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ANTI-ULCER AND ANTIOXIDANT EFFECT OF SIDA ACUTA ETHANOL LEAF EXTRACT; GCMS AND MOLECULAR DOCKING STUDY

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ABSTRACT
Anti-ulcer and antioxidant effect of Sida acuta ethanol leaf extract, GCMS and Molecular docking was studied using mice as animal model. Thirty (30) mice were used for the research and were grouped into five (5) of six (6) mice each. Group 1 was the negative control induced with Diclofenac but not treated.Group 2 was positive control induced and was treated with standard drug Omeprazole. Groups 3, 4 and 5 (the low, middle and high doses) received 125, 250 and 500 mg/kg body weight of the extract of Sida acuta respectively after induction with Diclofenac. Gas chromatogram showed 17 peaks indicating 17 compounds in ethanol extract of S. acuta leaves. The most prominent compound was 9-Octadecenoic acid (Z)-, methyl ester with 92.0616 % composition. The whole extract in vivo produced inhibition at 500 mg/kg dose (94.44 %), while 250 mg/kg b.w. (84.44 %) and 125 mg/kg b.w. (80.27%). Distilled water group (0.00 %). The standard drug, Omeprazole, 20 mg/kg, b.w. inhibited (68.61%). Therefore S. acuta whole extract extract was a better antiulcer drug than Omeprazole in vivo. The extract showed better antioxidant effect when compared with the negative control. The 2D interactions of ODM-2X08 and ASC-2X08 shows that binding affinity of ODM-2X08 was -7.5 Kcal/mol while ASC-2X08 was -5.5 Kcal/mol. This suggested that ODM possessed higher antioxidant property than ascorbic acid. The 2D interactions of 9-Octadecenoic acid (Z)-, methyl ester (ODM-2XZB) and Omeprazole (OME -2XZB) shows that the binding affinity of ODM-2XZB was -6.0 Kcal/mol while OME-2XZB was -7.5 Kcal/mol. This suggested that ODM possessed lower anti-ulcer properties than omeprazole. ADMET Predicted Profile showed that the lead compound is soluble in water, biodegradable and are less toxic. The lead compound, 9-Octadecenoic acid (Z)-, methyl ester (ODM) had only one violation, according to Lipinski rule of 5 (RO5) hence it is likely to have the chemical and physical properties to be orally bioavailable hence a good drug candidate

KEYWORDS: Anti-ulcer, Antioxidant, Sida acuta, GCMS, Molecular docking.

INTRODUCTION
Injurious agents exert damaging effects through reactive chemical species known as free radicals. (Rubin and Strayer, 2005; Kumar *et al*, 2005; Chuyanyu *et al*, 2002). Free radicals are highly reactive chemical species with an unpaired electron in the outer orbit (valence shell) of the molecule. In the literature, the unpaired electron is denoted by a dot, for example NO. The unpaired electron causes free radicals to be unstable and highly reactive so that they react nonspecifically with molecules in their vicinity, Kerr *et al*, (1996). In cell and tissues, free radicals react with proteins, lipids and carbohydrates thereby damaging cell membranes, inactive enzymes and nucleic acids that make up DNA and may damage cells and tissues, McCord (2000).

Reactive Oxygen Species (ROS) are oxygen-containing molecules that include free radicals such as superoxide (O_2^-), hydroxyl radical (OH) and nonradicals such as hydrogen peroxide (H_2O_2) these molecules are produced endogenously by normal metabolic processes or cell activities such as the metabolic burst as a result of

phagocytosis. Exogenous causes including ionizing and UV radiation and can cause ROS production in the body. Oxidative stress is a condition that occurs when the generation ROS exceeds the ability of the body to neutralize and eliminate it, Finkel (2003).

Oxidative stress is an imbalance between the production of free radicals and the body's ability to neutralize or detoxify their harmful effects through antioxidants, Brenneistein *et al* (2005). Free radicals are highly reactive molecules that contain oxygen and can damage cells, proteins, and DNA within the body, McCord (2000). This damage can lead to a variety of diseases such as neurological disorders (eg Alzheimer's disease, Parkinson's disease etc.), kidney disease, inflammatory disorders, and contribute to the aging process. Antioxidants are molecules that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. They can also repair damage already incurred by the free radicals.

Antioxidants are natural and synthetic molecules that inhibit the reaction of ROS with biologic structures or prevent the uncontrolled formation of ROS. Antioxidants include enzymatic and nonenzymatic compounds. Enzymes known to function as antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase and thioreductase. SOD forms hydrogen peroxide from superoxide. Catalase can catalyze the reaction that form water from hydrogen peroxide. Nonenzymatic antioxidants include carotenes (vitamin A), tocopherols (vitamin E), ascorbate (vitamin C), glutathione, and flavonoids, as well as micronutrients such as selenium and zinc, Brenneistein *et al* (2005). Nonenzymatic oxidants often directly react with oxidants to neutralize them. For example, vitamin C directly scavenges superoxide and hydroxyl radicals therefore can be used as standard drug for antioxidant studies. Oxidative stress from free radicals has been implicated in many diseases and play an important role in the development of cancer. (Comhair and Erzuum, 2005; Johnson and Giulivi, 2005; Klaunig and Kamendulis, 2004). Peptic ulcer is a term used to describe a group of ulcerative disorders that occur in areas of the upper gastrointestinal tract that are exposed to acid-pepsin secretions (Liu, 2005; Mitos and Rubbin 2008; Saad and Scheiman, 2004). The most common form of peptic ulcer are duodenal and gastric ulcers. Peptic ulcer diseases is a chronic health problem Mitos and Rubbin (2008). Duodenal ulcers occurs five times more commonly than gastric ulcers Mitos and Rubbin (2008). Gastric ulcers are open sores that develops in the lining of the stomach which can occur as a result of various factors including infections with *Helicobacter pylori* bacteria, prolonged use of Non-steroidal anti-inflammatory drugs (NSAIDs) or excessive stomach acid production. A variety of risk factor has been shown to have an association with peptic ulcer disease. The two most important are infection with the bacteria *H. pylori* and use of aspirin and other NSAIDs. Both *H. pylori* infection and exposure to NSAIDs have been shown to impair the mechanism that protect the gastric mucosa from the destructive effects of the corrosive acid. *H. pylori's* ability to induce inflammation and stimulate the release of cytokines and other mediators of inflammation contributes to mucosal damage. The pathogenesis of NSAIDs-induced ulcers is thought to involve mucosal injury and inhibition of prostaglandin synthesis. Aspirin appears to be the most ulcerogenic of the NSAIDs, Saad and Scheiman (2004). Ulcer development in NSAID users is dose dependent, but some risk occur even with aspirin dose of 81mg/day. In contrast, the peptic ulcer from other causes, NSAID-induced gastric injury is often without symptoms and life threatening complications can occur without warning, Saad and Scheiman (2004). Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human, animal's diseases and infections (Caceres *et al*., 1991; Nweze *et al.*, 2024; Vineela and Elizabeth, 2005). Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active principles or photochemical substances include terpenses, fiavonoid, bioflavonoid, benezophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthraxquinones (Iwu, 1993; Asaolu, 2003) or phytocompounds separated by GCMS (Igwe *et al*, 2016). *Sida acuta* is known as the common wirewood, is a species of flowering plant in the mallow family, Malvaceae. It originate in Central America, but has a pantropical distribution. It is considered a weed in some areas. *S. acuta* is viewed as an astringent, tonic which is used in treating urinary diseases and blood disorders, bile, liver and for treatment of nervous diseases, Karou *et al* (2007). Use of plant for medicinal purposes is important in the culture and tradition in Africa. Thus, up to 80% of the population depend directly on the traditional medicine for the primary health care (Kirby, 1996). *S. acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. In Central America, the leaves are used to treat asthma, renal inflammation, colds, fever, headache, ulcers and worms (Caceres *et al*., 1987; Coee and Anderson, 1996). In Colombia the plant is known to treat snake bites. Otere *et al*. (2000 a, b) demonstrated that the ethanolic extract of the plant had an effective moderate activity against the venom of *Bothrox athrox*. In West Africa, particularly in Burkina Faso. *S. acuta* is an erect, branched and perennial shrub with a woody tap root, hairy branded up to 1m high and is reproduced from their seeds, the stem is woody rounded and slender and is fibrous and hairy especially when young. The leaves are simple and alternate while the flowers are yellow with five petals. The fruit is capsuled with 5- 6 carpels. *S. acuta* is a tropical weed of cultivated crops pastures, roadsides and waste areas. Research carried out revealed that juice from leaf of *S. acuta* is antihermitic for intenstinal worms (Sofowora, 1982). Natural products provide a rich source of

bioactive molecules used for treating a wide range of animal and human diseases, Newman and Crag, (2007). These natural products (eg Plants) provide large number of lead compounds used for developing new drugs, Reayi and Arya (2005). Natural product drugs include aromatic polyketides, polyethers, coumarins, flavonoids, terpenoids, alkaloids and aminoglycosides Dewick, (2002). Flavonoids are known to have medicinal properties and play a major role in the successful medical treatments from ancient times and their use has preserved till date Dixon *et al* (1998). They are potent water-soluble antioxidants and free radial scavengers, which prevent oxidative cell damage and have strong anti-cancer activity (Del-Rio *et al*; Okwe and Okue, 2004; Okue *et al* 2006). They are anti-inflammatory, anti-spasmodic, and anti-allergic, anti-microbial agents (Mills and Bone, 2000; Robbers and Tyler, 2000; Harborne, 2000). The plant is used for various medicinal purposes such as malaria, ulcer, fever, gonorrhea, abortion, breast cancer, (Kayode 2006; Edeoga *et al*, 2005). *S. acuta* is known as the common wirewood, is a species of flowering plant in the mallow family. Malvaceae. It originate in Central America, but has a pantropical distribution. It is considered a weed in some areas. *S. acuta* is viewed as an astringent, tonic which is used in treating urinary diseases and blood disorders, bile, liver and as treatment for nervous diseases, Karou *et al* (2007). The use of plant for medicinal purposes is an important part of the culture and the tradition in Africa. Thus, up to 80% of the population depend directly on the traditional medicine for the primary health care (Kirby, 1996). This traditional medicine uses numerous plants among them, *Sida acuta* Burm f. (Malvaceae). *S. acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. The aerial part of the plant is the most frequently used part. In central America, the plant is used to treat asthma, reneal inflammation, colds, fever, headache, ulcers and worms (Caceres *et al*., 1987; Coee and Anderson, 1996). In Colombia the plant is known to treat snake bites. Otere *et al*. (2000 a, b) demonstrated that the ethanolic extract of the plant had an effective moderate activity against the venom of *Bothrox athrox*. In West Africa, particularly in Burkina Faso.Gas Chromatography Mass Spectrometry (GC-MS) is the technique used to separate the compounds in the plant. GCMS has been used by researchers to separate plant compounds and biomolecules (Igwe *et al.*, 2016, Ikpeazu, 2020, Otuokere, 2016). Virtual and *in silico* studies have been used by researchers to predict the activities of compounds found in plants and other chemical compounds Igwe *et al* (2020). This research work is designed to check the antiulcer and antioxidant potentials of *Sida acuta* and molecular docking of the most abundant compound identified by GCMS analysis. Figure 1 shows the picture of *S. acuta.*

Figure 1: shows the picture of leaves, flowers seed capsule of *Sida acuta.*

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Sida acuta*, were collected from University environment in Umudike, Abia State in Nigeria and was identified using Google plant identifier and confirmed by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract

The identified leaves of *Sida acuta* were air dried for 1 week and ground into coarse powder using a manual blender. The ground leaves were immersed in ethanol using cold maceration technique for 48hrs and then sieved using a Whatman filter paper. The sample was concentrated using hot air oven at 30^0C.

Different doses of 125, 250 and 500 mg/kg body weight was prepared and administered to the mice in groups 3, 4 and 5 respectively. These doses were calculated from the stock solution dissolved in distilled water.

Experimental Animals

Mice (15-30g) were purchased from University Farm. Approval was obtained from College of Vet. Medicine, Michael Okpara University of Agriculture Umudike, Nigeria, in line with the guidelines for the care and use of laboratory animals as given by the National Research Council (N.R.C, 1985). The mice were acclimatized and fed *ad libitum.*

Experimental Design

Thirty (30) mice were used for this research, they were grouped into five (5) of six (6) mice each. Group 1 was the negative control induced with Diclofenac but not

treated.Groups 2 was positive control induced and was treated with standard drug Omeprazole.Groups 3, 4 and 5 were the low, middle and high doses which received 125**,** 250 and 500 mg/kg body weight of the extract of *Sida acuta* respectively. The standard drug was Omeprazole administered at 20 mg/kg b.w. Induction agent for ulcer was Diclofenac at 50 mg/kg b.w. The mice were dosed for 5 days, thereafter were sacrificed by cervical dislocation and samples (Stomach) collected for analysis. All results in treatment groups were compared with the untreated groups at statistical confidence of 95% ($p<0.05$).The normal control group served as reference point.

Antioxidant Methods

Determination of total Protein

The total protein content of the homogenates was assayed using commercially available total protein kit (Randox Laboratories, UK), employing direct Biuret method.

Catalase activity

The catalase activity in the homogenate of the various organs were determined as described by **Goth (1991).**Volume of 0.2 ml of the homogenate was incubated in 1.0 ml substrate (65 pmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37 °C for 2 minutes. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate ($(NH_4)_6\ Mo_7O_{24}\ 4H_2O$) and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank 3.

serum catalase activity (kU/l) =

$$\frac{Abs(blank\ 1)-Abs(sampe)}{Abs(Blank\ 2)-Abs(Blank\ 3)} \times 271$$

Blank 1 contained 1.0 ml substrate, 1.0 ml molybdate and 0.2 ml serum; blank 2 contained 1.0 ml substrate, 1.0 ml molybdate and 0.2 ml buffer; blank 3 contained 1.0 ml buffer, 1.0 ml molybdate and 0.2 ml buffer.

Lipid Peroxidation

The level of thiobarbituric acid reactive substance (TBARS) was measured as index of lipid peroxidation and malondialdehyde (MDA) production as described by **Draper and Hadley (1990).** The homogenate (100 µL) was deproteinized by adding 2 mL of 14% trichloroacetic acid and 2 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula,

Concentration of TBARS = $\frac{Absorbance}{\Sigma L}$

where $\boldsymbol{\Sigma}$ = molar coefficient, and L = path length. The results were expressed in nmol/mg of protein.

Reduced glutathione (GSH) estimation

The Ellman (1959) method as described by Alam *et al.* (2013) and Sapakal *et al.* (2008) was used in the estimation of reduced glutathione level. The tissue homogenate 150 µL (in 0.1 M phosphate buffer pH 7.4) is taken and added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture is allowed to stand for 5 min prior to centrifugation for 10 min at 2000 rpm. The supernatant (200 µL) is then transferred to a new set of test tubes and added with 1.8 mL of the Ellman's reagent (5,50-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes are made up to the volume of 2 mL. After completion of the total reaction, solutions are measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH.

Superoxide Dismutase

The activity of the superoxide dismutase was evaluated as described by Sun and Zigman (1978). 50 µL of the homogenate was first added to 50 mM sodium carbonate buffer at the desired pH (10.2), and 2 ml of 10 mM epinephrine in the buffer was added at time zero. Matched controls at the same pH but without SOD were measured at time intervals in parallel with those of the experimental sample. Activity of SOD was expressed as the percentage of inhibition of the control absorption at 320 or 480 nm.

GC-MS analysis

GC-MS analysis test was carried out on a 7890A GC-MS Triple Quad instrument (Agilent Technologies, Santa Clara, USA). Chemically coupled with a 5% diphenyl, 95% dimethylpolysiloxane cross-linked stationary phase (0.25 mm film thickness), an HP-5MS 30 m–250 mm (i.d.) fused silica capillary column (Agilent J&W Scientific, Folsom, CA USA) was employed. Exactly 1.5 µL of the sample was manually inserted in the split less mode, Helium was used as a carrier gas at 1.0 mL/min in split mode. The injector and supply were both at 250°C. The oven's temperature was initially set at 40°C, and then gradually raised to 300°C at a rate of 10°C/min per minute, for a total of 60 minutes. The temperature was set to 305°C after the run and stayed for 1 minute. The mass spectrometer was operated in EI mode (70 eV). Data was collected in full scan mode with a scan time of 0.5 seconds from m/z 50 to 650. Agilent Mass Hunter Qualitative Analysis was used to evaluate the data (Version B.04.00). By comparing the average peak area of each component to the total areas, the relative percentage amounts of each component were computed.

Identification of phytochemical components of the GC-MS

The compounds from the GC-MS spectra were identified by comparing mass spectral data and retention indices with the Wiley Registry of Mass Spectral Data 8th edition and the National Institute of Standards and

Technology (NIST) Mass Spectral Library and compounds were identified. Calculation of retention indices (RI) relative to a homologous sequence of n-alkanes under identical experimental conditions, as well as comparison with the literature, further verified the identification.

Preparation of receptors and most prominent compound

Antioxidant

Cytochrome c peroxidase Protein Data Bank ID (PDB ID: 2X08) was obtained from the RCSB Protein Data Bank. Water molecules and the substrate ligand were removed using Molecular Molegro viewer software. The PDB structure of the most abundant compound, 9-Octadecenoic acid (Z)-, methyl ester was downloaded from PubChem and was abbreviated (ODM). Ascorbic acid abbreviated (ASC) (standard drug) was also downloaded and docked to 2X08.

Antiulcer

Pig Gastric H,K-ATPase (PDB ID: 2XZB) was obtained from the RCSB Protein Databank. Water molecules and the substrate ligand were removed using Molecular Molegro viewer software. The PDB of Omeprazole (OME) (Standard drug) was also docked to 2XZB.

ADMET properties

The ADMET profile of ODM was predicted using Admet SAR online server, Cheng *et al.*, (2012).

Docking protocol

ODM was loaded onto Pyrx virtual screening tool, Dallakyan and Olsonn, (2015). The energies were minimized and converted to PDBQT format using the PyRx virtual screening tool (Dallakyan and Olsonn, 2015). The binding conformation of the ligands complexed with protein were visualized using Biovia Discovery Studio, **BIOVIA, (2024).**

Statistics

The data were analysed using statistical package of social sciences (SPSS) version 23. Data were expressed as Mean± Standard Error of mean. The data were subjected to one-way analysis of variance (ANOVA). The different doses were compared and separated using post-hoc analysis (Duncan test) to check mean that is significant. The statistical confidence was placed at 95 %($p \leq 0.05$).

RESULTS AND DISCUSSION

Antioxidant Result and discussion.

Table 1: Antioxidant results with enzymatic tests of S. acuta.

Treatment	Catalase (IU/g protein)	Superoxide dismutase (IU/g protein)	malondialdehyde (nanomole/g protein)	Reduced glutathione (µg/L)
Distilled water, 5 ml/kg (Negative control)	64.57 ± 3.97	17.95 ± 0.34	85.83 ± 2.01	28.59 ± 2.33
Omeprazole, 20 mg/kg (Std Drug)	4.96 ± 1.37*	50.33 ± 0.34*	256.32 ± 5.10*	14.58 ± 0.15*
Sida acuta, 125 mg/kg (Low dose)	13.24 ± 0.24*	50.70 ± 0.70*	248.46 ± 1.54*	16.52 ± 0.52*
Sida acuta, 250 mg/kg (Middle dose)	6.78 ± 0.83*	21.69 ± 0.09*	241.33 ± 2.30*	20.53 ± 0.26*
Sida acuta, 500 mg/kg (High dose)	6.37 ± 0.58*	33.63 ± 0.32*	214.75 ± 6.67*	13.54 ± 0.15*

$_{*P}$<0.05 when compared with distilled water treated group

Catalase (IU/g protein): The higher the value (13.24 ± 0.24*); (6.78 ± 0.83*) and (6.37 ± 0.58*) the better the antioxidant effect when compared with the control (64.57 ± 3.97). The standard drug Omeprazole, (4.96 ± 1.37*) was used as a standard reference check. Therefore the extract is better than standard drug, Omeprazole. Table 1.

Superoxide dismutase (IU/g protein): The higher the value (50.70 ± 0.70*); (21.69 ± 0.09*) and (33.63 ± 0.32*) the better the antioxidant effect when compared with the control (17.95 ± 0.34). The standard drug Omeprazole, (50.33 ± 0.34*) was used as a standard reference check. Table 1

Malondialdehyde (nanomole/g protein). The lower the value (248.46 ± 1.54*); (241.33 ± 2.30*) and (214.75 ± 6.67*) the better the antioxidant effect when compared with the control (85.83 ± 2.01). The standard drug Omeprazole, (256.32 ± 5.10*) was used as a standard reference check. Therefore the extract is better than standard drug, Omeprazole. Table 1.

Reduced glutathione (µg/L): The higher the value (16.52 ± 0.52*); (20.53 ± 0.26*) and (13.54 ± 0.15*) the better the antioxidant effect when compared with the control (28.59 ± 2.33). The standard drug Omeprazole, (14.58 ± 0.15*) was used as a standard reference check. Therefore the extract is better than standard drug, Omeprazole at low and mid doses. Table 1.

Antiulcer results and discussion

Table 2: Anti-ulcer score with percentage inhibition of *Sida acuta*.

Treatment group	Ulcer score	Ulcer index	Percentage inhibition
Untreated, 5 ml/kg D/W	15.60±2.71[a]	1.79±0.01[a]	0.00±0.00[d]
Omeprazole, 20 mg/kg	4.60±0.67[b]	0.66±0.00[b]	68.61±4.72[c]
125 mg/kg Extract	2.60±0.24[b]	0.46±0.00[c]	80.27±5.11[b]
250 mg/kg Extract	2.20±0.58[b]	0.42±0.00[d]	84.44±4.10[ab]
500 mg/kg Extract	1.00±0.44[b]	0.22±0.02[e]	94.44±2.32[a]

Note: Values are presented as mean ± S.E (Standard error of mean). Different superscript letters along treatment groups shows significant ($p<0.05$) differences.

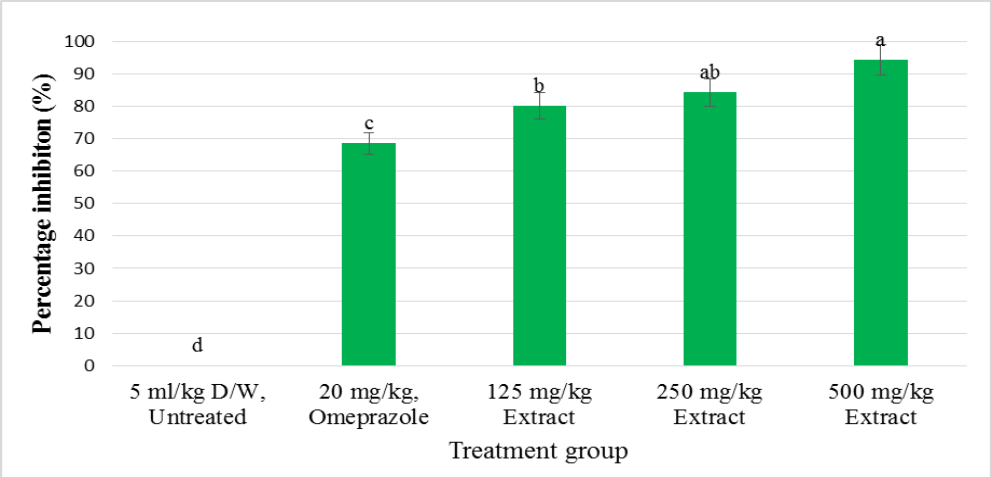

Figure 2: Graph showing percentage inhibition and ulcer index.

Antiulcer activity of the extract produced significant ($p < 0.05$) dose dependent decrease in ulcer score and index in treated rats induced with Diclofenac. (Table 2) compared with distilled water group. The extract produced its greatest inhibition at 500 mg/kg dose (94.44 %), while 250 mg/kg b.w. (84.44 %) and 125 mg/kg b.w. (80.27 %). Distilled water group (0.00 %). The standard drug 20 mg/kg, b.w. Omeprazole drug inhibited (68.61 %). Therefore *S. acuta* extract was a better antiulcer drug than Omeprazole in this study.

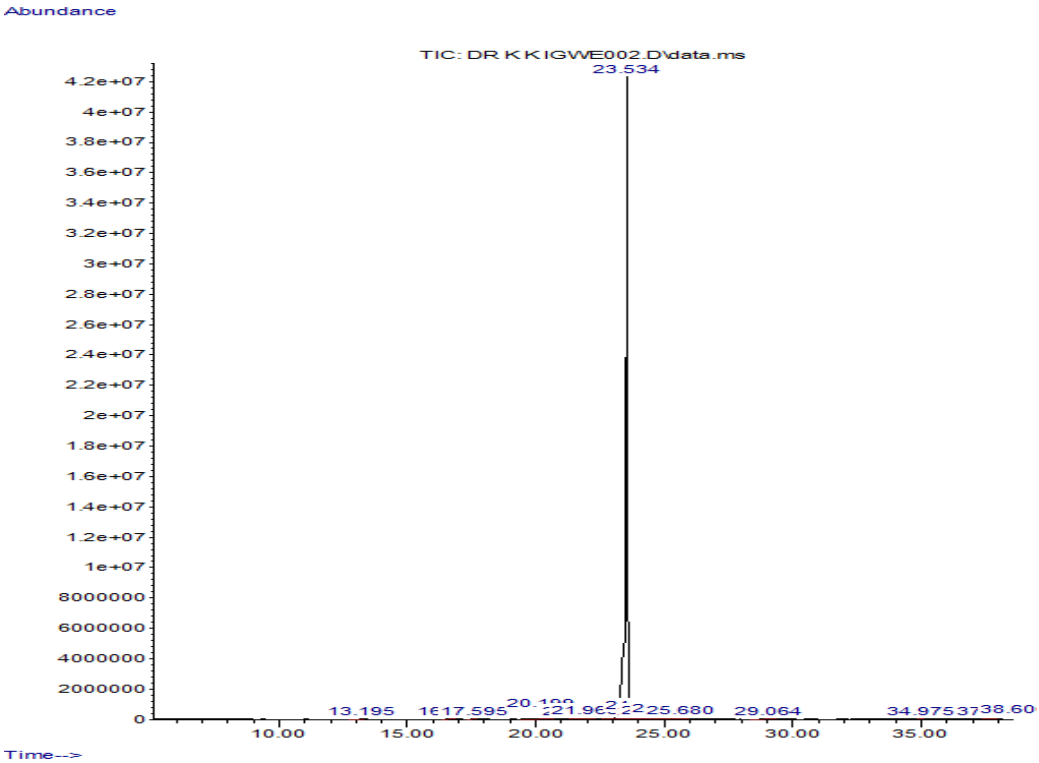

Figure 3: Gas Chromatogram of ethanol extract of *Sida acuta* leaves.

Table 3: Compounds present in ethanol extract of *Sida acuta* leaves.

Compound No	RT	Composition (%)	Compounds
1	13.1951	0.047	Pentafluoropropionic acid, undecyl ester
2	16.7207	0.0774	Beta.-Hexachlorocyclohexane
3	17.5948	0.0576	1-Docosene
4	20.1995	2.1961	Pentadecanoic acid, 14-methyl-, methyl ester
5	21.5395	0.1307	Hexadecanoic acid, ethyl ester
6	21.6297	0.1093	5-Eicosene, (E)-
7	21.9628	0.6201	n-Hexadecanoic acid
8	23.5345	92.0616	9-Octadecenoic acid (Z)-, methyl ester
9	24.0415	1.4414	Methyl stearate
10	24.5029	0.0348	9,12-Octadecadienoyl chloride, (Z,Z)-
11	24.6579	0.2049	(E)-9-Octadecenoic acid ethyl ester
12	25.1181	1.9093	trans-13-Octadecenoic acid
13	25.6795	0.6979	Pentadecanoic acid
14	29.0637	0.4306	9,17-Octadecadienal, (Z)-
15	34.9751	0.0485	2,6-Octadiene, 1-methoxy-3,7-dimethyl-, (E)-
16	37.734	0.0009	1-Cyclohexylnonene
17	38.6057	0.0681	Oleic Acid

Gas chromatogram of ethanol extract of *S. acuta* shows 17 peaks indicating 17 phytocompounds identified (Table 3). The most abundant compound is, 9-Octadecenoic acid (Z)-, methyl ester with highest composition of 92.0616 %. Structure (Fig 4).

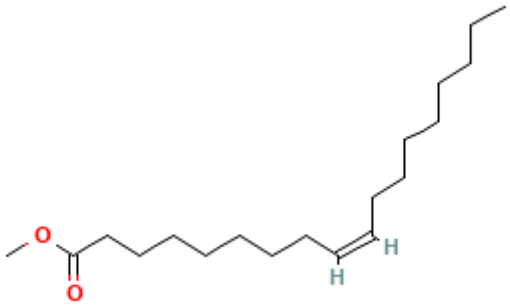

Figure 4: Structure of 9-Octadecenoic acid (Z)-, methyl ester (ODM) (most prominent compound).

Table 4: ADMET Predicted Profile for ODM.

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9838
Human Intestinal Absorption	HIA+	0.9941
Caco-2 Permeability	Caco2+	0.8177
P-glycoprotein Substrate	Non-substrate	0.6747
P-glycoprotein Inhibitor	Non-inhibitor	0.8472
	Non-inhibitor	0.6780
Renal Organic Cation Transporter	Non-inhibitor	0.8934
Distribution		
Subcellular localization	Plasma membrane	0.6788
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8474
CYP450 2D6 Substrate	Non-substrate	0.8876
CYP450 3A4 Substrate	Non-substrate	0.6171
CYP450 1A2 Inhibitor	Inhibitor	0.5466
CYP450 2C9 Inhibitor	Non-inhibitor	0.9433
CYP450 2D6 Inhibitor	Non-inhibitor	0.9530

CYP450 2C19 Inhibitor	Non-inhibitor	0.9415
CYP450 3A4 Inhibitor	Non-inhibitor	0.9705
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.8518
Excretion		
Toxicity		
AMES Toxicity	Non AMES toxic	0.9321
Carcinogens	Carcinogens	0.5217
Biodegradation	Ready biodegradable	0.8105
Acute Oral Toxicity	III	0.6390

ADMET properties

The ADMET results (Table 4) showed that ODM can penetrate the BBB. It was also found that ODM could be absorbed by the human intestine and can also penetrate to Caco-2 cells (Table 4). Nevertheless, the tested compounds proved to be non-potential substrates for P-glycoprotein (P-gp) which effluxes drugs and various compounds to undergo further metabolism and clearance (Amin, 2013) resulting in therapeutic failure because the drug concentration would be lower than expected (Levin, 2012). Many of the human microsomal P_{450} S aromatase catalyze the metabolism of a wide variety of compounds including xenobiotics and drugs (Ghosh *et al.*, 2012). Thus, inhibition of cytochrome P_{450} isoforms might cause drug-drug interactions in which co-administered drugs fail to be metabolized and accumulate to toxic levels (Lynch, and Price, 2007). Results showed that most of the cytochrome P_{450} isoforms cannot be inhibited by ODM. Fortunately, ODM did not show any acute toxicity and carcinogenic effect with respect to the Ames test data. The test compound was classified into Category III (compounds with LD_{50} values greater than 500mg/kg but less than 5000mg/kg) based on the criterion of WHO. Hence, the compounds should be administered within the safe dosages). ADMET Predicted Profile showed that the lead compounds are soluble in water, biodegradable and are less toxic.

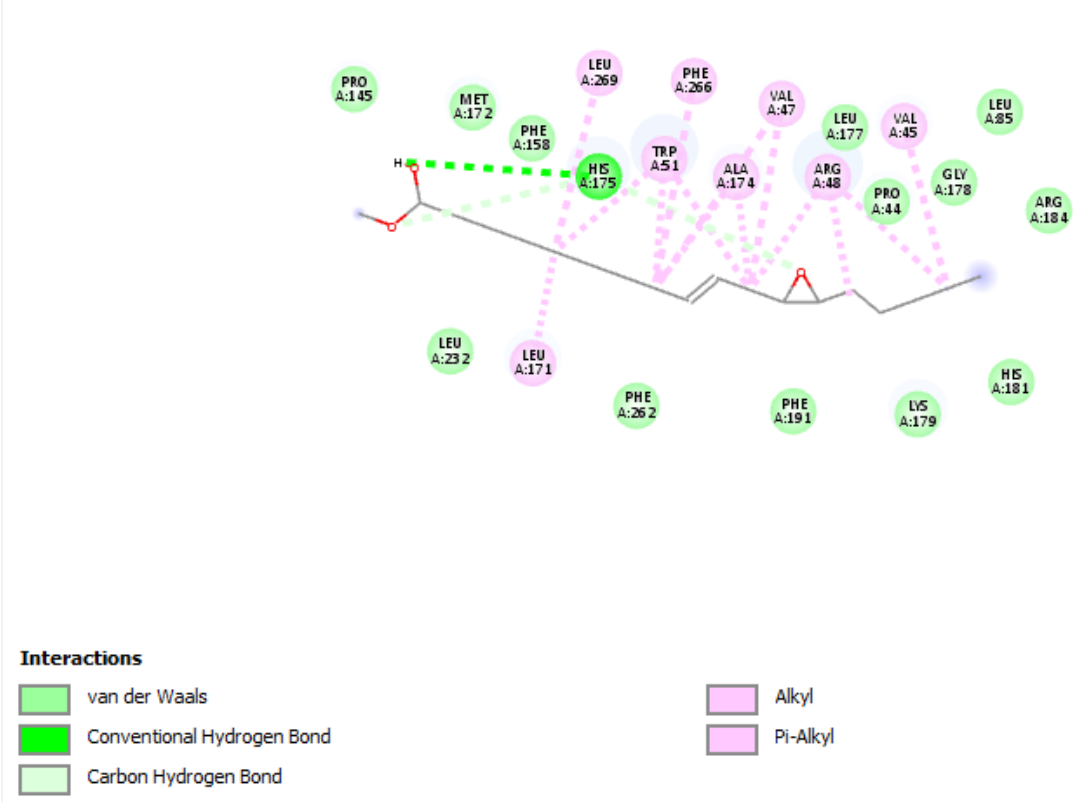

Figure 5: ODM-2X08 2D interactions, docking score = -7.5 Kcal/mol.

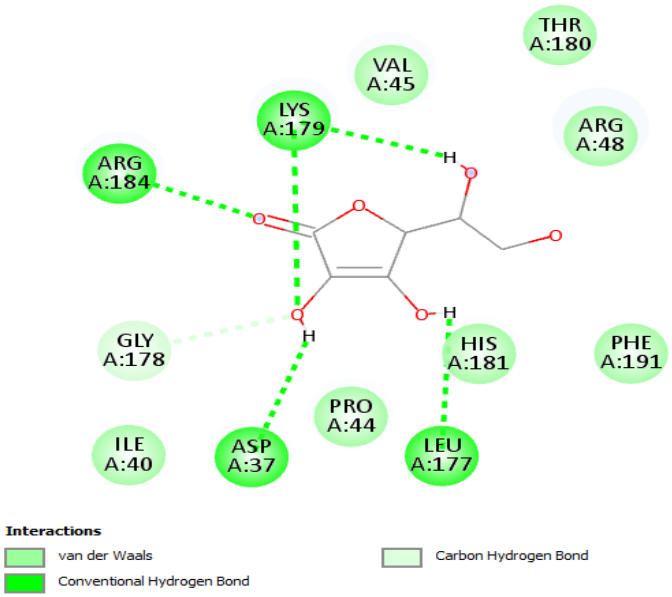

Figure 6: ASC-2X08 2D interactions, docking score = -5.5 Kcal/mol.

The 2D interactions of ODM-2X08 and ASC-2X08 shows that binding affinity of ODM-2X08 was -7.5 Kcal/mol (Fig 5) while ODM-ASC was -5.5 Kcal/mol (Fig 6).This suggested that ODM possessed higher antioxidant property than ascorbic acid.

The 2D interactions of ODM-2X08 showed conventional hydrogen bonds with HIS 175A. The van der Waals interactions were observed with PRO 145A, MET 172A, PHE 158A, ARG 184A, GLY 178A, LEU 85A and LEU 177A. Carbon hydrogen bonds were observed with LEU 232A, PHE 262A, PHE 191A, LYS 179A and HIS 181A, Alkyl and pi-alkyl bonds were observed with LEU 268A, PHE 266A, TRP 51A ALA 174A, VAL 47A, ARG 48A, VAL 45A and LEU 171A. Conventional hydrogen bond, carbon hydrogen bonds and van der Waals interactions were also observed in the 2D interactions of ASC-2X08 (Figure 6). The alkyl and pi-alkyl bonds were absent in ASC-2X08 2D interactions, hence lesser negative binding affinity was observed.

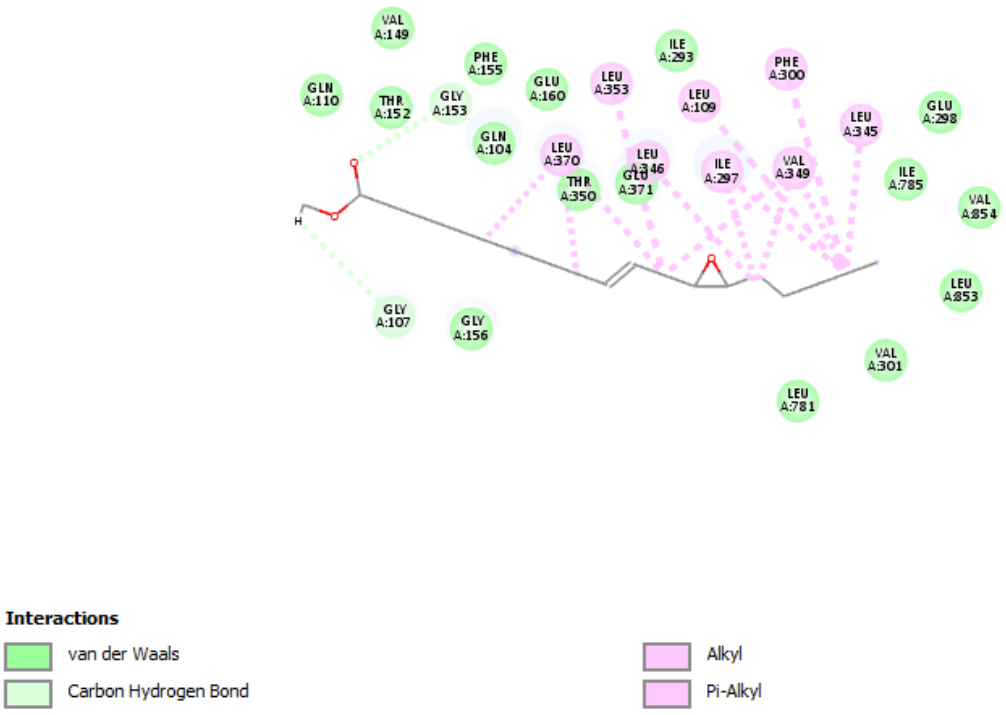

Figure 7: ODM-2XZB 2D interactions, Docking score = -6.0 Kcal/mol.

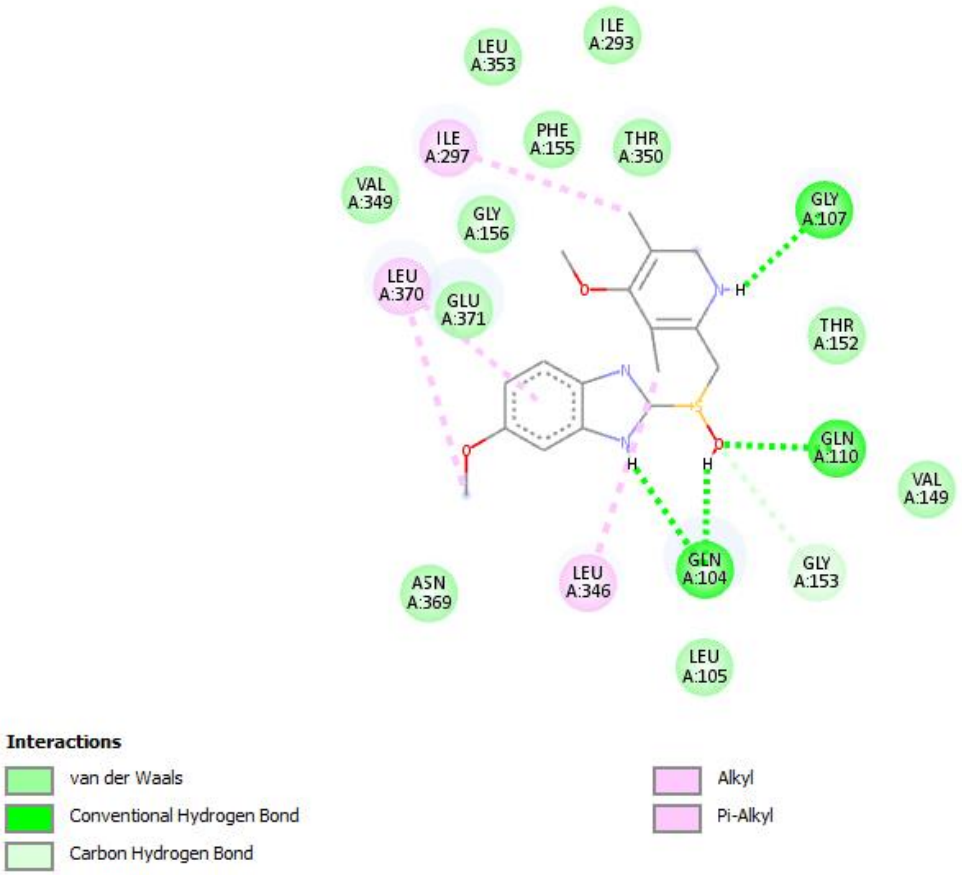

Figure 8: OME-2XZB 2D interactions, docking score = -7.5 Kcal/mol.

The 2D interactions of ODM-2XZB and | OME -2XZB are presented in Figures 7 and 8 respectively. The binding affinity of ODM-2XZB was -6.0 Kcal/mol while OME-2XZB was -7.5 Kcal/mol. This suggested that ODM possessed lower anti-ulcer property than omeprazole. The 2D interactions of ODM-2XZB showed carbon-hydrogen bond with GLY 107A. The van der Waals interactions were observed with VAL 149A, GLN 110A, THR 152A, GLY 153A, PHE 155A, GLY 160A, GLN 104A, ILE 293A, THR 350A, GLU 371A, GLU 298A, ILE 785A, VAL 854A, LEU 853A, GLY 156A, VAL 301A and LEU 781A. Alkyl and pi-alkyl bonds were observed with LEU 370A, LEU 346A, LEU 353A, LEU 109A, VAL 349A, PHE 300A, LEU 345A and ILE 297A. The carbon hydrogen bond, alkyl/pi-alkyl bonds and van der Waals interactions were also observed in the 2D interactions of ODM-2XZB (Figure 5). The conventional hydrogen bonds were absent in OME-2XZB 2D interactions, hence less negative binding affinity value compared OME-2XZB complex.

Drug-likeness prediction of ODM is presented in Table 5.

Table 5: Drug-likeness prediction of ODM.

Compound	Mol. weight (g/mol)	HB Acceptor	HB Donor	Lipophilicity LogP	Molar refractivity	No. of violations
ODM	296.49	2	0	5.43	102.14	1 LOGP>5

Lipinski's rule of five is a concept frequently used in drug discovery. This rule helps to predict if a biologically active molecule is likely to have the chemical and physical properties to be orally bioavailable. The Lipinski rule is based on pharmacokinetic drug properties such as absorption, distribution, metabolism and excretion. The rule is specific on physicochemical properties such as: Not more than 5 hydrogen bond donors; Not more than 10 hydrogen bond acceptors; Molecular mass less than 500 Da; Partition coefficient not greater than 5, (Lipinski, 2004).

According to Lipinski's rule of five, (Lipinski, 2004), an orally active drug can have not more than one violation of these conditions. From Table 5, the lead compound, 9-Octadecenoic acid (Z)-, methyl ester (ODM) had only one violation, hence it is likely to have the chemical and physical properties to be orally bioavailable.

The Gas chromatogram showed 17 peaks indicating 17 compounds in ethanol extract of *S. acuta* leaves. The most prominent compound was 9-Octadecenoic acid (Z), methyl ester with 92.0616 % peak area percentage.

CONCLUSION

The 2D interactions of ODM-2X08 and ASC-2X08 shows that binding affinity of ODM-2X08 was -7.5 Kcal/mol (Fig 5) while ODM-ASC was -5.5 Kcal/mol (Fig 6).This suggested that ODM possessed higher antioxidant property than ascorbic acid. Antiulcer activity of the extract produced significant ($p < 0.05$) dose dependent decrease in ulcer score and index in treated rats induced with Diclofenac. (Table 2) compared with distilled water group. The whole extract *in vivo* produced inhibition at 500 mg/kg dose (94.44%), while 250 mg/kg b.w. (84.44%) and 125 mg/kg b.w. (80.27 %). Distilled water group (0.00%). The standard drug 20 mg/kg, b.w. Omeprazole drug inhibited (68.61 %). Therefore *S. acuta* whole extract extract was a better antiulcer drug than Omeprazole *in vivo*. The extract showed better antioxidant effect when compared with the negative control. The 2D interactions of 9-Octadecenoic acid (Z)-, methyl ester (ODM-2XZB) and Omeprazole (OME - 2XZB) shows that the binding affinity of ODM-2XZB was -6.0 Kcal/mol while OME-2XZB was -7.5 Kcal/mol. This suggested that ODM possessed lower anti-ulcer property than omeprazole as a lead compound. ADMET Predicted Profile showed that the lead compounds are soluble in water, biodegradable and are less toxic. The lead compound, 9-Octadecenoic acid (Z)-, methyl ester (ODM) had only one violation, according to Lipinski rule of 5 (RO5) hence it is likely to have the chemical and physical properties to be orally bioavailable hence a good drug candidate.

Diclofenac causes cell death and exfoliation of gastric mucosa via increased membrane permeability to sodium and water (gastric accumulation), as well as intracellular accumulation of calcium (Khare *et al.,* 2008). It stimulates gastric acid secretion, enhance free radical release, down-regulate bicarbonate secretion and deplete the protective mucus and makes the gastric mucosa prone to the destructive effects of pepsin and hydrochloric acid leading to gastric ulceration, Adinortey *et al.,* (2013). The antiulcer activity of extract might be via the inhibition of proton pump or scavenging of free radicals, Cuevas *et al.,* (2011), as antioxidants scavenge free radicals and enhance membrane stability, thus protecting against cellular injury, Morais *et al.,* (2010). The reduced ulcer lesion in the extract treated groups implies that, it may have impaired any of the processes through which Diclofenac produced gastric ulceration and hence, possess antiulcer properties.

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