

Gas chromatography-mass spectrometry profiling and assessment of antiulcer and antioxidant activities of methanol extract from *Dioscorea praehensilis* rhizomes

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Peptic ulcer is a common disease in gastroenterology clinics. This study aimed to investigate the chemical composition, antiulcer, and antioxidant activities of methanol extract of *Dioscorea praehensilis* rhizomes. The rhizomes were extracted by macerating in 80% methanol and subjected to chemical profiling using Gas Chromatography-Mass Spectrometry (GC-MS). The assessment of anti-ulcer activity was done using ethanol and indomethacin-induced gastric ulcer models in rats while the antioxidant studies were conducted using quantitative analysis of phenolic (total phenols and total flavonoid) compounds, 1,1-diphenyl-2-picrylhydrazyl (DPPH), total antioxidant capacity (TAC) and ferric ion reducing antioxidant power (FRAP) assays. *D. praehensilis* extracts at 125, 250, and 500 mg/kg 'per os' (PO) exhibited a significant ($p < 0.01$) dose-dependent decrease in gastric lesions with the percentage inhibition of 65.16, 68.20, and 79.64% respectively in the ethanol model and 81.89, 90.44, and 95.35% in the indomethacin model compared with the control group. *D. praehensilis* extract contained high phenolic and flavonoid content. The extract also showed considerable antioxidant activity in the DPPH, TAC and FRAP assays. Furthermore, *D. praehensilis* contains bioactive constituents such as Pentadecanoic acid, 9-Eicosene, n-Hexadecanoic acid, 4-tert-butylphenol and Pentacosane. In conclusion, *D. praehensilis* possesses antiulcer and antioxidant activity, supporting its traditional use in treating ulcers and dyspepsia.

Keywords: Antiulcer. Gastric ulcer. *Dioscorea praehensilis*. Antioxidant. GC-MS.

INTRODUCTION

Peptic ulcer; either gastric or duodenum ulcer, is caused by an imbalance between the damaging factors, including hydrochloric acid and pepsin, and protective factors such as bicarbonate buffer, mucus secretion, antioxidants (vitamins and other compounds), and synthesis of prostaglandins in the stomach (Harold, Grant, Mitchel, 2007; Tytgat, 2011; Abdoulrahman, 2023). It is one of the most common diseases in gastroenterology

clinics, with a rising global prevalence (Ren *et al.*, 2022). For instance, in 2019, Peptic Ulcer Diseases (PUD) had a global prevalence of 8.09 million, which shows a 25.82% increase compared to the 6.4 million reported in 1990 (Ren *et al.*, 2022; Xie *et al.*, 2022).

PUD has various causes, including *Helicobacter pylori*, excessive use of non-steroidal anti-inflammatory drugs (NSAID), reactive oxygen species produced via oxidative stress, smoking, and excess alcohol consumption (Byeon *et al.*, 2018; Abdoulrahman, 2023). NSAID use ranks second only to *H. pylori* infection as the most common cause of PUD (Lanas *et*

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al., 2015). The rising prevalence of PUD is associated with changes in some of the risk factors related to its aetiology, especially the use of non-steroidal anti-inflammatory drugs (Xie *et al.*, 2022).

Current pharmacological management of gastric ulcers, such as proton pump inhibitors, prostaglandin analogues, cytoprotective agents, and histamine type 2 antagonists' aims to offset the imbalance between gastric acid production and the defensive mechanisms. Although effective, these agents are expensive and associated with reoccurrence, drug interactions, and a variety of side effects (Yismaw *et al.*, 2020). This has made herbal medicines popular as they are considered acceptable, economical, effective, relatively safe, and readily available (Onyeka *et al.*, 2020). According to Yismaw *et al.* (2020), extracts from medicinal plants and other natural products have become widely accepted sources of therapeutic agents for treating peptic ulcers.

D. praeheensis Benth belongs to the family Dioscoreaceae. Traditional healers use its tubers to treat various diseases, including stomach aches, diarrhoea, dyspepsia, and dysentery, and apply them to ulcers (Ngelinkoto *et al.*, 2021), inflammatory conditions, and scorpion bites (Adebisi *et al.*, 2018a). However, no literature has documented the scientific basis for these ethnomedicinal uses. This study aims to investigate the antiulcer and antioxidant abilities of *D. praeheensis* and ascertain the bioactive compounds.

MATERIAL AND METHODS

Material

Drugs, chemicals, solvents and equipment

The 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, diethyl ether, formalin, and ascorbic acid (AA), were obtained from Sigma-Aldrich Chemical Co (Darmstadt Germany), methanol (Guangdong Guanghua Sci-Tech Co., Ltd. Guangdong, China), Omeprazole (Surmount Laboratories Private Limited, Gujarat India), normal saline (Unique Pharmaceutical

Limited, Lagos Nigeria) and Indomethacin (Krishat Pharm. Industries Ltd. Ibadan Nigeria), were obtained from a local pharmacy premise. All other reagents, chemicals, and solvents used for this study were of analytical grade. The equipment includes a UV-Vis Spectrophotometer (Thermo Fisher), Agilent Intuvo 9000 GC, a Hot water bath (Genlab Ltd), a Microscope (Zeiss) and a weighing balance (Mettler Toledo).

Collection, identification, and extraction of plant material

D. praeheensis rhizomes were collected from Wammako, Sokoto Nigeria in August 2022. It was identified and authenticated in the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy Usmanu Danfodiyo University where a voucher specimen (PCG/UDUS/Dios/0002) was deposited. The rhizomes were allowed to dry under shade and powdered using a pestle and mortar. Seven hundred and fifty grams (750 g) of the dried plant material was extracted by maceration with 2.3 litres of 80% methanol. It was allowed to stand for 3 days, after which the mixture was filtered with Whatman filter paper. The resulting filtrate was evaporated to dryness in a water bath at 45°C.

***In vivo* antiulcer studies**

Experimental animals

Fifty Sprague Dawley rats of both sexes aged 8-10 weeks old, weighing 160-180 g were obtained and housed at the animal house facility located at the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. The animals were maintained under uniform husbandry conditions having natural photoperiod, humidity, temperature ($26 \pm 2^\circ\text{C}$), and free access to water and standard commercial chow (Chikun®), manufactured by Crown Flour Mill Ltd Lagos Nigeria, containing crude protein (15.00%), crude fat (4.00%), crude fiber (6.00%), calcium (1.00%), phosphorous (0.45%), Lysine (0.70%),

methionine (0.35%), metabolizable energy (2600 kcal/kg) and coccidiostats (60 mg/kg diet). The institution's guidelines for the care and use of laboratory animals in research with approval number NHREC/UDU-HREC/25/06/2023 were followed in all experiments.

Ethanol-induced gastric ulcer model

Twenty-five Sprague Dawley rats were divided into five groups of five rats each and fasted for 24 hours with free access to water until 2 hours before the commencement of the experiment. Group 1 received the vehicle, distilled water 5 mL/kg (negative control). Group 2 received omeprazole 20 mg/kg (positive control) while groups 3-5 received 125, 250, and 500 mg/kg of methanol extract of *D. praehensilis* respectively. All treatments were given by oral gavage. One hour after treatment, absolute ethanol (8 mL/kg) was used to induce gastric lesions according to the method of Ugwah *et al.* (2019). After 2 hours, rats were sacrificed humanely using diethyl ether, and their stomach was removed and opened along the greater curvature. The doses used for this study were selected based on the LD₅₀ value of >2000 mg/kg previously reported by our team (Adebisi *et al.*, 2018b).

Indomethacin-induced gastric ulcer model

Twenty-five rats were similarly pretreated as described above. An hour after treatments, gastric lesions were induced with 100 mg/kg indomethacin. Four hours later, rats were sacrificed and their stomach was excised as described in the ethanol-induced model.

Gross assessments of stomachs

The stomach tissues were rinsed in normal saline. The gastric ulcers were photographically documented. The sum of all lesions in each stomach was designated as the ulcer index and the percentage inhibition was calculated using the expression:

% Inhibition = (Ulcer index of control / Ulcer index of the test) × 100

Histopathological analysis

The stomachs of the animals were immediately fixed in a 10% buffered formalin solution and left for at least 24 hours. Sections of the stomach membrane and stomach disc were made from each stomach tissue. All the fixed tissues were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax melting at 60°C. Serial sections (5 µm thick) were then mounted on 3-aminopropyl trisilane-coated slides and dried at 37°C for 24 hours. Slide sections were deparaffinized, hydrated, and stained with Mayer's hematoxylin and eosin dyes and examined microscopically at 200 magnification.

In vitro antioxidant study

Quantitative analysis of phenolic compounds

The total flavonoid (TFC) and total phenolic (TPC) contents of the methanol extract of *D. praehensilis* were measured spectrophotometrically. TFC was determined as described by Miliauskas, Venskutonis and Van Beek (2004). Two millilitres of *D. praehensilis* extract (1.0 mg/mL) in methanol were added to a 2% AlCl₃ in ethanol. After one hour of incubation at room temperature, the absorbance was measured at 420 nm. The positive control consisted of similar concentrations of quercetin. The TFC was calculated as mg quercetin equivalent per gram of extract.

The method of Folin and Ciocalteu (1927) was used to determine the TPC. Briefly, gallic acid or extract concentrations ranging from 200 to 1000 µg/mL were prepared in methanol. Afterwards, 0.5 mL of these was mixed with 4.5 mL of distilled water followed by 0.5 mL of a ten-fold diluted Folin-Ciocalteu reagent. Next, five millilitres of 7% sodium carbonate followed by an additional 2 mL of distilled water were then added to the tubes. After ninety minutes of incubation at room temperature, the absorbance was measured at 760 nm. All experiments were performed in triplicates with gallic acid as the positive control. TPC was measured as gallic acid equivalent.

Determination of antioxidant activity by DPPH assay

The ability of *D. praeheensis* to scavenge free radicals was investigated using a DPPH assay as described by Hussen and Endalew (2023). Four milligrams of DPPH crystalline solid were dissolved in 100 mL of methanol to make a 0.1 mM solution. Similarly, a 500 µg/mL stock solution of the extract in methanol was prepared by dissolving 5 mg in 10 mL. Subsequently, the necessary concentrations (100, 200, 300, 400, 500, and 600 µg/mL) were prepared by serial dilution with methanol. Two millilitres of the plant extract from each concentration were then taken into a test tube, and 3 mL of DPPH solution was added. After thirty minutes of dark incubation, the absorbance was recorded at 517 nm using a spectrophotometer. Methanol was used as the blank, while the control consisted of a mixture of three millilitres of 0.1 mM DPPH and 100 µL methanol. The same concentrations of ascorbic acid was used as the standard. All measurements were performed in triplicate and the 50% inhibitory concentration (IC₅₀) was estimated from the plot of percentage inhibition against concentration. This percentage inhibition was calculated using the expression:

$$\text{DPPH \% Inhibition} = (\text{ABS of control} - \text{ABS of sample}) / \text{ABS of control} \times 100$$

ABS of control is the absorbance of methanol and DPPH solution

ABS of sample is the absorbance of the DP and DPPH solution.

Determination of total antioxidant capacity of the extract

Total antioxidant capacity (TAC) of *D. praeheensis* was investigated using the phosphor molybdenum assay as described by Prieto, Pineda and Aguilar (1999). This assay is based on the reduction of Molybdenum (VI) cation to Molybdenum (V) with a consequent formation of green phosphate Molybdenum (V) complex at acidic pH. In summary, 1 mL each of 0.6 M tetraoxosulphate (IV) acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added into a twenty millilitre of distilled

water and the volume made up to fifty millilitres with additional distilled water. Afterwards, 0.3 mL of *D. praeheensis* extract in different concentrations ranging from 100 to 600 µg/mL were added to different test tubes individually containing three millilitres of the reagent solution and incubated at 95°C for ninety minutes, cooled to room temperature and the absorbance was measured at 695 nm using a spectrophotometer. Methanol was used as the blank, while a mixture of the molybdate solution (0.3 mL) was used as a control. Ascorbic acid was used as the standard. All experiments were done in triplicate, and the antioxidant ability of *D. praeheensis* was estimated using the expression

$$\text{Antioxidant effect \%} = (\text{ABS of control} - \text{ABS of sample}) / \text{ABS of control} \times 100$$

ABS of control is the absorbance of the control.

ABS of sample is the absorbance of the sample.

Ferric reducing antioxidant power assay

FRAP assay as described by Benzie and Strain (1996) was adopted. The assay assesses the extract's capacity to convert ferric tripyridyltriazine complex into ferrous tripyridyltriazine at low pH levels. Briefly, One milliliter of the extract at a concentration of 1.0 mg/mL was mixed with 1.5 mL of freshly prepared FRAP solution (Twenty-five milliliter of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridyls- triazine in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride solution). The reaction mixtures were then incubated at 37°C for thirty minutes and the absorbance was measured at 593 nm using a spectrophotometer. Ferrous sulfate was used for the calibration curve, and the FRAP values (expressed as milligrams of ferrous ion (Fe²⁺) per gram of the extract) were then extrapolated from the curve.

GC-MS analysis of the extract

Agilent Intuvo 9000 GC system coupled with detector system 5977B MSD with split/splitless injector and equipped with a DB-5 MS fused silica capillary column (5% phenyldimethylsiloxane, 30.0 m x 320 µm,

film thickness 0.25 μm) was used. The carrier gas was helium gas, at flow rates of 1.2 mL per minute. The inlet temperature was set at 300°C, MS Source at 230°C, and MS Quad at 150°C while the oven temperature was set as follows: 50°C for 5 minutes, increased to 200°C at 20°C per minute and held for 7 minutes, and thereafter increased to 300°C at 20°C per minute and held for 6 minutes. Using Agilent Automated Liquid Sampler (ALS) G4513A, 1 μL of the extract and standard was injected into the GC system in splitless mode. The relative percent amount was determined by comparing each constituent's average peak area to the total areas. GCMSD/Enhanced MassHunter Software was used for data acquisition and processed with GCMSD data analysis software incorporated with the 2017 Version of the National Institute of Standards and Technology (NIST) Library. The obtained spectra of the unknown constituents of the *D. praehensilis* extract were compared with the standard mass spectra of known constituents stored in the NIST library.

Statistical analysis

Data obtained in the antiulcer studies were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed by One-way analysis of variance followed by the Dunnett test using GraphPad Prism version 8.4.3 (Graph Pad Software Inc., CA, USA). The level of significance was set at $p < 0.05$. For the

antioxidant studies, the IC_{50} was calculated from the dose-response curves ($n=3$).

RESULTS

Percentage yield of the plant material

The total weight of the crude methanol extract obtained from 750 g of the dried rhizomes of *D. praehensilis* was 44 g, hence, the percentage yield of the rhizomes of *D. praehensilis* after extraction with 80% methanol was 5.87%.

In vivo Antiulcer studies

Ethanol-induced gastric ulcer model

In this study, the administration of absolute ethanol in rats produced damage to the stomach and severe gastric lesions on gross examination as in Figure 1A. However, rats treated with Omeprazole and *D. praehensilis* extract suffered fewer gastric lesions compared to the untreated animals (Figures 1B, C, D, and E). *D. praehensilis* extract at doses of 125, 250, and 500 mg/kg and omeprazole 20 mg/kg exhibited a significant dose-dependent reduction in the mean ulcer index (Figure 2A) and a percentage inhibition of 65.16, 68.20, 79.64 and 49.01% respectively (Figure 2B).

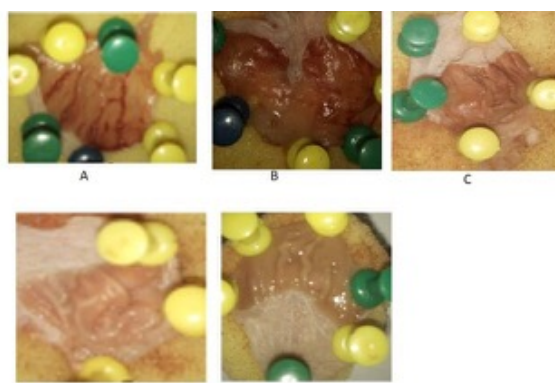


FIGURE 1 - Effect of *D. praehensilis* on the macroscopic appearance of ethanol-induced stomach damage. A. Ulcer control group displayed extensive haemorrhagic lacerations of the stomach epithelium. B. Omeprazole group demonstrated slight injuries to stomach mucosa. C, D, & E. Experimental groups (125, 250, and 500 mg/kg respectively) showed a reduction in gastric ulcers.

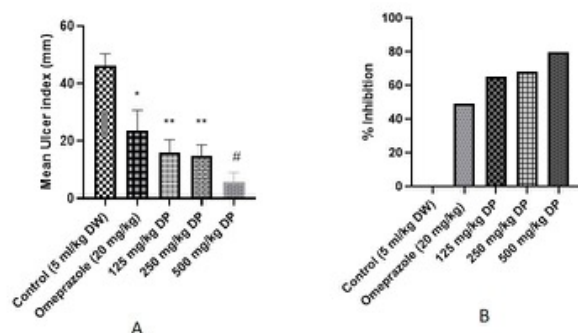


FIGURE 2 - Effect of *D. praehehensis* on ethanol-induced ulcer showing the mean ulcer index (A) and percentage ulcer inhibition (B). DW=Distilled water, DP=*D. praehehensis*. Data presented as mean $\pm$ SEM, N=5, * $p < 0.05$, ** $p < 0.01$, # $p < 0.0001$.

Indomethacin-induced gastric ulcer model

In this study, we observed that indomethacin also induced gastric ulcers, albeit not as severe as those observed in the ethanol model (Figure 3A). Rats treated with Omeprazole and *D. praehehensis* extract suffered fewer gastric lesions compared to the untreated rats (Figures 3B, C, D, and E). *D. praehehensis* at 125, 250, and 500 mg/kg and omeprazole at 20 mg/kg produced a reduction in mean ulcer index (Figure 4A). The percentage inhibition of ulceration obtained were 81.89, 90.44, 95.35 and 74.78% respectively compared to the vehicle-treated (control) group (Figure 4B).

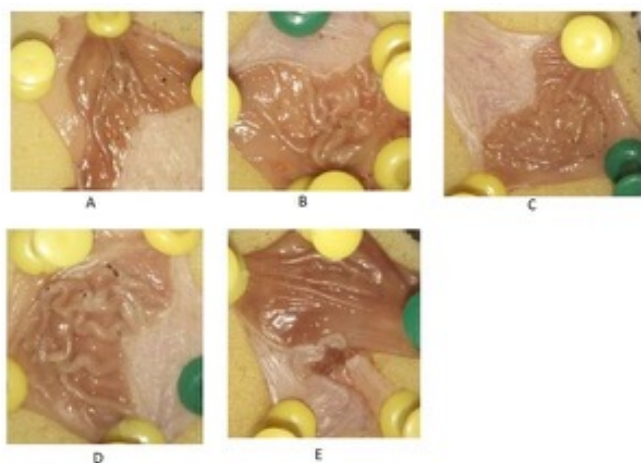


FIGURE 3 - Effect of *D. praehehensis* on the macroscopic appearance of indomethacin-induced stomach damage. A. Ulcer control group

displayed haemorrhagic lacerations of the stomach epithelium. B. Omeprazole group showed only slight injuries to stomach mucosa. C, D, & E. Experimental groups (125, 250, and 500 mg/kg respectively) showing a decrease in gastric ulceration.

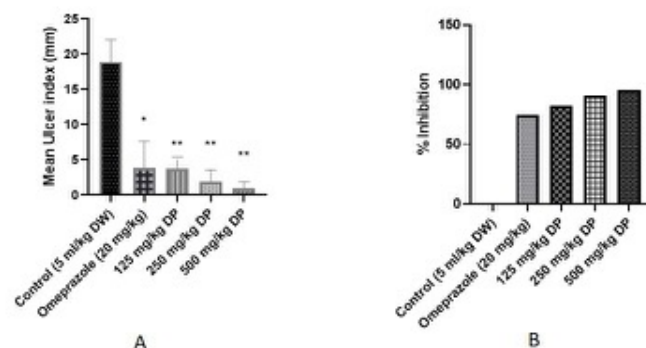


FIGURE 4 - Effect of *D. praehehensis* on indomethacin-induced ulcer showing mean ulcer index (A) and percentage ulcer inhibition (B). DW= Distilled water, DP=*D. praehehensis*. Data presented as mean $\pm$ SEM, N=5 for all groups. * $p < 0.05$, ** $p < 0.01$.

Histological analysis

Histological examination of the stomach samples of the rats showed the presence of ulceration in the control groups for both ethanol and indomethacin-induced ulcers. Ethanol and indomethacin-induced gastric damage is seen as a disruption of the surface epithelium and mucosal erosion strands (Figures 5A and 6A). Pretreatment with *D. praehehensis* methanol extract prevented severe ethanol and indomethacin-induced histological damage with the 250 mg/kg groups showing a healing gastric mucosa with epithelial regeneration and refilling of the ulcer crater (Figures 5C and 6C). The same was observed in the group that received omeprazole 20 mg/kg in both models (5B and 6B). The group that received 500 mg/kg *D. praehehensis* showed a re-epithelized gastric mucosa (healed ulcer) (Figure 5D and 6D).

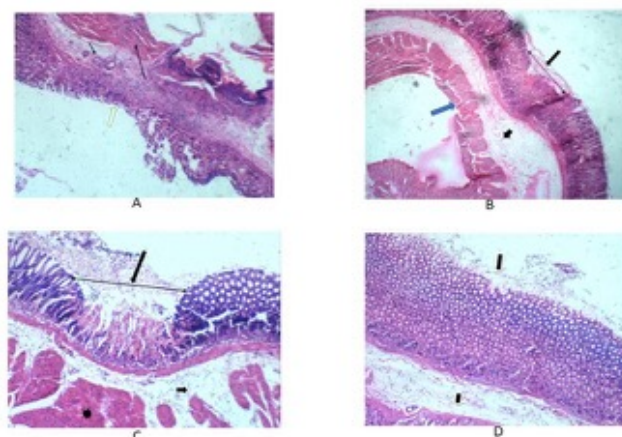


FIGURE 5 - Histological analysis of the stomach of rats treated with methanol extract of *D. praehensilis* in the ethanol-induced gastric ulcer model. A: Histological slide of the control group in the ethanol-induced model showing: Gastric ulcer (white arrow), Sub mucosa (short black arrow), and muscularis propria (long black arrow) H & E X 200.

B: Histological slide of the omeprazole group in the ethanol-induced model showing: Healing ulcer edge (Black long arrow), Sub mucosa (Short black arrow), and muscularis propria (Blue arrow) H & E X200.

C: Histological slide of the 250 mg/kg group in the ethanol-induced model showing: Epithelial regeneration (Black arrow) with refilling of the ulcer crater, Sub mucosa (short black arrow), and muscularis propria (Black star) H & E X 400.

D: Histological slide of the 500 mg/kg in the ethanol-induced model showing: Re-epithelized gastric mucosal (Long black arrow), Sub mucosa (Short black arrow) H & E X 400.

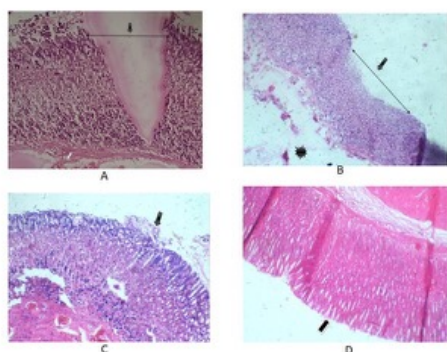


FIGURE 6 - Histological analysis of the stomach of rats treated with methanol extract of *D. praehensilis* in the indomethacin-induced gastric ulcer model

A: Histological slide of the control group in the indomethacin-induced model showing: Ulcer crater (Black arrow), and Muscularis mucosa (White arrow) H & E X 200.

B: Histological slide of the omeprazole 20 mg/kg group in the indomethacin-induced model showing healing gastric ulcer (black arrow) Sub mucosa (Black star) H & E X 200.

C: Histological slide of the 250 mg/kg group in the indomethacin-induced model showing: Healing gastric mucosa ulcer (Black arrow) H & E X 400.

D: Histological slide of the 500 mg/kg group in the indomethacin-induced model showing: Healed gastric mucosa ulcer (Black arrow) H & E X 400.

In vitro antioxidant study

Quantitative analysis of phenolic compounds

Results of the analysis of the phenolic compounds

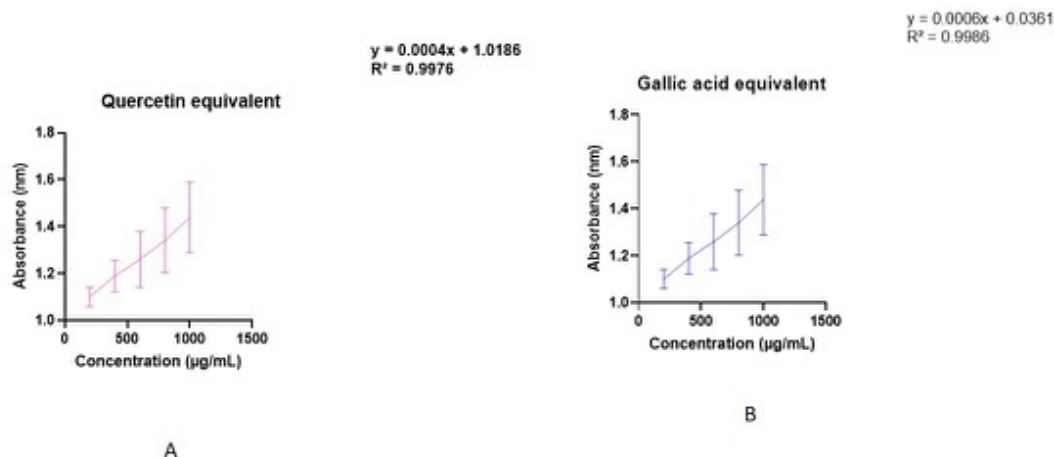


FIGURE 7A - Standard curve of the total flavonoid content of quercetin in methanol extract of *D. praehensilis*. Values expressed as mean $\pm$ SD, N = 3.

FIGURE 7B - Standard Curve of the total phenolic content of gallic acid in methanol extract of *D. praehensilis*. Values expressed as mean $\pm$ SD, N = 3.

DPPH, TAC and FRAP assays

In the DPPH assay *D. praehensilis* exhibited antioxidant activity at varying concentrations tested (100, 200, 300, 400, 500, and 600 µg/mL) with a percentage inhibition of $42.00 \pm 2.37\%$ at 100 µg/mL and $95.23 \pm 1.69\%$ at 600 µg/mL and an IC_{50} of 189.87 µg/mL. The standard ascorbic acid however showed better activity, with a percentage inhibition of $70.05 \pm 2.31\%$ and $99.58 \pm 2.10\%$ at these same concentrations and IC_{50} of 76 µg/mL (Figure 8).

In the phosphomolybdenum assay, the extract showed a good antioxidant capacity though lower than the standard ascorbic acid as observed with the DPPH assay. The percentage inhibition obtained for the *D. praehensilis* extract was $27.03 \pm 7.08\%$ at 100 µg/mL and $66.20 \pm 9.39\%$ at the highest concentration of 600 µg/mL with an IC_{50} of 340.05 87 µg/mL while

showed that the methanol extract of *D. praehensilis* contains high flavonoid and total phenolic contents. The TFC was 65.17 ± 11.54 mg of quercetin /g of the extract while that of TPC was 144.28 ± 14.94 mg gallic acid equivalent/g of the extract. The standard curves are shown in Figures 7A and 7B.

that of ascorbic acid was $69.29 \pm 3.41\%$ and $98.63 \pm 3.41\%$ with an IC_{50} of 124.02 µg/mL at these same concentrations (Figure 9).

In the FRAP assay, the extract was found to have a remarkable reducing power with a FRAP value of 77.33 ± 13.22 mg ferrous ion per gram of the extract. The standard curve is presented in Figure 10.

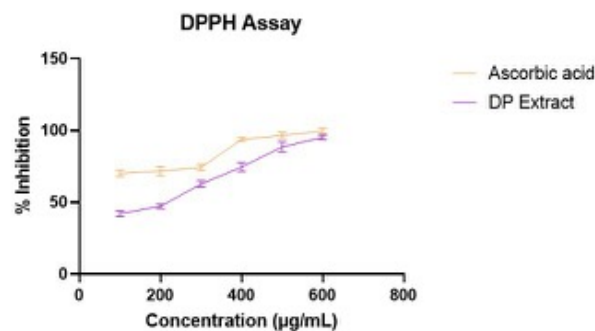


FIGURE 8 - DPPH scavenging property of methanol extract of *D. praehensilis* represented as percentage inhibition. N=3.

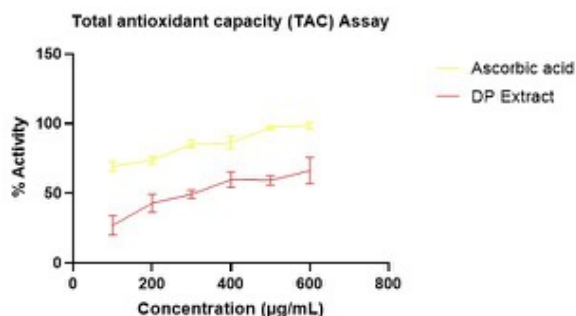


FIGURE 9 - Phosphomolybdenum reducing property of methanol extract of *D. praehensilis* N=3.

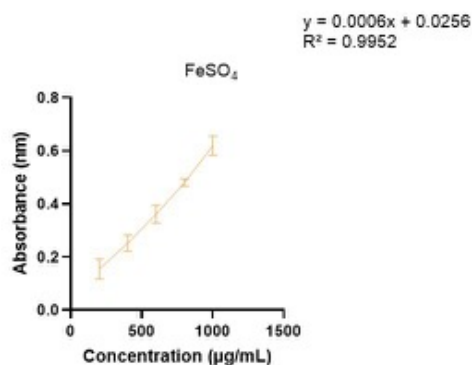


FIGURE 10 - Ferric reducing property of methanol extract of *D. praehensilis* N=3.

GC-MS profiling of the methanol extract of *D. praehensilis*

The GC-MS analysis of the methanol extract of *D. praehensilis* rhizomes yielded a total of twenty-one (21) compounds. Figure 11 depicts the chromatogram while Table I is a list of the chemical constituents with their retention time (RT), percentage quality, peak area (*i.e.* concentration percent), molecular formula, and molecular weight. The compounds identified include: Benzene, 1,1'-oxybis[3-phenoxy-; 3,4,5-Trimethyldihydrofuran-2-one; Toluene; Hexadecane, 2,6,10,14-tetramethyl-; Cyclohexane, hexyl-; Tridecane; Cyclohexane, 1,1'-(2-methyl-1,3-propanediyl)bis; Cyclooctane, cyclohexyl-; Heptylcyclohexane; Pentacosane; 4-Piperidineacetic acid, 5-ethylidene-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-.alpha.-methylene-, methyl ester, [2S-(2.alpha.,4.alpha.,5E)]; 9-Eicosene, (E)-; Sulfurous acid

butyl dodecyl ester; 1-Octadecene; Pentadecanoic acid, 14-methyl-, methyl ester; n-Hexadecanoic acid; 5-Eicosene, E; 11-Octadecenoic acid, methyl ester; 1,2-Bis(trimethylsilyl)benzene; Tetrasiloxane, decamethyl- and 4-tert-Butylphenol.

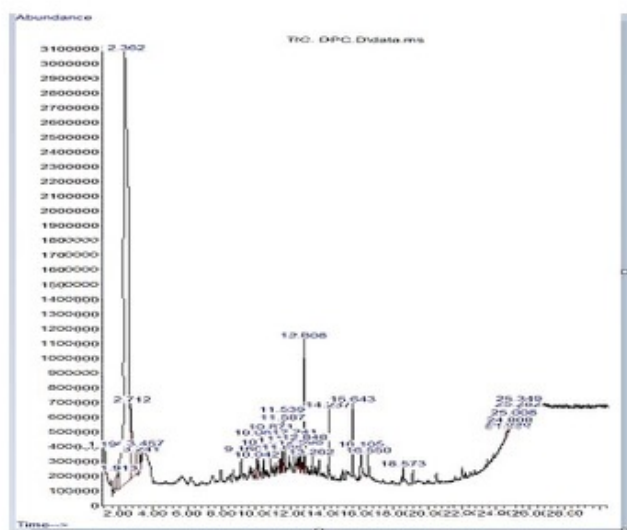
TABLE I - GC-MS spectral analysis of methanol extract of *Dioscorea praehensilis* (continue)

S/ No.	RT (Min)	Quality %	Peak Area %	Name of compound	Molecular formula	Molecular weight g/mol
1	1.196	94	1.35	Benzene, 1,1'-oxybis[3-phenoxy-	C ₃₆ H ₂₆ O ₅	538.6
2	1.913	47	1.14	3,4,5-Trimethyl-dihydrofuran-2-one	C ₇ H ₁₂ O ₂	128.169
3	3.241	60	0.74	Toluene	C ₆ H ₅ CH ₃	92.14
4	10.081	76	0.93	Hexadecane, 2,6,10,14-tetramethyl-	C ₂₀ H ₄₂	282.5
5	10.443	87	0.55	Cyclohexane, hexyl-	C ₁₂ H ₂₄	168.32
6	10.871	93	0.61	Tridecane	C ₁₃ H ₂₈	184.36
7	11.406	53	0.4	Cyclohexane, 1,1'-(2-methyl-1,3-propanediyl)bis	C ₁₆ H ₃₀	222.41
8	11.69	55	0.26	Cyclooctane, cyclohexyl-	C ₁₄ H ₂₆	194.36
9	11.947	86	0.3	Heptylcyclohexane	C ₁₃ H ₂₆	182.35
10	12.241	93	0.41	Pentacosane	C ₂₅ H ₅₂	352.7
11	12.598	53	0.51	4-Piperidineacetic acid, 5-ethylidene-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-.alpha.-methylene-, methyl ester, [2S-(2.alpha.,4.alpha.,5E)]	C ₂₁ H ₂₆ N ₂ O ₃	354.4
12	12.808	95	1.43	9-Eicosene, (E)-	C ₂₀ H ₄₀	280.5
13	12.848	83	0.55	Sulfurous acid, butyl dodecyl ester	C ₁₆ H ₃₄ O ₃ S	306.5

TABLE I - GC-MS spectral analysis of methanol extract of *Dioscorea praehensilis* (continuation)

S/ No.	RT (Min)	Quality %	Peak Area %	Name of compound	Molecular formula	Molecular weight g/mol
14	14.237	99	0.88	1-Octadecene	C ₁₈ H ₃₆	252.5
15	15.643	98	1.45	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
16	16.105	95	1.24	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
17	16.55	81	0.5	5-Eicosene, E	C ₂₀ H ₄₀	280.5
18	18.573	99	0.45	11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
19	24.633	47	0.32	1,2-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222.47
20	24.664	47	0.06	Tetrasiloxane, decamethyl-	C ₁₀ H ₃₀ O-Si ₄	310.68
21	24.698	47	0.06	4-tert-Butylphenol	C ₁₃ H ₂₂ OSi	222.40

RT =Retention time (minutes)



ulceration obtained with this extract is greater than that obtained with the standard drug in both models.

Oxidative stress, which results from a disruption of the balance between reactive oxygen species and antioxidants is implicated in the pathophysiology of peptic ulcer disease (Mohamed *et al.*, 2024) and studies have shown that the two gastric ulcer models used in this study are related to the generation of free radicals. Ethanol mediates gastric ulcers by inducing oxidative stress with a resultant generation of hydrogen peroxide (H_2O_2) which is then converted to hydroxyl free radical (OH) which consequently induces lipid peroxidation and Malondialdehyde (MDA) formation that attacks the gastric tissues, eroding the protective layer (Li *et al.*, 2018). Qiu *et al.* (2020) reported that there is a correlation between an increase in oxidant levels and a decrease in antioxidant levels with the degree of damage to gastric mucosa in an indomethacin model of gastric ulcer. Several recent studies have shown that agents with antioxidant and anti-inflammatory properties may play an important protective role in mitigating the effect of these gastric ulcer-inducing agents (Altuner, Kaya, Suleman, 2020; Shaik, Eid 2022; Mahmoud *et al.*, 2023; Danisman *et al.*, 2023).

In this study, we have reported the presence of a high amount of phenolic and flavonoid contents and antioxidant properties of the methanol extract of *D. praehensilis* in all the tests performed in a concentration-dependent manner. Plant secondary metabolites which have a range of physiological effects on the human body, are responsible for their medicinal value (Adebisi *et al.*, 2018a) and bioactive compounds and secondary metabolites derived from plants have a significant impact on preventing various human diseases (Wang *et al.*, 2023). For instance, the presence of one or more aromatic rings with varying numbers of hydroxyl groups allows for the good radical scavenging properties of phenolic and flavonoid compounds (Barreto *et al.*, 2020). These phytochemicals have the potential to mitigate free radical accumulation and prevent oxidative gastric mucosal damage; an effect which may be related to their high reactivity as

electron donors. Moreover, flavonoids can also boost mucosal prostaglandin, enhance mucus secretion, and prevent the growth of *H. pylori* (Mekonnen, Asrade Atnafie, Wahab Atta, 2020; Al-Mamary, Moussa, 2021; Bešlo *et al.*, 2023). Tannins have been reported to offer protection to the outmost layer of mucosa to resist chemicals and mechanical injury. Saponins on the other hand can mediate increased mucus production. (Mekonnen, Asrade Atnafie, Wahab Atta, 2020; Adane *et al.*, 2021). A previous analysis of the phytochemical constituents of *D. praehensilis* by our team revealed the presence of flavonoids, tannins, saponins, and steroids (Adebisi *et al.*, 2018a). The antiulcer properties being reported in this study may be due to the presence of these phytochemicals.

Other species of *Dioscorea* such as *Dioscorea bulbifera* are used in India to cure ulcers (Dutta, 2015), *Dioscorea oppositifolia* shows anti-ulcer activity in adult Wistar rats (Singh, 2022). These species have been reported to possess phytochemicals including phenolic acids; flavonoids, phenanthrene derivatives, and flavonoids (Adoménienė, Venskutonis, 2022). In a similar study in DR Congo, Bukatuka *et al.* (2016) reported the antioxidant properties of *D. praehensilis* tubers with an IC_{50} lower than that obtained in our study and the absence of flavonoids as against that reported in this study. This may be due to differences in the geographical locations of the plants as geographical locations have been shown to affect the phytochemical constituents and bioactivity of medicinal plants (Liu *et al.*, 2018; Dewanjee, Saha, Mishra, 2021)

The GC-MS analysis of the methanol extract of *D. praehensilis* showed the presence of some volatile phytochemicals that have been reported to possess some pharmacological activities. Among the identified compounds, n-Hexadecanoic acid and pentadecanoic acid have been shown to possess antioxidant, Anti-inflammatory, antimicrobial and antifungal properties (Hema, Kumaravel, Alagusundaram, 2011; Akpuaka *et al.*, 2013; Hadadi, Nematzadeh, Ghahari, 2020; Chirumamilla, Dharavath, Taduri, 2022); Octadecenoic acid possesses anti-inflammatory activity (Gomathi *et*

al., 2015); 4-*tert*-Butylphenol possesses antioxidant properties (Shakira *et al.* 2022); Pentacosane has shown remyelinating and anti-inflammatory properties (Senol *et al.*, 2023) while 1,2-Bis(trimethylsilyl)benzene possesses antioxidant, antibacterial, anti-inflammatory and antimicrobial effects (Susheela, Rosaline, Radha, 2018; Ali *et al.*, 2021).

The antiulcer activity of the extract of *D. praeheensis* may therefore be attributed to its antioxidant properties and the activities of the phytochemicals present in the rhizome. Further studies are needed to determine the precise mechanism(s) of action of this extract.

CONCLUSION

The data from this study showed that the methanol extract of *D. praeheensis* significantly decreased gastric lesions in ethanol and indomethacin-induced gastric ulcers, demonstrating its antiulcer properties. The extract also showed considerable antioxidant activity in some *in-vitro* antioxidant assays and contains bioactive constituents such as n-Hexadecanoic acid, pentadecanoic acid, 4-*tert*-Butylphenol and 1,2-Bis(trimethylsilyl)benzene that has been previously reported to possess antioxidant, anti-inflammatory and antimicrobial properties. These findings lend support to its use in the folkloric treatment of ulcers and dyspepsia. *D. praeheensis* may therefore be a source of new phytotherapeutic formulations for treating gastric ulcers.

COMPETING INTEREST

The authors declare no conflict of interest.

AUTHORSHIP CONTRIBUTION STATEMENT

Iyabo M. Adebisi: Conceptualization, Performed the experiments, Analysed data, Wrote the manuscript, and critically revised the manuscript

Abdullahi Suleiman: Performed the experiments and critically revised the manuscript.

Chinenye J. Ugwah-Oguejiofor: Performed the experiments and critically revised the manuscript.

Sunday J. Fajobi: Performed the experiments and critically revised the manuscript

Amina Yusuf Jega: Performed the experiments and critically revised the manuscript.

Mohammed Umar: Performed the experiments, and critically revised the manuscript.

DATA AVAILABILITY STATEMENT

Data available from the corresponding author upon reasonable request.

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