetate fraction produce a significant reduction in the intestinal motility in Wistar rats compared with the negative control group. The data revealed that the percentage reduction in gastrointestinal transit of charcoal meal was 28% (*p*<0.05, *p*<0.01), 39.43% (*p*<0.05, *p*<0.01, *p*<0.001) and 41.14% (*p*<0.005, *p*<0.01, *p*<0.001) at doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively, and a more significant effect was produced by Loperamide 52.57% (*p*<0.05, *p*<0.01, *p*<0.001) compared to negative control.

**Table 4:** Effects of ethyl acetate fraction of *O. gratissimum* leaves on Castor oil-induced gastrointestinal motility in rats.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dose Administered** | **Length of Small Intestine (cm)** | **Distance moved by the Charcoal Meal (cm)** | **% Charcoal Meal Transit (Peristaltic Index)** | **% Inhibition** |
| Diarrhea untreated (negative Control) | 88.02 ± 3.61 | 35.00 ± 2.65 | 39.68 | - |
| 3mg/kg loperamide (Positive Control) | 86.20 ± 9.57 | 16.60 ± 1.21a123 | 19.26 | 52.57 |
| 200mg/kg extract | 90.00 ± 2.21 | 25.20 ± 1.53a12 | 28.00 | 28 |
| 400mg/kg extract | 85.40 ± 2.11 | 21.20 ± 0.97a123 | 24.82 | 39.43 |
| 800mg/kg extract | 88.80 ± 2.63 | 20.60 ± 0.68a123 | 23.20 | 41.14 |

Data are expressed as mean ± SEM (n=5); ACompared with negative control; 1*p*< 0.05, 2*p*< 0.01, 3*p*< 0.001.

**3.2.3 *Effect on Castor Oil-Induced Enteropooling***

Compared to the negative controls, the three graded doses of the ethyl acetate fraction of the leaves of *O. gratissimum* significantly inhibited castor oil-induced enteropooling in rat at doses of 200, 400, and 800 mg/kg as revealed by the reduction in volume and weight of intestinal contents. As the data revealed in Table 5, the ethyl acetate fraction decreased the mean volume of the intestinal content by 7.81% , 23.44% , and 32.81% (*p*<0.05), and the weight of the intestinal content by 9.17 % , 25.83%, and 35.00 (*p*<0.05), at 200, 400, and 800 mg/kg doses, respectively, compared to the negative controls. However, the highest percentage of inhibition was recorded in Loperamide 43.75% (*p*<0.005, *p*<0.01) and 47,50% (*p*<0.005, *p*<0.01, *p*<0.001), for percent reduction of volume and weight of intestinal contents respectively.

**Table 5:** Effects of 50% ethanol leaves extract of *O. gratissimum* on Castor oil-induced enteropooling in rats.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dose Administered** | **Mean Volume of Small Intestinal Content (ml)** | **% Inhibition** | **Mean Weight of Small Intestinal Content (g)** | **% Inhibition** |
| Diarrhea untreated (negative Control) | 1.28 ± 1.02 | - | 1.20 ± 0.08 | - |
| 3mg/kg loperamide (Positive Control) | 0.72 ± 0.07a12 | 43.75 | 0.63 ± 0.06a123 | 47.50 |
| 200mg/kg extract | 1.18 ± 0.10 | 7.81 | 1.09 ± 0.10 | 9.17 |
| 400mg/kg extract | 0.98 ± 0.10 | 23.44 | 0.89 ± 0.10 | 25.83 |
| 800mg/kg extract | 0.86 ± 0.05a1 | 32.81 | 0.78 ± 0.05a1 | 35.00 |

Data are expressed as mean ± SEM (n=5); ACompared with negative control; 1*p*< 0.05, 2*p*< 0.01, 3*p*< 0.001.

**3.2.4 *In vivo Antidiarrheal Index***

The *in vivo* antidiarrheal index (ADI) was measured by considering three parameters as shown in Table 6. These are delays in defecation (Dfreq), gut meal travel distance (Gmeq), and purging frequency in the number of wet stools (Pfreq). The ethyl acetate fraction of *O. gratissimum* showed a dose-dependednt antidiarrheal index of 38.02, 62.95, and 79.83 at doses of 200, 400, and 800 mg/kg, respectively, while standard drug produced a maximum index of 135.35.

**Table 6:** *In vivo* Antidiarrheal indices of ethyl acetate fraction of *O. gratissimum* leaves on Castor oil-induced diarrheal model in rats.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dose Administered** | **Delay in Defecation (Time of Onset in Min. Dfreq) (%)** | **Gut Meal Travel Distance(Gmeq) (%)** | **Purging Frequency in Number of Wet Stools (Pfreq) (%)** | **Antidiarrheal Index (ADI)** |
| Diarrhea untreated (negative Control) | - | - | - | - |
| 3mg/kg loperamide (Positive Control) | 481.86 | 51.46 | 100.00 | 135.35 |
| 200mg/kg extract | 51.33 | 29.44 | 36.36 | 38.02 |
| 400mg/kg extract | 133.19 | 37.45 | 50.00 | 62.95 |
| 800mg/kg extract | 179.65 | 41.53 | 68.18 | 79.83 |

1. **DISCUSSION**

“Diarrhea is a common global problem that continues to be a significant cause of morbidity and mortality worldwide” (Mansour *et al*., 2014). “*Ocimum gratissimum* (OG), commonly called scent leaf, is a plant employed by different ethnic groups in Nigeria and other parts of the world as a culinary spice and vegetable to prepare soup and stew” (Ugbogu *et al*., 2021). “It is also applied in folklore medicine to manage and treat several pathologies such as common cold, body aches, pneumonia, diarrhoea, anemia, inflammation, and bacterial and fungal infections” (Akara *et al*., 2021). “Phytochemical analysis revealed that *O. gratissimum* leaf contains flavanoids, phenolics, tannins, phlobatamims, alkaloids, terpenoids, phytic acid and steroids in varying concentrations which is consistent with components of the plant reported by some studies” (Ojo *et al*., 2013).

An acute toxicity study was carried out to determine possible adverse reactions to a low dose and high dose administration, its observed that the lower the fraction of ethyl acetate administration the low tendency of mortality rate, while the higher the fraction dose the higher tendency of the mortality and signs of overt toxicity. The LD50 value of the ethyl acetate fraction of *O. gratissimum* leaves is 3,807.89. This entails that the fraction is not very toxic as the LD50 value happens to be high. Low LD50 value will indicate adverse toxicity but, in this case, the LD50 value was observed to be high.

In this study, diarrhea was induced experimentally by using castor oil, as this model is the general model to test the antidiarrheal activities of different substances. The ethyl acetate fraction of *O. gratissium* leaves was found to be effective against castor oil-induced diarrhea in terms of delaying the onset of diarrhea at 200 mg/kg, 400 mg/kg, 800 mg/kg tested doses as well as reduced the number and weight of wet and total stools) compared to the negative control (table 3). As presented in Table 4, the graded doses of the ethyl acetate fraction produce a significant reduction in the intestinal motility in Wistar rats compared with the negative control group. According to Hedge *et al*. (1994), rats were considered protected against diarrhea if they formed well-shaped wet feces and considered unprotected against diarrhea if they formed shapeless feces or unshaped feces with a large amount of liquid.

“In the castor oil-induced diarrhea model, the extract of *O. gratissimum* leaves at all tested doses significantly delayed the onset of defecation, reduced the number and weight of both wet and total fecal output. The highest dose, 800 mg/kg, significantly delayed the onset of diarrhea caused by castor oil when compared with the negative control, and the percentage of inhibition of defecation produced was closer to the inhibition produced by the standard drug. Furthermore, the fraction displayed a dose-dependent reduction in the percentage of weight of wet fecal output and weight of total fecal output. The reduction in the frequency of defecation, the weight of wet stools, and total stools indicates the efficacy of ethyl acetate fraction of *O. gratissimum* as an antidiarrheal agent. The results obtained support the report done in Nigeria” (Olayemi *et al*., 2016; Okere *et al*., 2015). “Castor oil also causes diarrhea by increasing oxidative stress on the intestinal epithelium which in turn alters the movement of electrolytes and water through the intestinal mucosa” (Jabri *et al*., 2016). “Liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandin E2 (PGE2), which results in the stimulation of motility and secretion, and the prevention of reabsorption of NaCl and water” (Tangpu *et al*., 2014).

“To prove the antidiarrheal activity of the ethyl acetate fraction of *O. gratissimum,* the possible mechanism of action was tested on intestinal motility and enteropooling models. In the castor oil-induced gastrointestinal motility model, it was observed that the fraction significantly suppresses the movement of the charcoal marker at 200 mg/kg, 400 mg/kg, and 800 mg/kg doses as compared to the negative control. The higher percentage of inhibition 41.14% (*p*<0.05, *p*<0.01, *p*<0.001), of the marker perceived at maximum dose was almost comparable to the standard drug (loperamide) 52.57% (*p*<0.05, *p*<0.01, *p*<0.001) at the dose of 3 mg/kg). These findings revealed that the fraction can influence the peristaltic movement of the intestine, thereby indicating the presence of intestinal antimotility activity. Concerning this, several plants have shown antidiarrheal activities by reducing the gastrointestinal motility and its secretions” (Mekonnen *et al*., 2018), “Antidiarrheal activity has been found in plants possessing tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids” (Havagiray *et al*., 2004).

“Tannins and flavonoids are suggested to be responsible for antidiarrheal activity by increasing colonic water and electrolyte reabsorption, and tannins could also decrease the irritability of the bowel, thereby reducing the peristaltic index” (Tadesse *et al*., 2014). “Tannins that are present in antidiarrheal plants could denature proteins in the intestinal mucosa by forming protein tannins, which may reduce secretion” (Jia *et al*., 2008). “Tannins are astringent, bitter plant polyphenols, which either bind and precipitate or shrink proteins” (Ashok and Upadhyaya, 2012). “Moreover, herbs with astringent properties are recommended as a treatment for diarrhea” (Palombo *et al*., 2006). “Other studies suggested that the anti-motility properties of herbs are mostly due to flavonoids” (Labu *et al*., 2015) by inhibiting the release of autacoids and prostaglandins results in inhibiting motility and hydro-electrolytic secretions induced by ricinoleic acid (Meite *et al*., 2009).

1. **CONCLUSION**

Results obtained in this study revealed that the ethyl acetate fraction of *O. gratissimum* administered at the investigated doses showed antidiarrheal activity in an animal model by decreasing the number of wet feces, gastrointestinal motility, and intraluminal fluid accumulation in the intestine. The presence of bioactive secondary metabolites such tannins, flavonoids, alkaloids, and saponins may be responsible for the antidiarrheal effects. These results offer scientific backing for the long-held belief that O*. gratissimum* can be used to cure diarrhea. The biochemical mechanism underlying the antidiarrheal efficacy of the ethyl acetate fraction of *Ocimum gratissimum* leaves will require more research.

Ethical approval

All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Nnamdi Azikiwe Animal Care Ethics Committee.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, Meta etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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