Bioactive constituents and antiulcer activity of the unripe fruit peel of *Musa Paradisiaca* L. (Musaceae)

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ABSTRACT

Background: *Musa paradisiaca* is used traditionally in treating diarrhoeal, wounds and infections and also in the management of diabetics and ulcer. This work aimed to evaluate the proximate contents, phytochemical constituents and the antiulcer potential of the peel of *Musa paradisiaca.*

Methods: Proximate parameters and phytochemical constituents was evaluated using standard procedures and gas chromatography-mass spectrometry (GC-MS), while the effect of the extract on ethanol induced stomach ulcer was study in adult Wister rats.

Results: Result revealed ash value (8.70 ± 0.50) %, crude fibre (10.50 ± 0.70) % and moisture content (20.30 ± 0.40) % respectively. Phytochemicals present included alkaloids, tannins, flavonoids, triterpenoids, steroids and saponins. GC-MS analysis identified twenty-three compounds including gamma tocopherol. The methanol extract exhibited significant antiulcer activity at 400 mg/kg and its activity increases as the dose increases.

Conclusion: The study showed that *Musa paradisiaca* contains important phytoconstituents and validated the ethno-medicinal claim of antiulcer effect.

***Keywords*:** *Musa paradisiaca* peel, Antiulcer, Phytochemicals, GC-MS analysis, Proximate analysis.

1. INTRODUCTION

Musa paradisiaca also known as plantain belongs to family Musaceae, it is an important food crop in several countries, though it grows mainly in South America, Asia and tropical Africa [1]. In Nigeria, it is widely grown in the south-south and south-east regions and the pulp unripe or ripe is made into different delicacies, while the peel is either thrown away as waste or used as herb when the need arises. The plant is made up of lengthy, overlying leafstalks with a stem height measuring between 1 m to 6.20 m [2] and can last for about 15yrs [3]. It fruits grow in bunch of circular clusters and an average diameter of 2.54 cm for each fruit. A complete season for plantain varies between 8 to 12 months [4]. Important minerals such as magnesium, nitrogen, phosphorus and potassium have been reported in plantain [5]. Nitrogen is utilised in the amino acid formation, potassium is necessary for the proper development of the muscular tissues, phosphorus determines how the body metabolise fat and carbohydrate and magnesium is needed for appropriate intercellular communication. Thirty percent of the plantain fruit is the peel which contains minerals and phytochemicals [6]. The herbal potential of the peel can be seen in its traditional uses of lowering blood pressure and prevention of muscle cramp [7]. Juice from the leaves are used as abortifacient, while its sap is used as a treatment for watery and bloody stool and convulsion. Aqueous decoction of the root is used venereal diseases and shortage of blood in the body, while the pulp are used as sexual enhancer, diabetics, ulcer and diuretics [8]. Studies have shown that the aqueous extract of plantain peels had antiulcer potential [9] but there is need to evaluate the methanol extract of the peel for the phytochemicals present using GC-MS and check the extract for antiulcer property. Thus this study aimed to evaluate the proximate parameter, phytochemical composition, identify the compounds present and determine the effect of the extract on ethanol induce gastric ulcer in rats.

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2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Material

Plantain fruits were obtained from Agekpanu village in Ovia North East of Edo State in January and identified by Dr H.A. Akinnibosun of Department of Plant and Biotechnology with voucher sample number of UBH-M486. They were carefully cut, peeled off, air dried for two weeks and pulverised using mechanical blender. The powdered material weighing 500 g was soaked for 3 days using 2.5 L of methanol, filtered and the filtrate concentrated *in-vacuo* at 50 ^oC. The extract was kept in a refrigerator at a temperature of 4 ^oC until when needed.

2.2 Methods

2.2.1 Proximate analysis

The dried powdered peel of *Musaparadisiaca* was evaluated by the technique of Association of Official Analytical Chemist [10]. Procedures for testing the different parameters such as ash 942.05 (4.1.10), crude fat 920.39 (4.5.01), crudefibre (962.09 (4.6.01), crude protein 955.04 (2.4.03) and moisture 934.01 (4.1.03) are as provided.

2.2.2 Phytochemical screening

These tests were conducted by the methods of Sofowora, (1993) [11] and Trease and Evans, (2002) [12]. The phytochemicals tested for include alkaloids, anthraquinone, flavonoids, tannins, cardiac glycosides, saponin, steroids and triterpenoids.

2.2.3 GC-MS analysis:

This was based on the method described previously by Odion *et al*., (2020)[13] with slight modification. Briefly, analysis of the sample was executed on QP 2010 SE Shimadzu, Japan. GC workingsettings were as follows; the column oven temperature was set at 60 ^oC, while the temperature for injection was at 250 ^oC. The temperature was held at 60 ^oC for 1.50 min and then increases to 260 ^oC at a rate of 14 min and held for there for 1.5 min. This was finally increased to 300 ^oC at a rate of 14 min and held for 3.30 min. helium was used as the carrier gas. The sample was injected in the split mode at 250 ^oC injector temperature. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 45 to 700 Dalton. Data were acquired and processed using ChemStation software. Compounds were identified by comparing their base peak and molecular weight with data from National Institute of Standard Technology (NIST) library.

2.2.4 Ethanol induced ulceration

The effect of methanol extract of *Musa paradisiaca* on ethanol-induced gastric ulcer in adult rats was evaluated by procedure described by Mbagwu *et al*., 2011[14]. Thirty rats (male and female) of weight 150-200 g were purchased from the Animal House of the Department of Pharmacology and were kept for two weeks prior to study for proper acclimatization, during which they had access to water and feed and were exposed to the night and day cycle (12 hr). The experimental protocol of the internationally accepted principle on the handling, care and use of laboratory animals were strictly adhered to.A day before the experiment the rats were fasted for 18 hrs and were randomly divided into 5 groups of 6 animals each; the first group treated with ranitidine 100 mg/kg (Positive control) orally, the second, third and fourth group were administered with the extract at 200mg/kg, 400mg/kg and 800mg/kg, orally. Fifth group was administered with distilled water 2 ml/kg body weight (negative control group). The drugs were administered 1 hr before intra gastric administration of 1 ml absolute ethanol. The rats were sacrificed and the stomachs were excised and cut open along the line of greater curvature to expose the walls. The stomachs contents were carefully removed and washed with distilled waterand the stomach contents examined macroscopically (X100) and fixed in 10 % formalin for histological analysis. The diameter of the ulcers was measured using a vernier caliper and scored accordingly.

$$\% \text{ Inhibition of ulcer} = \frac{C-T}{C} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where C= Total number of ulcer spots in control group (water)

T= Total number of ulcer spots in test sample

2.3 Statistical analysis

Results were presented as the mean± standard deviation (SD) as triplicate measurement. Analysis of variance was done by GraphPad Instat version 3.06. Level of significance was set at P≤0.05.

3.0 RESULT AND DISCUSSION

Proximate parameter revealed ash, crude fat, crude fibre, crude protein and moisture content as (8.70 ± 0.50) %, (0.08± 0.00) %, (10.50 ± 0.70) %, (2.00 ± 0.30) % and (20.30 ± 0.40) % respectively (Table 1). Evaluation of the proximate values of *Musa paradisiaca* is the determination of the nutritive content of the peel. Ash value determination aids in qualifying the bulk inorganic components in plantain peel. This include various minerals present and their presence have been shown to slow the process of spoilage. The value reported in this work are quiet high, when compared to what was reported by Oyeyinka and Afolayan (2019) [5]. Fat content in fruits are known to be low and this have been used to modify fat concentration in the body with the advantage of reduced heart diseases. The low level of fat in the peel of plantain is in coherence with other studies by other researchers [9].The importance of fibre in diet cannot be over emphasized, apart from facilitating digestion which encourages gastrointestinal health, they have been shown to be used in weight management, lowering of cholesterol, reduce heart diseases and colon cancer.The fibre content observed in this study were high and showed that plantain peel are rich in fibre component and thus could be used to enhance bowel motility. The protein content of plantain peel was observed to be low when it was compared to previous study. The crude protein of plantain peel was observed to be low considering the relevance of protein as the building block. Additionally crude protein is believe to curtail age related diseases [15] and reduce the effect of type II diabetes [16]. The moisture content was observed to be high due to the high level of water in the peel of *Musa paradisiaca*, this is also indicative of high level of nutrients. The values recorded in this study are for unripe *Musa paradisiaca* and the values for ripe plantain are quiet low, as reported by Okerah and co-workers (2015) [9]. High moisture content also imply short shelf life and the possibility of been effected by microbes.

Table 1. Proximate analysis of *Musa paradisiaca* powder peel

Proximate parameter	Percentage
Ash value	8.70 ± 0.50
Crude fat value	0.08± 0.00
Crude fibre value	10.50 ± 0.70
Crude protein	2.00 ± 0.30
Moisture content	20.30 ± 0.40

Phytochemical evaluation revealed alkaloids, flavonoids, saponins, tannins, steroids and triterpenoids while anthraquinone and cardiac glycosides were absent (Table 2). This result partly agrees with study conducted by Alisi and co-workers (2008)[17], where tannins, glycosides and flavonoids were identified in the fruit peel.One of the aim of this study was determination of the presence of the plant chemicals by testing the powdered fruit peel, this was achieved in aqueous and organic solvent (methanol). The pharmacological activity exhibited by herbs could be linked to the presence of phytochemicals in the plants [18]. Alkaloids and flavonoids have been reported to showmyriad of biological activities varying from antioxidant, anticancer, antiulcer, antivirusand antimicrobial activities [19]. Tannins have been reported to show anti-haemorrhagic property [20]. Saponins with its ability to froth, can be used as cleansing agent and thus exhibit antimicrobial potential [18] and as precursor for steroids biosynthesis [21]. Flavonoids are subgroups of polyphenols that contains hydroxyl groups in their structure which have been shown to scavenge for free radicals while alkaloids are basic molecules with nitrogen atom in its heterocyclic structure that confers some of its important properties.

Table 2. Phytochemical analysis of the peel of *Musa paradisiaca*

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Saponin	+
Tannins	+
Anthraquinone	-
Steroids	+
Cardiac glycosides	-
Triterpenoids	+

GC chromatogram revealed twenty-three compounds (figure 1), they include 2-Methyl[1,3,4]oxadiazole, Propanedioic acid, propyl-,Nonanoic acid,alpha.-D-Glucopyranoside, methyl 3,6-anhydro-,3-Pentanol, 2-chloro-4-methyl-,Decanoic acid, methyl ester,n-Hexadecanoic acid,9,12-Octadecadienoic acid(Z,Z),11,14,17-Eicosatrienoic acid, methyl ester, methyl ester,(Z,Z,Z),9,12-Octadecadienoic acid, methyl ester,Octadecanoic acid,Butanamine, 2-methyl-,9-Octadecenamide(Z),Eicosanoic acid,Hexadecanoic acid, 2 hydroxyl-1-(hydroxylmethyl)ethyl ester,n-Decanoic acid,9,12-Octadecadienoic acid(Z,Z), 2,3-dihydroxypropyl ester,Butyl 9,12,15-octadecatrienoate,Octadecanoic acid 2,3-dihydroxypropyl ester,7,11-Dimethyldodeca-2,6,10-trien-1-

ol,Methoxy-6-(4-trifluoromethylphenyl)naphthalene,Gamma-Tocopherol and2-Trifluoromethylbenzoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester. The identification was based on the retention time of the individual compounds, this means of identification was not conclusive until after the selected compounds were feed into the MS for their specific fragmentation pattern.

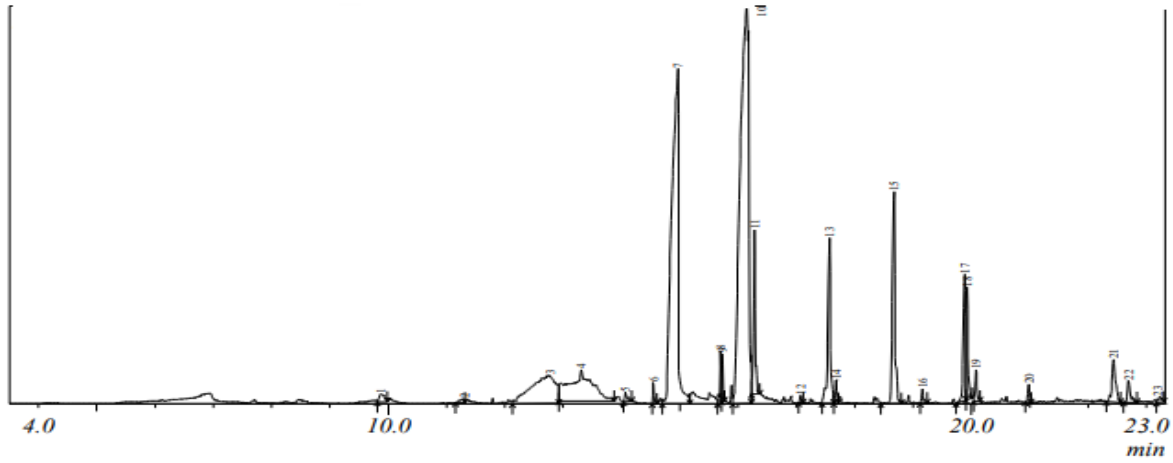


Figure 1. Chromatogram of extract of Musa paradisiaca peel.

These were then compared or matched with NIST data, the compounds identities were matched with their molecular weight from the molecular ion, base peak and their splitting pattern (Table 3). Base on their functional group the identified compounds were placed in the following groups; esters, fatty acids (saturated and unsaturated), amines, amide and alcohol. 9,12-octadecadienoic acid (Z,Z)- and n-hexadecanoic acid were identified as the most prominent compounds with percentage area of 24.01 % and 36.25 %. These group of compounds are found in most plantsdue to the protective effect they confer on them [22].

Table 3. Compounds identified from GC-MS determination of peel of *Musaparadisiaca*

S/N	RT	PA	MF	BP	M.W	Name of Compounds
1	9.870	0.45	$C_3H_4N_2O$	84	84	2-Methyl[1,3,4]oxadiazole
2	11.300	0.20	$C_6H_{10}O_4$	60	146	Propanedioic acid, propyl-
3	12.769	7.26	$C_9H_{18}O_2$	60	158	Nonanoic acid
4	13.309	7.22	$C_7H_{12}O_5$	57	176	alpha.-D-Glucopyranoside, methyl 3,6-anhydro-
5	14.066	0.23	$C_6H_{13}ClO$	73	136	3-Pentanol, 2-chloro-4-methyl-
6	14.545	0.32	$C_{11}H_{22}O_2$	74	186	Decanoic acid, methyl ester
7	14.964	24.01	$C_{16}H_{32}O_2$	73	256	n-Hexadecanoic acid
8	15.699	0.84	$C_{18}H_{32}O_2$	81	280	9,12-Octadecadienoic acid(Z,Z)
9	15.727	0.45	$C_{21}H_{36}O_2$	79	320	11,14,17-Eicosatrienoic acid, methyl ester
10	16.147	36.25	$C_{18}H_{32}O_2$	67	280	9,12-Octadecadienoic acid (Z,Z)-
11	16.281	2.97	$C_{18}H_{36}O_2$	73	284	Octadecanoic acid
12	17.070	0.12	$C_5H_{13}N$	58	87	Butanamine, 2-methyl-
13	17.565	4.56	$C_{18}H_{35}NO$	59	281	9-Octadecenamide(Z)
14	17.679	0.43	$C_{18}H_{36}O_2$	73	312	Eicosanoic acid
15	18.672	5.71	$C_{19}H_{38}O_4$	43	330	Hexadecanoic acid, 2 hydroxyl-1-(hydroxylmethyl)ethyl ester
16	19.157	0.21	$C_{10}H_{20}O_2$	73	172	n-Decanoic acid
17	19.882	3.36	$C_{21}H_{38}O_4$	67	354	9,12-Octadecadienoic acid(Z,Z), 2,3-dihydroxypropyl ester
18	19.924	1.86	$C_{22}H_{38}O_2$	79	334	Butyl 9,12,15-octadecatrienoate
19	20.078	0.58	$C_{21}H_{42}O_4$	98	358	Octadecanoic acid 2,3-dihydroxypropyl ester
20	20.982	0.28	$C_{14}H_{24}O$	69	192	7,11-Dimethyldodeca-2,6,10-trien-1-ol
21	22.437	1.77	$C_{18}H_{13}F_3O$	302	302	Methoxy-6-(4-trifluoromethylphenyl)naphthalene
22	22.695	0.71	$C_{28}H_{48}O_2$	151	416	Gamma-Tocopherol
23	23.235	0.20	$C_{18}H_{19}F_3O_2$	173	324	2-Trifluoromethylbenzoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester

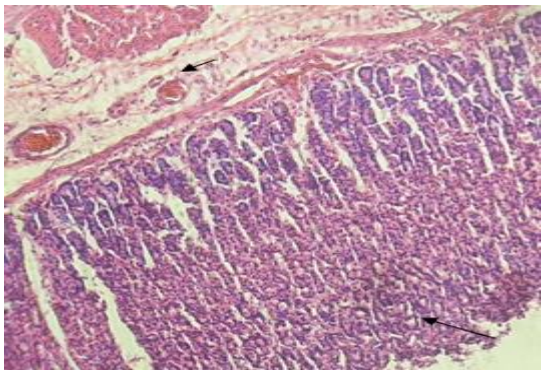
Key: RT=Retention time, MF= Molecular formula, MW= Molecular weight, BP= Base peak, PA=percentage area

The 1,3,4-oxadiazole moiety in 2-methyl[1,3,4]oxadiazole have shown moderate thymidine phosphorylase inhibitory activity, it has also inhibit Ca2þ/calmodulin simulated cyclic adenosine 3', 5'-monophosphate (cAMP) formation in cells and have shown activity against glucuronidase [23].Hexadecanoic acid, 2 hydroxyl-1-(hydroxylmethyl)ethyl esterhas been reported to have antioxidant, hypocholesterolemic, antiandrogenic,

haemolytic, and 5- alpha reductase inhibitory activities. 9,12-Octadecadienoic acid(Z,Z) possess anti-secretory property which enhances the activity of antibiotics administered against *H. pylori* bacteria. Other uses include antispermigenic, antitonsilitic, anti-tubercular, choleretic, and contraceptive activities [24]. From the antioxidant and anti-inflammatory potentials of γ-tocopherol, itis may likely have protective effect against ulcerogenic agents [25].

X100 MAGNIFICATION

Control (C1) Stomach



Control (C2) Stomach

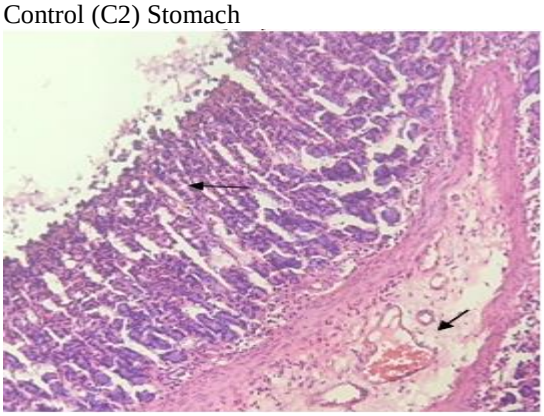
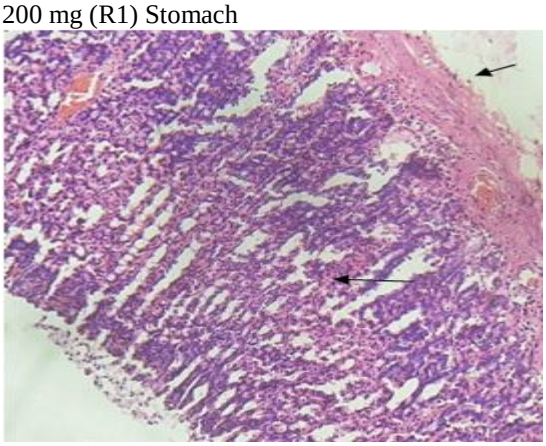


Figure 2: C1 Stomach reveals visible mucosa (long arrow), which appears lined with columnar epithelium below the muscularis mucosa are visible blood vessels and the submucosa contains connective tissue (short arrow). C2 reveals scanty leukocyte infiltration in sub mucosa (long arrow). There is also presence of infiltrates in the overlying muscularis mucosa and mild mucosal damage (short arrow).

X100 MAGNIFICATION

200 mg (R1) Stomach



200 mg (R2) Stomach

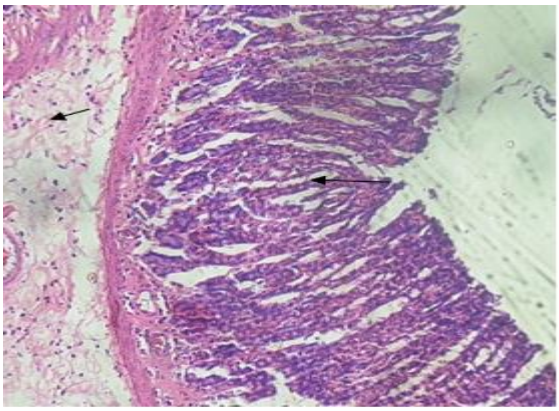
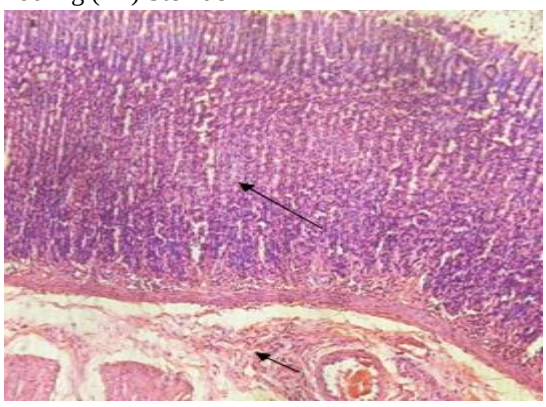


Figure 3: R1 reveals edema with leukocyte infiltration (long arrow) in sub mucosa disruption of surface epithelium mucosa and visible mucosal damage with mild infiltrates (short arrow). R2 reveals mild disruption of surface epithelium mucosa (long arrow) and damage with visible infiltrates and mild ulcerative mucosal damage (short arrow)

X100 MAGNIFICATION

400 mg (B1) Stomach

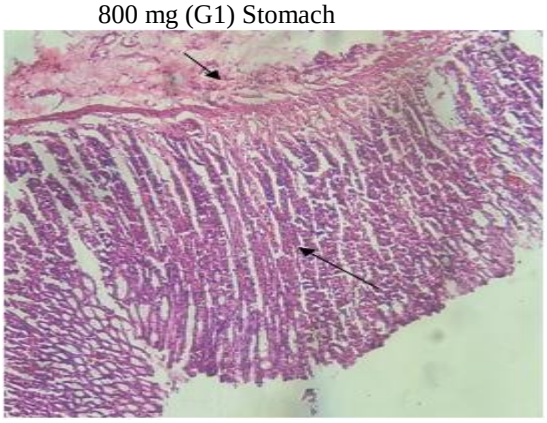


400 mg (B2) Stomach

Figure 4: B1 Stomach reveals visible mucosa (long arrow), which appears lined with columnar epithelium below the muscularis mucosa are visible blood vessels and the submucosa contains connective tissue (short arrow). B2 reveals scanty leukocyte infiltration in sub mucosa (short arrow). There is also presence of infiltrates in the underlying thickened muscularis mucosa and mild mucosal damage (long arrow).

X100 MAGNIFICATION

800 mg (G1) Stomach



800 mg (G2) Stomach

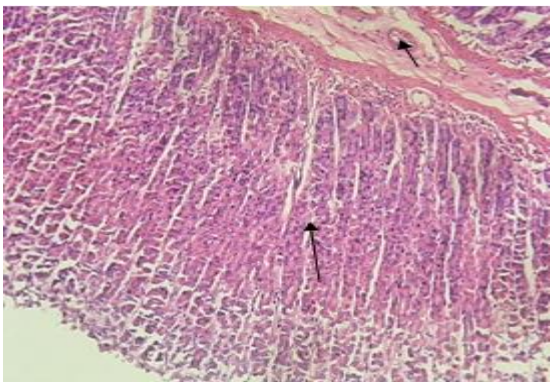
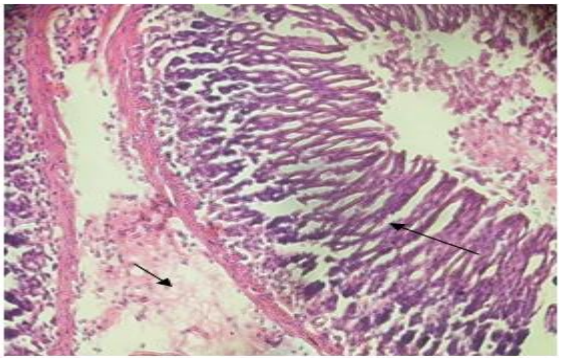


Figure 5: G1 Stomach reveals visible elongated mucosa (long arrow), which appears lined with columnar epithelium below the muscularis mucosa are visible connective tissue (short arrow). G1 Stomach reveals visible mucosa (long arrow), which appears lined with columnar epithelium below the muscularis mucosa are visible blood vessels and the submucosa contains connective tissue (short arrow).

X100 MAGNIFICATION

Antiulcer Drug (S1) Stomach



Antiulcer Drug (S2) Stomach

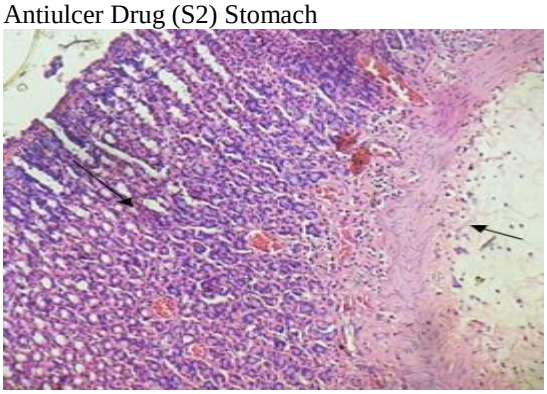


Figure 6: S1 stomach reveals visible mucosa (long arrow), which appears lined with columnar epithelium below the muscularis mucosa are visible blood vessels and the submucosa contains connective tissue (short arrow).S2 reveals scanty leukocyte infiltration in sub mucosa (short arrow). There is also presence of infiltrates in the overlying muscularis mucosa and mild mucosal damage (long arrow).

Distilled water showed the highest number of ulcerative spot 29.30±3.02, while 800 mg/kg of extract had the lowest number of ulcer spot of 8.01±2.32. Ranitidine produced 10.00±3.33 number of spot which is slightly higher than that produced by the 800 mg/kg (Table 4). Statistically, the number of ulcer spots were significantly different P≤0.05 when compared to the negative control (distilled water).

Table 4. Effect of methanol extract of *Musaparadisiaca* peel on ethanol induce ulceration in Wister rat

Group	No. of ulcer spots	% Ulcer inhibition
Distilled water	29.30 ± 3.02	Nil
200mg/kg	17.32 ± 4.05	40.89
400mg/kg	15.02 ± 2.36	48.74
800mg/kg	8.01 ± 2.32	75.23
Ranitidine	10.00 ± 3.33	65.87

From the result above, it can be seen that a higher dose of 800 mg/kg of the extract was needed to produce considerable ulcer inhibition of 75.23 % in the rats compared to the other doses of 200mg/kg and 400 mg/kg of extract of percentage inhibition of 40.89 % and 48.74 % respectively. Ranitidine had a lower percentage ulcer inhibition 65.87 %, compared to 800 mg/kg of extract and this could be attributed to the presence of multiple compounds in the extract and could be exerting their effects synergistically or additively. Ethanol induces ulcer in the gastric mucosa by to its corrosive nature and by rapid penetration which causes cells and plasma membrane damage and these might lead to increased membrane permeability to sodium and water. This leads to a massive intracellular accumulation of calcium which leads to cell death. It is also associated with increased purine degradation which leads to increased oxygen radical production and ROS-mediated increased lipid peroxidation. The extract (400 mg/kg and 800 mg/kg) were both effective in reducing the development of ethanol induced gastric ulcerations and hence protecting the gastric mucosa.

4. CONCLUSION

Musa paradisiaca peel have high level of minerals, water and different phytochemicals which could be responsible for the antiulcer property exhibited. This study have justify the claim made by traditional medicine healer that peels from plantain can be used for treating ulcer. There is need for further study on the peel of *Musa paradisiaca* to isolate and characterise the compounds responsible for the antiulcer activity and determine its mechanism of action.

Acknowledgement

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Conflict of interest

None

Contribution of authors

Emmanuel E. Odion wrote and supervised the work, Philip A. Obarisiagbon supervised the antiulcer activity in the experimental animals, Racheal O. Ogboru assist in the write-up of the work while Osaigbovo J. Oboigba performed the work.

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