

Phytochemical Constituents of *Ocimum gratissimum* extract and its effect against gastro-enteric bacteria on the intestinal linings of an albino Wister rats

Amarachi Chukwuma Offor ^{1,*}, Toyosi Deborah Adebola ² and Chizoba Enemchukwu ²

¹ Department of Pharmaceutics and Pharmaceutical Technology, University of Port-Harcourt, Rivers State, Nigeria.

² Department of Pharmaceutics and Industrial Pharmacy, Madonna University Nigeria.

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Abstract

Enteritis is the inflammation of the small intestines which could lead to diarrhea. Bacterial gastroenteritis is a digestive system problem caused by bacteria. The symptoms include nausea, vomiting, fever, abdominal cramps and diarrhea. These can be treated with antibiotics, but in recent times, especially in economically developing countries like Nigeria, these drugs have become extremely costly resulting to the low purchasing power of the greater populace. Antibiotic resistance due to abuse also necessitated a growing need for alternative and affordable source of anti-bacteria medication. *Ocimum gratissimum* leaves were collected, selected and pulverized, it was macerated in ethanol for 3 days with intermediate agitation, filtered, and concentrated. The extract was subjected to LD₅₀. Phytochemical analysis were done on the plants leaves to check for secondary metabolites. A pilot study of *E.coli* was carried out with 8 albino rats grouped into four. Different titrations of *E.coli* were administered intraperitoneal to the rats to determine the minimum amount of *E.coli* capable of infecting the rats, the stool sample was inoculated in a sterile broth and then cultured in an EMB agar plate selective for *E.coli*. After 24 hours culture, a 0.8 mL was selected as it showed the least colony. The extract of *Ocimum gratissimum* was formulated using 0.5%w/v, 1.25%w/v and 0.9% normal saline as control in Group A, B and C respectively. These were used to treat the 20 albino wistar rats grouped A to D (at n = 5). Group D Negative control was not infected with 0.8 mL of *E.coli* broth. The results showed that *Ocimum gratissimum* is rich in flavonoids, tannins, saponins, resins, terpenoids, alkaloids, fats and oils. Pictorial results of the histopathology shown that albino rats of group A and C have normal to mild intestinal tract, not erupted or distorted but that it is dose dependent as group B with 1.25%w/v extract restored the intestinal linings of the albino rats to that of the Negative control which was not infected intraperitoneal. Results proved that *Ocimum gratissimum* has activity against gastroenteritis bacteria *E. coli* and can serve as an alternative in treatment of bacteria enteritis as it is readily available and affordable.

Keywords: *Ocimum gratissimum*; Gastroenteritis; Phytochemical; Intestinal Linings; Histopathology; Extract

1. Introduction

Gastroenteritis is a digestive system problem caused by bacteria. It can affect one person or group of persons who ate the same food, normally called food poisoning [1]. The symptoms of acute bacterial infection are usually from mild, moderate to severe cases which causes deterioration of the patient's condition. It has symptoms like vomiting, severe abdominal pain, fever, intestinal bleeding- resulting in bloody stool and diarrhea [2].

One of the most common causes of intestinal bacteria are *Escherichia coli*, *Salmonella* species, *Shigella* species and *Campylobacter jejuni*. Some symptoms depends on the type of bacteria that caused the sickness and could be mild discomfort to severe life-threatening complications such as dehydration, electrolyte imbalance [3].

* Corresponding author: Amarachi Chukwuma Offor

Treatment of gastroenteritis includes taking in lots of fluids, manage diarrhea, and getting plenty of rest. Children under the age of five years are mostly at the risk of dehydration, therefore requires close monitoring. The use of antibiotics is only recommended in severe cases [1].

Recent studies have also explored the antioxidant properties of *Ocimum gratissimum*, which are largely attributed to its high content of phenolic compounds and flavonoids [4] Oxidative stress is known to exacerbate inflammatory responses in the gastrointestinal tract, and antioxidants play a crucial role in mitigating this damage. By reducing oxidative stress, *Ocimum gratissimum* may help protect intestinal tissues and promote healing as stated in Ajayi *et al.*, (2019) [5]. This makes it a promising candidate not only for treating acute diarrhea but also for managing chronic gastrointestinal conditions. Studies have shown that *Ocimum gratissimum* have different phytochemicals present in the plant of which include; terpenes, flavonoids, sterols, alkaloids, glycosylate, saponin, polyphenols, volatile compounds and resin [6].

In this research, the effect of *Ocimum gratissimum* against the effect of *Escherichia coli* on the intestinal linings of the albino wistar rats were analyzed, and compared to the intestinal linings of a non-infected albino rats.

2. Materials and methods

2.1. Materials

Ocimum gratissimum leaves, Ethanol (Central drug house Ltd, India), *Escherichia coli* (Pharmaceutical microbiology lab. Faculty of Pharmacy Madonna University), Albino Wistar rats (Pharmacology Animal farm of Madonna University), Distilled water, EMB agar, sterile broth, Analytical Balance (Shimadzu ATX224 USA), water bath (Medline Scientific), incubator, Manual blender, Weighing balance (Ohaus Corp, Pine Brook, NJ USA), Rotor Evaporator (Barnstead, UK).

2.2. Methods

Twenty albino wistar rats of both sex (90-100g) were purchased and acclimatized to normal laboratory condition for four weeks prior to study, appropriate feed and clean water were given to them. The Institution's Ethical Committee approved the protocol for this study under the ethical number MAU/DRC/HD/E/PHARM/2024/020 and the animals were handled according to guideline of National Institute of Health Guide for Care and Use of Laboratory Animal.



Figure 1 Picture of *Ocimum gratissimum* plant

The plant *Ocimum gratissimum* was bought from oil mill market in Port-Harcourt Rivers State, it was identified by a Pharmacognost in Madonna University, inspected and selected out for discoloration and mottling of the leaves, the leaves were separated from the stems with hands, adequately sorted out, cleaned and air dried under a shade. It was coarsely milled using a manual blender, then stored in an airtight container for future use. A 300 g were macerated in 500 mL of ethanol for three days with intermittent agitation every 24 hours interval.

2.3. Phytochemical analysis

A weighed amount of the powdered milled sample was subjected to phytochemical screening using stipulated procedure described by Harborne (1998) [7], Trease and Evans (2009)[8].

Test for alkaloids, flavonoids, Saponins, carbohydrate, tannins, terpenoids, reducing sugar, fats and oils, carbohydrate, steroid, resin, glycosides and acidic compounds were carried out [9].

2.4. For Glycoside

50mg of the aqueous *Ocimum gratissimum* leaf extract was hydrolyzed with 5ml concentrated HCL for 2 hours in water bath and filtered. A 2 mL of hydrolyzed were filtered, hydroxylate were taken in a test tube and 3 mL of chloroform was added to the test tube. The appearance of pink color which indicated the presence of glycoside [10], [11].

2.5. Pilot Study

A pilot study was carried out to determine the minimum amount of the *E. coli* capable of causing an infection in the albino wistar rats. This was done after infecting the rats with the stipulated amount of *E.coli* broth, after 24 hours, stool from each group was put into four different test tube containing a sterile broth and was stricken on an EMB agar plate and incubated for a period of 24 hours.

After 24 hours, growth was seen in groups 2 to 4 indicating the presence of *E.coli* in the stool of the albino wistar rats. Group 2 (0.8 mL) was selected as the best and least concentration to cause an infection in the rats. A 0.8 mL was used to infect the wistar rats via intraperitoneal.

Table 1 The Pilot Test

| Albino Wistar rats | Group 1 | Group 2 | Group 3 | Group 4 |
|----------------------|---------|---------|---------|---------|
| Amount of Broth (mL) | 0.4 | 0.8 | 1.0 | 2.0 |

A 20 rats of both sex were divided into four groups of four rats each (n-5) and was labelled group A to D respectively, in separate cages lined with white paper, and treatment was conducted as follows. Groups A to C were infected with *E. coli* via intraperitoneal, while Group D was not infected and served as Negative control. This procedure was repeated for a period of 3days. The animals were observed for the presence of diarrhea defined as more frequent passage of stool than the normal or passage of loose and watery stool.

After the observation, the animals were sacrificed to evaluate the effect of the *E.coli* on the gastro-Intestinal tract (GIT) of the Wistar rats and to evaluate if *Ocimum gratissimum* was able to inhibit its effect [12].

Table 2 The antibacterial activities of *Ocimum gratissimum* against *E. coli* bacteria

| Groups | A | B | C |
|---------------------|--------------------------------------|--------------------------------------|---------------|
| Formulation content | Extract of <i>Ocimum gratissimum</i> | Extract of <i>Ocimum gratissimum</i> | Normal saline |
| Extract amount (g) | 0.2 | 0.5 | - |
| % age strength | 0.5%w/v | 1.25%w/v | 0.9%w/v |

3. Results

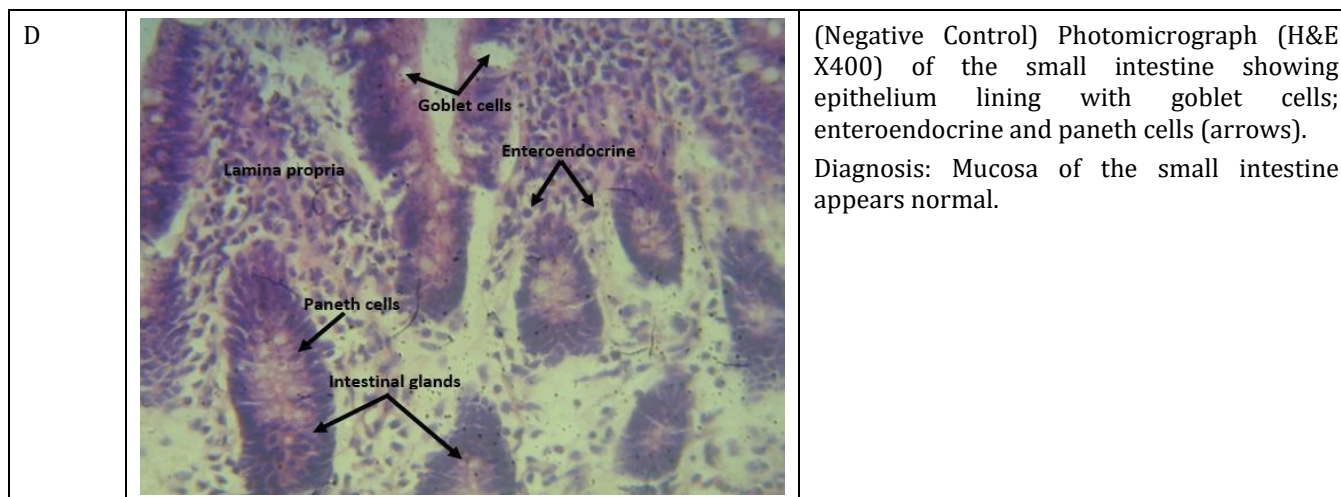
Table 3 The result of the qualitative phytochemical screening of the plant leaf *Ocimum gratissimum*

| Phytochemicals | Result |
|-----------------|--------|
| Alkaloid | + |
| Flavonoid | ++ |
| Saponin | ++ |
| Terpenoid | +++ |
| Reducing sugar | + |
| Tannin | +++ |
| Fats and Oil | ++ |
| Acidic compound | + |

| | |
|-----------|-----|
| Steroid | +++ |
| Resin | +++ |
| Glycoside | - |

Table 4 The Histology Result of the H & E staining on the intestinal mucosa lining

| Groups | Histopathology pictorial | Report |
|--------|--------------------------|--|
| A | | <p>Extract (0.5%w/v) Photomicrograph (H&E X400) of the mucosa with mild intestinal gland atrophy and mucosa cells degeneration (arrows)</p> <p>Diagnosis: Mucosa of the small intestine appears mildly distorted.</p> |
| B | | <p>Extract (1.25%w/v) Photomicrograph (H&E X400) of the small intestine showing the mucosa with intestinal glands, Paneth and enteroendocrine cells (arrows)</p> <p>Diagnosis: Mucosa of the small intestine appears normal.</p> |
| C | | <p>(Normal saline) Photomicrograph (H&E X400) of the small intestine of the mucosa showing Intestinal gland atrophy and mild mononuclear activities (arrows).</p> <p>Diagnosis: Distortion of the small intestine.</p> |



4. Discussion

This research has shown the antibacterial effect against *E.coli* which inhibited its detrimental action on the intestinal wall of the albino wistar rats. Also the studies shown a dose dependent activity of the plant as concentrations of 0.5%w/v did not completely inhibit the *E.coli* as some distortion of the cells were seen unlike 1.25%w/v concentration which completely neutralized the bacterial effect of the *E.coli* on the intestinal linings. These results confirmed its use by ancient people in the treatment of different ailments [12].

The activity of *O. gratissimum* was due to rich phytochemical constituents present in the plant as shown from the phytochemical analysis [9]. The results also indicated that our extract has anti-bacterial activity and is safe for use in gastro-intestinal infections and enteritis as confirmed histologically where the small intestine showed no distortion, normal intestinal linings when compared with that of the Negative control group D which were not infected with the gastro-enteric bacteria. This indicates that *O. gratissimum* at 1.25% w/v concentration is effective, safe and can be used as an alternative source in gastro-enteric bacteria treatment.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

Ethical approval was sought and obtained according to the Institution's Ethical committee.

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