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ANTIOXIDANT AND ANTI-ULCER EFFECT OF CLEOME RUTIDOSPERMA ETHANOL LEAF EXTRACT; GCMS AND MOLECULAR DOCKING STUDY

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ABSTRACT

Antioxidant and Anti-ulcer effect of *Cleome rutidosperma* ethanol leaf extract, GCMS and Molecular docking was studied in rats. Twenty (20) rats were used for this research and were grouped into four (4) of five (5) rats 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 and 4 (the low and high doses) received 125**,** 500 mg/kg body weight of the extract of *C. rutidosperma* respectively and were also induced with Diclofenac. The Gas chromatogram showed 14 peaks indicating 14 compounds in ethanol extract of the plant. The most prominent compound was Oleic acid with 22.6 % peak area percentage. *C. rutidosperma* showed a better antioxidant effect compared with the negative control treated with distilled water when tested with catalase, supreroxide dismutase, malondialdehyde and reduced glutathione. At 500 mg/kg b.w. *C. rutidosperma* extract had better reduction of ulcer index (82.08 %). The negative control treated with distilled water had no reduction (0.00 %) while standard drug Omeprazole had (64.70 %). This indicates that the extract contain compound that can be an alternative of both ulcer and antioxidant than synthetic drug in the protection of Diclofenac induced ulceration and antioxidant study. The 2D interactions of Oleic acid (OLA-2X08) and Ascorbic acid (ASC-2X08) showed that binding affinity of OLA-2X08 was -7.3 Kcal/mol while ASC-2X08 was -5.5 Kcal/mol. This suggested that OLA possessed higher antioxidant property than ascorbic acid. The A D M E T results showed that Oleic acid (OLA) can penetrate the Blood Brain Barrier (BBB). It was also found that OLA could be absorbed by the human intestine and can also penetrate to Caco-2 cells (for permeability assessment) and result demonstrated relatively high success. Biotransformation and elimination results showed that most of the cytochrome P_{450} isoforms were not inhibited by OLA so can be eliminated easily thus OLA did not show toxicity. OLA had only one violation, according to Lipinski rule of 5 (RO5) therefore it is likely to have the chemical and physical properties to be orally bioavailable, hence a good drug candidate.

KEYWORDS: Antioxidant, Anti-ulcer, *Cleome rutidosperma*, 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. 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). Free radicals can establish chain reactions that can generate new free radicals. 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. The actions of free radicals may disrupt and 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 can lead to breakdown in cell components, activation of signal transduction pathways and changes in the gene and protein expression. DNA modification and damage can occur as a result of oxidative stress and causes cell and tissue damage, Van-Hauton *et al* (2006).

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, cancer and contribute to the aging process (Comhair and Erzuum, 2005; Johnson and Giulivi, 2005; Klaunig and Kamendulis, 2004). 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. 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 *Helicobacter pylori* and use of aspirin and other Non Inflamatory Anti Inflamatory Drug (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, Khare *et al,* (2008).

Pathogenesis of NSAIDs-induced ulcers 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 asprin dose of 81mg/day, Cuevas *et al* (2011). 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).

Medicinal plants are natural gifts to humanity and evidence suggests that they have been used since antiquity.The plant *Cleome rutidosperma* also known "spider flower" or "African bee plant" (Family-*Capparidaceae*) is a low-growing herb that grows up to 70 cm creeping and is found in landfills, water logged areas and grassy places with trifoliate leaves and small violet blue flower which turns pink in West Africa. It has elongated capsules of seed pods with asymmetrical, dull black seeds. Bose1 *et al* (2007).

It has also been discovered in various parts of tropical America as well as South East Asia. Various components of the plants of the *Cleome* genus are employed as stimulants, antiscorbutic, anthelminthic, vesicant, rubefacient, antiulcer, antioxidants. Diuretic and laxative action have also been documented. Bidla *et al* (2004).

The roots of this plant have also been shown to have hypoglycemic properties Mondal *et al* (2009). In separating compounds in *Cleome rutidosperma*, Gas Chromatography Mass Spectrometry (GC-MS) technique was used. Researchers have used GCMS to separate compounds in plants (Igwe *et al,* 2015; Otuokere *et al,* 2016; Ikpeazu *et al* 2020). GC-MS is considered an effective technique for separating plant compounds and biomolecules (Igwe *et al.*, 2016, James and Norby 2003). 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)

Pharmacologically, *C. rutidosperma* produces observable analgesic activity Anindya *et al* (2007) and depressed locomotory activity in animals treated with morphine, aspirin and chlorpromazine. Bose *et al* (2004). Anti-convulsant activity, Jena *et al* (2009). Anti-microbial properties Bose1 *et al* (2005). The extracts of *C. rutidosperma* roots in wound healing, Mondal and Suresh (2012). Anti-bacterial and anti-oxidant activities, Prabha *et al* (2017), decreases the level of blood glucose, Okoro *et al* (2010) and antiathritic activity, Chakraborty *et al* (2010). Anti-plasmodial effect, (Bidla *et al* 2004; Bose *et al* 2010). Immune Boosting, (Bidla *et al* 2004; Bose *et al* 2010). Research study shows that methanolic extracts of *C. rutidosperma* exhibited significant anti-cancer activities, Pabhra *et al* (2017). This research is designed to investigate the antioxidant and antiulcer properties of *C. rutidosperma*, exploring its potential as a natural source of bioactive compounds for cellular protection and therapeutic applications. This research work is aimed at checking the antiulcer and antioxidant potentials of *Cleome rutidosperma*, GCMS and molecular docking of the most abundant compound found in the plant. Figure 1 shows the pictures of the seed pods, flowers and leaves of *C. rutidosperma.*

Figure 1: Shows *Cleome rutidosperma* leaves, flowers and long seed pods.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *C. rutidosperma*, were collected from University environment in Umudike, Abia State in Nigeria and was identified using Google plant identifier then 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 *C. rutidosperma* 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 48 hrs and then sieved using a Whatman filter paper. The sample was concentrated using hot air oven at 30°C.

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

Experimental Animals

Albino rats weighing between 150-300g were purchased from the University Farm. Approval was obtained from the 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 rats were acclimatized and fed *ad libitum.*

Experimental Design

Twenty (20) rats were used for this research, they were grouped into four (4) of five (5) rats each. Group 1 was the negative control induced with Diclofenac (for ulcer) but not treated. Group 2 was the positive control induced and was treated with standard drug Omeprazole. Groups 3 and 4 were the low and high doses which received 125 and 500 mg/kg body weight of the extract of *C. rutidosperma* 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 rats 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.

Methods of Antioxidant Determination

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). The homogenate (0.2 mls) 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 Σ = 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 (SOD)

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.

Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis 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 comparisons with the literature, further verified the identification.

Preparation of receptors and most prominent compound

Antioxidant

Cytochrome c peroxidase (PDB ID: 2X08) was obtained from the RCSB Protein Databank. Water molecules and the substrate ligand were removed using molecular molegro viewer software. The crystal structure (PDB file) of the most abundant compound, oleic acid was downloaded from PubChem. It was abbreviated as OLA. Ascorbic acid (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 crystal structure of the standard drug, Omeprazole (OME) in the PDB file was also docked to 2XZB.

ADMET properties

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

Docking protocol

OLA 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 were significant. The statistical confidence was placed at 95 %($p \leq 0.05$)

RESULTS AND DISCUSSION

Table 1: Antioxidant Result of *Cleome rutidosperma.*

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) Induced,treated with DW	5.28 ± 3.84	21.42 ± 2.21	508.50 ± 34.11	9.67 ± 0.39
Omeprazole, 20 mg/kg (Std drug)	8.73 ± 4.04	23.58 ± 10.36	382.58 ± 96.50	14.29 ± 0.68*
Cleome rutidosperma, 125 mg/kg (Low dose)	16.85 ± 2.75*	37.29 ± 17.88	336.65 ± 60.73	14.73 ± 0.41*
Cleome rutidosperma, 500 mg/kg (High dose)	10.04 ± 3.31	48.38 ± 10.38	345.24 ± 60.56	12.77 ± 0.48*

*$p<0.05$ when compared with distilled water treated group

Catalase (IU/g protein): The higher the value (16.85 ± 2.75) and (10.04 ± 3.31) the better the antioxidant effect when compared with the negative control (5.28 ± 3.84). The standard drug Omeprazole, (8.73 ± 4.04) was used as a standard reference check.

Superoxide dismutase (IU/g protein): The higher the value (37.29 ± 17.88) and (48.38 ± 10.38) the better the antioxidant effect when compared with the negative control (21.42 ± 2.21). The standard drug Omeprazole, (23.58 ± 10.36) was used as a standard reference check.

Malondialdehyde (nanomole/g protein). The lower the value (336.65 ± 60.73) and (345.24 ± 60.56) the better the antioxidant effect when compared with the negative control (508.50 ± 34.11). The standard drug Omeprazole, (382.58 ± 96.50) was used as a standard reference check.

Reduced glutathione (µg/L): The higher the value (14.73 ± 0.41) and (12.77 ± 0.48) the better the antioxidant effect when compared with the negative control (9.67 ± 0.39). The standard drug Omeprazole, (14.29 ± 0.68*) was used as a standard reference check.

Ulcer Result

Table 2: *Cleome rutidosperma.*

Treatment group	Ulcer score	Ulcer index	Percentage inhibition
Untreated, 5 ml/kg D/W	22.20 ± 2.63^{a}	2.39 ± 0.01^{a}	0.00 ± 0.00^{c}
Omeprazole, 20 mg/kg	7.40 ± 0.24^{b}	0.92 ± 0.00^{b}	64.70 ± 4.40^{ab}
125 mg/kg Extract	9.20 ± 2.57^{b}	1.10 ± 0.07^{b}	59.78 ± 9.57^{b}
500 mg/kg Extract	4.00 ± 1.22^{b}	0.46 ± 0.11^{c}	82.08 ± 5.68^{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.

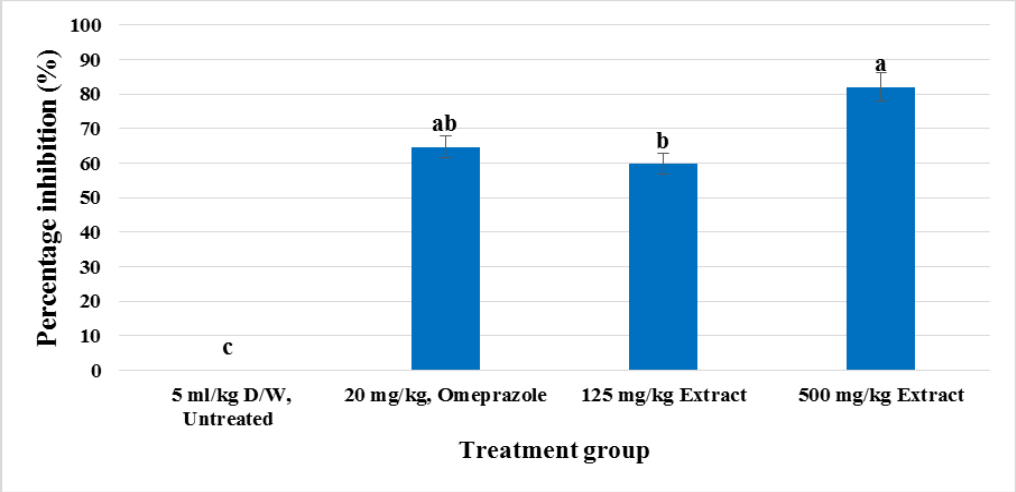

Figure 2: Graph showing percentage reduction in ulcer index with different treatments.

Antiulcer Activity of the *Cleome rutidosperma* produced significant ($p < 0.05$) dose dependent decrease in ulcer score in Diclofenac-induced rats (Table 2) compared with the standard (Omeprazole). Similarly, there were significant ($p<0.05$) reduction in the ulcer index (UI) of the *Cleome rutidosperma* treated group compared with the standard drug. The *Cleome rutidosperma* extract produced its maximum (82.08%) at 500 mg/kg dose, followed by the 59.78% achieved in lowest (125 mg/kg) dose. However, the 64.70% antiulcer activity of the Omeprazole drug was comparable with both doses of the *Cleome rutidosperma* extract, indicating that the extract could effectively be a better herbal alternative to synthetic drug in the protection of Diclofenac-induced ulceration

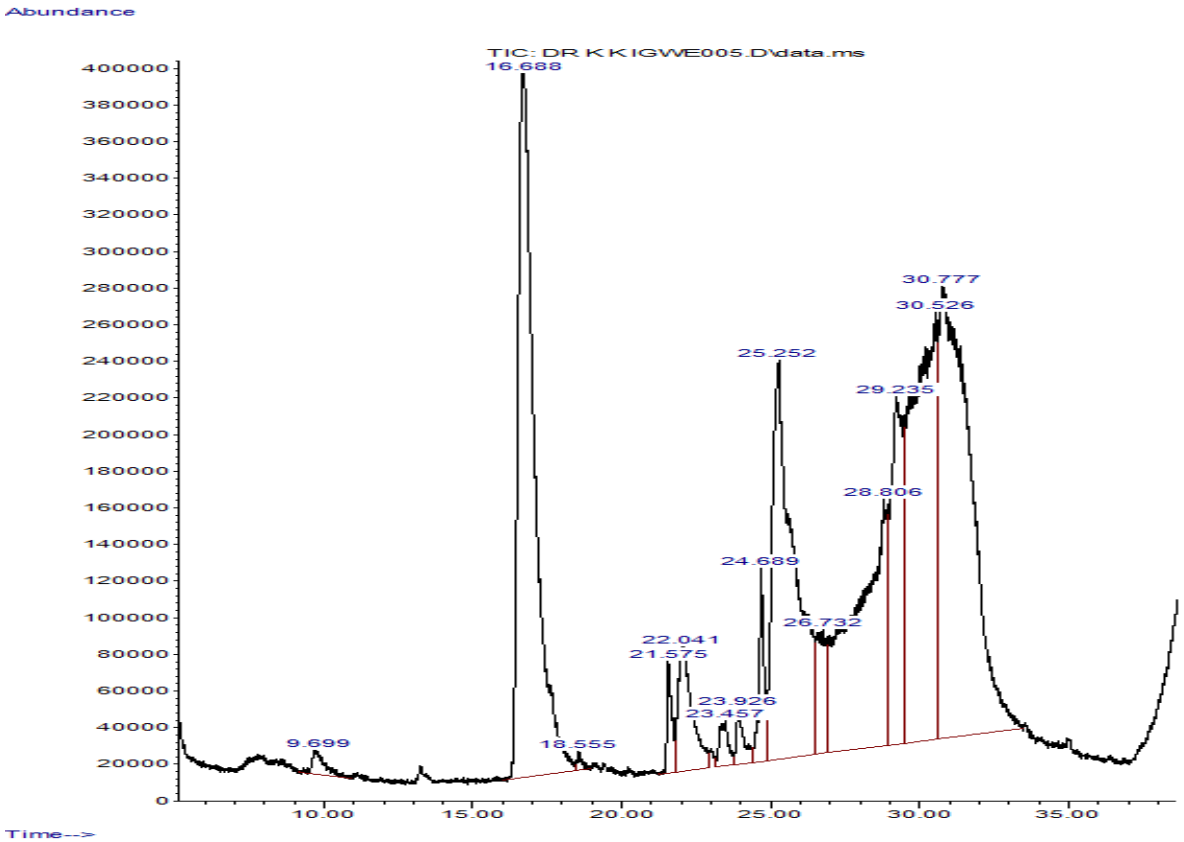

Figure 3: Gas Chromatogram of ethanol extract of *Cleome rutidosperma* leaves.

Gas Chromatogram (GC) of ethanol extract of *C. rutidosperma* leaves is presented in Figure 3. The GC shows the presence of 14 peaks indicating 14 compounds in ethanol extract of *C. rutidosperma* leaves. The compounds have been listed in Table 3. The structure of the most prominent compound (22. 566 % composition) is presented in Figure 2.

Table 3: Compounds present in ethanol extract of *Cleome rutidosperma* leaves.

Compound No	RT (Mins)	Composition (%)	Compounds
1	9.699	0.4629	Delta.-Pentachlorocyclohexene
2	16.6878	18.5962	Lindane
3	18.5552	0.1458	Acetoxyacetic acid, undec-2-enyl ester
4	21.5755	0.8458	Hexadecanoic acid, ethyl ester
5	22.0409	2.823	n-Hexadecanoic acid
6	23.4565	0.6446	Undec-10-ynoic acid, undecyl ester
7	23.9264	0.6612	Phytol
8	24.6893	1.5688	Ethyl Oleate
9	25.2515	13.7547	cis-Vaccenic acid
10	26.7315	2.0435	Undec-10-ynoic acid, tetradecyl ester
11	28.8064	12.5285	5-Eicosene, (E)-
12	29.2346	6.1811	9-Octadecenal, (Z)-
13	30.526	16.8779	Octadec-9-enoic acid
14	30.7765	22.866	Oleic Acid

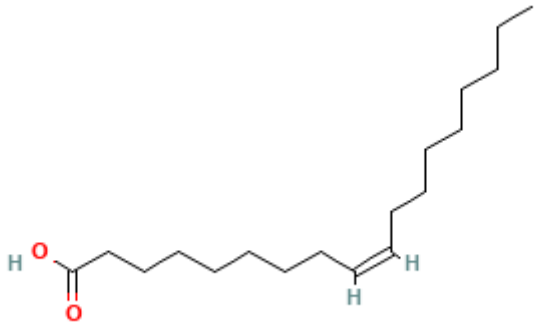

Figure 4: Structure of oleic acid (most prominent compound).

Table 4: ADMET Predicted Profile for Oleic acid.

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9539
Human Intestinal Absorption	HIA+	0.9945
Caco-2 Permeability	Caco2+	0.8371
P-glycoprotein Substrate	Non-substrate	0.5962
P-glycoprotein Inhibitor	Non-inhibitor	0.9487
Distribution		
Subcellular localization	Plasma membrane	0.5465
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.7643
CYP450 2D6 Substrate	Non-substrate	0.8954
CYP450 3A4 Substrate	Non-substrate	0.6678
CYP450 1A2 Inhibitor	Inhibitor	0.9107
CYP450 2C9 Inhibitor	Non-inhibitor	0.8972
CYP450 2D6 Inhibitor	Non-inhibitor	0.9545
CYP450 2C19 Inhibitor	Non-inhibitor	0.9467
CYP450 3A4 Inhibitor	Non-inhibitor	0.9295
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9349
Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9133
	Non-inhibitor	0.9103
AMES Toxicity	Non AMES toxic	0.9674
Carcinogens	Non-carcinogens	0.6568
Fish Toxicity	High FHMT	0.9712

Tetrahymena Pyriformis Toxicity	High TPT	0.9999
Honey Bee Toxicity	High HBT	0.7123
Biodegradation	Ready biodegradable	0.8110
Acute Oral Toxicity	IV	0.8289
Carcinogenicity (Three-class)	Non-required	0.7021

ADMET properties

The ADMET results (Table 4) showed that OLA can penetrated the BBB. It was also found that OLA could be absorbed by the human intestine, and can also penetrate to Caco-2 (Table 4). Nevertheless, the tested compounds proved to be non-potential substrates for Pglycoprotein (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_{450s} aromatase catalyze the metabolism of a wide variety of compounds including xenobiotic 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 OLA. Fortunately, OME did not show any acute toxicity and carcinogens effect with respect to the Ames test data. The test compound was classified into Category IV (harmful if swallowed, 300 $LD_{50} \leq 2000$ mg/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.

Table 5: Drug-likeness prediction of OLA.

Compound	Mol. weight (g/mol.)	HB Acceptor	HB Donor	Lipophilicity LogP	Molar refractivity	No. of violations
OLA	282.00	2	1	6.10	87.08	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 bases pharmacokinetic drug properties such as absorption, distribution, metabolism and excretion on specific physicochemical properties such as: No more than 5 hydrogen bond donors; No 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, an orally active drug can have no more than one violation of these conditions. From Table 5, OLA had only one violation, hence it is likely to have the chemical and physical properties to be orally bioavailable, (Lipinski, 2004)

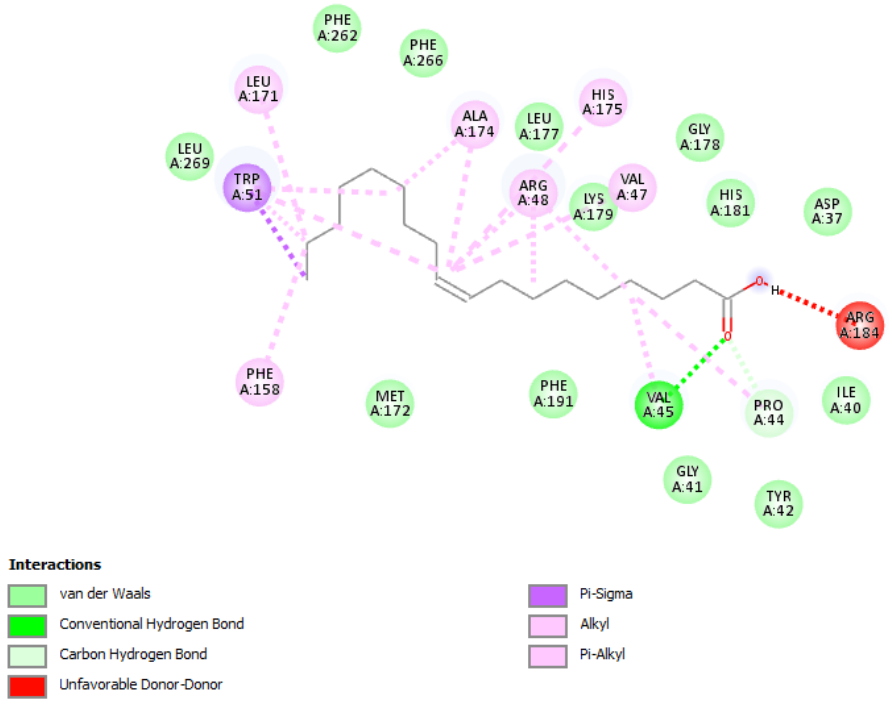

Figure 5: 2D Interactions of 2X08-OLA, docking score = -7.3 Kcal/mol

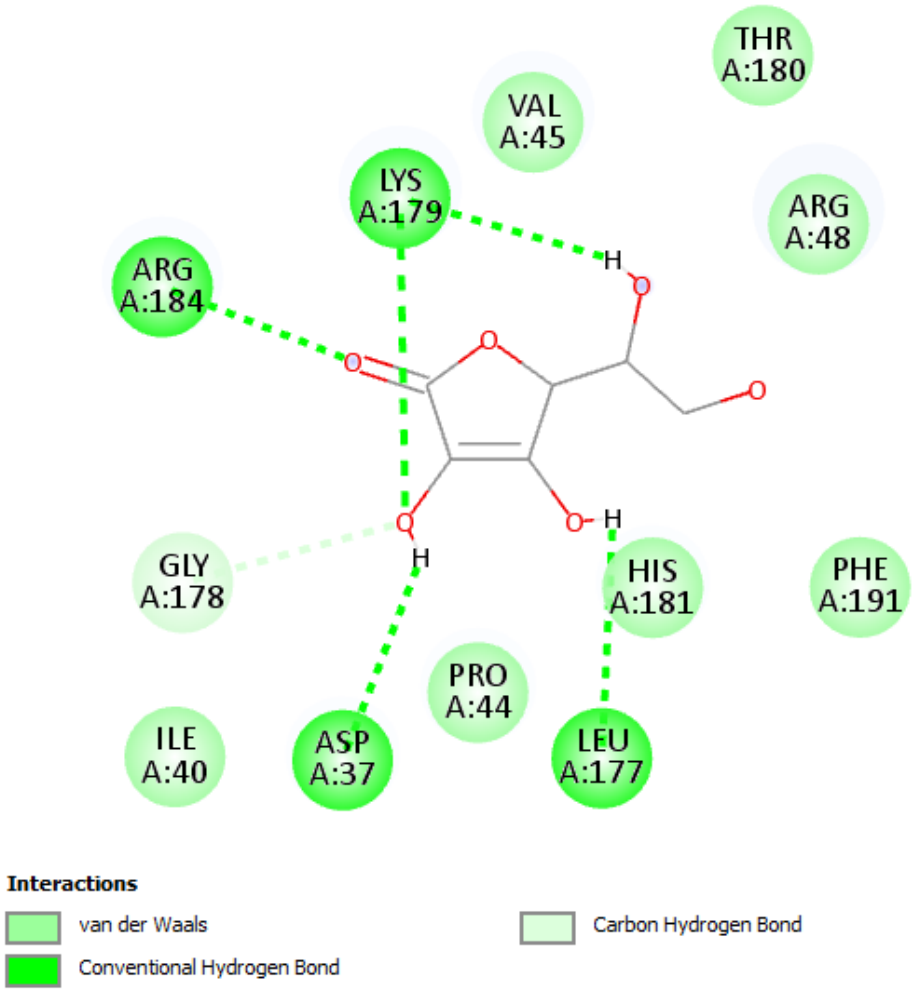

Figure 6: 2D interactions of 2X08-ASC, docking score = -5. 5 Kcal/mol.

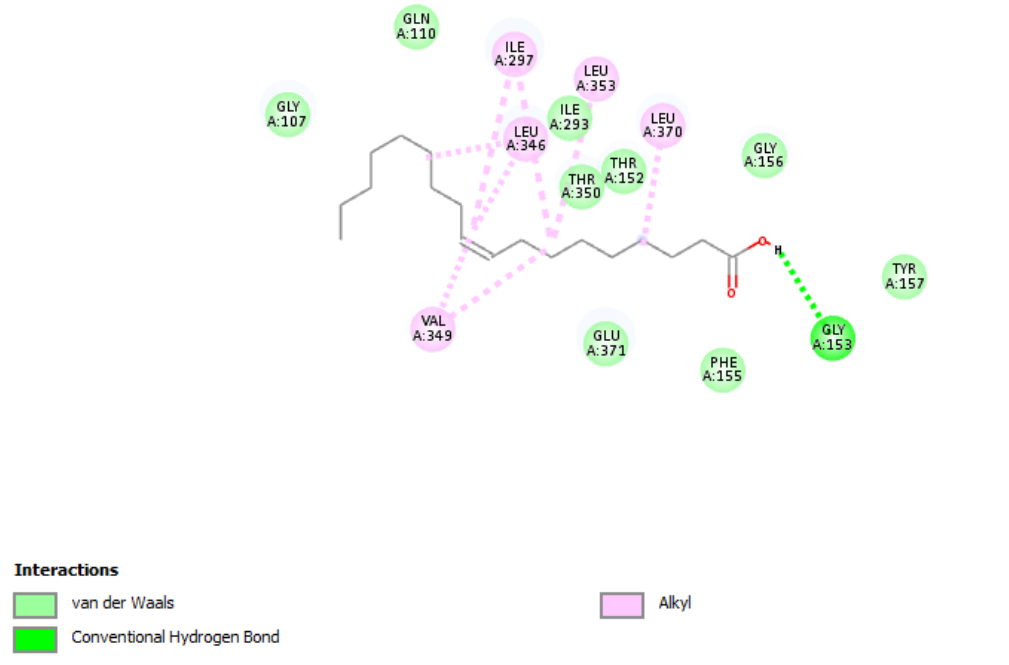

Figure 7: 2D Interactions of 2XZB-OLA, docking score = -5.5 Kcal/mol.

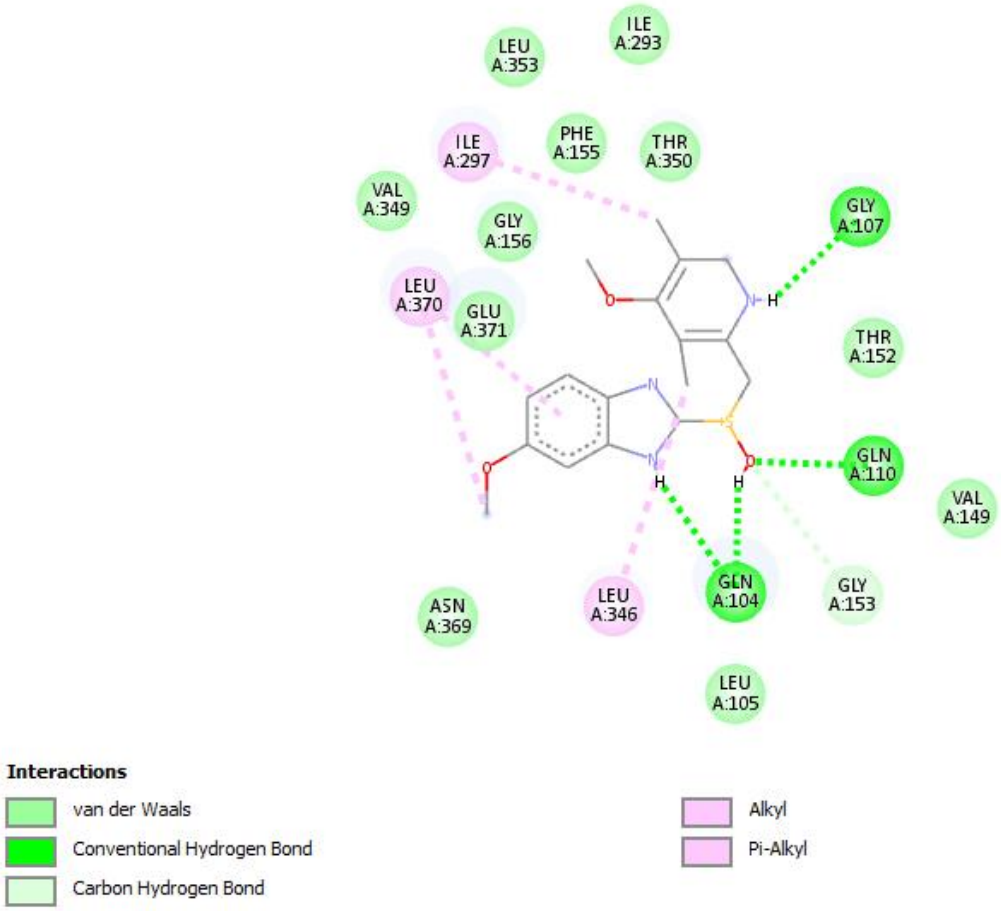

Figure 8: 2D interactions of 2XZB-OME, docking score = -7. 5 Kcal/mol.

The 2D interactions of OLA-2X08 and |ASC-2X08 are presented in Figures 5 and 6 respectively. The binding affinity of OLA-2X08 was -7.3 Kcal/mol while ASC-2X08 was -5.5 Kcal/mol. This suggested that OLA possessed higher antioxidant property than ascorbic acid. The 2D interactions of OLA-2X08 showed van der Waals interactions through LEU 269A, PHE 262A, PHE 266A, LEU 177A, LYS 179A, GLY 178A, HIS 181A, ASP 37A, ILE 40A, TYR 42A, GLY 41A, PHE 191A and MET 172A. Carbon hydrogen bonds were observed with PRO 44A. Alkyl and pi-alkyl bonds were observed with PHE 158A, ARG 48A, VAL 47A, HIS 175A, ALA 174A and LEU 171A. Pi-sigma bond was observed through TRP 51A. Unfavorable donor-donor bond occurred with ARG 184A. Conventional hydrogen bond, carbon hydrogen bonds and van der Waals interactions were also observed in the 2D interactions of ASC-2X08 (Figure 6). The Pi-sigma bond, alkyl and pi-alkyl bonds were absent in ASC-2X08 2D interactions, hence lesser negative binding affinity was observed.

The 2D interactions of OLA-2XZB and |OME-2XZB are presented in Figures 7 and 8 respectively. The binding affinity of OLA-2XZB was -5.5 Kcal/mol while OME-2XZB was -7.5 Kcal/mol. This suggested that OLA possessed lower anti-ulcer property than OME (Standard drug). The 2D interactions of OLA-2XZB showed van der Waals interactions with GLN 110A, GLY 107A, ILE 293A, THR 350A, THR 152A, GLY 156A, TYR 157A, PHE 155A and GLU 371A. Alkyl and pi-alkyl bonds were observed with VAL 349A, ILE 297A, LEU 346A, LEU 353A and LEU 370A. Conventional hydrogen bond was observed through GLY 153A. The conventional hydrogen bond, alkyl/pi-alkyl bonds and van der Waals interactions were also observed in the 2D interactions of OME-2XZB **(Figure 8).** The carbon hydrogen bonds were absent in OME-2XZB 2D interactions.

CONCLUSION

The Gas chromatogram showed 14 peaks indicating 14 compounds in ethanol extract of *Cleome rutidosperma* leaves. The most prominent compound was Oleic acid with 22. 566 % peak area percentage. *C. rutidosperma* showed a better antioxidant effect compared with the negative control treated with distilled water when tested with catalase, supreroxide dismutase, malondialdehyde and reduced glutathione. At 500 mg/kg b.w. *C. rutidosperma* extract had better reduction of ulcer index (82.08 %). The negative control treated with distilled water had no reduction (0.00 %) while standard drug Omeprazole had (64.70 %). This indicates that the extract contain compound that can be an alternative of both ulcer and antioxidant than synthetic drug in the protection of Diclofenac induced ulceration and antioxidant study. The 2D nteractions of Oleic acid (OLA-2X08) and Ascorbic acid (ASC-2X08) showed that

binding affinity of OLA-2X08 was -7.3 Kcal/mol while ASC-2X08 was -5.5 Kcal/mol. This suggested that OLA possessed higher antioxidant property than ascorbic acid. The A D M E T results showed that Oleic acid (OLA) can penetrate the Blood Brain Barrier (BBB). It was also found that OLA could be absorbed by the human intestine, and can also penetrate to Caco-2 cells (for permeability assessment) and result demonstrated relatively high success. Biotransformation and elimination results showed that most of the cytochrome P_{450} isoforms were not inhibited by OLA so can be eliminated easily thus OLA did not show toxicity. OLA 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.

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