

**STUDY OF ANTI-ULCEROGENIC EFFECT OF METHANOL EXTRACT AND FRACTIONS OF THE LEAVES OF *OCIMUM GRATISSIMUM* (LAMIACEAE), ON ETHANOL-INDUCED ULCER IN RATS**

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ABSTRACT

Peptic ulcer has been a ravaging gastrointestinal disorder in clinical practice. The symptomatic effect is the perforation of the stomach lining. The study was aimed at evaluating the anti-ulcer activity and phytochemical constituents of the leaves of *Ocimum gratissimum*. The powdered leaves were macerated in 95% v/v of methanol for 72 h, and the dried extract obtained was washed with n-hexane (Hex), ethyl acetate (EtOAc) and butanol (Buta) successively using the solvent partition method to fractionate. Phytochemical constituents were evaluated using validated methods. Acute toxicity was performed using a modified Lorke's method. Ant ulcer studies were conducted using an ethanol-induced ulcer in the rat model. The rats received 200, 400 and 20 mg per kg body weight of *Ocimum .gratissimum* extract, its fractions and omeprazole respectively once daily for 14 days. The extraction yielded 3.3 % methanol extract, 16.8 % n-hexane 24.4 % ethyl acetate and 5.2 % butanol fractions. The phytochemical constituents of the extracts are alkaloids, flavonoids, tannins, terpenoids, carbohydrates, steroids and saponin. There was no mortality even at an oral dose of 5000 mg of extract/kg of mouse in the acute toxicity evaluation. The extract and its fractions (200 and 400 mg/kg doses) exhibited dose-dependent ulcer curative. The Hex fraction (400 mg/kg) demonstrated the best anti-ulcer effect with 78.5 % and ulcer index (UI) of 0.60 ± 0.24 when compared to the standard with 50% and 1.40 ± 0.24 respectively. A significant reduction ($p < 0.05$) in the UI was observed in rats treated with the *Ocimum .gratissimum* extract and its fractions.

Keywords: anti-ulcer, ethanol-induced ulcer, *Ocimum gratissimum*, Omeprazole, peptic ulcer, toxicity, rats

INTRODUCTION

The study of peptic ulcer disease, its management and treatment has been of huge concern in the clinical field. Studies have revealed *Helicobacter pylori* bacteria and pain-relieving nonsteroidal anti-inflammatory drugs (NSAIDs) as the major risk factors for ulcers (Anthony, 2018). Other disease conditions associated with peptic ulcers include severe abdominal pains, weight loss, vomiting, bloating, and heartburn. (Tarasconi *et al.*, 2020; Okorie *et al.*, 2022; Okorie *et al.*, 2023)

There is an increased search for treatment for this ravaging disease because Pylori infections affect about half of the world's human population. Due to the considered advantages of products from natural sources, it has become pertinent for researchers to search for the treatment of peptic ulcers from plant sources (Al- Sayed *et al.*, 2020; Ahmad *et al.*, 2019). The effectiveness of the bioactive ingredients found in herbal medicines to act as natural healers and their accessibility, perceived safety, availability and affordability may be responsible for the rising preference for using herbal medicines over conventional medications. (Diovu *et al.*, 2023; Ugwuokpe *et al.*, 2022). One of the recently found medicinal plants, *Ocimum gratissimum*, popularly called smell leaf, has served as a potential source of novel medication or as a complementary therapy for a variety of illnesses.

Ocimum gratissimum (*O. graissum*), is well distributed across Africa, Asia, and South America and is also a member of the Lamiaceae family (Akara *et al.*, 2021). It is a perennial

herbaceous plant with a high economic value and has a potent aroma. Vital bioactive substances contained in the plant include tannins, flavonoids, phenols, calcium, phosphorous, iron, vitamin A, and more, all of which are essential for human health (Venuprasad *et al.*, 2014; Melo *et al.*, 2019). In traditional medicine, *O. graissum* is used pharmacologically to treat a variety of illnesses, including cough, fever, pneumonia, diarrhoea, inflammation, aches, anaemia and bacterial and fungal infections (Akara *et al.*, 2021). It is used in the production of foods including fish, soup, meat and stew as a natural flavouring ingredient, condiment, or vegetable. Due to its use in traditional medicine in the management of gastric ulcers and related ailments, this study, therefore, evaluated the qualitative phytochemical constituents and anti-ulcer properties of *O. graissum*. The methanol extract and its fractions were used to assay the anti-ulcer activity of *O. gratissimum* using *in-vivo* models.

MATERIALS AND METHODS**Materials****Chemicals and reagent**

All the reagents used are of the analytical standard, and includes methanol, n-hexane (GHTECH Guandong Sci-Tech., Co.), acetic acid, ethyl acetate (BDH Chemicals., Poole England), mercuric iodide, potassium iodide, hydrochloric acid, chloroform (JHD), ferric chloride, Million's reagent, alpha naphthol, Benedict's solution, cupric ions, dilute hydrochloric acid (HCl), ammonia, sodium

hydroxide (NaOH), Mayer reagent, sulphuric acid, omeprazole (Ciron drugs and pharmaceuticals Pvt. Ltd, India), Fehling Solution B (BDH Chemicals Ltd., Poole England).

Experimental animals

The guidelines from the National Research Council (NRC) for the care and use of laboratory animals 8th edition were followed while carrying out animal experiments (NRC 2011). Healthy mixed sexes of Wistar Albino rats were procured from Animal Farm of the University of Nigeria, Nsukka. The animals were acclimatized to the environmental conditions for 14 days, housed in aluminum cages in softwood shavings as beddings at a temperature/humidity of 25 ± 2 °C/24.5-26%, with unrestricted access to pelleted poultry feed and drinking water *ad libitum*.

Methods

Collection and authentication of plant material

The fresh leaves of *O. grattissimum* were purchased from Eke Agbani, Enugu, Nigeria in September 2022 and were authenticated by Mr. Patrick Obi, a taxonomist at the Pharmacognosy Department, Enugu State University of Science and Technology, Nigeria. A voucher specimen, ID FP/Cog/17020, was deposited at the herbarium of the Department. The leaves were subjected to air-drying for 21 days at 25 ± 2 °C and the reduced into powder using a mechanical grinder. The fine powder was preserved in a moisture-free air-tight container and used for phytochemical analysis and anti-ulcer evaluation.

Preparation of plant extract

A 750 g pulverized sample was macerated in 3 liters of methanol (analytical grade) for 72 h and agitated intermittently. The mixture was pre-filtered using a funnel clogged with cotton wool, filtered through a Whatman No. 1 filter paper and the filtrates concentrated under vacuum at a reduced temperature to furnish methanol extract. The dried extract was then stored in an airtight container.

Fractionation of methanol extract

A 25 g of dried methanol extract was dispersed in 150 ml of 10 % aqueous methanol using liquid-liquid fractionation, and 200 ml of the solvents starting with n-hexane, ethyl acetate and butanol respectively were introduced into the dissolved extract. Shaken very well and allowed to separate into two layers. The fraction of each solvent was collected at each point and was concentrated under a vacuum at a reduced temperature using a rotary evaporator to obtain a dried fraction. The crude fractions were tested for the presence of secondary metabolites such as alkaloids, steroids, tannins, flavonoids, glycosides, anthraquinone, reducing sugar, carbohydrates, terpenoids and saponins using a standard method. (Harbourne, 1992).

Pharmacological evaluation

Acute toxicity study:

The extract was tested for acute toxicity in mice using the Lorke (1983) protocol. The tests were conducted in two phases. Phase one evaluated the toxic range of the extract. The mice were randomly placed in 3 groups (n = 3) and each group received 10, 100 and 1000 mg of the extract per kg body weight of the mouse. The mice were closely monitored for 24 h for behavioral changes and any death. With no death encountered in phase one, the second phase became inevitable. Since there were no deaths recorded in phase one, three fresh groups of mice; (n=1), were dosed 1600, 2900, and

5000 mg of the extract per kg body weight of mouse. The mice were monitored for signs of acute intoxication or death for 24 h. The median lethal dose (LD₅₀) was calculated using Lorke's formula (Abu et al., 2022).

$$LD_{50} = \sqrt{a \times b}, \quad (1)$$

(a = least lethal dose, b = highest safe dose).

Preparation of stock solutions

A 1 g of crude methanol extract, n-hexane and ethyl acetate fractions of *O. grattissimum* leaves were weighed out into three different glass containers with a stopper. 10 ml of distilled water was poured into each of the glass bottle containers corked tight and then shaken thoroughly until the extract was completely dissolved in the vehicle. A 20 mg of omeprazole in 5 ml vehicle of stock solution was prepared. The bottle was corked tight, and shaken until the powder was completely dissolved.

Induction of ulcer using ethanol model

Fifty-six adult albino rats of both sexes (150-180 g) were randomly distributed into 8 groups (n = 7) and were kept in 8 different cages labeled as group 1 to group 8. The rats were fasted but allowed free access to water, 24 h before the day of ulcer induction. The rats were orally administered 1 ml of ethanol to induce gastric ulceration. After an hour, two rats were randomly picked from each group and sacrificed to check for gastric ulceration. The gastric juice in the stomach of each rat was emptied into the dispensing bottle where the respective pH was taken using the pH meter and then the ulceration in the stomach was observed using a 10x magnifier lens. The rats were treated with the calculated doses of omeprazole (positive control), plant extract and fractions (200 mg and 400 mg) once daily for 14 days.

7 h after the last treatment, all the treated rats were sacrificed by cervical dislocation, and dissected following standard protocol. The gastric juice in the stomach of each rat was emptied into a dispensing bottle and its pH was taken using the pH meter.

The grouping and administration of the extract, fractions and control drug are as follows:

- Group 1: Negative control; 1 ml ethanol alone
- Group 2: Positive control; 1ml ethanol + 20 mg of omeprazole/kg body weight of the mouse
- Group 3: 1 ml ethanol + 200 mg extract/kg body weight of the mouse
- Group 4: 1 ml ethanol + 400 mg extract/kg body weight of the mouse
- Group 5: 1 ml ethanol + 200 mg of Hex fraction per kg body weight of the mouse
- Group 6: 1 ml ethanol + 400 mg of Hex fraction per kg body weight of the mouse
- Group 7: 1 ml ethanol + 200 mg of EtOAc fraction per kg body weight of the mouse
- Group 8: 1 ml ethanol + 400 mg of EtOAc fraction per kg body weight of the mouse

Measurement of pH

A 1 ml aliquot of gastric juice was mixed with distilled water (1 ml) and pH of the solution was measured using a pH meter.

Measurement of free acidity

One millilitre of gastric juice was pipetted into a conical flask (100 ml). Three drops of phenolphthalein indicator were added and titrated with 0.25N NaOH until the pink colour disappeared and the colour of the solution turned to yellowish orange.

$$\text{Free acidity} = N_A V_A = N_B V_B \quad (2)$$

Estimation of total gastric acidity

A 1 ml of gastric juice in a conical flask was titrated against 0.10 mol L⁻¹ sodium hydroxide solution using a phenolphthalein indicator until the colourless solution changed to light pink. The volume of sodium hydroxide that neutralized the gastric juice was recorded. The total acidity of gastric juice was calculated thus:

$$\text{Gastric acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.10 \times 100 \text{ mEq per litre}} \quad (3)$$

Measurement of ulcer score and ulcer index (UI)

The blood clots and the gastric contents of the opened stomachs of the rats were removed by rinsing with distilled water and thereafter examined for the formation of ulcers using a 10x magnification lens. The Ulcer score, Ulcer index (UI) and percentage curative were obtained from the number of ulcers counted (Alok et al., 2012) using subjective indices of 0 to 5 (Table 1).

Table 1: Ulcer score categorization

Score	Ulcer severity
0	No visible lesions
1	Oedema of the mucosa
2	Small lesions of 1-2mm in size)
3	Small or intermediate lesions of 3-4mm in size
4	Intermediate lesions or 1 gross lesions of > 4mm in size
5	Perforated lesions

$$\text{Ulcer index (UI)} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}} \quad (4)$$

$$\text{Curative (\%)} = \frac{\text{UI of untreated} - \text{UI of treated}}{\text{UI of untreated}} \times 100 \quad (5)$$

Statistical analysis

Data were expressed as mean \pm standard error of the mean and statistically evaluated using one-way analysis of variance (ANOVA), followed by a 2-sided Dunnett post hoc comparison test. A $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION**Results****Yields of *O. gratissimum* extraction**

A 750 g of *O. gratissimum* yielded 25 g of extract. The 25 g of the extract furnished 4.2, 6.1 and 1.3 g of EtOAc, Hex and Buta fractions respectively. The yield of the methanol extract, ethyl acetate and butanol fraction was calculated to be 14.7, 3.0 and 7.8% respectively as seen in Table 2.

Table 2: Yield of extraction and fractionation

Solvents	Weight of extract/ fractions (g)	Yield (%)
Methanol	25	3.3
n-hexane	4.2	16.8*
Ethyl acetate	6.1	24.4*
Butanol	1.3	5.2*

*yield was calculated based on the 25 g of extract

Phytochemical analysis of *O. gratissimum*

The phytochemical studies on *O. gratissimum* revealed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, saponins and carbohydrates as observed in Table 3.

Table 3: Phytochemical constituents of *O. gratissimum*

Phytoconstituents	Extract	Hex	EtOAc	Buta
Alkaloids	+	-	-	-
Flavonoids	+	-	+	+
Tannins	+	-	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	-
Glycosides	-	-	-	-
Saponins	+	-	+	+
Anthraquinone	-	-	+	-
Reducing Sugar	-	-	+	-
Carbohydrates	+	+	+	-

- = absent; + = present

Acute toxicity of *O. gratissimum*

It is shown in the result below that the methanol extract was not toxic even at a dose of 5000 mg/kg as there was no sign

of behavioral changes or physical alteration that led to the death of any of the mice.

Table 4: Acute toxicity of *O. gratissimum*

Group	Dose (mg/kg)	Lethality rate
Phase one		
Group A	10	0/3
Group B	100	0/3

Group C	1000	0/3
Phase two		
Group A	1600	0/1
Group B	2900	0/1
Group C	5000	0/1

Anti-ulcer activity of *O. gratissimum*

The curative effect of *O. gratissimum* on the ethanol-induced ulcer model reported in Table 4 shows that the methanol

extract and fractions significantly reduced gastric lesions when compared with the positive control.

Table 5: Ulcer curative effect of *O. gratissimum*

Group	Treatment	Ulcer Index		Curative (%)
		After Induction	After treatment	
1.	Negative control	3.00±0.00	2.80±0.20	0.0
2.	Positive Control	3.00±0.00	1.40±0.24*	50.0
3.	200 mg/kg extract	3.00±0.00	1.80±0.20*	35.7
4.	400 mg/kg extract	3.00±0.00	1.20±0.37*	57.1
5.	200 mg/kg Hex	3.00±0.00	1.40±0.24*	50.0
6.	400 mg/kg Hex	3.00±0.00	0.60±0.24*	78.6
7.	200 mg/kg EtOAc	3.00±0.00	1.60±0.24*	42.9
8.	400 mg/kg EtOAc	3.00±0.00	1.20±0.20*	57.1

Results are expressed as mean ± SEM. A significant difference is set at *p<0.05 level representing a significant difference in UI compared with the untreated control. One-way, ANOVA followed by Dunnett

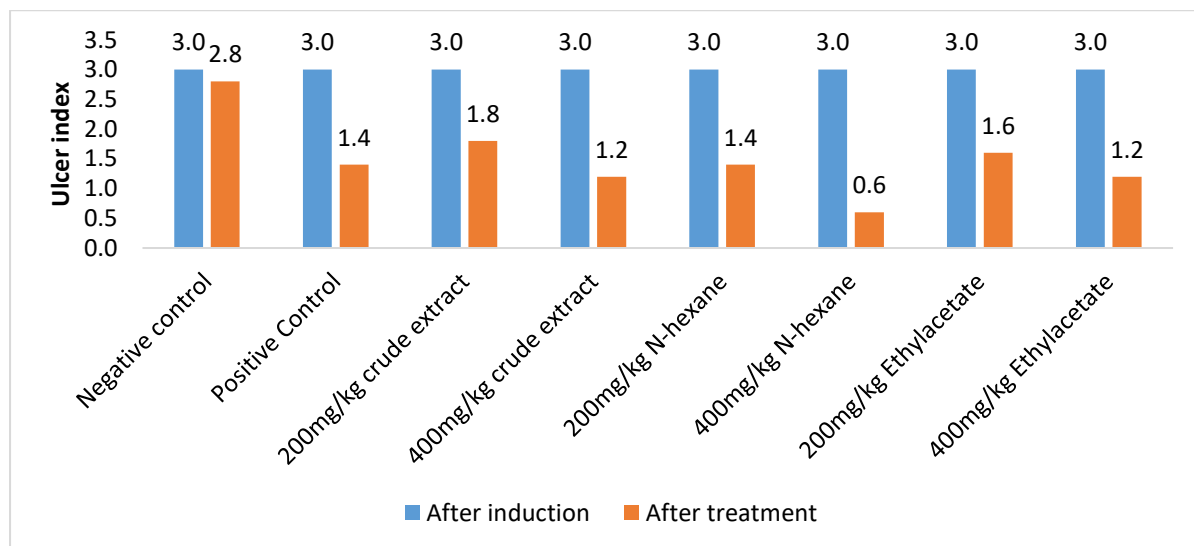


Figure 1: Ulcer index of *O. gratissimum* on an ethanol-induced ulcer in albino Wistar rats.

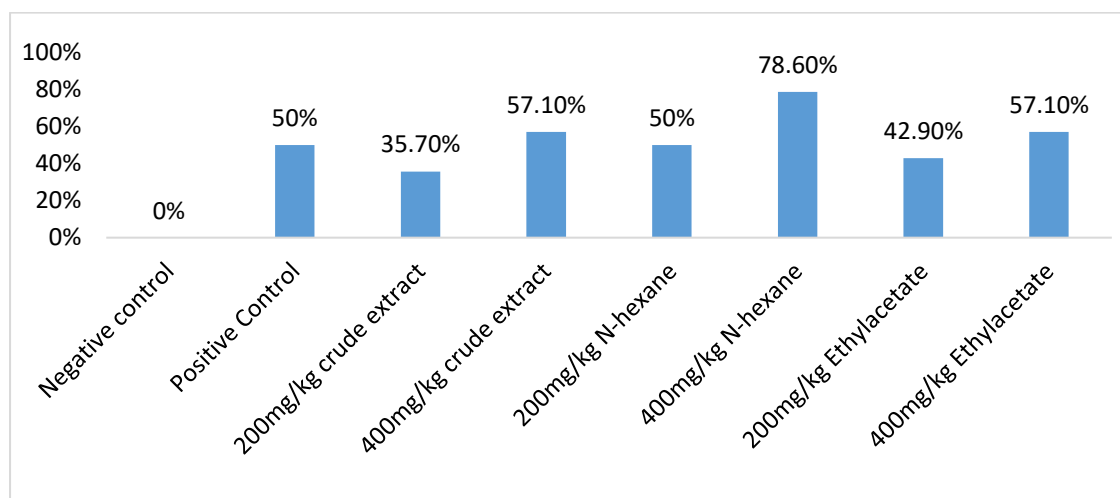


Figure 2: Ulcer curative effect of *O. gratissimum* in albino Wistar rats.

Effect of *O. gratissimum* on the mean potential of hydrogen

Table 6 below shows an increase in the pH as a result of the treatment with the methanol and fractions of *O. gratissimum*.

Table 6: Effect of *O. gratissimum* on the mean pH.

Group	Treatment	Potential of hydrogen (pH)	
		After Induction	After treatment
1.	Negative control	2.81±0.30	2.39±0.08
2.	Positive control	2.86±0.36	4.68±0.22*
3.	200mg/kg crude extract	2.68±0.05	6.00±0.49*
4.	400mg/kg crude extract	2.78±0.22	6.19±0.51*
5.	200mg/kg n-hexane	2.94±0.30	6.50±0.26*
6.	400mg/kg n-hexane	3.11±0.10	6.49±0.37*
7.	200mg/kg ethyl acetate	2.55±0.23	5.81±0.46*
8.	400mg/kg ethyl acetate	2.77±0.45	6.36±0.20*

Results are shown as mean ± SEM. A significant difference is set at *p<0.05 level representing a significant difference in the mean potential of hydrogen (pH) when compared with the negative control. One-way, ANOVA followed by Dunnett

Effect of *O. gratissimum* on the free and total gastric acidity

Table 7 below shows a decrease in the free and total acidity as a result of the treatment with the extract and fractions of *O. gratissimum*.

Table 7: Effect of *O. gratissimum* on the free and total gastric acidity.

Group	Treatment	Free acidity		Total acidity (mEq/L)	
		After Induction	After treatment	After induction	After treatment
1	Negative control	12.8	12.52	1.28	1.25
2	Positive Control	11.15	9.90	1.13	0.99
3	200 mg/kg extract	11.42	9.60	1.14	1.01
4	400 mg/kg extract	11.2	9.05	1.20	0.91
5	200 mg/kg Hex	13.05	9.83	1.31	0.98
6	400 mg/kg Hex	12.27	9.05	1.23	0.88
7	200 mg/kg EtOAc	12.08	9.85	1.21	0.96
8	400 mg/kg EtOAc	8.55	8.82	0.86	0.91

Discussion

One of the ulcer-inducing models in experimental rats is absolute ethanol and it was adopted in this study. The induction normally causes extensive damage to the gastric mucosa and other biological processes mediated by free radicals (Adebimpe et al., 2020). These events ultimately lead to the destruction of lipid membranes (Ayoka et al., 2023a; Ayoka et al., 2023b, Ekeanyanwu et al., 2016).

Medicinal plants have shown promising results in handling peptic ulcers from previous studies (Okorie et al 2022a; 2022b; 2023). The yields of the extraction and fractionation methods obtained were 3.3 % of the powdered plant material, 16.8, 24.4 and 5.2 % of the methanol extract. The yields showed that there were more non-polar constituents of the plant which could be attributed to volatile oils and other terpenes than polar constituents as shown in Table 2. The low yield of butanol fraction could be attributed to the presence of some volatile compounds. (Melo et al., 2019)

The results of phytochemical screening of *O. gratissimum* extract and its fractions in Table 3, revealed the presence of tannins, alkaloids, saponins, flavonoids, steroids, carbohydrates and terpenoids. These findings were similar to a previous report by Talabi and Makanjuola (2017), where the *O. gratissimum* water extract revealed an abundance of tannins, flavonoids, saponins, alkaloids, steroids and phlorotannin, glycoside, chalcones, anthraquinone and phenols. Hence, the significant ant-ulcer effect of *O. gratissimum* may be associated with these metabolites present. (Jayachitra et al., 2018; Lozano et al., 2016)

The acute toxicity study of the extract of *O. gratissimum* revealed that there was no lethality in the first and second phases as shown in Table 3. This justifies the use of the leaves

for cooking different kinds of food in Nigeria and also the treatment of different health conditions. (Akara et al., 2021; Ayoka et al., 2022)

The effect of *O. gratissimum* on the ulceration index of ethanol-induced ulcers in rats is shown in Figure 3. The manifestation of ulcer through ethanol induction was inhibited to a very reasonable degree by pretreatment of the experimental animals with omeprazole (the reference drug) and the different doses of *O. gratissimum* extract and fractions as evident in the reduction of the ulcer index. The results show that after induction of the ulcer, all the rats in all the groups had an ulcer index of 3.00±0.00. The plot shows that after the fourteen-day treatment with the different fractions and the extract of the plant, a dose-dependent decrease for both the extract and its fractions was observed in all the treated groups. The same was also observed for the group treated with the standard drug. The results further revealed that the group which received 400 mg/kg body weight of n-hexane fraction had the least ulcer index (0.60±0.24), thus the highest antiulcer effect followed by 400 mg/kg body weight of ethyl acetate and crude extract which obtained a mean of 1.20±0.20 and 1.20±0.37 respectively. This dose-dependent anti-ulcer activity of the extract, ethyl acetate and n-hexane is in agreement with other previous works done on the anti-ulcer property (Jayachitra et al., 2018; Okorie et al., 2022). The negative control group however continued to record a high ulcer index by the end of the treatment indicating that the ulcer did not resolve. The result reveals that there was a significant reduction in the mean ulcer index of all the groups treated with the plant extract and the standard drug when compared to the untreated control group that was not treated but induced with ethanol at p<0.05 level of significance.

The higher reduction in UI observed for 400 mg/kg body weight dose of the n-hexane fraction compared with omeprazole 20 mg agreed with a report by Mostofa *et al.*, (2017) where the 400 mg/kg extract of *Phyllanthus niruri* L. leaves elicited a more significant ulcer inhibition than omeprazole.

The result of Figure 3 shows that the group treated with Hex fraction recorded the highest percentage curative effect on gastric erosion (78.6 %), followed by the EtOAc (400 mg/kg body weight), extract (57.10 %), omeprazole and 200 mg/kg body weight Hex (50.0 %), ethyl acetate fraction (200 mg/kg body weight) gave 42.90 % and extract 200 mg/kg body weight (35.70 %) reduction. Finally, the negative control recorded no cure for gastric erosion. The extract, Hex and EtOAc fractions showing higher anti-ulcer activity when compared with omeprazole is also supported by other previous works (Okorie *et al.*, 2022b; 2023)

Table 6 shows the effect of *O. gratissimum* on the potential of hydrogen (pH) of induced ulcers in rats. The results show that after induction of the ulcer, all the rats in all the groups had relatively uniform low pH. However, the plot shows that after the fourteen-day treatment with the different fractions and the extract of the plant, an increase in the mean pH was observed in all the treated groups. The same was also observed for the group treated with omeprazole. This indicates that the plant can inhibit gastric secretion which consequently protect the stomach environment from ulceration. Meanwhile, the negative control group, however, continued to record low pH by the end of the treatment indicating that the ulcer did not resolve. (Ahmed, 2022)

The efficacy of the *O. gratissimum* was also evaluated in terms of acidity value (Table 7). The values for the free and total gastric acidity were relatively the same in all the groups' post-ulcer induction; however, there was a significant reduction after treatment with the doses of *O. gratissimum* and omeprazole. The untreated control groups were the highest with 12.52N and 1.25 mEq L⁻¹ in the other groups. The free acidity for each treated group was lower than the positive control group 9.90N treated with 20 mg/kg omeprazole. The treated groups for the total gastric acidity also had lower values than omeprazole except for the 200 mg/kg body weight crude extract (1.01 mEqL⁻¹) with slightly higher values. This agrees with the previous report of Lee *et al.* (2022) where the total gastric acidity of each treatment group treated with an extract of *Cinnamomum cassia* was lower than the control group.

CONCLUSION

The study has shown that *O. gratissimum* demonstrated a significant ulcer healing effect on ethanol-induced gastric ulcers. This is evident as there was a comparable antiulcer activity of omeprazole and the extract and its fractions against the ethanol-induced ulcer in rats. Traditionally, *O. gratissimum* has been useful in the management of gastric ulcers and related ailments. The study validates this ethno-medicinal information and further elucidates the connections between the ulcer-healing properties of *O. gratissimum* and its constituent secondary metabolites.

Recommendations

The findings of this study can be a base reference standard for further work on the plant to discover and isolate the actual phytochemicals responsible for the antiulcer activity because there is enough evidence to support the claim based on the result obtained

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