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Identification of the Active Ingredients in Methanolic Leaf Extract of *Aspilia africana* (Pers.) C.D. Adams and their Effects on Ibuprofen Induced Ulcer Model in Wistar Rats

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Abstract: ***Peptic ulcer is one of the most dreadful diseases in the world today. However, the present study was investigated to identify the active ingredients in the methanolic leaf extract of Aspilia africana and their possible effects on Ibuprofen induced ulcer model in Wistar rats using standard analytical methods. The GC-MS spectral analysis revealed thirty three (33) active ingredients with 9,12,15-Octadecatrienoic acid, methyl-ester(z,z,z)-, stigmasterol, hexadecanoic acid, methylester(z)-, Methyl linolelaidate, n-Hexadecanoic acid, squalene, phytol and vitamin E possessing important pharmacological and cytoprotective functions. The oral acute (LD_{50}) toxicity study divulged that the extract did not cause mortality to any of the experimental animals even at the highest dose of 5000mg/kg. Other studies disclosed that the induction of ulcerogenesis by ibuprofen led to alterations in the mucosal membrane and this was evidenced by severe distortions in gastric contents, serum concentrations of blood glucose, antioxidant enzymes, lipid peroxidation marker, hepatocellular enzymes, renal function and haematological indexes. Pretreatment with the natural antidote (A. africana leaf extract) remediated this imbalance significantly more than a standard antiulcer drug (omeprazole). From these observations, we conclude that the methanolic leaf extract of A. africana offers more promising gastroprotection than omeprazole and due to low toxicity; it should be incorporated in traditional medicine for the management and treatment of peptic ulcer disease.***

Keywords: *Aspilia africana*, active ingredients, NSAIDs, antiulcerogenicity, GC-MS.

1. Introduction

Peptic Ulcer Disease (PUD) is a sore on the lining of the duodenum or stomach which occurs when the presence of some aggressive factors overwhelm that of the protective factors (mucin secretion, prostaglandins, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover) of the gastrointestinal tracts (GITs), which leads to the interruption of their mucosal functionality and integrity [1, 2, 3, 4]. It has been disclosed as a high ranking global health problem affecting about 8 to 10% of the world's population [5]. As a major cause of concern in the past three to four decades, it has aroused much interest among clinicians, experimentalists and regulators [6, 7]. Abdominal pain, bloating and abdominal fullness, water-brash, nausea, copious vomiting, loss of appetite, weight loss, hematemesis, melena and anemia are possible clinical signs and symptoms of PUD [8]. Even though, the exact pathophysiology of PUD is still questionable, the aggressive factors that have been documented to play pivotal roles in the induction or facilitation of PUD include: alcoholism, stress, cigarette smoking, sedentary lifestyle, bacterial infection (*helicobacter pylori*), non-steroidal anti-inflammatory drugs (NSAIDs) and spicy food intake [2, 4]. NSAID is a class of synthetic drugs widely used, at both over the counter and prescription level dosages, as anti-inflammatory, anti-analgenic or anti-pyretic therapies in the treatment of rheumatic and other musculo-skeletal conditions, dental pain and surgery, accidental sports injuries, juvenile idiopathic arthritis, menstrual pain and acute fever in children [9, 10, 11, 1]. The identified therapeutic target of these drugs is the inhibition of cyclooxygenase enzyme (COX) which has been attested to inhibit the synthesis of prostaglandins from arachidonic acids, thus reducing smooth muscle contraction of the uterus during menstruation/labor and decreasing elevated body temperature that produces pain and fever [12]. Despite these glories so far achieved in clinical studies, the use of NSAIDs has posed several ills to humans that include PUD. This occurs when the absence of prostaglandins leads to: increase release of a vasoconstrictor (endothelin-1) which has been authenticated to induce mucosal injury, by activating the release of neutrophils and reactive oxygen species that initiate gastric injury or by reducing mucosal blood flow, mucus and bicarbonate secretions, epithelial cell renewal or increased leukocytes that are all responsible for the pathogenesis of ulcerations [13, 14, 15]. To remediate this imbalance, several regiments (conventional drugs and complementary alternative medicine) have been adopted.

The use of conventional drugs such as: antacids (magnesium hydroxide, aluminum hydroxide); H_2-receptor blockers (ranitidine, cimetidine, famotidine); anticholinergics (porenzepine, telezepine) and proton pump blockers (omeprazole, lansoprazole) for the management of PUD presents limited efficacies and are often associated with severe side effects and relapse [16]. These shortcomings have led to the search for more effective therapies with better protection and decrease in the incidence of relapse [3]. Complementary alternative medicine (CAM) has gained substantial applause in the world today as the most effective clinical regiment for the management of PUD owing to the following criteria: bioavailability, higher safety margin, efficacy, quality, affordability, microbiomes, and

bioaccumulation of chemicals that targets metabolic pathways [17, 18, 4]. Over the years, several high profile techniques like gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FT-IR), gas chromatography -flame ionization detector, (GC-FID), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) have been adopted in the screening of biological samples to identify the active ingredients resident in them especially medicinal plants [18]. GC-MS adopted in this study was used to identify phyto-constituents (active ingredients), thus elucidating the molecular structure, molecular formulae, % peak area and retention time of possible bioactive compounds. Many tropical herbs have been scientifically reported to possess potent antiulcer activity [19]. *Aspilia africana* (Pers.) C.D. Adams, belonging to the family *Asteraceae* and commonly known as Orangila in Igbo, Tozalin in Hausa and Yunyun in Yoruba, is a semi wood herb occurring throughout the regions of the savannah and tropical Africa on waste lands [20]. Several ethnomedical potentials have been documented in the pharmacopeia about the plant. Included in the list are antimicrobial, antifertility, anti-inflammatory and gastroprotective activities [21, 22, 23, 24]. There is paucity of documented information on the assessment of *Aspilia africana* as potential agent in the amelioration of ibuprofen induced ulcerogenesis in wistar rats with special reference to the negative impacts of this ulcerogen on blood glucose, haematological, antioxidant, lipid peroxidation marker, hepatocellullular and renal function indexes. However, the thrust of the present study was to identify the active ingredients resident in methanolic leaf extract of *Aspilia africana* (Pers.) C.D. Adams using the technique of GC-MS and their effects on ibuprofen induced ulcer model in wistar rats.

Plate 1: Fresh leaves of *Aspilia Africana*

2. Materials and Methods

Collection, identification and processing of the plant sample

Fresh leaves of *Aspilia africana* (Plate 1) were harvested from the premises of Abia State University Uturu, Abia State, Nigeria. The plant was identified at the Department of Plant Science and Biotechnology, Faculty of Biological and Physical Sciences, Abia State University Uturu, Abia State, Nigeria. Upon identification, the fresh leaves of the plant were transported to the laboratory of the Department of Biochemistry of the university. The leaves of the plants were sorted to remove extraneous materials, washed in distilled water and air dried under shade. On drying, the leaves were milled to fine powder using electric blended. The powdered sample was stored in a polythene bag prior to use.

Preparation of plant extract

Exactly 1000g of powdered leaves of *Aspilia africana* was soaked in 3.0L of methanol for 24 hours, strained with muslin cloth and then filtered utilizing Whatman No. 1 filter paper. The filtrate was permitted to dry in open air and a dull greenish extract was formed. The resultant concentrate was divided into two portions. The first portion was stored in a labeled sterile bottle for GC-MS analysis while 20g of the left over concentrate was disintegrated in 10mL of 3% Tween-80, made up to 100ml with distilled water and this was kept in a refrigerator at 4^0C prior to use.

Procedure for Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The methanolic leaf extract of *Aspilia africana* was analyzed through GC-MS for the identification of different compounds. The GC-MS analysis was carried out using Clarus 500 (Perkin - Elmer) Gas chromatograph equipped and coupled to a mass detector Turbo mass gold (Perkin - Elmer) spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane, 30m x 0.25 mm x 0.25 µmdf) of capillary column. The oven was set to an initial temperature 110ºC for 2 min, further increased up to 200^{o}C at the rate of 10°C/min. Finally temperature was raised up to 280ºC at the rate of 5ºC/min for 9 min. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min. An aliquot of 2µl of sample was injected into the column with the injector temperature at 250ºC (Split ratio of 10:1). The electron ionization system with ionizing energy of 70 eV was used. Mass spectral scan range was set at 45-450 (m/z). Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 82,000 patterns. The spectrum of the unknown component was compared with the spectra of the known components stored in the NIST library. The name, molecular weight,

molecular formula and structure of the components of the test materials were ascertained.

Laboratory animals

Exactly Thirty two (32) male Wistar rats aged 7 weeks, weighing between 70-130g were used for this research. They were procured from the Animal House of the Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus. The rats were kept in the animal house of the Department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu. These animals were allowed to acclimatize for 14 days (2 weeks) under standard laboratory conditions with free access to commercial feed and clean water *ad libitum*. They were kept in well ventilated rooms with 12/12 h light/dark condition and ambient room temperature. The experimental procedures used in this study conform to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [25].

Acute toxicity test

The LD_{50} of the extract was determined using Lorke's method [26] with modifications. Briefly, the test was carried out in two phases. In phase 1, nine (9) rats were divided into three groups of three rats each. The groups were administered orally with graded doses (10, 100 and 1000 mg/kg respectively) of the extract. The animals were placed under observation for 24hours and their behaviours monitored as well as mortality. Whereas, in phase 2, three (3) rats were divided into three groups of one rat each, which received graded doses (1600, 2900 and 5000 mg /kg) of the extract respectively. The animals were also put under perception for 24hours and their behaviour and mortality were determined. On the off chance that mortality happens, LD_{50} will be calculated with the formula:

D_0 = higher dose that gave mortality

D_{100} = lowest dose that gave mortality

$LD_{50} = \sqrt{D_0} \times D_{100}$

Ibuprofen-induced ulcer study

The anti-ulcerogenic potential was investigated using modified method of Deepanjana *et al*. [27]. A total of twenty (20) wistar rats were starved for 24 hours and grouped into four (I, II, III, and IV) of five (5) rats each based on their body weights. Each administration in the various groups was done according to the specification below:

Groups (n=5)	*Substance Administered*	*Dosage (mg/kg body weight)*
I (Normal Control)	Distilled water mixed with 0.03ml of 3% Tween 80	0.25ml
II (Negative control)	Ibuprofen	400
III (Reference drug)	Omeprazole	20
IV(Plant sample)	*Aspilia africana*	500

After 30 minutes of treatment, groups III and IV animals received 400mg/kg of Ibuprofen orally. Four (4) hours later, all the animals were anaesthetized with chloroform and dissected. The stomachs were excised and carefully opened along the line of greater curvature to expose the walls. The stomachs' contents were then washed off and the stomach walls viewed with the aid of hand lens (x10) to determine the ulcer scores using the method of Raju *et al.* [28]. The ulcerative lesions were counted and scored as follows:

1	Normal stomach	0
2	Pinhole	1.0
3	Spot ulceration	1.5
4	Haemorrhagic streaks	2.0
5	Small erosion	2.5
6	Large erosion	3.0
7	Perforation	3.5

Calculation of ulcer index and percentage inhibition

Ulcer Index (UI) = Mean of ulcer scores per rats

Percentage Ulcer inhibition:

The percentage ulcer protection was determined using the formula of Suziki *et al*. [29].

$$\text{Protection Index} = 1 - \frac{\text{Ulcer index with extract}}{\text{Ulcer index with distilled water}} \times 100$$

Determination of acid secretory indexes

The animals were sacrificed by sinus puncture, stomach was dissected out and the gastric juice collected was centrifuged for 5 minutes at 200rpm and the volume of the supernatant was expressed as ml/100g and pH was measured using pH meter. Total and free acidity was estimated by the method of Kore *et al*. [30].

Determination of pepsin activity

Pepsin was assayed according to the method of Shay *et al*. [31], using haemoglobin as substrate. The pepsin content was expressed as µM of tyrosine liberated/ml.

Biochemical determinations

Blood was collected using syringe and needle into plain and Ethylenediaminetetraacetic acid (EDTA) bottles for liver function and haematological analyses respectively. The bottles were allowed to stand for 15 minutes, spun at 12,000 rpm for 5 minutes and serum and plasma were decanted respectively using a Pasteur pipette into another set of plain test tubes, covered and stored in a refrigerator prior to further analysis.

Blood glucose determination

This test was carried out using a glucose enzymatic-colorimetric test kit (GOD-POD), produced by Cypress Diagnostics, Belgium.

Total protein determination

Total protein was determined according to the method described by Tietz [32].

Determination of lipid peroxidation

Lipid peroxidation otherwise known as malondialdehyde (MDA) was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Buege and Aust [33].

Assay of antioxidant enzymes

Catalase (CAT) activity was determined according to the method described by Sinha [34], Superoxide Distimutase (SOD) activity was determined according to the method described by Marklund and Marklund [35], Glutathione

Peroxidase (GPx) activity was determined according to the method described by Fergusson and Chance [36] and reduced Glutathione (GSH) was determined according to the method described by Ellman [37].

Assay for haematological indexes

Standard operating procedures as described by Afia and Momoh [38] using the BC-3200 Auto-Haematology Analyzer was used for estimation of the haematological parameters. Namely: white blood cells (WBC), neutrophils, eosinophil, basophil, lymphocyte, monocyte, red blood cells (RBC) and hemoglobin (Hb) were then calculated.

Assay for liver enzymes and kidney function indexes

The liver function enzymes; Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were spectrophotometrically determined using standard for the kits of Randox Laboratory Ltd, Co. Antrim, United Kingdom. The renal function indexes: urea, creatinine, sodium, potassium, chloride and bicarbonate ions were spectrophotometrically determined using Teco test kits (Teco Diagnostics, USA). The manufacturer's instructions for the entire biochemical test were strictly adhered to.

Statistical analysis

Results were expressed as mean ± SD (standard deviation). Statistical analysis was performed by One-way analysis of variance (ANOVA) with the SPSS Statistic software package, version 20. One-way ANOVA with a Tukey's multiple comparisons test was used to identify statistical differences among groups. A *p*-value of ≤ 0.05 was considered statistically significant.

3. Results

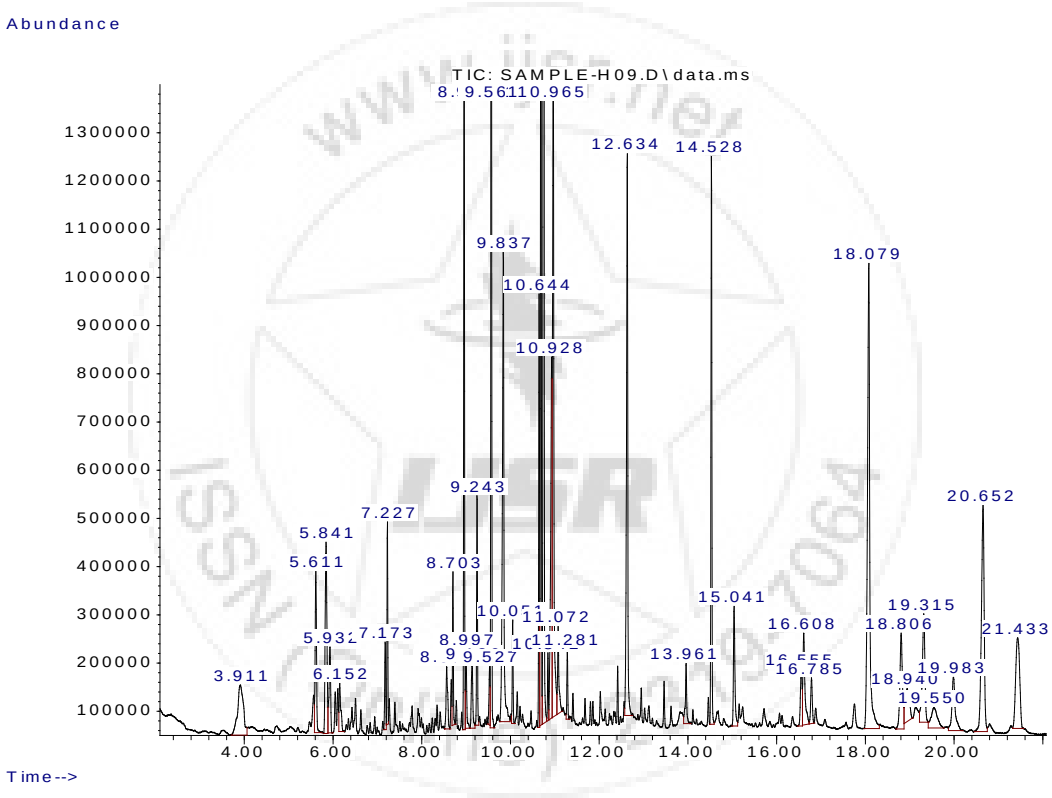

Figure 1: Mass Chromatogram of methanolic leaf extract of *Aspilia Africana*

Table 1: List of compounds identified at various retention times from methanolic leaf extract of *Aspilia africana* by GC-MS

S/N	RT	Compound name	MW	Formula	Area (%)
1	3.910	Ether, 6-methylheptylvinyl	156	$C_{10}H_{20}O$	2.06
2	5.611	Hydroxylamine,O-decyl	173	$C_{10}H_{23}NO$	1.55
3	5.841	β-Ylangene	204	$C_{15}H_{24}$	2.34
4	6.152	1-Cyclopenta[1,3]Cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,	204	$C_{15}H_{24}$	0.54
5	7.173	Humulene (3as-(3aα,3b)	204	$C_{15}H_{24}$	0.60
6	7.227	Caryophyllene oxide	220	$C_{15}H_{24}O$	1.14
7	8.703	5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	208	$C_{13}H_{20}O_2$	0.88
8	8.954	Tetradecane	198	$C_{14}H_{30}$	4.74
9.	8.977	1-Octadedelyne	250	$C_{18}H_{34}$	0.63
10.	9.136	3-Decyn-2-ol	154	$C_{10}H_{18}O$	0.70
11.	9.234	Phthalic acid, isobutyl octa-decyl ester	474	$C_{30}H_{50}O_4$	1.48
12.	9.527	3,7,11,15-Tetramethyl-z-hexadecen-1-ol	296	$C_{20}H_{40}O$	0.44
13.	9.569	Hexadecanoic acid, methylester (z)-	268	$C_{17}H_{32}O_2$	6.60
14	9.837	Hexadecanoic acid, methylester	270	$C_{17}H_{32}O_2$	5.22

15	10.051	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	0.63
16	10.687	Methyl linolelaidate	294	$C_{19}H_{34}O_2$	6.52
17	10.746	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-	292	$C_{19}H_{34}O_2$	13.52
18	10.842	Phytol	296	$C_{20}H_{40}O$	0.41
19	10.928	Cyclopentaneundecanoic acid methyl ester	268	$C_{17}H_{32}O_2$	3.77
20	10.965	Octadecynoic acid	280	$C_{18}H_{32}O_2$	6.22
21	11.072	9,12,15-Octadecatrienoic acid (z,z,z)-	278	$C_{18}H_{30}O_2$	0.84
22	11.281	Cyclopentaneundecanoic acid	254	$C_{16}H_{30}O_2$	0.42
23	12.634	Octadecane,6-methyl	268	$C_{19}H_{40}$	5.14
24	13.961	Doconexent	328	$C_{22}H_{32}O_2$	0.59
25	15.041	Squalene	410	$C_{30}H_{50}$	1.25
25	16.608	1-Ericosanol	298	$C_{20}H_{42}O$	1.27
27	16.785	Vitamin E	430	$C_{29}H_{50}O_2$	0.51
28	18.079	Stigmasterol	412	$C_{29}H_{48}O$	7.55
29	18.806	Trans-Z-α-Bisabolene epoxide	220	$C_{15}H_{24}O$	1.72
30	18.940	3-Trifluoroacetoxypentadecane	324	$C_{17}H_{31}F_3O_2$	0.92
31	19.315	Naphthalenone,3,5,6,7,8,8a-hexa-hydro-4,8a-dimethyl-6-(1-methylethenyl)-	218	$C_{15}H_{22}O$	1.98
32	19.983	2H-Pyran,2(7-heptadecynyloxy)tetrahydro-	336	$C_{22}H_{40}NO_2$	1.24
33	20.652	Urs-12-ene-24-orc acid, 3-oxo-methyl ester, (+)-	468	$C_{31}H_{48}O_3$	4.42

The results of the gas chromatography combined with mass spectrometry (GC-MS) for the methanolic extract of *Aspilia africana* leaves are presented in Figure 1 and Table 1. The results revealed the presence of thirty three (33) bioactive compounds with 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)- (13.52%), Stigmasterol (7.55%), Hexadecanoic acid, methylester (z)- (6.60%), Methyl linolelaidate (6.52%) and Octadecynoic acid (6.22%) as the most predominant active ingredients in the leaf extract of the plant. The minor comprised compounds include phytol (0.41%), 3,7,11,15-Tetramethyl-z-hexadecen-1-ol (0.44%) and vitamin E (0.51%).

Table 2: Acute (oral) toxicity study of wistar rats after 24h of administration of methanolic leaf extract of *Aspilia africana*

Group	Dose (Mg/Kg)	D/T	Signs of Toxicity
A	0.25 ml (H_2O)	0/3	No toxic effects observed
B	10	0/3	No toxic effects observed
C	100	0/3	No toxic effects observed
D	1000	0/3	No toxic effects observed
E	1600	0/1	No toxic effects observed
F	2900	0/1	Dullness and quietness was observed after 5 minutes
G	5000	0/1	Restless but calm after a while

D/T: Number of albino rat deaths/Total number of albino rats used

The result of the acute (oral) study of wistar rats post 24h of administration of methanolic leaf extract of *A. africana* is presented in Table 2. The result showed that the ingestion of the plant extract did not cause any mortality in the experimental animals throughout the period of the experiment.

Table 3: Effects of methanolic leaf extract of *Aspilia africana* on ulcerogenic indexes of Ibuprofen-induced ulcer rats.

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
Gastric volume (mL)	$1.23\pm0.0.28^b$	2.39 ± 0.49^a	0.90 ± 0.09^b	0.75 ± 0.04^b
pH	3.24 ± 0.71^b	1.75 ± 0.50^a	2.53 ± 0.35^b	2.90 ± 0.23^b
Free acidity (mEq/L)	120.15 ± 1.79^b	142.18 ± 3.68^a	102.32 ± 3.61^c	107.29 ± 1.21^c
Total acidity (mEq/L)	133.91 ± 2.63^b	213.21 ± 6.63^a	142.31 ± 3.07^b	127.90 ± 0.114^c
Pepsin activity (µmol/Tyr/mL)	141.71 ± 1.84^b	216.69 ± 6.93^a	113.05 ± 4.52^c	126.29 ± 1.49^c
Ulcer Index	0.00 ± 0.00	1.83 ± 0.00^a	0.83 ± 0.00^b	$0.64\pm.0.00^b$
Percentage Inhibition	100.00	0.00	54.00	65.00

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p< 0.05).

The result of the effects of the methanolic leaf extract of *Aspilia africana* on ulcerogenic indexes of Ibuprofen-induced ulcer model in wistar rats is presented in Table 3. The result showed that the induction of ulcer on the rats resulted in the distortions of the architectural design of the gastrointestinal mucosa and this was evidenced by manifold increase in gastric juice, pH, free acidity, total acidity, pepsin activity and ulcer index. The result revealed that there was a significant difference ($p<0.05$) in the serum level of gastric juice, pH, free acidity, total acidity and pepsin activity between the induced group and the test groups (group treated with omeprazole and plant extract). Omeprazole (20mg/kg) and 500mg/kg body weight of *A. africana* drastically lowered the ulcerogenic indexes (gastric juice, pH, free acidity, total acidity and pepsin activity) in the level comparable to that of the normal control. The percentage ulcer inhibition for group III (omeprazole) and Group IV (*A. africana*) were recorded as 54.00% and 65.00% respectively; with the plant extract proffering better prophylactic measures.

Table 4: Effect of methanolic leaf extract of *Aspilia africana* on blood glucose concentration of Ibuprofen-induced ulcer rats

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
Blood Glucose at 0hr (mg/dl)	84.60±6.43^{b}	111.80±21.78^{a}	73.40±3.78^{c}	82.80±3.03^{c}
Blood Glucose at 2hrs (mg/dl)	82.40±6.66^{b}	93.83±4.79^{a}	69.39±1.71^{c}	68.84±1.71^{c}
Blood Glucose at 4hrs (mg/dl)	65.67±2.36^{b}	68.23±1.54^{a}	64.80±3.05^{b}	50.18±28.10^{c}

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p< 0.05).

The result of the effect of the methanolic leaf extract of *Aspilia africana* on blood glucose concentration of Ibuprofen-induced ulcer model in wistar rats is presented in Table 4. The result revealed that the induction of ulcer on the experimental rats resulted in an increase in blood glucose level of the experimental rats. This raise in the level of blood glucose was lowered following pretreatment with standard drug (omeprazole) and 500mg/kg body weight of the extract. The result also revealed that there was a significant difference ($p<0.05$) between the grouped induced with 400mg/kg body weight of Ibuprofen (negative control) and other experimental groups (Normal control, omeprazole and *A. africana)* at 0 to 2h of exposure. Omepgrazole (standard drug) and 500mg/kg of *A. africana* significantly ($p<0.05$) inhibited the increase in the level of blood glucose between 0-2h of exposure. At 4h of exposure, the blood level of the experimental animal further dropped to 65.67mg/dL, 68.23mg/dL, 64.80mg/dL and 50.18mg/dL in Groups I, II, III and IV respectively.

Table 5: Effects of methanolic leaf extract of *Aspilia africana* on lipid peroxidation marker and antioxidant indexes of Ibuprofen-induced ulcer rats.

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
SOD (µI/L)	30.43±0.92^{d}	17.50±0.33^{a}	21.72±0.73^{b}	24.80±0.96^{c}
GSH (nM/mg protein)	17.47±0.73^{a}	18.75±0.27^{a}	20.35±0.93^{b}	25.00±1.04^{c}
MDA (nM/mg protein)	1.82±0.11^{c}	4.33±0.47^{a}	2.25±0.13^{b}	2.04±0.12^{bc}
GPx (µI/L)	18.22±1.12^{a}	23.37±1.95^{a}	16.86±9.43^{a}	20.11±0.45^{c}
Catalase (nM/mg)	40.23±0.92^{a}	19.56.±1.09^{d}	22.27±1.54^{c}	27.18±2.43^{b}

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p> 0.05). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, Malondialdehyde; GPx, Glutahione peroxidase.

The result of the effects of the methanolic leaf extract of *Aspilia africana* on lipid peroxidation marker and antioxidant indexes of Ibuprofen-induced ulcer model in wistar rats is presented in Table 5. The result showed that there were drastic declines in the serum levels of SOD, GSH and catalase following the induction of ulcer on the wistar rats. This distortion was countered following pretreatment with standard drug (omeprazole) and 500mg/kg body weight of *A. africana*. The increase in the level of MDA (lipid peroxidation marker) as observed in the negative control group was corresponding decreased with administration of omeprzole and plant extract. The preventive measures of the different ulcerogenic antidotes were statistically significant ($p<0.05$).

Table 6: Effects of methanolic leaf extract of *Aspilia africana* on renal function indexes of Ibuprofen-induced ulcer rats.

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
Urea (mg/dL)	30.43±3.45^{d}	48.09±1.13^{a}	43.33±1.90^{b}	38.71±1.03^{c}
Creatinine (mg/dL)	0.39±0.12^{c}	0.87±0.09^{a}	0.61±0.06^{b}	0.47±0.40^{c}
Na^{+}(mEq/L)	147.95±1.35^{c}	150.08±1.54^{a}	149.72±0.42^{a}	149.31±0.48^{ab}
K^{+}(mEq/L)	3.13±0.53^{c}	5.13±1.33^{a}	3.44±0.52^{b}	3.81±0.26^{ab}
Cl^{-}(mEq/L)	103.12±1.61^{b}	168.43±139^{a}	106.43±0.57^{b}	108.51±118^{b}
HCO^{-}_{3} (mmol/L)	29.35±0.63^{b}	31.62±1.90^{a}	30.75±0.52^{ab}	29.27±1.17^{b}

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p< 0.05).

The result of the effects of methanolic leaf extract of *Aspilia africana* on renal function indexes of Ibuprofen-induced ulcer model in wistar rats is presented in Table 6. The result showed that induction of ulcer in the wistar rats led to severe imbalance in the functionality of the body fluid filter (kidney) as evidenced by marked variations in the serum levels of urea, creatinine, sodium, potassium, chloride and bicarbonate ions. However, this imbalance was regulated following pretreatment with different regiments of omeprazole (20mg/kg) and 500mg/kg body weight of *A. africana* respectively. There was a significant difference ($p<0.00$) in the measured renal parameters between the induced and the treated groups. The result also revealed that the amelioration of ulcerogenesis by 500mg/kg body weight of *A. africana* was statistically significant at $p<0.05$ when compared to that of omeprazole. The effectiveness of *A. africana* in restoring balance in serum levels of urea and creatinine, sequel to the induction of ulcer, was evidenced by declines in their serum concentrations, which were comparable to those of normal control.

Table 7: Effects of methanolic leaf extract of *Aspilia africana* on hepatocellular indexes of Ibuprofen-induced ulcer rats.

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
AST (U/L)	13.30 ± 0.61^{a}	23.55 ± 4.36^{b}	16.55 ± 0.67^{c}	10.68 ± 1.01^{c}
ALT (U/L)	21.84 ± 0.92^{a}	29.50 ± 1.89^{b}	27.33 ± 0.39^{b}	26.53 ± 0.91^{b}
ALP (U/L)	103.20 ± 2.63^{a}	97.69 ± 1.73^{a}	84.64 ± 2.36^{a}	90.97 ± 2.44^{a}
Total Protein (mg/dL)	7.34 ± 0.16^{a}	6.52 ± 0.21^{a}	5.72 ± 1.26^{a}	6.82 ± 0.21^{a}
Total Bilirubin (mg/dL)	1.24 ± 0.36^{a}	0.60 ± 0.06^{b}	0.62 ± 0.34^{b}	0.82 ± 0.72^{b}

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p> 0.05). Alanine aminotransferase (ALT), Aspartate aminotransaferase (AST) and Alkaline phosphatase (ALP).

The result of the effects of methanolic leaf extract of *Aspilia africana* on hepatocellular indexes of Ibuprofen-induced ulcer model in wistar rats is presented in Table 7. The induction of ulcerogenesis on the experimental rats by Ibuprofen resulted in significant increase ($p<0.05$) in the serum concentrations of AST, ALT and ALP on one hand and a simultaneous decrease in the level of total protein. These alterations in the hepatocellular indexes were effectively regulated following pretreatment with omeprazole and the methanolic leaf extract of *A. africana*. The restoration of hepatocellular balance was not statistically significant ($p<0.05$) between the group treated with omeprazole and 500mg/kg body weight of *A. africana* in the serum levels of AST, ALT, ALP and total bilirubin. The result further revealed that there was no significant difference ($p<0.05$) in the level of total protein between the induced and treated groups.

Table 8: Effects of methanolic leaf extract of *Aspilia africana* on haematological indexes of Ibuprofen-induced ulcer rats

Indexes	Group I (Normal control)	Group II (Ibuprofen, 400mg/kg)	Group III (Omeprazole, 20mg/kg)	Group IV (*A. africana*, 20mg/kg)
Hb (g/dL)	13.00 ± 1.00^{a}	10.40 ± 0.55^{b}	10.40 ± 0.55^{b}	11.40 ± 1.52^{b}
RBC $x10^{6}$ cells (mm^{3})	6.37 ± 0.65^{a}	4.26 ± 0.53^{a}	4.98 ± 0.57^{bc}	5.26 ± 0.87^{b}
WBC^{T} $x10^{3}$cells(mm^{3})	6.00 ± 1.14^{ab}	7.88 ± 2.33^{b}	4.14 ± 1.77^{b}	4.88 ± 1.06^{b}
Neutrophil	20.80 ± 7.69^{a}	27.20 ± 17.17^{a}	30.60 ± 5.18^{a}	31.40 ± 3.93^{a}
Lymphocyte	56.80 ± 19.34^{b}	83.40 ± 11.30^{a}	49.00 ± 16.73^{b}	59.00 ± 13.45^{b}
Eosinophil	0.00	0.00	0.00	0.00
Basophil	0.00	0.00	0.00	0.00
Monocyte	3.20 ± 2.59^{bc}	2.40 ± 0.55^{c}	5.20 ± 0.84^{ab}	7.20 ± 1.92^{a}

Values represent the mean ± SD for N =5. Values in the same rows bearing the same letter of the alphabet are not significantly different from each other (p> 0.05). Hb, Haemoglobin; RBC, Red Blood Cells; WBC^{T}, White Blood Cell Total.

The result of the effects of methanolic leaf extract of *Aspilia africana* on haematological indexes of Ibuprofen-induced ulcer model in wistar rats is presented in Table 8. The administration of 400mg/kg body weight of Ibuprofen resulted in ulcerogenesis and this was manifested by drastic decrease in the blood levels of red cells and haemoglobin. Treatment with the standard drug (omeprazole) and the plant extract (500mg/kg of *A. africana*) slightly raised the blood level of the experimental animals. The leaf extract of *A. africana* at the tested dose offered better haematological potential than that of the reference drug; although this therapeutic feat of *A. africana* over omeprazole was not statistically significant at $p<0.05$.

4. Discussion

Plant has remained man's companion from time immemorial, providing him with food, shelter and medicine. In South East of Nigeria, the decoction of the leaves of *Aspilia africana* is used to heal wounds and arrest bleeding [24]. It is against this background that the present study was investigated to identify the bioactive compounds resident in methanolic extract of *Aspilia africana* leaves and its effects on ibuprofen induced ulcer model in wistar rats by quantifying several biochemical indexes such as blood glucose concentration, ulcerogenic, antioxidant, lipid peroxidation marker, hematological, kidney and liver functions. The GC-MS spectral analysis of the methanolic extract of *A. africana* leaves revealed the presence of thirty three (33) bioactive compounds and according to Duke's ethnobotanical and phytochemistry database [39], some of the identified compounds possess several biological and pharmacological properties. The major comprised bioactive compounds were 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)- (13.52%), Stigmasterol (7.55%), Hexadecanoic acid, methylester (z)- (6.60%), Methyl linolelaidate (6.52%), Octadecynoic acid (6.22%); while minor compounds such as phytol (0.41%), 3,7,11,15-Tetramethyl-z-hexadecen-1-ol (0.44%), vitamin E (0.51%) were also identified (Figure 1 and Table 1). 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z) is a polyenoic fatty acid which possesses the following pharmaceutical activities: anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antiarthritic, anti-coronary, anti-eczemic, antiacne, 5-Alpha reductase inhibitor and antiandrogenic [40]. This observation was validated by an *in vivo* study carried out by Divakar *et al*. [41] on the evaluation of gastro-protective activity of fish oil (omega-3-fatty acids) against experimentally induced acute gastric ulceration in rats. According to their findings, fish oil exerted a comparable antiulcerogenic effect to that of Ranitidine (the reference drug). Stigmaterol is an

unsaturated plant sterol occurring in plant fats or oils and has been reported to have anti-inflammatory, inhibit tumor promotion and anti-HIV reverse transcriptase activities as opined by Venkata *et al.* [42] who screened and documented the possible active ingredients resident in *Eupatorium odoratum*. Hexadecanoic acid, methyl ester is a palmitic acid compound that possesses antioxidant, decrease blood cholesterol and anti-inflammatory activities [43]. Also, methyl linolelaidate has been observed to possess antioxidant and catalase activator properties [42]. Among the minor comprised bioactive compounds, phytol and vitamin E (ergesterol) have been observed to possess important biochemical and physiological functions. Phytol constitutes a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases and it has been screened to possess antimicrobial, anticancer, anti-inflammatory, antidiuretic, immunostimulatory and anti-diabetic activities [42] while vitamin E has antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant and antileukemic tendencies [44].

The result of oral acute (LD_{50}) toxicity of the methanolic extract of the plant showed no mortality at the highest concentration of 5000mg/kg (Table 2), thus establishing that the leaf extract of *A. africana* is safe [45] as exemplified by its use as food by domestic and wild animals [24]. However, for more precision, chronic toxicity study is recommended to be done to ascertain the safety of the extract when taken continuously for a long time. Ibuprofen, just like other NSAIDs has been described to induce ulceration by decreasing the GIT's pH, increasing free acidity, gastric volume, total acidity and pepsin activity which in turn led to the formation of sore in the gastric mucosal membrane and reduce its integrity and function [1]. This previous observation is in line with the current study. On the contrary, both Omeprazole and *A. africana* methanolic leaf extract have shown inhibitory effects on ibuprofen induced ulcer with percentage gastroprotection of 54% and 65% respectively (Table 3) and also reversed all the ulcerogenic indexes induced by it. This therefore, disclosed that the plant extract plays a more important role in preventing ulceration than omeprazole. This observation is in line with the findings of Ubaka *et al.* [24] who reported that antiulcer activity of aqueous extract of *Aspilia africana* in mice. Whittle [13] described the inhibitory effect of NSAIDs in prostaglandin synthesis to cause local release of reactive oxygen species (ROS). Similarly, it has also been observed to cause photo-cleavage through ROS generation [46]. ROS like other free radicals have been described to have the ability of destroying mitochondrial DNA in any part of the biological system thus resulting in mutation of lysyl-tRNA gene that causes some types of adult-onset (type II) diabetes mellitus which inhibit carbohydrate metabolism [12]. It is therefore not surprising when the current study described ibuprofen to cause hyperglycemia (Table 4), drastic suppression of the antioxidant system (superoxide dismutase, glutathione and catalase) and snowball the level of a malondialdehyde (MDA) which is a marker of lipid peroxidation caused by the ROS generated (Table 5). On the contrary, both omeprazole and *A. africana* leaf extract remediated all these alterations; which might be suggested that they both have some antioxidant potentials and therefore good products to serve as antiulcer agents. The antioxidant boost of the methanolic leaf extract of the plant was validated by the result of the GC-MS spectral analysis, which divulged preponderance of several bioactive compounds with proven antioxidant activities.

Ibuprofen has been known to possess anti-inflammatory and antipyretic potentials [10] hence, its applications in the treatment and amelioration of pains associated with rheumatic and other musculo-skeletal conditions. The intake of these synthetic products recently has been identified to pose serious threats to the liver, kidney and blood of animals. In this study, sustained alterations in the serum level of urea, creatinine and electrolytes (Table 6) and decrease in hepatocellular and haematological indexes (Tables 7 and 8) respectively supported earlier findings that Ibuprofen and other NSAIDs are potential hepatotoxic, nephrotoxic and ulcerogenic agents [47, 48, 49, 50]. The decrease in Hb and RBC is an indication of excessive blood loss due to bleeding from the mucosal membrane (anemia). Groups pretreated with omeprazole and 500mg/kg of the leaf extract of *A. africana* showed a rise in the blood level in the experimental animals. These findings are in line with the work of Dhanabalan *et al.* [51] who reported the gastroprotective effect of *Glycyrrhiz aglabra* on aspirin induced ulcer in albino rats. Omeprazole is a conventional proton pump inhibitor that is employed in the treatment of peptic ulcer disease. Biswas *et al.* [52] reported that omeprazole inhibited gastric ulcer by virtue of its antioxidant property (the scavenging of hydroxyl radicals). However, it is quite obvious from the results that the methanolic leaf extract of *A. africana* mimics the action of omeprazole in ameliorating peptic ulcers in wistar rats. However, the result obtained showed that the gastroprotective activity of omeprazole was lower than that of the plant extract owing to the presence of important active ingredients (bioactive compounds) such as 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-, stigmasterol, hexadecanoic acid, methylester (z)-, Methyl linolelaidate, n-Hexadecanoic acid, squalene, phytol and vitamin E which have been quantitatively demonstrated to possess better pharmacological and cytoprotective properties. The extract may have countered the effect of ibuprofen by reactivating prostaglandin synthesis and other defensive factors such as mucin, cellular mucus, bicarbonate, mucosal blood flow and cell turnover due to the bioaccumulation of certain active ingredients which are possible precursors or analogues of arachidonic acids. This observation is in line with the works of earlier researchers who investigated the antiulcer activities of plantain peels and fruits (fermented) on different ulcer models in wistar rats [53, 3].

5. Conclusion

The results of the present study revealed that ibuprofen (400mg/kg) caused significant breach or lesion in the mucosal cells of gastrointestinal tract (ulcerogenesis), increase in blood glucose level, reduction in antioxidant system with a corresponding increase in lipid peroxidation (MDA) and consequently, alterations in the normal serum concentrations of liver and kidney function parameters. This imbalance was effectively remediated by the administration of synthetic (omeprazole) and natural (leaf extract of *A.*

africana) antidotes at 20mg/kg and 500mg/kg body weight respectively. Owing to the preponderance of important active ingredients as showed by the technique of GC-MS, we conclude that the decoctions of the leaf extract of *A. africana* should be incorporated in ethnomedicine, on one hand for the management of pain and inflammation thus, preventing the negative impacts associated with NSAIDs and on the other hand for the prevention, treatment and management of PUD.

6. Future Scope

There is an ongoing research work to effectively investigate the effect of this *Aspilia africana* and other plant extracts on the synthesis of prostaglandin and expression of certain anti-inflammatory cytokines using different ulcer models in wistar rats.

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