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*Original Research Article*

# FORMULATION OF *Ocimum gratissimum* LEAVES EXTRACT SUSPENSION AND EVALUATION OF ITS ANTIDIARRHEAL AND HAEMATOLOGICAL ACTIVITY ON ALBINO WISTAR RATS

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## ABSTRACT

Diarrhoea is the third leading cause of death in children less than 5years according to the World Health Organisation (W.H.O) 2021. Its major complications are dehydration and malnutrition. This has necessitated the need for affordable, readily available sources of anti-diarrhoea medications from natural sources, especially in developing countries. *Ocimum gratissimum* leaves, known as African basil is widely available in Nigeria. They were macerated in ethanol for 3days with intermediate agitation, filtered, and concentrated. The extract was subjected to $LD_{50}$ to ensure its safe concentration for the formulation of suspensions. Suspension was formulated using standard procedure and maintaining good manufacturing practise (GMP). Diarrhoea was induced using castor oil and gastro-enteric bacteria *E. coli* separately. A pilot study showed 0.8 mL of *E.coli* broth as the least colony on an EMB agar plate to infect the rats. The suspensions were formulated using 0.5 %w/v and 1.25 %w/v of the *Ocimum gratissimum* leaves extract. Group C and D contained standard loperamide and normal saline, respectively. The results, only formulation A and B containing 0.5 %w/v and 1.25 %w/v of the *Ocimum gratissimum* extract showed 78.9 % and 89.5 % faeces inhibition when compared with the standard loperamide (Group C), which had the least frequent stool with 84.4 % faeces inhibition, while Group D had 0 % faeces inhibition. Haematological analysis of the albino rats showed increased platelet count, prominent in group D. It could be due to the increased diarrhoea and suggests the relationship of diarrhoea and thrombocytosis, if left untreated, could lead to blood clotting. Parameters of group A and B are comparable with the negative control, as there was no significant difference using statistical methods. These results show that *Ocimum gratissimum* leaves extract at 1.25 %w/v is a suitable alternative in the treatment of diarrhoea caused by gastro-enteric bacteria and it is safe.

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## INTRODUCTION

Diarrhoea, defined as the frequent passage of loose or watery stools in a day, remains a significant global health issue [1]. According to the World Health Organization (WHO, 2024), diarrhoea is the third leading cause of death among children under five years old, accounting for approximately 1.7 billion cases annually. Diarrhoea is a symptom of an intestinal tract infection which can be caused by a variety of bacterial, viral and parasitic organisms [2]. It can be classified into acute, persistent and chronic forms. The severity of diarrhoea depends on its duration and the underlying causes, ranging

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from mild dehydration to life-threatening electrolyte imbalances.

Diarrheal diseases disproportionately affect low- and middle-income countries due to poor sanitation, unsafe drinking water, and limited access to healthcare services. The condition contributes significantly to malnutrition and stunted growth, particularly in children [3] [2]. Now, septic bacterial infections are likely to cause an increased proportion of all diarrhoea-associated deaths [2].

In low-income countries, children under the age of three years experience an average of three diarrhoea episodes [2]. Infection is more common when there is a shortage of adequate sanitation and hygiene and safe water for drinking, cooking and cleaning. Among children under 5 years of age, the most common viral pathogens are rotavirus, norovirus, adenovirus and astrovirus. Bacterial pathogens include *Escherichia coli*, *Salmonella spp*., and *Shigella spp*.[1],[2],[3]. This results in widespread malnutrition and stunted growth, prompting the need to have an alternative source of remedy in the prevention and treatment of diarrhoea.

A Pharmaceutical Suspension are coarse dispersion in which the internal phase (extract) is dispersed into the external phase [4]. The route of administration can be used to classify suspensions. The oral suspension is given through the oral route [5]. The extract of *Ocimum gratissimum* leaves was used to formulate an oral suspension against gastro-enteric bacteria causing diarrhoea *E. coli* [15].

*Ocimum gratissimum*, commonly known as African basil or clove basil, is a perennial herb that belongs to the Lamiaceae family. It is widely distributed in tropical and subtropical regions, particularly in Africa, India, and Southeast Asia [6]. Known for its aromatic properties, *Ocimum gratissimum* has been extensively used in traditional medicine for various therapeutic purposes. Its leaves, seeds, and essential oils are believed to possess numerous medicinal properties, including antimicrobial, antidiarrheal, antioxidant, and anti-inflammatory effects [7], [8].

*Ocimum gratissimum* may help protect intestinal tissues and promote healing [9]. This makes it a promising candidate not only for treating acute diarrhoea but also for managing chronic gastrointestinal conditions. Furthermore, *Ocimum gratissimum* has been investigated for its anti-inflammatory effects. The plant's ability to modulate the immune system and reduce inflammation suggests that it may have applications beyond simple symptom relief, potentially addressing the underlying causes of diarrhoea [8]. The modulation of pro-inflammatory cytokines by *Ocimum gratissimum* flavonoids has been suggested as a mechanism by which the plant exerts its therapeutic effects [10].

## MATERIALS AND METHODS

### Materials

Fresh leaves of *Ocimum gratissimum*, *Escherichia coli* (Pharmaceutical Microbiology laboratory, Madonna University), Wistar rats, Loperamide (PHINODIOM), Glycerin, Distilled water, Ethanol (Central Drug House Ltd, India). Analytical Balance (Shimadzu ATX224 USA), Water bath (Medline Scientific), incubator, pH meter (HANA). Weighing balance (Ohaus Corp, Pine Brook, NJ, USA), Rotor Evaporator (Barnstead, UK)

**Preparation and extraction of leaves**

Fresh leaves of *Ocimum gratissimum* were purchased in a market in Port Harcourt, Nigeria. The plant was identified and authenticated by a Pharmacognosist and given a University Herbarium number MU/PHGSY/24/001. The plant leaves were hand separated from the stems and flowers, washed under a clean running tap water and air dried in the shade.

With the aid of a tabletop manual blender, the dried plant material was reduced to a coarse powder. A 250 g of *Ocimum gratissimum* powdered leaves were macerated in 1.2 L of Ethanol for 72 hours with intermediate shaking every 24 hours. It was filtered using a Muslin cloth to obtain a particle-free filtrate. The filtrate was concentrated at 30 $^{o}$C using a rotor evaporator. The extract was further prepared into a suitable suspension at various concentration 0.2 g (0.5 %w/v), 0.5 g (1.25 %w/v) and 1 g (2.5 % w/v).

**Percentage extract yield**

The percentage yield of the extract was calculated by weighing the total extract yielded and calculating it against the coarsely milled *Ocimum gratissimum* leaves.

**Toxicity Testing**

$LD_{50}$ of *Ocimum gratissimum* extract was carried out using different % strength of the extract [14], grouped into three batches A, B and C containing 0.5 %w/v, 1.25 %w/v and 2.5 %w/v, respectively. Fifteen albino rats were grouped into three cages of n-5 and were treated with the formulation. 2 mL of each was administered orally and observed.

**Formulation of Suspension**

Suspension of *Ocimum gratissimum* leaves extract was carried out using the United States Pharmacopoeia (USP) standard for the Toxicity testing and treatment of both sexes of the Albino Wistar rats.

**pH Evaluation of the Formulated *Ocimium gratissimum* extract Suspension:**

A 0.5 mg of the extract was dissolved in 50 ml of distilled water, a pocket pH meter was used to test for the pH of the suspension by dipping the pH meter into the mixture and allowed to stand for 2 minutes before the reading was taken.

**Evaluation of the antidiarrhea effect (castor oil induces diarrhoea):**

Twenty Wistar albino rats of both sexes (90 – 100g) were purchased and acclimatized to normal laboratory conditions for four weeks before the study. Appropriate feed and clean water were given to them. The Institution's Ethical Committee approved the protocol for this study under the number. MAU/DRC/HD/E/PHARM/2024/020, and the animals were

handled according to guideline of the National Institute of Health Guide for Care and Use of Laboratory Animals.
25 rats of both sexes were divided into four groups of five rats each (n-5) and were labelled group A to D, respectively, in separate cages lined with white paper. The animals were fasted for a period of 18 hours but with water, after which the treatment was started as stated below. Group A, was administered orally, with 2 ml of 0.2 g (0.5 %w/v) of plant suspension extract, Group B, was administered orally, with 2 ml of 0.5 g (1.25 %w/v) of plant suspension extract, Group C, was administered orally, with 0.5 ml of 16 g (40 %w/v) Loperamide, and Group D was administered orally, with 2 ml of 0.9 % normal saline while Group E left with no treatment.
An hour following this prophylactic treatment, 2 mL of castor oil was administered to each rat in various groups (group A to D) and were observed for 6 hours, then the results and effect was recorded for each group.

**Pilot study**
Eight Wistar rats of both sex was divided into four groups in different cages of two rats each (n-2), labelled group 1 to 4 respectively, to ascertain the minimum concentration of the diarrhoea-inducing bacteria (*E.coli*) to be used to induce diarrhoea in the rats.

**Evaluation of the anti-diarrhoea effect (*E. coli* Induced of Diarrhoea)**
Twenty-five Wistar albino rats of both sexes (90 - 100 g) were purchased and acclimatized to normal laboratory conditions for four weeks before the study, and appropriate feed and clean water were given. The animals were handled according to guideline of the National Institute of Health Guide for the Care and Use of Laboratory Animals. The animals were given only water for up to 18 hours, after which the treatment was started as stated below. Group A, was administered orally, with 2 ml of 0.2 g (0.5 %w/v) of plant suspension extract, Group B, was administered orally, with 2 ml of 0.5 g (1.25 %w/v) of plant suspension extract, Group C, was administered orally, with 0.5 ml of 16 g (40 %w/v) Loperamide, and Group D was administered orally, with 2ml of 0.9% normal saline.
After the prophylactic treatment, four groups, which are the Group A to D, were infected intraperitoneally with 0.8 mL of *Escherichia coli*, respectively. Group E, which is the Negative control group, was not administered *Escherichia coli*. This procedure was repeated for a period of 3 days. The treatment procedure is as shown in Table 1.

The animals were observed for the presence of diarrhoea, defined as more frequent passage of loose and watery stools than the normal [2].

**Collection of Blood Samples**
Haematological samples were collected from the animals using a capillary tube from the eye socket to check the effect of the diarrhoea on the haematological parameters.

**Statistical Analysis**
Data were analysed using SPSS Statistics 22.0; data are expressed as mean ± S.D. A one-way analysis of variance was also used, and differences in parameters were considered significant at $P > 0.5$.

## RESULTS
Table 2 showed percentage yield of *Ocimum gratissimum* leaves extract as 3.12 %.

**Toxicity Testing**
Results of $LD_{50}$ in Table 3 show that after 24 h, the rats in cages A and B were active, feeding well and didn't show any sign of toxicity, while the rats in cage C showed signs of toxicity and 50 % died, while after 48 hours, all of them died. Concentration 0.5 %w/v and 1.25 %w/v in formulation A and B, respectively, was suggested to be safe and was used for this work.

**Formulation of Suspension**
The suspensions were formulated as stated in the Pharmaceutical Codex and following good manufacturing practise as stated in the Pharmacopoeia. Two different Formulations Concentration A at 0.5 % w/v and Concentration B at 1.25 %w/v, were prepared as shown in Table 4.

**pH Evaluation of the Suspension**
The pH of the formulated *Ocimum gratissimum* extract Suspension is 6.8, which is the pH specific for the active ingredient in the formulation, as shown in Table 5.

**Evaluation of anti-diarrhoea Treatment (Castor oil induced)**
Results showed that *Ocimum gratissimum* has a significant antidiarrheal effect. The animals in group A, as shown in Table 6 and Figure 1, has 16 % diarrhoea severity, group B has 6.7 %, while group C is 0 % severity, suggesting that the plant extract has a dose-dependent antidiarrheal effect. The 1.25 %w/v (Group B) nearly matches the effectiveness of loperamide, with a high inhibition percentage of 89.5 % in *E. coli* induced diarrhoea. The result suggests that although Loperamide has a lower frequency of stool, the % diarrhoea inhibition is 84.4 % (in *E.coli* induced diarrhoea), which indicates its efficiency in reducing intestinal motility, but does not have antibacterial activity like *Ocimum gratissimum* extract, which also has higher antibacterial activity. This could be due to the bioactive components of *Ocimum gratissimum* that inhibit diarrhoea, possibly by reducing intestinal motility or by impacting water and electrolyte absorption, though the exact mechanism would require further investigation [11].
The 0.5 %w/v (Group A) also demonstrates antidiarrheal properties, but with a slightly lower inhibition rate of 78.9 %.

**Table 1: Treatment of Each Group**

| Group | A | B | C | D |
|---|---|---|---|---|
| Content(% strength) | Extract 0.5 | Extract 1.25 | Loperamide 40 | Normal saline 0.9 |
| Extract amount (g) | 0.2 | 0.5 | - | - |
| Product amount (g) | - | - | 16 | - |
| Quantity administered (mL) | 2 | 2 | 0.5 | 2 |

**Table 2**: **% Yield of the Leaves extract**

| Plant | Weight of plant (g) | Weight of extract (g) | % extract yield |
|---|---|---|---|
| *Ocimum gratissimum:* Leaves | 250 | 7.8 | 3.12 |

**Table 3**: **Formulation of Suspension for $LD_{50}$**

| Formulation Group | A | B | C |
|---|---|---|---|
| % Strength (w/v) | 0.5 | 1.25 | 2.5 |
| Extract (g) | 0.2 | 0.5 | 1.0 |
| Distilled water | qs | qs | qs |
| Raspberry (drops) | 2 | 2 | 2 |
| Chloroform $H_20$ (drops) | 2 | 2 | 2 |
| Glycerol (mL) to | 40 | 40 | 40 |

**Table 4: Formulation of Suspension**

| Formulation Group | A | B |
|---|---|---|
| % Strength (w/v) | 0.5 | 1.25 |
| Extract (g) | 0.2 | 0.5 |
| Distilled water | qs | qs |
| Raspberry (drops) | 2 | 2 |
| Chloroform $H_20$ (drops) | 2 | 2 |
| Glycerol (mL) to | 40 | 40 |

**Table 5**: **pH Reading**

| Formulation | Content | pH |
|---|---|---|
| A | 0.5 % w/v extract | 6.8 |
| B | 1.25 % w/v extract | 6.8 |
| C | 0.9 % Normal saline | 7.1 |

**Table 6: % Severity of Diarrhoea (Castor oil Induced diarrhoea)**

| Groups | Content | Number of Normal faeces | Number of wet faeces | Total number of faeces | % Severity of Diarrhoea |
|---|---|---|---|---|---|
| A | 0.5 %w/v extract | 21 | 4 | 25 | 16 % |
| B | 1.25 %w/v extract | 14 | 1 | 15 | 6.7 % |
| C | Loperamide | 10 | 0 | 10 | 0 % |
| D | Normal saline | 0 | 40 | 40 | 100 % |

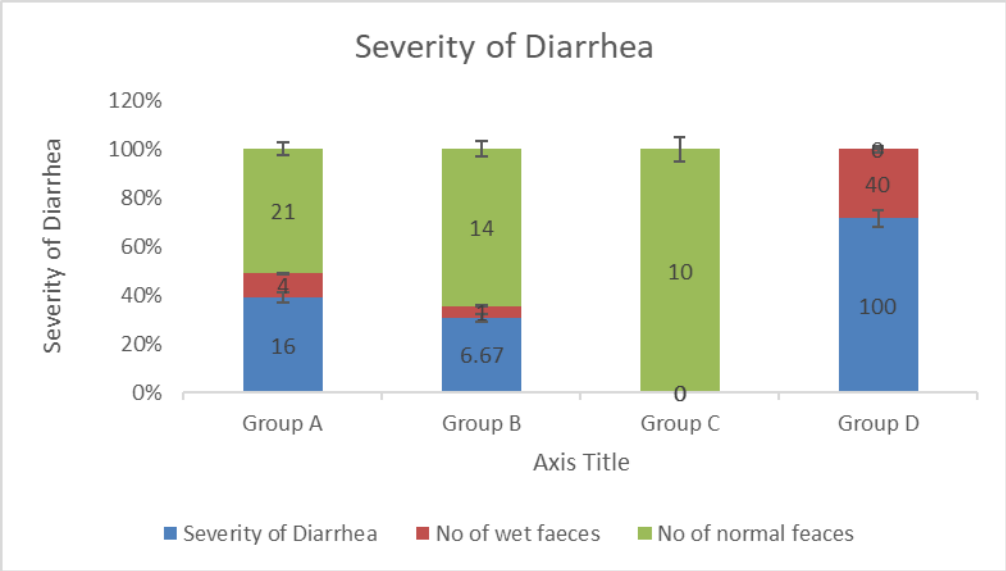


**Figure 1. Severity of Diarrhoea**

**Table 7: Showing %age wet faeces inhibition**

| Formulations | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Leaves of *Ocimium gratisimium* (g) | 0.2 | 0.5 | - | - |
| Loperamide (g) | - | - | 16 | - |
| Normal Saline (g) | - | - | - | 0.9 |
| Number of wet faeces | 4 | 2 | 1 | 19 |
| Number of normal faeces | 8 | 14 | 5 | 0 |
| Total Number of faeces | 12 | 16 | 6 | 19 |
| % Wet faeces inhibition | 78.9 | 89.5 | 84.4 | 0 |

**Table 8: Haematological parameters**

| Group | WBC ($\times10^{9/L}$) | LYP | GRAND (g/L) | RBC ($\times10^{12}$/L) | PLT ($\times10^{9}$/L) | HGB (g/L) |
|---|---|---|---|---|---|---|
| D Normal saline | 8.2 ±0 | 7.2±0.14 | 0.65±0.7 | 6.13±0.03 | 1259±2.83 | 12.0±0 |
| E Negative control | 8.0±2.4 | 6.20±1.41 | 1.35±0.78 | 6.95±0.02 | 692±35.36 | 14.05±0.49 |
| C Standard control | 10.80±0.85 | 8.95±1.91 | 1.30±0.85 | 7.01±0.77 | 738.5±244.66 | 14.35±1.34 |
| A {0.2 (g) (0.5% w/v)} | 11.15±1.63 | 8.45±1.26 | 2.00±0.28 | 6.53±0.11 | 715±244.66 | 13.50±09 |
| B {0.5 (g) (1.25% w/v)} | 8.15±0.21 | 6.95±0.64 | 0.75±0.21 | 6.68±0.83 | 814.5±55.86 | 13.75±1.48 |

This decrease in effectiveness at a lower dose indicates a dose-response relationship for *Ocimum gratissimum* leaves antidiarrheal effect.

**Antidiarrheal effect of the *Ocimum gratissimum* leaves suspension (*E. coli-induced* diarrheal)**

Table 7 shows the inhibition of diarrhoea by the number of wet faeces passed.

**Haematological Parameters**

Table 8 shows the results of the haematological parameters of Groups A to E.

## DISCUSSION

This research highlights the potential of *Ocimum gratissimum* as a safe, effective, and affordable alternative to synthetic antidiarrheal drugs. $LD_{50}$ indicate its safety at 1.25 %w/v and 0.5 %w/v as shown in Table 3 [16]. It investigates the antidiarrheal effects of *Ocimum gratissimum* (scent leaf) leaf extract suspension in albino rats. Diarrhoea, a leading cause of mortality in children under five, especially in resource-limited settings, necessitates accessible and effective treatments. The results showed that it has both the potential to treat diarrhoea induced by both castor oil and gastro - enteric bacteria *E. coli,* which signifies anti-bacterial activity. These results confirm its use by ancient people in the treatment of different ailments.

The activity of *O.gratissium* was due to rich phytochemical constituents present in the plant [12]. The result showed a reduction of severity of diarrhoea caused by Castor oil as 90% compared with 100 % standard loperamide anti-diarrhoea medication. Its inhibition of diarrhoea caused by *E. coli* is 89.5 % greater than that of loperamide, which is 84.4 %, this showed that it has potent antibacterial activity against

gastro-enteric bacteria more than loperamide, but loperamide showed less frequent stools, which may be due to a reduction of the intestinal motility. Studies suggest a relationship between diarrhoea caused by Inflammatory bowel disease (IBD) and thrombocytosis, unlike gastroenteritis [13]. The platelet counts of the animals in group D showed a significant difference from other groups, and the haemoglobin level (Hb) is significantly low at P > 0.5 % confidence interval. Group A and Group B showed an increased WBC count, unlike the group E (Negative control), which was not infected with *E. coli* bacteria; this could be an inflammatory response to the underlying cause of the diarrhoea to fight off the infection [17]. Hb level of the rats in group D treated with only Normal saline was significantly decreased at P > 0.05 %, which showed that prolonged diarrhoea can cause anaemia after fluid loss reduces Plasma volume. The results showed that Group C and Group B have the same inhibition of wet faeces, as the difference in the number of wet faeces passed was insignificant at P > 0.5 %

Haematological parameters also showed that albino rats treated with *O.gratissimium* leaves extract have no significant changes from the albino rats not infected with *E. coli* bacteria or induced with castor oil, which indicates antidiarrheal activity, restoration of plasma blood volume and safety of the product.

## CONCLUSION

This research has been able to demonstrate the anti-diarrhoea effect of Ocimum gratissimum leaves extract suspension, confirming its use in traditional medicine for diarrhoea management. It may be considered a promising complementary approach to managing diarrhoea and also beneficial to individuals at a 1.25 %w/v concentration to get a standard treatment. From the castor oil induced diarrhoea results above, data suggests that Ocimum gratissimum leaves extract exhibits strong antidiarrheal activity particularly at the 1.25 %w/v concentration. Its efficacy at inhibiting wet faeces production is closely to that of loperamide, a standard antidiarrheal medication. In fig 1, the % severity of diarrhoea is 0 % with Loperamide and 6.67 % with product B at 1.25 %w/v. Results shown a dose-dependent efficiency of the plant extract. Therefore, Ocimum gratissimum could potentially be developed as a natural remedy for diarrhoea, although additional studies would be needed to further understand its mechanism of action and to determine optimal dosing.

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## AUTHORS' CONTRIBUTION

ACO, developed the research ideas, methodology, design, sourced some of the materials, logistics, laboratory work and contributed time, energy and some financial resources required for the research. Proof read the work, edited and made valuable corrections and guidelines on reporting of the data generated from the research. She is the brain behind the work. TDA carried out the extraction, sourced some materials, laboratory onsite work under the supervision and guidance of ACO, she sourced funds used in this research and also engaged all her time, energy and resources in typesetting of the work. MO also gave some valuable contribution in study ideas and guideline towards reporting of the data generated in the work and assisted in proofreading the work.

## CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

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