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ANTIOXIDANT ACTIVITY OF METHANOL LEAF EXTRACT OF Chanca piedra *(Phyllanthus niruri* Linn.*)* AGAINST CCL_4-INDUCED OXIDATIVE STRESS IN WISTAR STRAIN RATS AND *IN-SILICO* DOCKING ANALYSIS

Ali Abdullahi Damasak[1]*, Maryam Ado Mahmud[3], Bawagana Kyari[1], Mohammed Yakubu[2] and Yagana Shettima Abba[2]
[1]Department of Nutrition & Dietetics, Faculty of Basic Medical Sciences,
[2]Department of Biochemistry, Faculty of Life Sciences, University of Maiduguri
[3]Department of Biochemistry, College of Medical Sciences, Yobe State University
E-mail: *aliadamasak@gmail.com*

ABSTRACT

Oxidative stress is referred to as the excessive production of reactive oxygen species (ROS) relative to antioxidant defense that initiates and advances liver injury. This study was carried out to determine the antioxidant activity of methanol leaf extract of Chanca piedra *(Phyllanthus niruri* Linn.*)* in CCl_4-induced oxidative stress in Wistar strain rats. The sample was obtained from Maiduguri, Borno State, Nigeria. The active components were identified using Gas Chromatography-Mass Spectrometry (GC-MS) and docked with Autodock 4.0 program. The catalase activity was assessed using standard method. Administration of the plant leaf extract at different doses of 100 mg, 200 mg and 400 mg/kg body weight significantly increased ($P<0.05$) the activity of catalase with values of 0.033±0.01, 0.038±0.03 and 0.040±0.02 µmol/min, respectively, compared to the negative control group 0.020±0.00 µmol/min. The results of the docking studies of the identified phytomolecules with catalase enzyme (2CAG) revealed that these compounds possessed distinct affinities with the catalase enzyme, ranging from -9.88 kcal/mol (6,10,14-trimethyl-pentadecan-2-ol (CID_530418) to -5.32 kcal/mol (1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID_616782). The results however, showed that 6,10,14-trimethyl-pentadecan-2-ol had best binding affinity for the enzyme (-9.88 kcal/mol) to act as antioxidant and was observed to interact with His362 (distance = 2.89Å) and Asp335 (distance = 2.60Å). Moreover, the *in silico* and *in vivo* antioxidant activity demonstrated by the extract of *Phyllanthus niruri* Linn. (*Chanca piedra)* might be attributed to bioactive compounds such as 6,10,14-trimethyl-pentadecan-2-ol which displayed potential hydrogen bond interactions with catalase enzyme (2CAG) *in silico*. Finally, the phytocompounds associated with Chanca piedra

(Phyllanthus niruri Linn*.)* plants might be regarded as valuable source of antioxidants, given their potentials for medicinal use and lack of side effects.

Keywords: *Chanca piedra*, oxidative stress, catalase, antioxidant activity.

INTRODUCTION

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of reactive oxygen species (ROS) that initiates and advances liver injury. Numerous risk factors, such as alcohol, drugs, environmental toxins, and irradiation, can cause the liver to experience oxidative stress, which in turn can lead to serious liver conditions such as alcoholic liver disease, diabetes, and cardiovascular disease. Recently, it has been shown that synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene are harmful to human health. The use of antioxidants denotes a logical therapeutic approach to treat and prevent oxidative stress-related liver disease (Arun and Balasubramanian, 2011). Hence, search for efficient, non-toxic natural substances with antioxidant activity for the treatment of liver disease is worthwhile. Acute or chronic hepatitis, hepatosis (non-inflammatory illnesses), and cirrhosis are the three main types of liver disease (degenerative disorder resulting in fibrosis of the liver). Its origin is primarily attributed to the use of specific medications and chemicals (such as chemotherapy with some antibiotics, aflatoxin, peroxidized oil, carbon tetrachloride, and chlorinated hydrocarbons), excessive alcohol use, bacterial infections, and autoimmune diseases (Bharti *et al.,* 2014). Cellular necrosis, a rise in tissue lipid peroxidation, and a decrease in tissue glutathione (GSH) levels are the typical symptoms of liver injury. Serum levels of numerous biochemical indicators, including triglycerides, cholesterol, bilirubin, serum glutamate oxalo-transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP), are also raised (Bharti *et al.,* 2014).

Due to their natural origin and apparent safety, herbal remedies in Ayurveda have been suggested for the treatment of liver problems in the absence of a dependable liver protective agent in modern medicine (Bapat *et al.,* 2013). Cellular macromolecules such as nucleic acids, proteins, and lipids can chemically react with reactive species created in the cell during normal

biological metabolism, resulting to their oxidative modification, which can change their composition and potentially harm their cellular functions. (Bapat *et al.,* 2013).

Phyllanthus niruri Linn. *(*also known as *Chanca piedra,* gale of the wind, stonebreaker or seed-under-leaf in English and Geron tsuntsaye in Hausa*)* is a plant possessing several pharmacological properties. It grows 50–70 cm tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. It is in the genus *Phyllanthus* of the family *Phyllanthaceae* (Patel *et al.,* 2011).

Phyllanthus niruri is taken for diuretic, hypoglycemia, and hypertensive situations in addition to treating kidney stones, gallbladder stones, liver-related ailments such liver cancer and jaundice, as well as having anti-inflammatory, anti-tumot and antinociceptive effect (Bagalkotkar *et al.,* 2006). This study therefore, focused on the antioxidant potentials of methanol leaf extract of *Phyllanthus niruri.*

MATERIALS AND METHODS

Reagents and Chemicals

The reagents and chemicals used in this research are of analytical grade and were obtained from British Drugs Houses (BDH) Chemicals Ltd. Poole, England.

Plant Collection and Identification

The sample (*Phyllanthus niruri* Linn. *(Chanca piedra)* was obtained from Maiduguri, Borno State, Nigeria, in December, 2021. The plant sample was identified by a Taxonomist at the Department of Biological Sciences and voucher sample (Vet212B2) was deposited at the Veterinary Pharmacology Laboratory herbarium, University of Maiduguri, Nigeria.

Preparation of Plant Material

After collection, the leaves of *Phyllanthus niruri* Linn. *(Chanca piedra)* were thoroughly washed and shade dried. The leaves were ground into a fine

powder using pestle and mortar, and then sieved to remove debris. Powdered form of the sample was stored at low temperature prior to extraction.

Methanol Extract Preparation

Five hundred grams (500 g) powder of *Phyllanthus niruri* Linn. *(Chanca piedra)* leaves was extracted with one liter of 70 % methanol using cold maceration method, the product was concentrated to dryness at low temperature and then stored in a refrigerator until used.

Experimental Animals

Healthy Wistar rats of both sexes weighing between 100 - 250 g were obtained from the Animal House of Department of Biochemistry, University of Maiduguri, Maiduguri, Borno State, Nigeria. The rats were handled in accordance with the guidelines for the care and use of laboratory animals (United States of America National Research Council, (US-NRC) (2003). Ethical clearance was obtained from the Animal Use and Ethics Committee (AUEC), Faculty of Veterinary Medicine, University of Maiduguri REF/FP/092020/PGVP/06.

Experimental Design for the Determination of *In vivo* Antioxidant Activity of Methanol Leaf Extract of *Phyllanthus niruri* Linn. *(Chanca piedra)*

A total of 36 Wistar rats were randomly divided into six (6) different groups of six (6) rats each and the following treatment was done once per day for fourteen (14) consecutive days (Kandimalla *et al.,* 2016).

Group I: served as normal control and were given feeds and water *ad libitum.*

Group II: served as negative control and received on the 14th day CCl_4 (1.5 ml/kg, i.p.) in 1:1 dilution with olive oil.

Group III: served as positive control and received silymarin (25 mg/kg body weight) for 14 days orally and on 14th day CCl_4 (1.5 ml/kg, i.p.) in 1:1 dilution with olive oil.

Group IV: served as treatment group and received methanol leaf extract (100 mg/kg body weight) of *Phyllanthus niruri* Linn. *(Chanca piedra)* for 14 days orally and on 14th day CCl_4 (1.5 ml/kg, i.p.) in 1:1 dilution with olive oil.

Group V: served as treatment group and received methanol leaf extract (200 mg/kg body weight) of *Phyllanthus niruri* Linn. *(Chanca piedra)* for 14 days orally and on 14th day CCl_4 (1.5 ml/kg, i.p.) in 1:1 dilution with olive oil.
Group VI: served as treatment group and received methanol leaf extract (400 mg/kg body weight) of *Phyllanthus niruri* Linn. *(Chanca piedra)* for 14 days orally and on 14th day CCl_4 (1.5 ml/kg, i.p.) in 1:1 dilution with olive oil (Kandimalla *et al.,* 2016).

The animals were administered daily with different doses of the extract for fourteen (14) days and observed for any changes or signs of toxicity or death throughout the study. Twenty four (24) hours after the last treatment, blood was collected through direct cardiac puncture (Kandimalla *et al.,* 2016).

Determination of Catalase Activity

The catalase activity was assessed in accordance with the method described by Atawodi, (2008).

Gas Chromatography-Mass Spectroscopic (GC- MS) Technique

The methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra)* was analyzed using GC-MS technique. GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with a HP-5 MSf used silica column (5% phenyl methyl siloxane 30.0m ×250 μm, film thickness 0.25 μm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min (Olivia *et al.,* 2021). Other GC-MS conditions are ion-source temperature, 250°C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 μl injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 36°C for 5 min and changed to 150 V at the rate of 4 °C/min. The temperature was raised to 250°C at the rate of 20 °C/min and held for 5 min. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data (Olivia *et al.,* 2021).

In silico Docking Analysis

Physicochemical Analysis

All the compounds obtained from the GC-MS analysis were subjected to virtual screening based on their physicochemical properties (the molecular weight, number of hydrogen bond donors (HBDs ≤5), number of hydrogen acceptors (HBAs≤10), Log P≤5 and Drug likeness) using the data warrior tools (Kesar *et al.,* 2020).

Pharmacokinetic Analysis

All the compounds that has good binding energy that were obtain from the docking studies were further screened based on their pharmacokinetics (Rate of absorption, distribution metabolism and excretion) properties using Data Warrior tool. Also, the toxicity of each compound was assessed with data warrior tool (Kesar *et al.,* 2020).

Molecular Docking Analysis

The three-dimensional (3-D) structure of the enzyme (catalase) was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) database. The Autodock 4.0 program was used to carry out the molecular docking investigations. All compounds with desired physicochemical properties were selected for the docking studies. The protein preparation process was applied in the first phase. This procedure carries out operations like removing water molecules, providing the hydrogen that is lacking, and erasing alternative conformations. In order to incorpo*r*ate all of the necessary amino acid residues of the allosteric site for interaction with ligands, the allosteric site for the protein structure was specified using a sphere object radius. Lamarckian genetic algorithm (GA) was used to perform the automated docking of the protein with each ligand. Torsion bonds and side chains of the ligands were allowed to rotate freely, while the PDB file was kept rigid. The bioactive substances were docked into the allosteric site of catalase. As can be seen from the docking score and probable interactions between the phytoconstituents and catalase, the chemicals only docked in the allosteric site (Leechalsit *et al.,* 2019; Kesar *et al.,* 2020).

Data Analysis

Results were expressed as mean ± Standard Error of Mean (SEM). Differences among mean of the groups was determined with one-way Analysis of Variance (ANOVA) followed by post-hoc test using SPSS software version 23.0. $P< 0.05$ was considered statistically significant (Mead and Curnow, 1982).

Results

Determination of Catalase Activity

The results of *in vivo* antioxidant assay as presented in Table 1 showed that carbon tetrachloride (CCl_4) caused a significant ($P<0.05$) decrease in the level of catalase in the negative control group as compared to treatment groups where the plant extract of Chanca piedra *(Phyllanthus niruri* Linn.*)* caused a significant ($P<0.05$) increase in the catalase activity.

Table 1: Catalase Activity

Experimental Groups	Catalase (µmol/min)
Normal control (Dist. Water)	0.032 ± 0.01^{d}
Negative control (CCl_4 (1.5 ml/kg)	0.020 ± 0.00^{a}
Positive control (Sylimarin, (25 mg/kg body weight)	0.035 ± 0.01^{d}
100 mg/kg extract + CCl_4	0.033 ± 0.01^{d}
200 mg/kg extract + CCl_4	0.038 ± 0.03^{c}
400 mg/kg extract + CCl_4	0.040 ± 0.02^{c}

Data are expressed as mean ± SEM, of three determinations. Values with different superscripts vertically down the column are significantly different. ($P < 0.05$)

Key: CCl_4= Carbon tetrachloride (1.5 ml/kg)

Bioactive Compounds Found in Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra)*

A total of twenty six (26) compounds were identified from the GC-MS analysis of methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra)* exhibiting various phytochemical activities. The chemical constituents with their retention time (RT), molecular formula and molecular weight (MW) are presented in Table 2. The following bioactive compounds were found to be

present in the methanol leaf extract of *Chanca piedra*: 1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl] bis [2-ethyl-, N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine, Carbonic acid, 2-chloroethyl 2-methoxyethyl ester, Dimethyl palmitamine, 2-Dodecen-1-ol, 12-chloro-, Octane, 1-(ethenylthio)-, Cyclohexanone, 4-hydroxy-, 1H-Imidazole, 4,5-dihydro-2-methyl-, 2-Butene, 2-nitro-, 1,3-Benzodioxol-2-one, hexahydro-, cis-, Oxazole, 4,5-dihydro-2,5-dimethyl-, Benzeneacetic acid, 2-hydroxy-, methyl ester, 1-Allyl-cyclohexane-1,2-diol, 6,10,14-Trimethyl-pentadecan-2-ol, 10-Heneicosene (c,t), 2-Dodecanol, 3-Methyl-2-(2-oxopropyl) furan, Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methyl pentyl)-, 2-Heptadecenal, 7-Hexadecenal, (Z)-, 1-Decanol, 2-hexyl-, 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester), 1-Hexyl-2-nitrocyclohexane, Cholestan-3-ol, 2-methylene-, (3β,5α)-, 3-Methyl-2-(2-oxopropyl) furan, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and Phytol.

Table 2: Bioactive Compounds Found in Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra)*

S/No.	Compound Name	Formula	Molecular Weight	Retention Time (Min.)	Peak
1.	1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl-	$C_{27}H_{27}N_3$	393.22	8.62	2
2.	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	$C_{10}H_{24}NP$	189.16	9.82	3
3.	Carbonic acid, 2-chloroethyl 2-methoxyethyl ester	$C_6H_{11}ClO_4$	182.03	10.13	3
4.	Dimethyl palmitamine	$C_{18}H_{39}N$	269.31	10.13	4
5.	2-Dodecen-1-ol, 12-chloro-	$C_{12}H_{23}ClO$	218.14	12.05	4
6.	Octane, 1-(ethenylthio)-	$C_{10}H_{20}S$	172.13	12.66	4
7.	Cyclohexanone, 4-hydroxy-	$C_6H_{10}O_2$	114.07	12.66	4
8.	2-Butene, 2-nitro-	$C_4H_7NO_2$	101.05	12.05	6
9.	1,3-Benzodioxol-2-one, hexahydro-, cis-	$C_7H_{10}O_3$	142.06	12.66	6
10.	1H-Imidazole, 4,5-dihydro-2-methyl-	$C_4H_8N_2$	84.07	12.05	7
11.	Oxazole, 4,5-dihydro-2,5-dimethyl-	C_5H_9NO	99.07	13.37	8
12.	Benzene acetic acid, 2-hydroxy-, methyl ester	$C_9H_{10}O_3$	166.06	17.44	9
13.	6,10,14-Trimethyl-pentadecan-2-ol	$C_{18}H_{38}O$	270.30	17.97	10
14.	10-Heneicosene (c,t)	$C_{21}H_{42}$	294.33	18.55	11
15.	2-Dodecanol	$C_{12}H_{26}O$	186.20	18.55	11

16.	1-Allyl-cyclohexane-1,2-diol	$C_9H_{16}O_2$	156.12	21.11	12
17.	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	$C_{20}H_{40}$	280.31	21.11	12
18.	2-Heptadecenal	$C_{17}H_{32}O$	252.25	22.41	14
19.	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238.23	18.55	15
20.	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$	138.07	18.55	16
21.	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.26	29.79	22
22.	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	$C_7H_9FN_2O_2$	172.06	31.23	23
23.	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.17	32.02	24
24.	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400.37	32.02	24
25.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.31	32.25	25
26.	Phytol	$C_{20}H_{40}O$	296.31	32.25	25

Physicochemical Properties of the Bioactive Compounds Found in Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra)*

The results of the physicochemical properties of the bioactive compounds found in methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra)* are shown in Table 3. The phytoconstituents were screened for physicochemical properties based on their molecular weight (<500DA), number of HBA (≤10), number of HBD (≤5), MolLogP (≤5) and drug likeness.

Table 3: Physicochemical Properties of the Bioactive Compounds Found in Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra)*

S/No.	PubChem ID	Molecular Weight(≤500)	Number of HBA (≤10)	Number of HBD (≤5)	MolLogP (≤5)	Druglikeness
1.	CID_10798	84.1215	2	1	-0.388	3.7798
2.	CID_543562	99.1325	2	0	0.0802	1.1851
3.	CID_543706	114.143	2	1	0.4915	-2.7749
4.	CID_91692254	182.602	4	0	0.8751	-7.9385
5.	CID_534521	172.158	4	0	0.7781	-1.9363
6.	CID_534521	172.158	4	0	0.7781	-1.9363
7.	CID_697893	142.153	3	0	0.7777	-5.4682
8.	CID_545772	138.165	2	0	1.3531	-1.9776
9.	CID_89719	166.175	3	1	1.225	-6.8927
10.	CID_534396	156.224	2	2	1.3691	-10.136
11.	CID_283728	172.335	0	0	4.125	-20.988
12.	CID_546995	189.282	1	0	2.2356	-18.174
13.	CID_5364498	218.767	1	1	4.5716	-19.024
14.	CID_544017	213.320	3	0	3.1361	-21.133

15.	CID_25045	186.337	1	1	4.4994	-24.047
16.	CID_5364438	238.413	1	0	5.9476	-26.022
17.	CID_95337	242.445	1	1	6.176	-22.455
18.	CID_5363335	252.440	1	0	6.402	-27.622
19.	CID_530418	270.499	1	1	6.5175	-8.8795
20.	CID_41687	280.538	0	0	7.3351	-3.8753
21.	CID_5280435	296.537	1	1	7.4212	-3.7661
22.	CID_5366244	296.537	1	1	7.4212	-3.7661
23.	CID_5364553	294.564	0	0	9.3586	-24.054
24.	CID_16221	269.515	1	0	6.6323	-16.915
25.	CID_616782	393.532	3	2	6.0935	1.456

Key:
HBA = Hydrogen Bond Acceptors
HBD = Hydrogen Bond Donors
CID = Compound Identity

Pharmacokinetic Properties of the Bioactive Compounds Found in Methanol Leaf Extract of *Phyllanthus niruri* Linn. (*Chanca piedra)*

The pharmacokinetic properties of the bioactive compounds (toxicity) are presented in Table 4. The compounds were screened for the following properties which include: blood brain barrier (BBB), cytochrome P2D6 inhibitor, gastrointestinal absorption (GIA), mutagens, tumorigens, reproducibility and irritant.

Table 4: Pharmacokinetic Properties of the Bioactive Compounds Found in MethanolLeaf Extract of *Phyllanthus niruri*

S/No	Compound Name	BBB	CYP2D6 Inhibitor	GIA	Mutagens	Tumorigens	Reproducibility	Irritant
1.	1H-Imidazole, 4,5-dihydro-2-methyl-	No	Non Inhibitor	Low	None	None	None	None
2.	Oxazole, 4,5-dihydro-2,5-dimethyl-	No	Non Inhibitor	High	None	None	None	None
3.	Cyclohexanone, 4-hydroxy-	Yes	Non Inhibitor	High	None	None	None	None
4.	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	Yes	Non Inhibitor	High	None	None	None	None
5.	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	Yes	Non Inhibitor	High	None	None	None	None
6.	1,3-Benzodioxol-2-one, hexahydro-, cis-	Yes	Non Inhibitor	High	None	None	None	None
7.	3-Methyl-2-(2-oxopropyl)furan	Yes	Non Inhibitor	High	None	None	None	High
8.	Benzeneacetic acid, 2-hydroxy-, methyl ester	Yes	Non Inhibitor	High	None	Low	Low	None
9.	1-Allyl-cyclohexane-1,2-diol	Yes	Non Inhibitor	High	None	None	None	Low
10.	Octane, 1-(ethenylthio)-	Yes	Non Inhibitor	High	None	None	None	Low
11.	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	Yes	Non Inhibitor	High	None	None	None	None
12.	1-Hexyl-2-nitrocyclohexane	Yes	Non Inhibitor	High	None	None	None	None
13.	2-Dodecanol	Yes	Non Inhibitor	High	None	None	None	None
14.	1-Decanol, 2-hexyl-	Yes	Non Inhibitor	High	None	None	None	None
15.	2-Heptadecenal	No	Non Inhibitor	High	Low	High	None	Low
16.	6,10,14-Trimethyl-pentadecan-2-ol	No	Non Inhibitor	High	None	None	None	None
17.	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	No	Non Inhibitor	Low	None	None	None	None
18.	Phytol	No	Non Inhibitor	Low	None	None	None	None
19.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	No	Non Inhibitor	Low	None	None	None	None
20.	10-Heneicosene (c,t)	No	Non Inhibitor	Low	None	None	None	None
21.	Dimethyl palmitamine	No	Non Inhibitor	Low	None	None	None	High
22.	1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl-	No	Non Inhibitor	High	None	None	None	None

Key:

BBB – Blood Brain Barrier
CYP2D6 – Cytochrome P_{450}
GIA – Gastrointestinal Absorption

Docking Scores and Amino Acid Residues Involved in H-Bond Formation

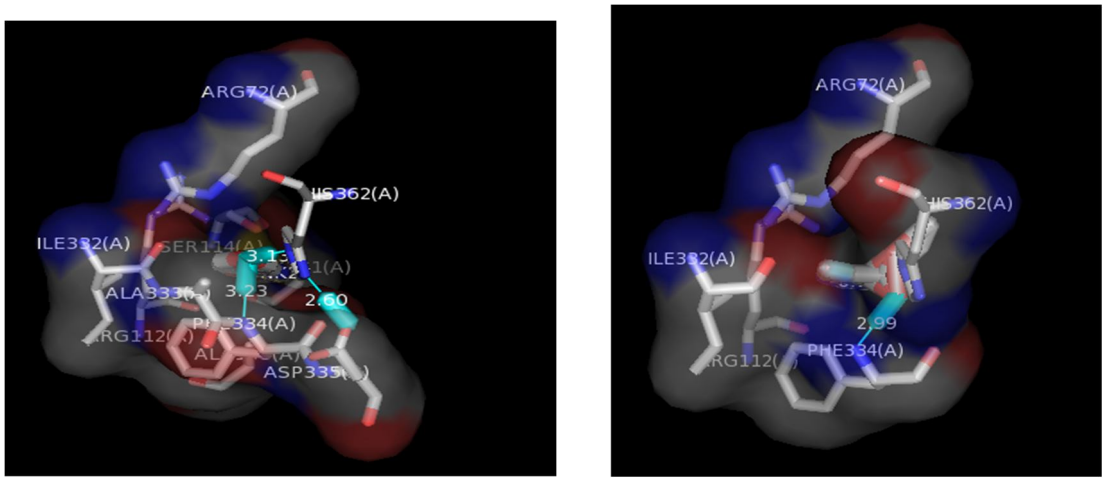
Molecular docking study of the bioactive compounds identified from methanol leaf extract of *Phyllanthus niruri* Linn. (*Chanca piedra)* was carried out on catalase enzyme with PDB code: 2CAG. Among the twenty five (25) compounds obtained from the GC-MS analysis of the plant, only ten (10) compounds with desirable properties were subjected to the docking analysis and found that cyclohexanone, 4-hydroxy- (CID_543706), 4-fluoro-1-methyl-5-carboxylic acid, ethyl(ester) (CID_534521), 1,3-benzodioxol-2-one, hexahydro-, cis-(CID_697893), 3-methyl-2-(2-oxopropyl) furan (CID_545772), benzeneacetic acid, 2-hydroxy-, methyl ester(CID_89719) were docked only in the allosteric site of catalase enzyme (2CAG) with docking scores of -7.16, -5.99, -6.17, -5.39, and -6.48 kcal/mol respectively. Similarly, 1-allyl-cyclohexane-1,2-diol (CID_534396), 1-hexyl-2-nitrocyclohexane (CID_544017), 2-dodecanol (CID_25045), 6,10,14-trimethyl-pentadecan-2-ol (CID_530418), and 1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID_616782) were however docked only in the allosteric site of catalase enzyme (2CAG) with docking scores of -5.58, -7.28, -5.42, -5.32 and -9.88 kcal/mol respectively.

Based on the docking results, out of the top ten (10) compounds, 1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID: 616782) had the best binding energy of -9.88 kcal/mol, and was observed to interact with His362 (distance = 2.89Å) and Asp335 (distance = 2.60Å), while 1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID_530418). (CID_530418) had the lowest binding energy of -5.32 kcal/mol. Following -Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl-, Cyclohexanone, 4-hydroxy- (CID_543706) and 1-Hexyl-2-nitrocyclohexane (CID_544017) showed the next highest binding affinity of –7.16 and-7.28 kcal/mol respectively. Cyclohexanone, 4-hydroxy- (CID_543706) formed hydrogen bond with Phe334 (distance = 3.23Å), His362 (distance = 3.13Å), and Asp335 (distance = 2.60Å), while 1-Hexyl-2-nitrocyclohexane (CID_544017) was found to

interact with Asp335 (distance = 2.60Å), His362 (distance = 3.20Å), Phe334 (distance = 2.73Å) and Arg365 (distance = 2.83Å). The top ten (10) compounds with their binding affinities values are shown in Table 5.

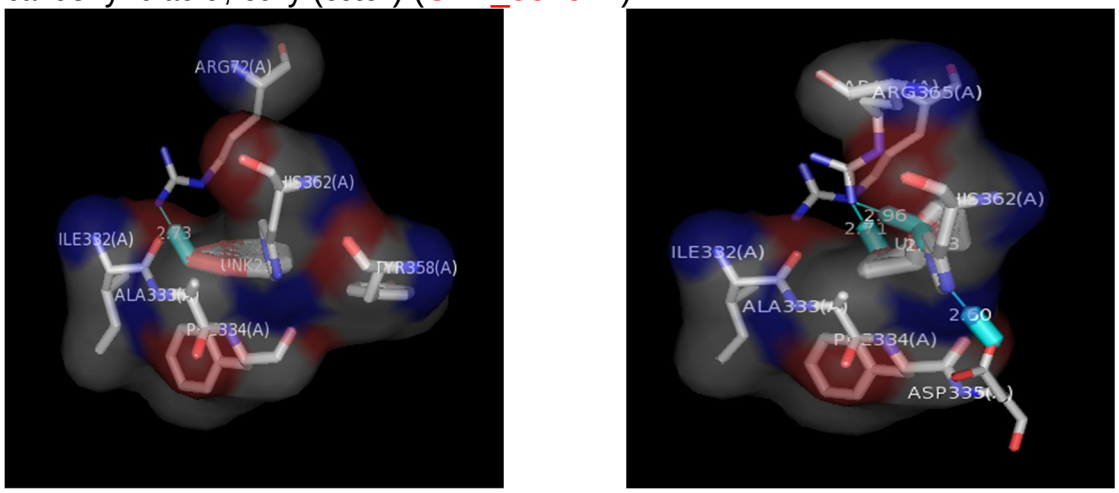
Table 5: Docking Scores and Amino Acid Residues Involved in H-Bond Formation

S/No.	Compound Id	Docking Score (kcal/mol.)	Residues Involve in H-bonds	Distance (Å)
1.	CID_543706	-7.16	Phe334	3.23
			His362	3.13
			Asp335	2.60
2.	CID_534521	-5.99	Phe334	2.99
3.	CID_697893	-6.17	Arg72	2.73
4.	CID_545772	-5.39	Arg72	2.71
			Arg365	2.96
			His362	2.78
			Asp335	2.60
5.	CID_89719	-6.48	Val74	3.17
			Val74	2.62
			Arg365	2.58
			His362	2.89
			Asp335	2.60
6.	CID_534396	-5.58	Phe334	3.20
			Asp335	2.60
			His362	2.86
			Arg365	2.99
7.	CID_544017	-7.28	Asp335	2.60
			His362	3.20
			Phe334	2.73
			Arg365	2.83
8.	CID_25045	-5.42	His362	2.99
			Asp335	2.60
			Arg365	3.15
9.	CID_530418	-5.32	Glu71	2.60
10.	CID_616782	-9.88	His362	2.89
			Asp335	2.60



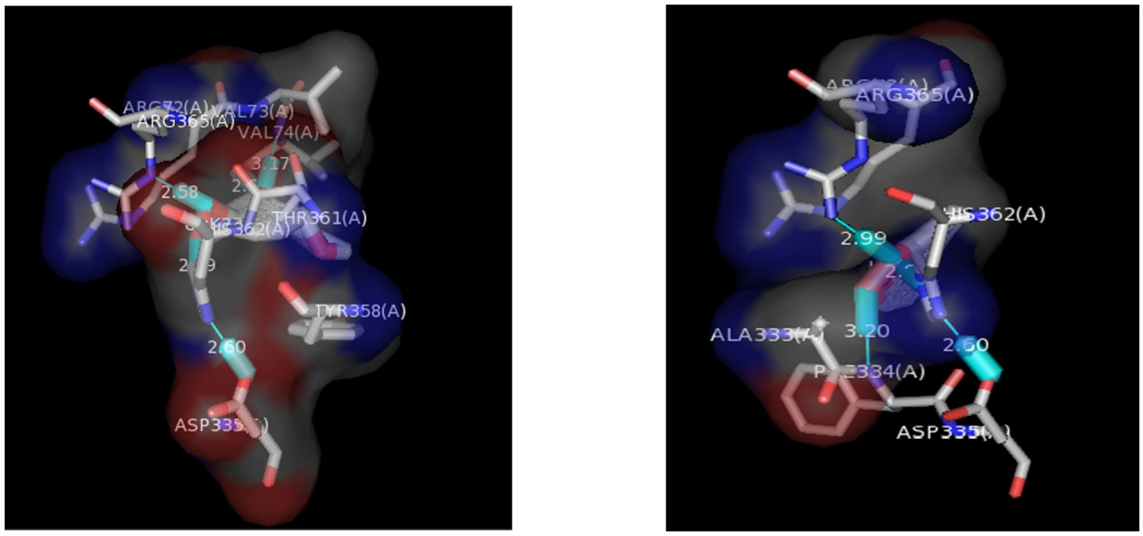
(a) (b)

Fig. 1. Ligand Interactions of catalase (2CAG) enzyme with: (a) Cyclohexanone, 4-hydroxy- (CID_543706) and (b) 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester) (CID_534521).



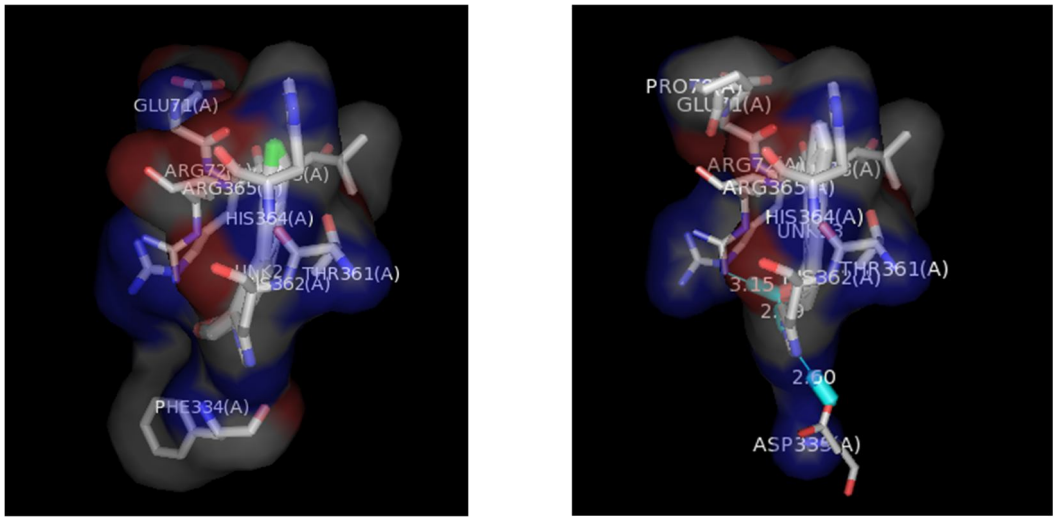
(c) (d)

Fig. 2. Ligand Interactions of catalase (2CAG) enzyme with: (c) 1,3-Benzodioxol-2-one, hexahydro-, cis- (CID_697893) and (d)3-Methyl-2-(2-oxopropyl)furan (CID_545772).



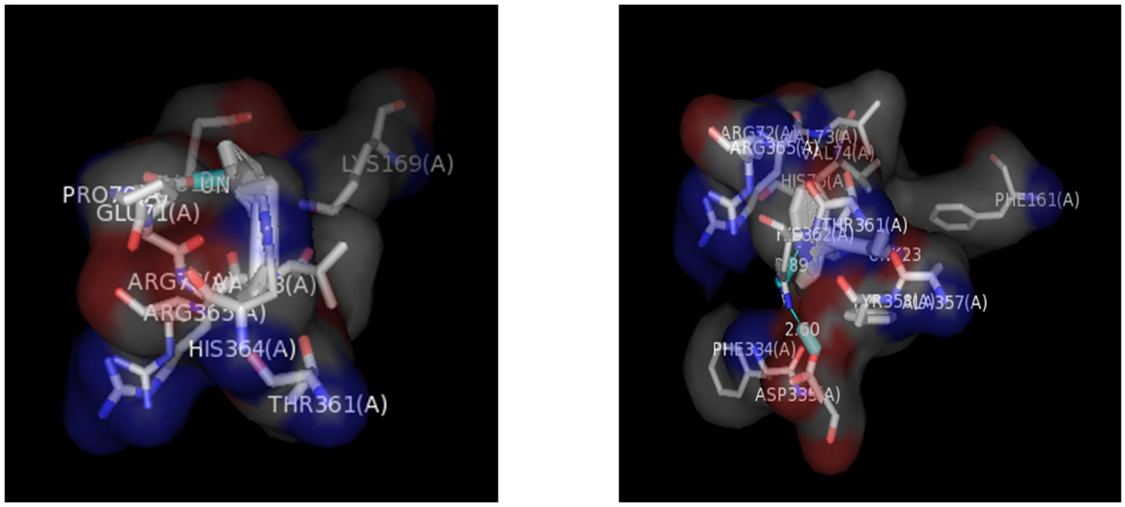
(e) (f)

Fig. 3. Ligand Interactions of catalase (2CAG) enzyme with: (e) Benzeneacetic acid, 2-hydroxy-, methyl ester(CID_89719) and (f)1-Allyl-cyclohexane-1,2-diol(CID_534396).



(g) (h)

Fig. 4. Ligand Interactions of catalase (2CAG) enzyme with: (g) 1-Hexyl-2-nitrocyclohexane (CID_544017)and (h)2-Dodecanol (CID_25045).



(i) (j)

Fig. 5. Ligand Interactions of catalase (2CAG) enzyme with: (i) 6,10,14-Trimethyl-pentadecan-2-ol (CID_616782) and (j)1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID_530418).

DISCUSSION

The *in vivo* antioxidant activity assay (catalase) revealed that there was a significant decrease in the catalase activity in the negative control group as compared to normal control group. Treatment with the plant extract significantly ($P<0.05$) increased the levels very close to that of the normal control group at different doses of 100 mg, 200 mg and 400 mg/kg body weights for catalase. These results showed that the methanol leaf extract of Chanca piedra *(Phyllanthus niruri* Linn.*)* has a significant effect on the activity of catalase (Table 1). Catalase exists in both eukaryotic and bacterial cells. Most of them are located in an oxidative particle of all types of mammalian cells except red blood cells where various hydrogen peroxide (H_2O_2) oxidases were created. Since H_2O_2 acts as a substrate for a specific reaction that generates highly hydroxyl radical, it is believed that the primary role of catalase in cellular antioxidant defense mechanisms is to reduce the accumulation of hydrogen peroxide (H_2O_2) (Ho *et al.,* 2004). The role of catalase in protecting cells and tissues from oxidation has been studied extensively. The over expression of catalase makes cells more resistant to H_2O_2 toxicity and oxidative-mediated damage (Ho *et al.,* 2004). To combat the destructive effects of ROS and make oxidative cellular metabolism possible, aerobic

organisms developed protective antioxidant enzymes such as catalase, superoxide dismutase, peroxiredoxin and glutathione peroxidase, among which, catalase is a well-known crucial enzyme to scavenge H_2O_2. This haem-containing protein is the most efficient enzyme and can decompose millions of hydrogen peroxide (~107 M/Sec) molecules every second into molecular oxygen and water without the production of free radicals (Young and Woodside, 2001). Various researchers reported a significant ($P<0.05$) decrease in the activities and expression of antioxidant enzymes such as catalase, in liver of animals treated with carbon tetrachloride (CCl_4) compared to normal control groups of experimental animals (Hamed *et al.,* 2016, Hafez *et al.,* 2014 and Zhang *et al.,* 2013).

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes (Lengauer and Rarey, 1996; Kitchen *et al.,* 2004). The GC-MS analysis of methanol leaf extract of *Chanca piedra* showed that twenty six (26) different compounds were present in the plant extract (Table 2). The bioactive compounds obtained from the *Phyllanthus niruri* sample were screened for physicochemical properties based on their molecular weight 500 DA, number of hydrogen- bond acceptors≤ 10, number of hydrogen bond donors 5, and logP5 (Table 3). It was noted that all the twenty five compounds had desirable physicochemical properties.

The pharmacokinetic properties of the individual compounds (toxicity) were determined. The properties predicted include: tumorigenicity, reproducibility, and irritability. Based on the results of the pharmacokinetics twenty two out of the twenty six compounds possessed desirable pharmacokinetic properties except for 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester), 2-Dodecanol and 6,10,14-Trimethyl-pentadecan-2-ol were found to be highly toxic (Table 3). The docking analysis for those bioactive compounds was examined to ascertain their binding affinities to the target protein (catalase with resolution of 2.70Å) as antioxidants. Catalase is an enzyme found in almost all living organisms that are exposed to oxygen which

catalyzes the breakdown of H_2O_2 (hydrogen peroxide) to O_2 (oxygen) and H_2O (water). It is an essential enzyme that acts in protecting the cell from oxidative stress by ROS. The binding interaction and conformation of each phytoconstituent with the target was ranked based on lowest energy and lowest RMSD, respectively, according to previous studies that indicated that the lower the binding energy score obtained, the better the ligand–protein binding affinity (Imana *et al.,* 2020).

The results of the docking studies of the identified phytomolecules with catalase enzyme (2CAG) revealed that these compounds possessed distinct affinities with the catalase enzyme, varying from -9.88 kcal/mol (6,10,14-trimethyl-pentadecan-2-ol (CID_530418) to -5.32 kcal/mol (1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl]bis[2-ethyl- (CID_616782) (Table 3). The results however, showed that 6,10,14-trimethyl-pentadecan-2-ol had best binding affinity for the enzyme (-9.88 kcal/mol) to act as antioxidant.
The interactions of the compound with the highest affinity (6,10,14-trimethyl-pentadecan-2-ol) revealed the presence of hydrogen bond interaction as H-acceptor with His362 and H-donor with Asp335 amino acid residues (Fig. 5). Similarly, the 3-D interactions showed direct involvement of hydrophobic interactions with Phe334 and Val74 amino acid residues. Moreover, the *in vitro* and *in vivo* antioxidant as well as the hepatoprotective effect demonstrated by the leaf extract of *Phyllanthus niruri* might be attributed to bioactive substances present in the plant such as 6,10,14-trimethyl-pentadecan-2-ol which displayed potential hydrogen bond interactions with catalase enzyme (2CAG) *in silico*. Finally, the phytocompounds associated with *Phyllanthus niruri* plant might be regarded as valuable sources for the large-scale industrial production of antioxidants, given their potential for medicinal use and lack of side effects.

CONCLUSION

In conclusion, the antioxidant activity of Chanca piedra *(Phyllanthus niruri* Linn.*)* was evaluated using *in vivo* assay suggesting that the plant methanol leaves extract have antioxidant activity in acute liver disease in animals, however, the results was validated using *in silico* molecular docking analysis and found that the active compounds of the plant interacts with the allosteric site of catalase enzyme suggesting its plausible role in exhibiting anti-oxidant

effect. This study, therefore, suggests that the phytoconstituents associated with Chanca piedra *(Phyllanthus niruri* Linn.*)* plants might be regarded as valuable source of antioxidants, given their potentials for medicinal use and lack of side effects.

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