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Chemical constituents responsible for antiulcer property of leaf extract of *Acioa barteri* (Hook. F. ex Oliv.) Engl. in Wistar Albino Rats

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Abstract
The discovery of a highly effective antiulcer drugs with limited side effects remains a necessity. This study evaluated the anti-ulcer activities of the phytochemicals in ethanol extracts as acioa leaf extracts with Wister albino rats that had been subjected to indomethacin-induced and pylorus-ligated induced gastric ulceration, respectively. Acioa leaf extracts resulted in lower ulcerative index or extent of ulceration in the rat guts, total acidity, acid volume, and higher pH. Similar effects were obtained with ranitidine and Lansoprazole. However, acioa extract dosed at 500 mg/kg concentration gave a better gastro-protective effect than at 250 mg/kg. High Performance Liquid Chromatography, Diode Array Detector identified Gallic acid, Quercetin, Kaempferol, and 3,4,6, tribromo-2-(2,4 dibromophenoxy) phenol as the chemicals responsible for antiulcer activities of acioa leaves. The result demonstrated that acioa leaf extract is a potential anti-ulcer agent.

Keywords: *Acioa barteri*, antiulcer, indomethacin, leaf extract, peptic ulcer High Performance Liquid Chromatography

1. Introduction
Peptic Ulcer Disease (PUD) is a prevalent disease condition encountered around the world [1]. The PUD is characterized by the breakdown of the lining of the gastrointestinal tract due to the release of gastric acid or pepsin. The hormone, pepsin, plays a crucial role in triggering mucosal breakdown [2]. Peptic ulcers can also be caused by the bacteria, *Helicobacter pylori*, which is most common in those 40 to 60 years of age [3]. Ulcers form when there is an imbalance in the gastrointestinal system primarily between the protective factors (mucus and bicarbonate) and the aggressive forces (acid and pepsin). This imbalance can be caused by diseases such as gastric cancer, *Helicobacter pylori* infection, or lifestyle (drug: alcohol, non-steroidal anti-inflammatory agent, stress, and smoking) [4].
Several chemical components that have played a significant role in human health since the dawn of time. More than fifty percent (50%) of modern drugs are derived from natural plant origin., For almost 80% of the world's population, medications derived from plants are their first line of basic health care, validating the hypothesis that plant extracts can be a good source of novel drugs [5].
Ideally a drug should have minimal side effects in which case, the benefits outweigh the risks. Most anti-ulcer drugs currently available produce myriad of toxicities and may also cause relapse, as a result, there is a need for alternatives drugs [6] with fewer side effects or re-occurrence of symptoms and natural products may potentially not less adverse side effects than synthetic products.
Acioa barteri synonym Acioa dactyladenia commonly known as monkey fruit or acioa, is a member of Chrysobalanaceae family [7]. Historically, phytochemicals that abound in extracts from leaves, stems, roots, flowers, and seeds of acioa are already known to be used locally to treat a variety of diseases [8]. A previous qualitative phytochemical examination confirmed the presence of alkaloids, steroids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and resins) in acioa leaves [9]. Thorough investigation and extraction of these phytochemicals from the leaves are needed so that those that demonstrate medicinal potential can be developed for commercial use.

2. Materials and Methods

2.1 Collection and preparation of plant extract

Acioa leaves were collected from Amudi Obizi village, Ezinihitte Mbaise, Imo State and were identified by Dr. Garuba Omosun, a taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The collected samples was air-dried at room temperature and pulverized to the desired size using a milling machine. The pulverized sample weighing a total of 1.5 kg was transferred into a beaker containing 1500 ml of ethanol (the extraction solvent). The mixture was stirred for ten minutes and left to stand for 72 hours. The mixture was agitated at intervals of five minutes. Subsequently, the mixture was sieved with muslin cloth and the filtrate was further filtered. The resultant filtrate was concentrated using a rotary evaporator maintained at 40-50 °C.

2.2 Animal and housing

Twenty-four Wistar albino rats used in this experiment were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria, and transferred to the Animal House of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University. The rats were housed in clean metal cages and allowed to acclimatize to the environment for one week. During this period, the rats were fed commercial pelleted feed and water and proper sanitation was maintained in the animal house. The animals were handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals [10].

2.3 Indomethacin-Induced Gastric Ulceration in Rats

The rats weighing between 120-150 g were randomly divided into four treatment groups of five rats each. The animals were starved of food for 24 hours before the commencement of experiment [11]. They were dosed as follows:

Group 1-60 mg/kg Indomethacin (by mouth dissolved in 5% Na_2CO_3), Group 2-250mg/kg acioa leaf extract plus Indomethacin as above Group 3-500 mg/kg *acioa leaf* extract plus Indomethacin as above while Group 4-20 mg/kg of ranitidine plus Indomethacin as above.

Indomethacin doses for treatment groups 2-4 were administered one hour after acioa leaf extract doses. After four hours, the animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline solution. Macroscopic examination of the tissues was carried out with a hand-held lens and presence of ulcer lesions was scored [12]. Ulcer index (UI) or extent of ulceration, and percent maximum protection (%MPU) of each of the groups pretreated with extract was calculated using standard methods [13, 14]. The percent MPU is a measure of the protective effects of the acioa leaf extracts treatments against gastric mucosal damage by indomethacin.

2.4 Pylorus-Ligated (Pl) Induced ulceration in rats

Twenty adult Wistar albino rats weighing between 120-150 g were divided into four treatment groups of five animals each and fasted for 18 hours but provided with free access to water. During this time, they were kept in restraining cages to prevent coprophagy.

The treatment groups

Group-1: Ethanol alone
Group 2: 250 mg/kg ethanol extract of acioa leaf extracts
Group 3: 500 mg/kg ethanol extract of acioa leaf
Group 4: 5 mg/kg Lansoprazole

Acioa leaf extracts were administered to the rats 60 minutes prior to pyloric ligation under light ether anesthesia. The abdomen was opened, and pyloric ligation was done without causing any damage to its blood supply. The animals were deprived of water during the post-operative period. After 6 hours, the stomachs were dissected and contents collected in test tubes. The stomachs were cut opened along the greater curvature and ulcers were scored and extent of protection was reported [15, 16].

Gastric juice was collected from each rat six hours after pylorus ligation and centrifuged for five (5) minutes at 2000 rpm and the volume of supernatant was recorded. The pH of the gastric juice was recorded, and the contents were subjected to analysis for free acidity. Free acidity and total acidity were determined using 0.01N NaOH and Topfers reagent containing phenolphthalein as indicator [14].

2.5 Histopathology Procedure

Tissues harvested from the rats were preserved using 10% neutral buffered formalin placed in pre-labeled universal containers. Tissues not exceeding 3-5 mm thickness were dissected and placed in labeled tissue cassettes. The tissues were subjected to automatic tissue processing using the Leica TP2010 automatic tissue processor for 18 hours passing them through the four stages of tissue processing namely, fixation (using 10% Neutral buffered formalin), dehydration (using ascending grades of isopropyl alcohol), clearing or de-alcoholisation (using xylene), and finally, impregnation or infiltration (using molten paraffin wax) [17].

The tissues were then embedded in paraffin wax using the Leica automated tissue embedder and sectioned to obtain ultra-thin sections of five (5) microns, using the thermo-scientific semi-automated rotary microtome. Tissues were floated out from the thermo-scientific digital floating bath on frosted end pre-labeled slides and dried on the thermo scientific digital slim line hot plate.

The tissues were further dried in the hot-air oven overnight and subjected to heamatoxylin and eosin staining to determine the general tissue structure. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope using X 400 magnification [18].

2.6 Vacuum Liquid Chromatography (VLC) of the ethyl acetate fraction

For the leaf extract, 2 g portions were dissolved in 1 mL of methanol and carefully triturated on about 25 g silica gel (200-400 mesh size) in a mortar to form a homogenous mixture. A glass column (diameter 2.5 cm x 30 cm height) was packed with silica gel (200-400 mesh size) to the bed size of 14 cm height. The mixture was introduced from the top of the column and a small amount of silica gel (in place of sea sand) was added on top of the adsorbed fungal extract mixture. Cotton wool was used to cover the silica gel to prevent distortion of the silica gel bed when the solvent system will be introduced, and vacuum pump was connected to the column. The column was eluted gradually with increasing order of polarity of solvent from non-polar to highly polar solvent system. The column was developed with a gradient mixture of 500 ml of n-hexane and ethyl acetate solvent system in the ratio of 10:0, 9:1, 8:2, 6:4, 4:6, 3:7, 2:8, 0:10 using negative pressure created by the vacuum pump.

Furthermore, the column was developed with a gradient mixture of 500 ml of dichloromethane and methanol solvent system in the ratio of 10:0, 9:1, 8:2, 6:4, 4:6, 2:8, 0:10 to give successive fractions. The column was allowed to run dry after each fraction was collected. The eluate was collected separately with a round bottom flask, evaporated using a rotary evaporator and labeled appropriately.

2.7 Analytical High Pressure Chromatography (HPLC)

The crude extract and each of the VLC fractions (2 mg) respectively was reconstituted respectively with 2 mL of HPLC grade methanol. The mixture was sonicated for 10 min and thereafter centrifuged at 3000 rpm for 5 min. About 100 µL of the dissolve sample was mixed with 500 µL of HPLC grade methanol. HPLC analysis was carried out using Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S). Detection was carried out at 235, 254, 280 and 340 nm. The separation column (125 mm length x 4 mm internal diameter) was prefilled with Eurospher C-18 and a linear gradient of nano pure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. Compounds were detected using diode array and identified based on similarity with data in the in-built library. The Chromeleon 6.30 software was used to create results for the HPLC chromatograms and ultraviolet (UV) spectra of the secondary metabolites.

2.8 Statistical Analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-25). Results were presented as mean±Standard error of mean (SEM) of sample replicates. Raw data were subjected to one way analyses of variance (ANOVA) followed by post hoc turkey's test. $p<0.05$ were considered to be statistically significant.

3. Results and Discussion

The ulcer index (UI) of the treatments is shown in Table 1. The UI of indomethacin alone of 4.17 was reduced by pre-treatment of rats with the aqueous extract of acioa at 250 and 500 mg/kg to 1.83 and 0.61, with relative reduction of ulceration (%MPU) of 56 and 85%, respectively (Table 1). The standard treatment, ranitidine, provided 88% protection. Thus, the antiulcer effect of acioa leaf extract increased with the higher dose, the 500 mg/kg of ethanol extract producing less UI (1.15±1.03) than the 250 mg/kg dose. Therefore, even though, at both doses, acioa leaf extract decreased the incidence and severity of gastric erosions, the effects appeared to be dose dependent.

Table 1: Effect of *Acioa barteri* ethanol leaf extract on indomethacin-induced gastric ulcers

	Dose (mg/kg live weight of rat)	Ulcer index (UI ± SE)	% maximal protection from ulceration (MPU)
Indomethacin (IND)	60	4.17±0.31	0
IND + Acioa barteri	250[1]	1.83±0.40	56
	500[1]	0.61±0.55	85
Ranitidine	20	0.50±0.34	88

[1]Dose of acioa leaf extract administered after Indomethacin Treatment, $p<0.05$.

A single dose of indomethacin at 60 mg/kg body weight was sufficient to cause significant mucosal injury. The gross examination of the excised stomach tissues revealed varying degrees of mucosal alterations.

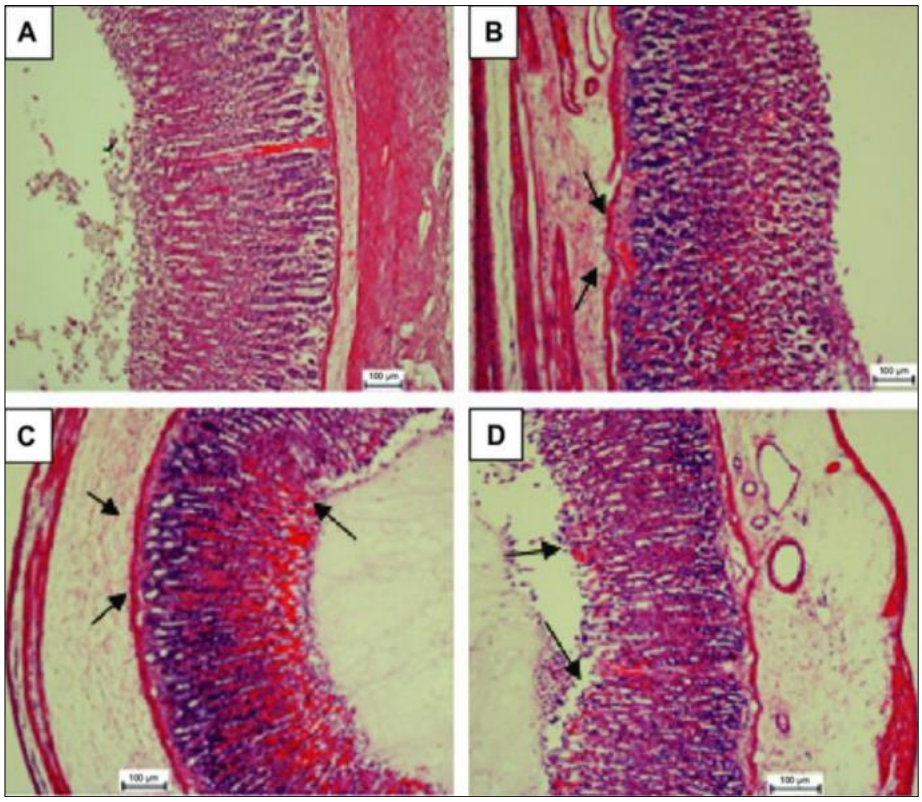


Fig 1: Histopathology of Indomethacin-Induced Gastric Ulceration in Rats. Analysis of Hematoxylin and Eosin staining of the gastric mucosa: The sections were cut parallel to the muscle layer, 400 x magnifications

Plate A (Negative control) revealed several changes in gastric mucosa, such as severe desquamation (white arrows) and loss of surface epithelial cell (red arrow), necrosis, vacuolization, edema and dilated gastric glands along with infiltration of inflammatory cells. Histoarchitecture deeply affected.
Plate B (Positive control) showed typical gastric histoarchitecture with intact epithelium and glands (black arrows). Histoarchitecture not affected.
Plate C (extract 250 mg/kg) showed few superficial surface of mucosa (black arrows), gastric glands appeared normal without inflammatory cells infiltration Histoarchitecture slightly affected.
Plate D (extract 500 mg/kg) displayed very mild erosion of superficial epithelial cells loss and mild hemorrhages (Black arrow) moderately affected.
The calculated Ulcer Index (UI) and % maximum protection from ulceration (%MPU) presented in Table 1 showed that group I (the negative control: albino rats administered indomethacin only) had the highest UI of 4.17±0.31 whereas the animals pre-treated with either Ranitidine (the positive control) or acioa leaf extracts had a significantly lower UI in comparison. The animals treated with Ranitidine had the least UI (0.5±0.34) and the highest %MPU (88%) showing that the standard anti-ulcer drug offered the highest protection against gastric ulcerations. The extract at 500 mg/kg offered better gastro-protection (UI=0.6±0.55 and % MPU=85.37%) which was comparable to the protection offered by the standard anti-ulcer drug (ranitidine UI=0.5±0.34 and % MPU=88.00%). The extract at 250 mg/kg gave a weaker gastro-protection (UI=1.83±0.40 and % MPU=56.12 %) compared to the ranitidine and the higher dose of the extract.
NSAIDs such as indomethacin are well known for their ability to cause gastric mucosal damage19 by inhibiting the enzyme, cyclooxygenase, which reduces prostaglandin production20. Prostaglandins are essential for maintaining the integrity of the stomach mucosa because they promote the formation of mucus and bicarbonate, increase mucosal perfusion, reduce acidity, and speed up gastric epithelial turnover21. Suppression of prostaglandin increases the susceptibility of the gastric mucosa to damage by causing gastric hypermotility, disrupting gastric blood flow, stimulating reactive oxygen species, lipid peroxidation and neutrophil infiltration [20].

It was observed that pre-treating with either ranitidine or acioa leaf extract reduced the degree of indomethacin-induced damage to the gastric mucosa. Compared to the negative control (indomethacin alone), the excised stomach of rats treated with acioa leaf extract showed less mucosal lesions. The protective of the leaf extracts appeared to be dose dependent with the higher dose providing better protection in terms of UI and higher %MPU as evidenced by mucosal lesions or damages.
Ranitidine is an active Histamine H2 receptor antagonist and is a standard anti-ulcer drug used to treat peptic ulcer and it does this by inhibiting the production of gastric acids [23]. The similar anti-acid properties of acioa leaf extract that has been shown may be explained by various mechanisms. Acioa leaves contain phytochemicals including alkaloids, steroids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and resins that may have gastro-protective and cytoprotective abilities and thus antiulcer properties. For instance, tannins can precipitate microproteins and promote vasoconstriction, and provide stomach protection by forming an additional protective layer on mucous membranes, making them less vulnerable to harm by irritants [24]. Additionally, anti-ulcer properties of saponins have been reported [25] possibly due to its surfactant-like properties. In the case of flavonoids, they are well-known antioxidants that can protect the gastric mucosa by clearing reactive oxygen species and stabilizing mucous membranes [26] and increase gastric mucosal prostaglandin [25]. The antiulcer property of acioa leaf extracts shown here provides scientific support for the folkloric use of A. barteri for treating stomach ailments28. There is, therefore, the potential of using action leaf extracts as a basis for developing alternative drugs for treating gastric ulcers.
The effects of the treatment on gastric acid secretion following pyloric ligation-induced ulcer in rats are shown in Table 2. One of the major disease pathways for stomach ulcer is increased volume of acidic gastric juice 21. The untreated (negative control) animals produced the highest volume of acid of 7.21 mL and acidity (98.89 mEq/L) and and lowest pH (2.2), and consequently, the highest UI (13.12). Treatment with 250mg/kg acioa leaf extract significantly reduced all the parameters. At 500 mg/kg, all the parameters were further reduced, indicating that the effect of the leaf extract may be dose dependent.

Table 2: Effect of *Acioa barteri* on gastric secretion following pyloric ligation induced ulcer in rats

Treatment	Dose	Index (± SE)	Total Acidity (mEq/L)	Acid Volume (mL)	pH
(Negative Control) Ethanol	(1 mL)	13.12±2.00	98.89±0.45	7.21±0.65	2.2±0.90
Ethanol extract	250 mg/kg[1]	3.12±0.22**	30.5±0.34**	4.13±0.44**	4.00±1.08**
Ethanol extract	500 mg/kg[1]	1.15±1.03**	22.5±0.66**	2.00±0.23**	5.00±1.00**
Lansoprazole	8 mg/kg[1]	1.10±0.52**	21.1±0.76**	2.11±0.10**	5.6±0.51**

[1]Liveweight of rats; Results are expressed as mean± S.E.M; $p< 0.05$.

The total acidity of the extract at 250 and 500 mg/kg were 30.5 and 22.5 mEq/L, respectively, compared to 21.1 mEq for the standard drug (Lansoprazole) is shown in Table 2. The volume of acid in the gastric secretion of animals on 250 and 500 mg/kg acioa leaf extract was 4.13 and 2.00, respectively. The pH of gastric secretion of animals in the ethanol group was lowest but was higher in the groups that received the leaf extracts, which had similar pH levels as the standard Lansoprazole treatment. Thus, the A. barteri leaf extract had a similar effect of stomach acidity as Lansoprazole, the standard drug for lowering gastric acid production [29]. The pH of the two levels of the leaf extract did not differ and therefore did not seem to be dose dependent.

Following pylorus ligation-induced ulcer in albino rats, the excised stomach tissues showed that both doses of A. barteri extracts produced significant ($p<0.05$) anti-ulcer activity (Table 2). Whilst the negative control (Ethanol alone) had the highest value for UI (13.12), total acidity (98.89), acid volume (7.2), and lowest pH (2.2), the pre-treated groups had significantly ($p<0.05$) lower UI, total acidity, acid volume as well as higher pH. The Lansoprazole treatment group had the least values of the paraments measured and highest which is expected as it's a drug of choice optimum protection from ulceration of the gastric walls. Dosing with 250 mg/kg A. barteri had significant (P>0.05) positive effect on acidity and pH but was not to the same extent as the 500 mg/kg dosing.

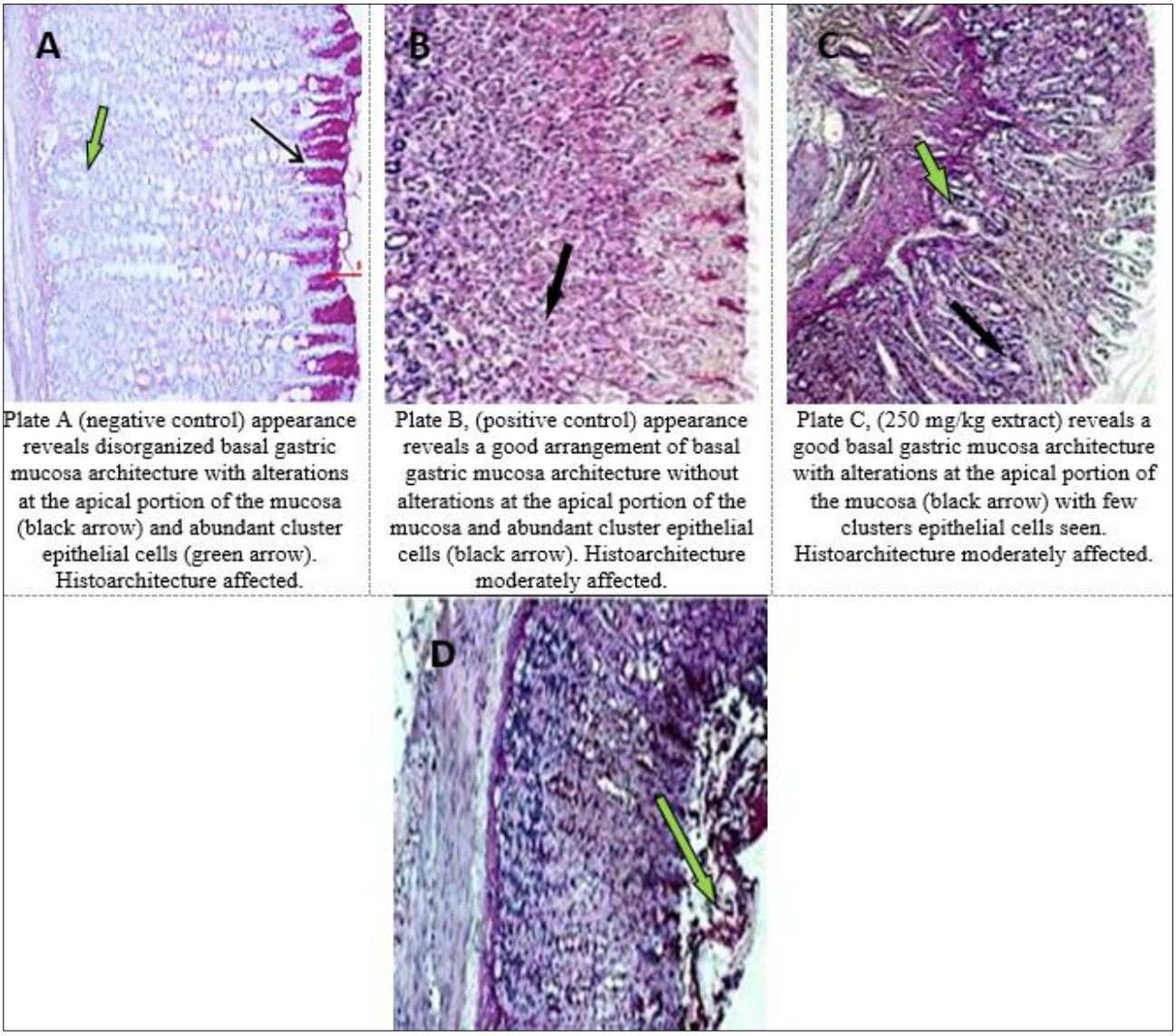


Fig 2: Histopathology (Pyloric Ligated induced) analysis of hematoxylin and eosin staining of the gastric mucosa, the sections were cut parallel to the muscle layer, 400 x magnifications.

The effects of the extracts appeared to be dose dependent with greater effect on UI and acidity from the higher than the lower dose. Indicates that the low dose of the extract was not adequate to provide sufficient protection of the stomach lining against ulcer lesions.

The study showed that acioa leaf extract of 500 mg/kg gave better reduction in total acidity (p<0.05), a better anti-secretory activity as evidenced by the observed reduction in gastric acid secretion and a higher rise in pH compared to the negative control. Both extract concentrations gave a significant reduction in ulcer index (500 mg/kg=1.15± 1.03, 250 mg/kg=3.12±0.22) when compared to the negative control (13.12±2.00). Based on UI, as a measure of ulcerated area, Lansoprazole 8 mg showed resulted in the least ulceration (1.10) and lower total acidity (21.1) than the extract, but the benefits did not surpass those of the 500 mg/kg acioa extract in terms of acid volume and pH (Table 2). Thus, the 500 mg/kg dose of the leaf extract exhibited similar antisecretory potential as the standard drug. Again, this potential is due to the cytoprotective and antisecretory properties of acioa leaves demonstrate the potential acioa leaf extract as a foundation for the development of substitute medications for the treatment of stomach ulcers.

The results of the analysis of methanolic acioa leaf extract using High Performance Liquid chromatography analysis are shown in Table 3 and Figure 3. These analyses revealed the presence of about four major peaks that were determined to be gallic acid (phenol), quercetin (flavonoid), kaempferol (flavonoid), and 3, 4, 6, tribromo-2-(2,4-dibromophenoxy) phenol (Figures 5-8).

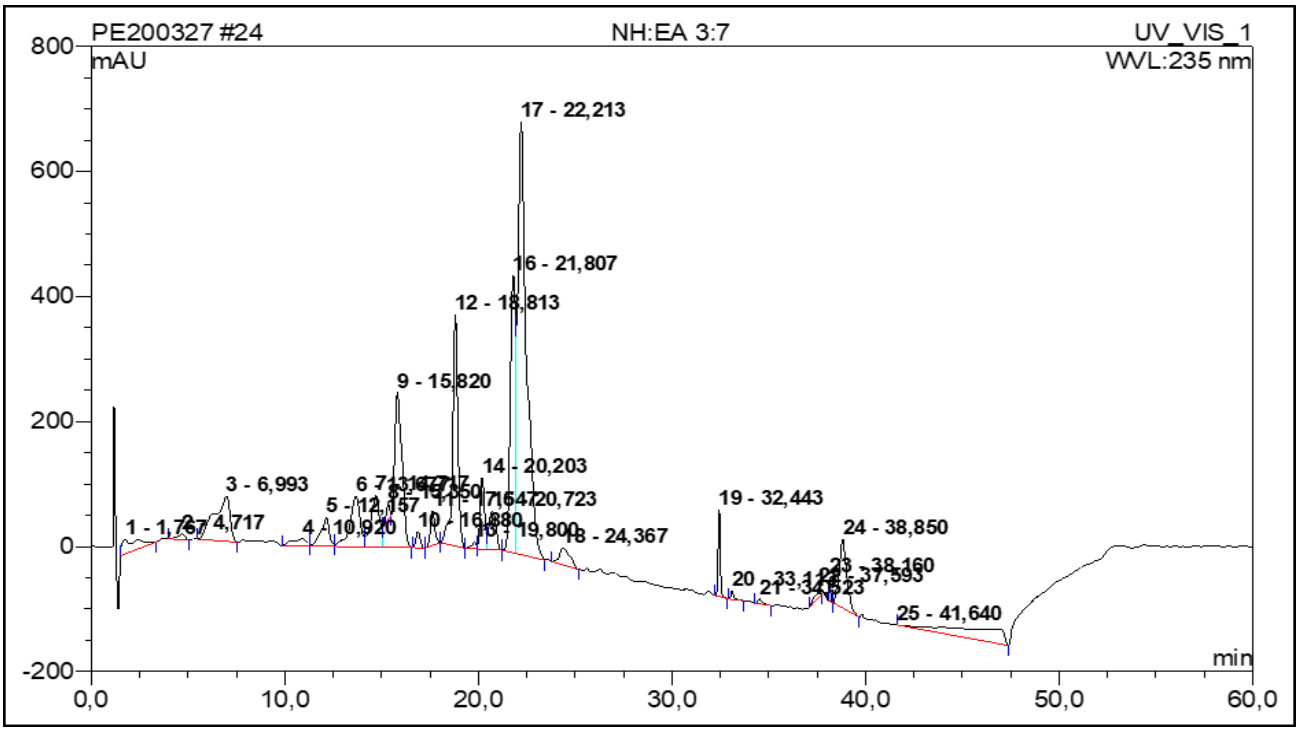
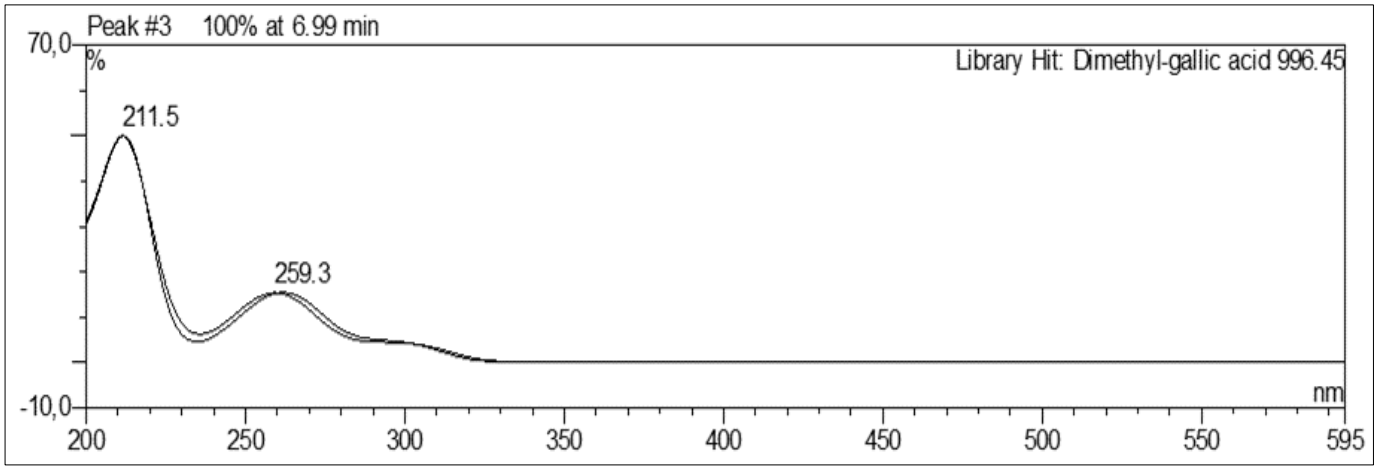


Fig 3: HPLC Analysis Result of the leaf extract (VLC fraction)

Table 3: Bioactive Compounds from VLC Fractions of *Acioa barteri* using HPLC-DAD Analysis

S/N.	Chemical Name	RT (min)	Molecular Formular	Molecular Weight	Class of Phytochemicals	Library Hit	Biological Activities	References
1.	Gallic acid	6.993	$C_7H_6O_5$	170	Phenol	996.45	Antiulcer	(Govindarajan *et al.*, 2006) [30].
2.	Quercetin	20.723	$C_{15}H_{10}O_7$	302	Flavonoids	980.38	Antiulcer	(Kahraman *et al.*, 2003) [28].
3.	Kaempferol	24.367	$C_{15}H_{10}O_6$	286	Flavonoids	953.09	Antiulcer	(Izzo *et al.*, 1994) [27].
4.	3,4,6, tribromo-2-(2,4-dibromophenoxy)phenol	10.920	$C_{12}H_5Br_5O_2$	580	Phenol	992.30	Antiulcer	(Sumbul *et al.*, 2011) [31].

UV spectra of detected compounds showing Gallic acid (4), Quercetin (5), Kaempferol (K, mpf3 Orhamnoglucsid) (6), 3, 4, 6, tribromo-2-(2,4 dibromophenoxy) phenol (7)



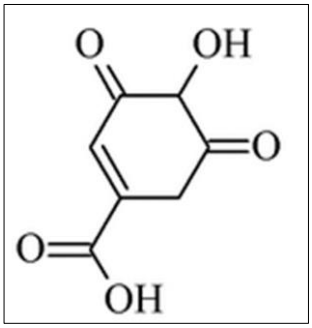
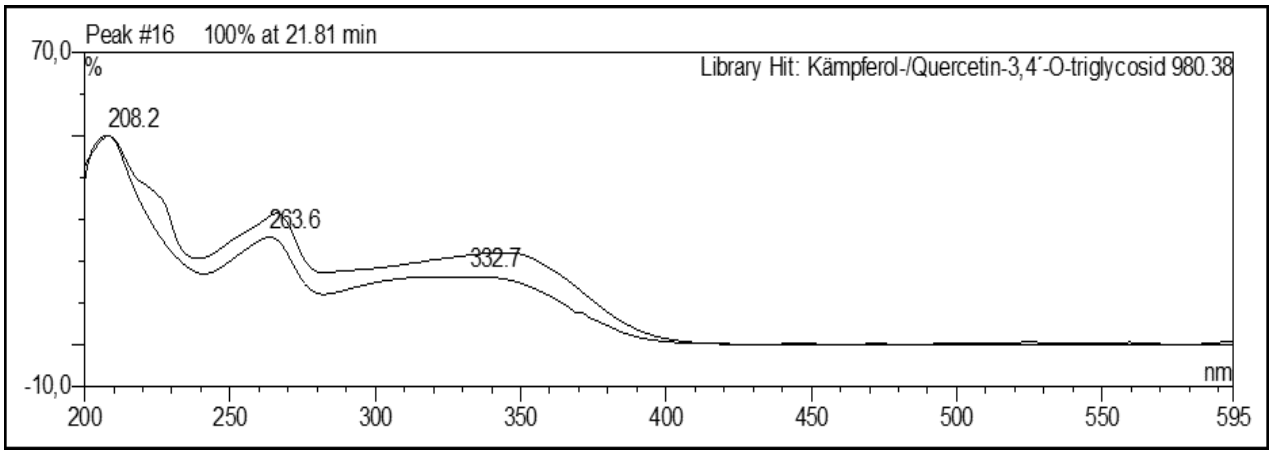


Fig 4: Gallic acid



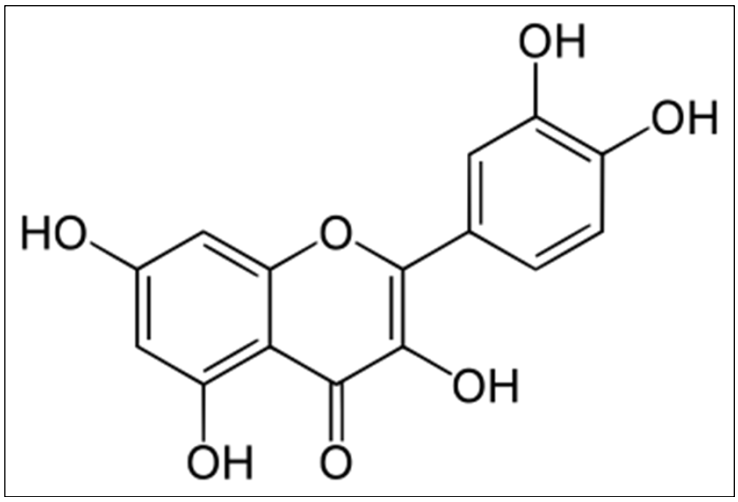
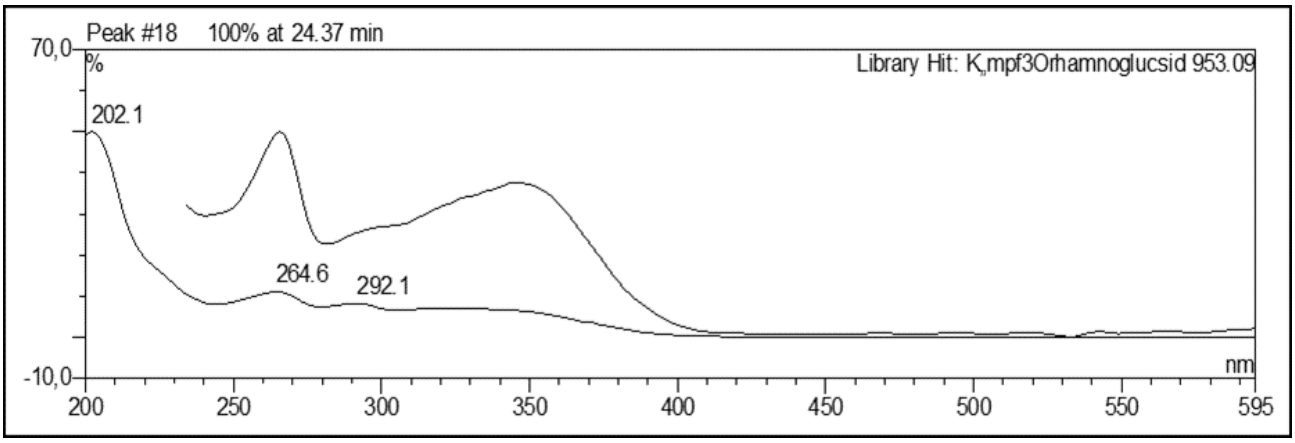


Fig 5: Quercetin



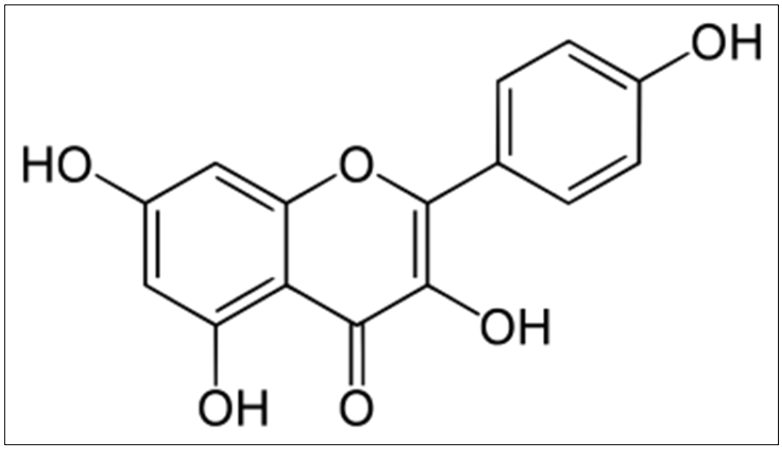
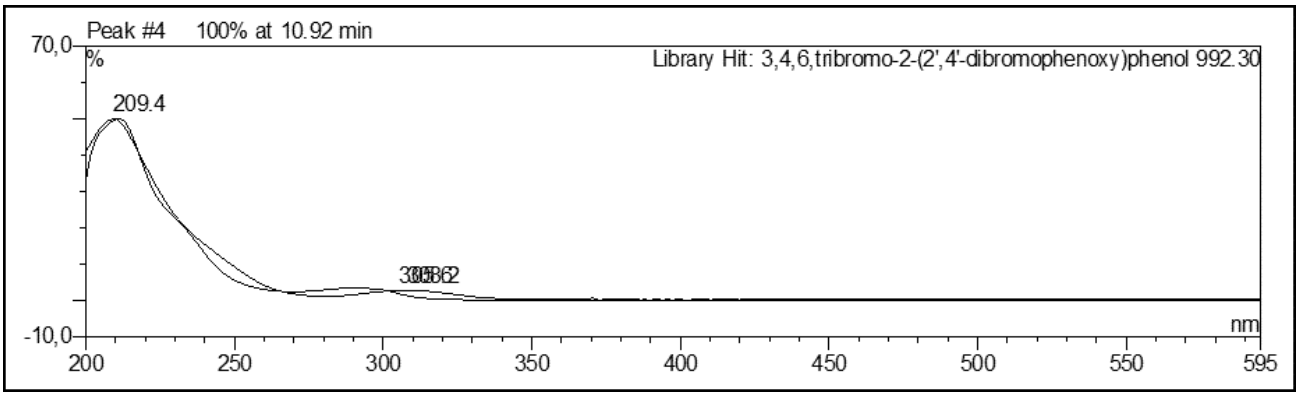


Fig 6: Kaempferol (K, mpf3Orhamnoglucsid)



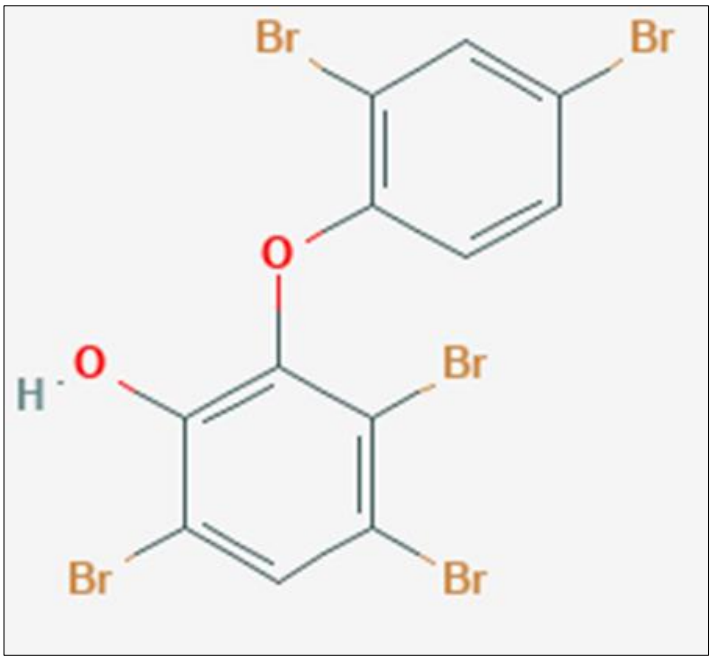


Fig 7: 3, 4, 6, tribromo-2-(2,4-dibromophenoxy) phenol

We attribute the anti-ulcer activities of the extract to these the components.

There is ample information in literature regarding flavonoids, phenols and gallic acid, which are major the constituents of acioa leaves that buttress the potential of acioa leaf extract as anti-acid and basis for the development of an anti-ulcer drug. In the gastrointestinal tract, flavonoids exhibit anti-secretory, antidiarrhoeal and antiulcer properties [30]. Quercetin (3,3',4', 5,7-pentahydroxyflavone) protected the gastrointestinal mucosa from acute lesions and ulcerations induced by different experimental models including necrotic agents and drugs (reserpine, aspirin, indomethacin), restraint stress pylorus-ligation including, and acid-ethanol [31] and ethanol-induced gastric ulcers[32]. The gastroprotective mechanism of quercetin is thought to involve endogenous PAF [31], an increase in mucus production, antihistaminic properties, which decrease histamine levels and reduction of the number of ethanol-induced mast cells. Another flavonoid contained in acioa leaf extract is kaempferol, a natural flavone derivative, reported to prevent gastric mucosal ulceration induced in animal through various models including reserpine, acidified ethanol and absolute and 50% ethanol [31]. The therapeutic effects of many plant-based traditional medicines have been linked to the presence of polyphenols [33]. Gallic acid a phenolic compound, also a component of A. barteri leaves, also possesses antiulcer activity [34]. A wide number of researchers have proven that phenolic compounds displayed a number of pharmacological properties in the gastrointestinal tract, acting as antisecretory, cytoprotective and antioxidant agents and can be an alternative for the treatment of gastric ulcers [35].

4. Conclusion

The anti-ulcer activity of the ethanolic crude extract of *Acioa barteri* leaf and its chemical compositions using HPLC-DAD were determined with respect to ulcer index, total acidity, PH, acid volume and % maximal protection from ulceration. The study showed that A. barteri leaves contained various bioactive components in good amounts that are responsible for its anti-ulcer pharmacologic activities, providing evidence to support the use of the leaves for the treatment of gastrointestinal diseases by traditional medical practitioners. The gastro-protective effects exhibited by the leaf extract (lower ulcer index, reduced total acidity, high pH, lower acid volume, and high % maximal protection of ulceration) were observed to be higher at the higher concentration of the extract. The efficacy of the extract at a higher concentration (500 mg/kg) was comparable to that of the standard drugs, ranitidine, and Lansoprazole. The anti-ulcer effects and gastro-protection reporrted were due both antisecretory and cytoprotective activities of gallic acid, quercetin, kaempferol, and 3,4,6, tribromo-2-(2,4-dibromophenoxy) phenol. The findings from this study support the use of *Acioa barteri* leaf extract in anti-ulcer therapy and as a basis for an alternative anti-ulcer drug.

5. Compliance with ethical standards

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6. Disclosure of conflict of interest

The authors declare no conflict of interest.

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8. Authors Contribution Statement

All authors were involved in the conceptualization of the research. Nnamdi M. Adione and Innocent O. Ajawobu, contributed to data collection, while Omoirri M. Aziakpono Innocent N. Okpoli, and Ogechi O. Anyanwu contributed to the analysis and development of the initial manuscript draft. Professor Festus BC Okoye supervised the project and reviewed successive drafts along with Dr. Ike V Ezenwa. Professor Festus BC Okoye and Dr Ike V Ezenwa reviewed the various drafts; Dr. Ike V. Ezenwa developed the final manuscript for publication.

9. Data Availability Statement

Data generated or analyzed during this study are provided in full within the published article and its supplementary

10. References

1. Monjur A. Digestive System; Recent Advances. In: Qi X, Ed; c2020.
2. Yuan Y, Leontiadis GI. Ulcer-related vs non-ulcer-nonvariceal upper gastrointestinal bleeding which has a worse outcome? Aliment Pharmacol Ther. 2019;49(6):818-819.
3. Smeltzer SC, Bore BG, Hinkle JK, Cheever KH. Brunner & Suddarth's Textbook of Medical Surgical Nursing. 12th ed. Philadelphia, United States: Lippincott Williams and Wilkins; c2010.
4. Nwachukwu CD, Okwuosa NC. Investigation of the Anti-Ulcer Activity of Chloroform Leaf Extract of Aspila Africana in Rats. Indian J Novel Drug Deliv. 2012;(1):52-56.
5. Panda V, Sonkamble M. Anti-ulcer activity of Ipomoea batatas tubers (sweet potatoes). Funct Foods Health Dis. 2012;2(3):48-61.
6. Lavnya A, Kumar MP, Anub J, Anjana A, Ayyasay S. Antiulcer activity of Canabalia virosa (ROXB) W&A leaves in animal model. Int J Life Sci Pharma Res. 2012;2(4):39-43.
7. Jongkind CCH. Plant Ecology and Evolution. 2012;145(1):126-128.
8. Cooper, Record; c1931.
9. Anyanwu OO, Bariko GT, Okoye F. Preliminary phytochemical and acute toxicity study of methanol leaf extract of Acioa barteri; c2020.
10. Alphin RS, Ward JW. Action of hexopyrronium bromide on gastric secretion in dogs and on gastric secretion and ulceration in rats. Arch Int Pharmacodyn Ther. 1967;270:128-140.
11. Nwafor PA, Effraim KD, Jacks TW. Gastroprotective effects of aqueous extract of Khaya senegalensis bark on indomethacin-induced ulceration in rats. West Afr J Pharmacol Drug Res. 1996;12:46-50.
12. Zaidi SH, Mukerji B. Experimental peptic ulceration. Part 1. The significance of mucus barrier. Indian J Med Res. 1958;46:27-37.
13. Nwafor PA, Okwuasaba FK, Binda LG. Antidiarrhoeal and antiulcerogenic effects of methanolic extract of Asparagus pubescens root in rats. J Ethnopharmacol. 2000;72:421-427.
14. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd Ed. New Delhi, India: Vallabh Prakashan; c1999. p. 148-150.
15. Shay H, Komarov SA, Fele SS, Meranze D, Gruenstein H, Siplet H. A simple method for uniform production of gastric ulceration in rat. Gastroenterology. 1945;5:43-61.
16. Lerch ML, Bauer DR, Theiss A, Chafin D, Otter M, Baird GS. Monitoring Dehydration and Clearing in Tissue Processing for High-Quality Clinical Pathology. Biopreserv Biobank. 2019;17(4):303-311. DOI: 10.1089/bio.2018.0122.
17. McSorley K. Osteogenic Potential and safety of ephrinb2 transfected with Polyethyleneimine gene activated matrices for use in bone tissue engineering [dissertation]. Royal College of Surgeons in Ireland; c2014.
18. Al Batran R, Al-Bayaty F, Al-Obaidi MM, Abdualkader AM, Hadi HA, Ali HM, et al. In vivo antioxidant and antiulcer activity of Parkia speciosa ethanolic leaf extract against ethanol induced gastric ulcers in rats. 2013;8(5).
19. Musumba C, Pritchard DM, Pirmohamed M. Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther. 2009;517-531.
20. Oluwabunmi IJ, Abiola T. Gastroprotective effect of methanolic extract of Gomphrena celosioides on indomethacin induced gastric ulcer in Wistar albino rats. Int J Appl Basic Med Res. 2015;5(1):41.
21. Jalilzadeh-Amin G, Najarnezhad V, Anassori E, Mostafavi M, Keshipour H. Antiulcer properties of *Glycyrrhiza glabra* L. extract on experimental models of gastric ulcer in mice. Iran J Pharm Res. 2015;14(4):1163.
22. El-Ashmawy NE, Khedr EHA, El-Bahrawy HA, Selim M. Nebivolol prevents indomethacin-induced gastric ulcer in rats. J Immunotoxicol. 2016;13:580-589.
23. Oche O, Sani I, Chiaka NG, Samuel NU, Samuel A. Pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of Vitex doniana in normal and streptozotocin-induced diabetic albino rats. Asian Pac J Trop Biomed. 2014;4(2):124-130.
24. Júnior Gd, de Souza Rodrigues T, Hiruma-Lima CA, Vilegas W. New steroidal saponins and antiulcer activity from *Solanum paniculatum* L. Food Chem. 2015;160-167.
25. Ahmed F, Rahman MS. Preliminary assessment of free radical scavenging, thrombolytic and membrane stabilizing capabilities of organic fractions of *Callistemon citrinus* (Curtis.) skeels leaves. BMC Complement Altern Med. 2016;16(1):247.
26. Kelly N, Porat D, Havsteen BH, Dahan A. Flavonoids acting in gastrointestinal tract, having antispasmodic, anti-secretory, antidiarrhoeal and antiulcer properties. J Nutr Biochem. 2009;2:9-10.
27. Izzo AA, Di Carlo G, Mascolo N, Capasso F. Antiulcer effect of flavonoids: role of endogenous PAF. Phytother Res. 1994;8:179-181.
28. Kahraman M, Izzo AA, Di Carlo G, Mascolo N, Capasso F. Gastrointestinal mucosa from acute lesions induced by various experimental models and against acid-ethanol

and ethanol-induced gastric ulcers. Phytother Res. 2003;8:17-19.
29. Manach C, Morand C, Demigne C, Texier O, Regerat F, Rémésy C. Bioavailability of rutin and quercetin in rats. FEBS Lett. 1997;409:12-16.
30. Govindarajan O, Krummen M, Potgeter H, Roth A, Siffrin C. Plant and fruit extracts contains gallic acid as an essential constituent with antiulcer activity. Phytother Res. 2006;8:79-81.
31. Sumbul A, Potgeter H, Roth A, Siffrin C. Phenolic compounds display a number of pharmacological properties in the gastrointestinal tract, and can be an alternative for the treatment of gastric ulcers. Biomed Inform. 2011;5(6):38-42.