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ANTI-ANAEMIC EFFECT OF *MANIHOT ESCULENTA* LEAVES ON PHENYLHYDRZINE INDUCED ANAEMIA, SERUM BIOCHEMISTRY; GCMS AND MOLECULAR DOCKING STUDY IN RATS

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ABSTRACT

Anti-anaemia effect, GCMS analysis and molecular docking study of ethanol extract of *Manihot esculenta* leaves was studied on Phenylhydrazine induced anaemia using Wistar rats as animal model. Twenty five (25) rats were used for the research, they were grouped into five (5) of five (5) rats each. Group 1 was the negative control group and received distilled water. Groups 2 was the anaemic non treated group, 3, 4 and 5 were the treatment groups received 250, 500 and 1000 mg/kg body weight of the *M. esculenta* extract respectively. The rats were dosed for 14 days, thereafter were sacrificed and blood collected by cardiac puncture for analysis. The effect of *M. esculenta*, extract was checked on haematological parameters and serum enzymes activities. All results in treatment groups were compared with the untreated anaemic group at statistical confidence of 95% ($p<0.05$). The normal control group saved as reference point. The result shows that *M.esculenta* restored the blood cells in a dose dependent manner compared with those untreated anaemic group. The leukogram indicated lymphopenia, neutrophilia, monocytosis and eosinophilia. AST, ALP and ALP were within normal reference range indicating liver safty while bilirubin, urea and creatinine showed no kidney problem. Total protein was recovered in dose dependant manner compared with untreated anaemic group. Gas chromatogram showed 34 indicating 34 phytocompounds present in *M.esculenta* and the three most abundant compounds were Oleic acid, Squalene and 2-Methyl-Z,Z-3,13-octadecadienol. The lead compounds had good absorption, distribution metabolism and excretion with no toxicity and can be good candidate for drug production.

INDEX TERMS: Anaemia, *Manihot esculenta*, Transaminases, Phosphatases, GC-MS, Molecular docking.

INTRODUCTION

Plant is a major source of compounds with nutrition and health and pharmaceutical potentials. Natural bioactive products are recognized for their beneficial effects in various human and animal diseases. They are of great interest to researchers due to their broad structural diversity and wide range of biological activities, Atanasov *et al* (2021). Therefore, cassava is mainly grown as a root crop for human consumption as a source of carbohydrates. In some regions, cassava leaves are consumed as green vegetables and are used in traditional medicines. Unlike roots, cassava leaves are an interesting source of nutrients such as proteins, vitamin A, vitamin C and fibers (Saragih *et al* 2020; FAO, 2022). *Manihot esculenta* is a shrub that grows to about 5 m high and is part of the Euphorbiaceae family, considered one of the most complex and diverse angiosperm families, with about 300 genera and 8000 species Ramalho *et al* (2018). As a function of the species studied, they are characterized by the presence of several secondary metabolites such as terpenoids, flavonoids and polyphenolic classes of compounds (Salehi *et al* 2019; Afoakwa, 2021; Lancaster *et al*, 1983). They contribute to various therapeutic properties, including anti-arthritic and anti-inflammatory activities Bani *et al*, (2000) as well as inhibition of digestive enzymes including α-amylase, glucosidase and lipases. Anaemia is a blood disorder in which the blood has a reduced ability to carry

oxygen due to a lower than normal number of red blood cells, a reduction in the amount of hemoglobin or hemoglobin abnormalities. (Janz *et al*, 2013; Ng *et al*, 2019) When anaemia comes on slowly, the symptoms are often vague, such as tiredness, weakness, shortness of breath, headaches, and a reduced ability to exercise, Janz *et al*,(2013) When anemia is acute, symptoms may include confusion, feeling like one is going to pass out, loss of consciousness, and increased thirst, Janz *et al*,(2013). Anaemia must be significant before a patient becomes noticeably pale, Janz *et al*,(2013) Symptoms of anaemia depend on how quickly hemoglobin decreases,(Ng *et al*,2019; Payne *et al*, 2015). Anaemia can be caused by blood loss, decreased red blood cell production, and increased red blood cell breakdown,.(Janz *et al*,2013) Causes of decreased production include iron deficiency, vitamin B_{12} deficiency, thalassemia and a number of bone marrow tumors, Janz *et al*,(2013) Causes of increased breakdown include genetic disorders such as sickle cell anemia, infections such as malaria, and certain autoimmune diseases, (Janz *et al*,2013; Payne *et al*, 2015).Anaemia can also be classified based on the size of the red blood cells and amount of hemoglobin in each cell, Janz *et al*,(2013). If the cells are small, it is called microcytic anaemia; if they are large, it is called macrocytic anaemia; and if they are normal sized, it is called normocytic anaemia,, Janz *et al*,(2013). Anaemia is the most common blood disorder, affecting about a fifth to a third of the global population.(Janz *et al*,2013;Vos *et al*. 2012).; Peyrin-Biroulet, 2015). Iron-deficiency anemia affects nearly 1 billion people, Vos *et al*. (2012; Qaseen *et al* 2013). In 2013, anemia due to iron deficiency resulted in about 183,000 deaths – down from 213,000 deaths in 1990. This condition is most prevalent in children, WHO, (2022) with also an above average prevalence in elderly, Janz *et al*,(2013) and women of reproductive age especially during pregnancy,Vos *et al*. (2012; WHO, 2021).Anaemia is one of the six WHO global nutrition targets for 2025 and for diet-related global targets endorsed by World Health Assembly in 2012 and 2013. Efforts to reach global targets (contribute to reaching Sustainable Development Goals (SDGs), WHO, (2016) with anaemia as one of the targets in SDG 2 for achieving zero world hunger, (Payne *et al*, 2015).The compounds in *M. esculenta* were separated using Gas Chromatography Mass Spectrometry (GCMS). Chromatography has been and will continue to be the most effective technique for isolating and purifying all types of biomolecules and plant compounds (Igwe *et al* 2016, 2016a). It is widely used as an analytical tool example GCMS, (Igwe *et al* 2016, 2016a; James and Nordby, (2003). Many researchers have used GCMS to separate compounds in plants (Igwe *et al* 2020; Ikpeazu *et al* 2020; Otuokere, 2016). This research is therefore designed to check the anti-anaemic potentials, serum biochemistry, haematology and thereafter dock the most abundant compound separated in *M. esculenta* with α-amylase (1B2Y) complexes to establish its binding energy and drug likeness prediction. Figure 1 shows the picture of *M. esculenta.*

This study was designed to examine changes in activities of serum enzymes, GCMS and molecular docking study of ethanol extract of *M. esculenta* and toinvestigate the haematopoetic potential of the leaf extract on phenylhydrazine induced haemolytic anaemia in albino Wistar rats. It is therefore important to document the bioactive activities of this plant hence this research. Fig 1 shows the picture of *Manihot esculenta* leaves.

Fig. 1: Picture of *Manihot esculenta* leaves.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *M. esculenta*, were collected from Ohafia, 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 *M. esculenta*, were shade dried for 10 days and pulverized to a coarse powder using mechanical grinder (Corona-Landers C 1A SA). The plant extract was prepared using Soxhlet method described by (Jensen 2007). Thirty five grams (45g) of coarse powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at 70^0C throughout the extraction period of 48 hours. At the end of the extraction period the extract was concentrated using hot air oven at 30^0C to obtain dried extract which was weighed and kept in a well labelled sterile specimen bottle.

Different doses of 250, 500 and 1000 mg/kg body weight was prepared and administered to rats in group 2, 3, and 4 respectively. These doses were calculated from the stock solution dissolved in distilled water.

Haematology and Biochemical Investigation

Haematological investigations performed by manual methods include red blood cell count (RBC), white blood cell count (WBC) Differential count (DC) Hemoglobin concentration (Hb), packed cell volume (PVC) or hematocrit as described by Cole (1986).

PCV was measured by the micro-hematocrit method using capillary tubes while RBC and WBC were measured manually using an improved Neubauer counting chamber. The differential count was measured manually using a thin blood film stained with Leizhman stain. Haemoglobin concentrations was determined by cyanomathemoglobin method, Kachmar (1970).

Using RBC, PCV and Hb, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Lewis *et al*., 2006; Igwe *et al*., 2019; Hoffbrand and Moss, 2011).

$$MCV = \frac{PCV}{RBC} \times 10 \text{ fl}$$

$$MCH = \frac{Hb}{RBC} \times 10 \text{ pg}$$

$$MCHC = \frac{Hb}{PCV} \times 100 \text{ g/dl}$$

[Lewis *et al* 2006, Hoffbrand and Moss 2011, Igwe *et al* 2019a].
Biochemical investigation was performed using ELISA reagent kits. The measure included alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), determined by the method of (Reitman and Frankel, 1957). Using serum enzyme levels to determine liver and kidney state Klein *et al*., (1960), Urea by (Fawcett and Scott, 1960) and Creatinine by (Blass *et al*., 1996). Total protein and Albumin were determined by the Biuret method as described by Lubran (1978). Samples were analyzed immediately to avoid artifactual changes (Ihedioha and Onwubuche, 2007).

Experimental Animals
Albino rats (130 to 250 g) 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 rats were acclimatized and fed *ad libitum.*

Induction of Anaemia with Phenylhydrazine (PHZ)
This was done according to the modified method described by Ihedioha and Onwubuche (2007). Haemolytic anaemia was induced in the rats intraperitoneally with 2.5% phenyl hydrazine hydrochloride (Fisher Scientific Company, New Jersey, USA) at a dose of 30 mg/kg body weight. The anaemia was maintained by the administration of 15 mg/kg body weight of 2.5% phenylhydrazine hydrochloride at interval of 3 days, for the duration of the experiment.

Experimental Design
Twenty five (25) rats were used for the research, they were grouped into five (5) of five (5) rats each. Group 1 was the normal control group and was administered distilled water. Groups 2 was anaemic non treated. Groups 3, 4 and 5 were the treatment groups which received 250, 500 and 1000 mg/kg body weight of the *M. esculenta* extract respectively. The rats were dosed for 14 days, thereafter was sacrificed by cardiac puncture and blood collected for analysis. All results in treatment groups were compared with the untreated anaemic group at statistical confidence of 95% ($p<0.05$).The normal control group saved as reference point.

Gas Chromatography - Mas Spectrometry (GCMS) 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 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 activin receptor type IIB (ActRIIB) and prominent compounds
ActRIIB (PDB ID: 2QLU) was obtained from the RCSB Protein Databank. Water molecules and the substrate ligand (adenine) were removed using Molecular Molegro viewer software. The PDB of three most abundant compounds; oleic acid, squalene and 2-Methyl-Z,Z-3,13-octadecadienolwere downloaded from PubChem. They

have been abbreviated as OLA, SQL and MOD respectively.

ADMET properties

The ADMET profile of the lead Chemicals were predicted using Admet SAR online server (Cheng *et al.*,2012).

Docking protocol

OLA, SQL MOD and ferrous ammonium citrate (FAC) were loaded onto Pyrx virtual screening tool (Dallakyan and Olsonn, 2015). The energies were minimized and converted to PDBQT format. Ligands screened in the second round were energy minimised 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 PLIP server (Adasme *el al*., 2021).

RESULTS

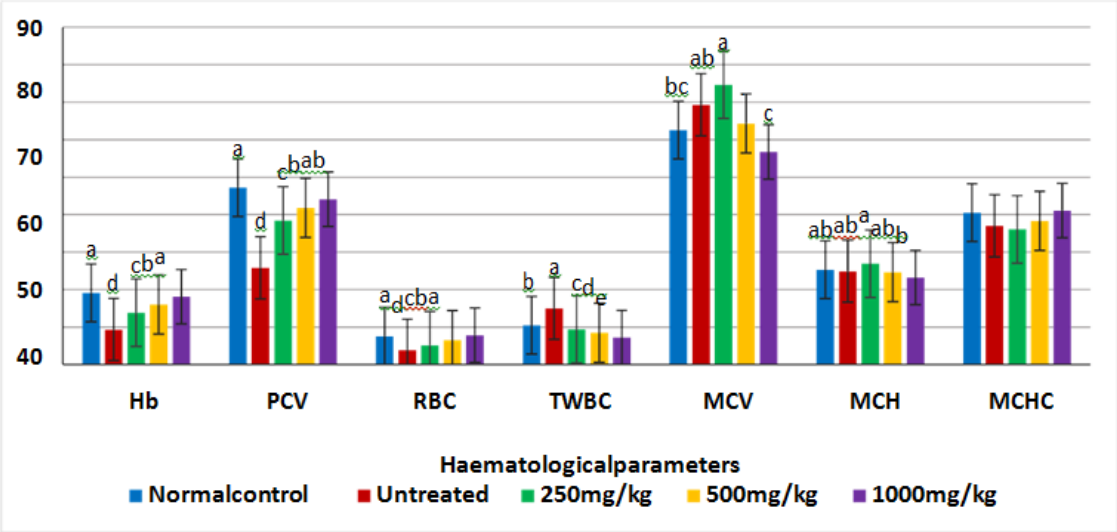


Figure 2: Effect of *Manihot esculenta* extract on the haematological parameters of PHZ induced anaemia in Wistar rats.

Valuesare presentedasmean±S.E(Standarderror).Differentsuper scriptlettersalong treatment groups represent significant (p<0.05).

Hb(g/dl)

Normal control: 19.12±0.55^{a}; **Untreated anaemic**9.36±0.46^{d};**250 mg/kg:** 13.86±0.06^{c};**500 mg/kg:** 16.02±0.29^{b};**1000 mg/kg:** 18.10±0.14. Hb was restored compared with untreated anaemic group with normal control as check for recovery.

PCV (%)

Normal control:47.20±1.39^{a}; **Untreated anaemic**28.20±0.91^{c};**250 mg/kg:**38.46±0.53^{c};**500 mg/kg:** 6.52±0.22^{b};**1000 mg/kg:** 44.10±0.55^{ab}. PCV was restored compare with untreated anaemic group and normal control as check for recovery.

RBC ($\times10^{6}$mm^{3})

Normal control: 7.56±0.15^{a}; **Untreated anaemic** 3.80±0.29^{d};**250 mg/kg:** 5.16±0.11^{c};**500 mg/kg:** 7.26±0.05^{b};**1000 mg/kg:** 7.82±0.26^{a}. RBC was restored compare with untreated anaemic group and normal control as check for recovery.

TWBC ($\times10^{3}$mm^{3})

Normal control: 10.48±0.36^{b}; **Untreated anaemic** 15.00±0.28^{a};**250 mg/kg:** 8.46±0.08^{d};**500 mg/kg:** 8.46±0.08^{d}; **1000 mg/kg:** 7.20±0.10^{e}. Affected TWBC (leukocytosis) was restored compare with untreated anaemic group and normal control as check for recovery.

MCV(fl): Normal control 62.56±2.45^{bc}:; **Untreated anaemic**69.31±6.17^{ab};**250 mg/kg:**74.68±1.93^{a};**500 mg/kg:** 64.33±1.81^{abc};**1000 mg/kg:**56.73±2.54^{c}. There was no significant change on mean corpuscular volume of the RBC mass compared with untreated anaemic group and normal control.

MCH (pg): Normal control 25.30±0.71^{ab}:; **Untreated anaemic**24.92±1.08^{ab};**250 mg/kg:**26.90±0.49^{a};**500 mg/kg:** 24.65±0.68^{ab};**1000 mg/kg:**23.23±0.69^{b}. There was no significant change on mean corpuscular haemoglobin compared with untreated anaemic group and normal control.

MCHC (g/dl): Normal control 40.54±0.85:; **Untreated anaemic**37.02±3.49;**250 mg/kg:** 36.06±0.54;**500 mg/kg:** 38.33±0.43;**1000 mg/kg:** 41.08±0.82. There was no significant change on mean corpuscular haemoglobin concentration compared with untreated anaemic group and normal control.

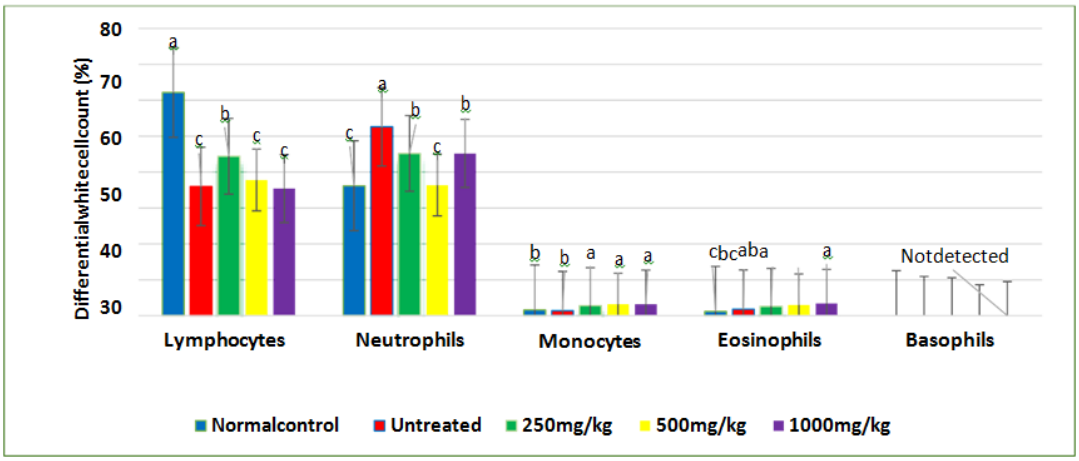


Figure 3: Effect of *Manihot esculenta* extract on differential white cell count of PHZ induced anaemia in Wistar rats.

Values are presented as mean ± S.E (Standard error). Different superscript letters along treatment groups represent significant (p<0.05) differences.
Lymphocytes (%): Normal control 62.20±0.73^{a}:; **Untreated anaemic**36.00±0.70^{c};**250 mg/kg:** 44.40±1.28^{b};**500 mg/kg:** 37.80±3.80^{c};**1000 mg/kg:** 35.40±2.08^{c}. The extract induced lymphopenia as the dose increased.
Neutrophils (%): Normal control 36.20±1.28^{c}:; **Untreated anaemic**52.60±2.50^{a};**250 mg/kg:** 45.20±0.73^{b};**500 mg/kg:** 36.40±2.61^{c};**1000 mg/kg:** 45.20±1.39^{b}. The extract induced neutrophilia as the dose increased.
Monocytes (%): Normal control :1.60±0.24^{b}; **Untreated anaemic**1.40±0.24^{b};**250 mg/kg:**2.80±0.48^{a}; **500 mg/kg:** 3.20±0.20^{a};**1000 mg/kg:** 3.20±0.48^{a}. The extract induced monocytosis as the dose increased.
Eosinophils (%): Normal control: 1.20±0.20^{c}; **Untreated anaemic** 1.80±0.37^{bc};**250 mg/kg:** 2.60±0.24^{ab};**500 mg/kg:** 2.60±0.24^{ab};**1000 mg/kg:** 3.40±0.24^{a}. The extract induced mild eosinophilia as the dose increased.

Basiphils (%): Normal control:0.00±0.00; **Untreated anaemic** 0.00±0.00;**250 mg/kg:**0.00±0.00 ;**500 mg/kg:** 0.00±0.00 ;**1000 mg/kg:** 0.00±0.00, within normal reference range.

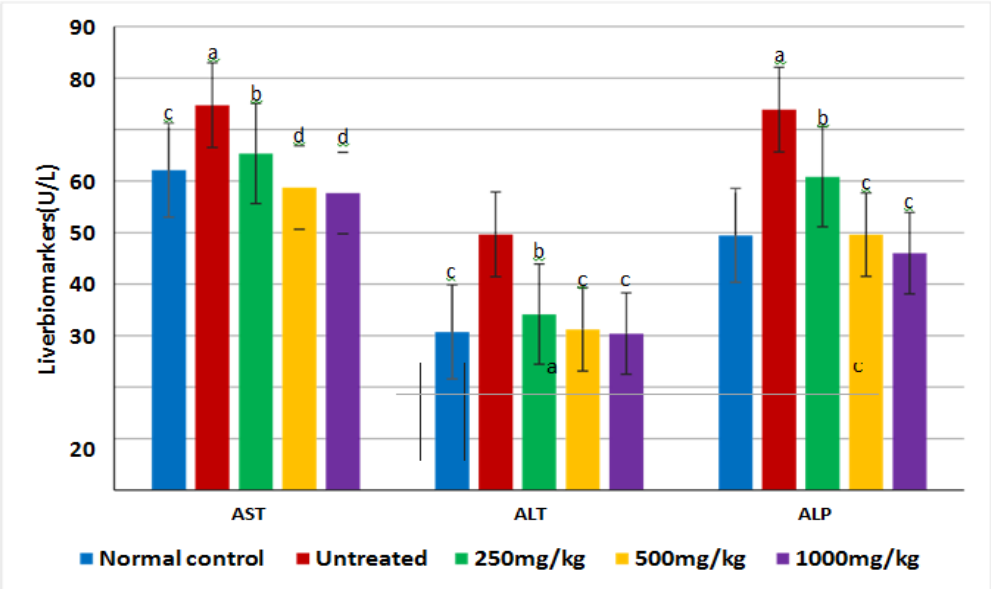


Figure 4: Effect of *Manihot esculenta* extract on serum enzymes of PHZ induced anaemia in Wistar rats.

Values are presented as mean ± S.E (Standard error). Different superscript letters along treatment groups represent significant (p<0.05) differences.
AST (U/L)**: Normal control:** 62.12±0.87^{c}**: Untreated anaemic**; 74.74±1.37^{a};**250 mg/kg:**65.36±0.83^{b};**500 mg/kg:** 58.74±1.14^{d};**1000 mg/kg:**57.68±0.99^{d}.

ALT (U/L)**: Normal control:** 30.72±0.56[c]**: Untreated anaemic**; 49.66±0.29[a];**250 mg/kg:** 34.18±1.22[b];**500 mg/kg:** 31.22±0.43[c];**1000 mg/kg:**30.40±0.81[c].

ALP (U/L)**: Normal control:** 49.48±0.31[c]**: Untreated anaemic**; 73.88±1.35[a];**250 mg/kg:** 60.86±0.41[b];**500 mg/kg:** 49.60±2.37[c];**1000 mg/kg:** 46.00±1.09[c].

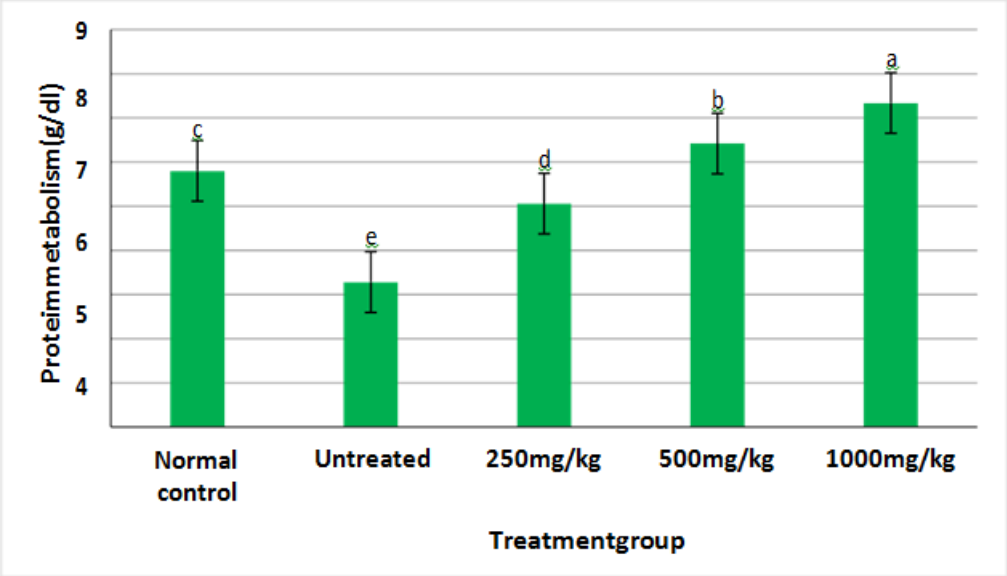


Figure 5: Effect of *Manihot esculenta* extract on Serum Total Proteinon PHZ induced anaemia in Wistar rats.

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

Total Protein (mg/kg): Normal control: 5.80±0.08[c]**: Untreated anaemic**; 3.28±0.09[e];**250 mg/kg:** 5.06±0.08[d]; **500 mg/kg:** 6.42±0.12[b];**1000 mg/kg:**7.34±0.18[a]

The result of total protein showed significant ($p<0.05$) reduction caused by PHZ agent, while treatment with the extract did not only restored the serum mean level to normal level, but also significantly($p<0.05$) elevated the TP serum level asthe extract doses were increased (Figure5). This is an indication that *M. esculenta* extract enhances serum protein metabolism.

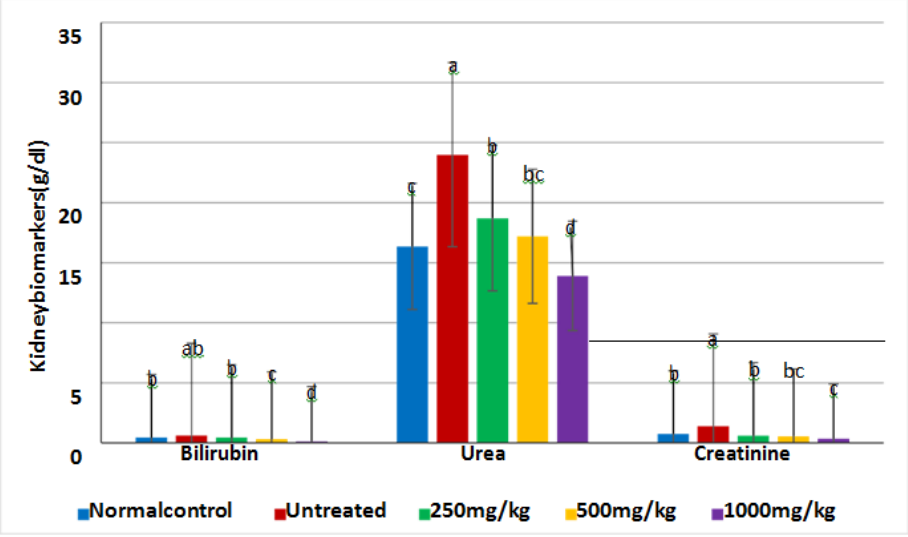


Fig. 6: Effect of *Manihot esculenta* extract on Serum kidney biomarkers on PHZ induced anaemia in Wistar rats.

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

Bilirubin (mg/dl)**: Normal control:** 0.44±0.01[b]**: Untreated anaemic**; 0.62±0.02[ab];**250 mg/kg:** 0.44±0.02[b];**500 mg/kg:** 0.32±0.01[c]; **1000 mg/kg:** 0.14±0.15[d].

Urea (mg/dl)**: Normal control:** 16.36±0.58[c]**:Untreated anaemic**; 24.00±0.70[a];**250 mg/kg:** 18.72±0.80[b];**500 mg/kg:** 17.20±0.68[bc]; **1000 mg/kg:** 13.90±0.13[d].

Creatinine (mg/dl)**: Normal control:** 0.75±0.06[b]**: Untreated anaemic**; 1.41±0.15[a];**250 mg/kg:** 0.61±0.01[b]; **500 mg/kg:** 0.56±0.00[bc]; **1000 mg/kg:** 0.35±0.17[c].

Similarly, Fig 6 showed nephro toxicity of PHZ agent on Wistar rats, with significant ($p<0.05$), release of serum kidney biomarkers (Bilirubin, urea and creatinine) as shown in the untreated group compared with the normal serum level, while the mean values in the extract treated groups suggest both ameliorative and nephron-protective ability of the *M. esculenta* extract as the doses were increased (dose dependently).

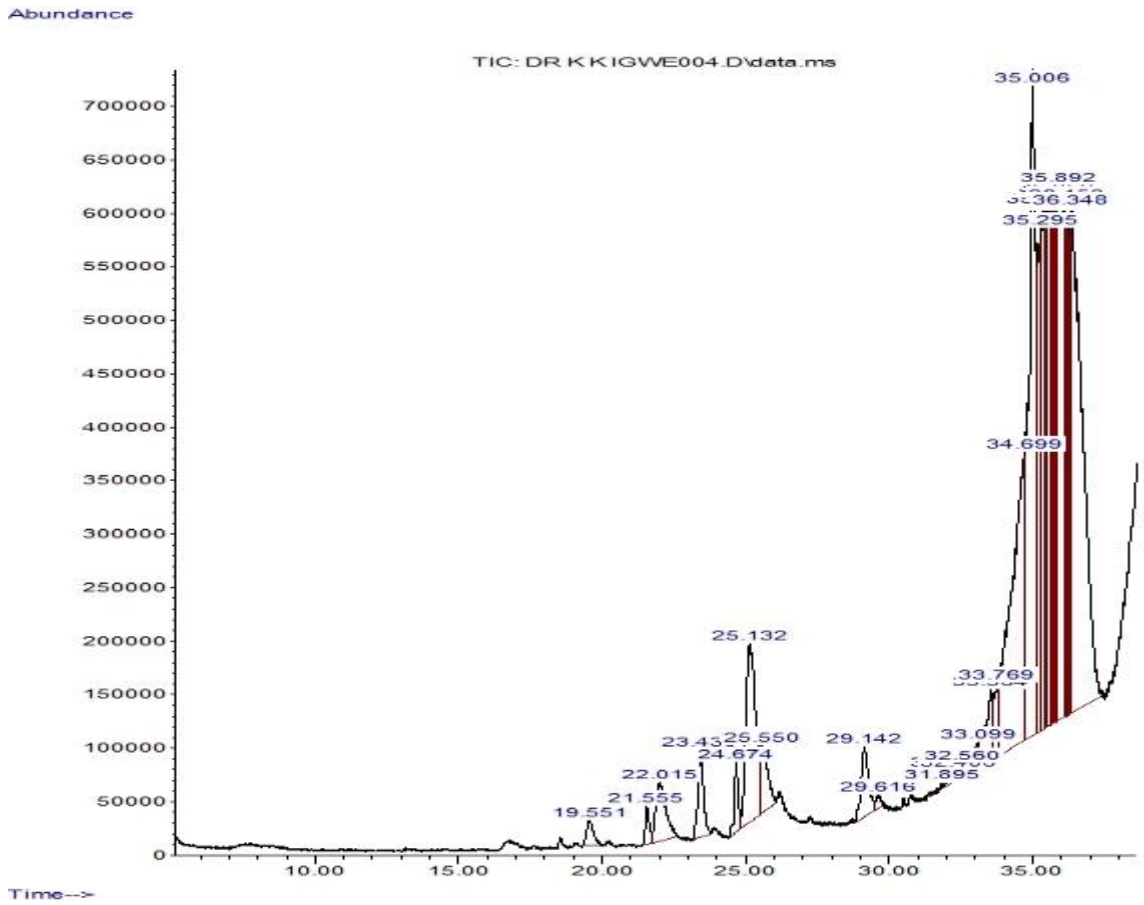


Figure 7: Gas Chromatogram of ethanol extract of *Manihot esculenta.*

The gas chromatogram of ethanol extract of *Manihot esculenta* revealed a total of 34 peaks corresponding to bioactive compounds. Figure 7 depicts the gas chromatogram. The compounds have been listed in Table 1. Structures of the three most abundant compounds isolated from GC-MS of ethanol extract of *M. esculenta* are presented in Figure 8. ADMET Parameters prediction of the lead compounds are listed in Table 2.Table 3 shows the docking score (Kcal/mol) of *M. esculenta* phytocompounds/ActRIIB complexes. PLIP molecular docking interactions of ActRIIB with *M. esculenta* phytocompounds (OLA, SQL, MOD and FAC (standard drug) are presented in Figure 4.

Table 1: Identified compounds from the GC-MS of ethanol extract of *M. esculenta.*

S/N	RT(Mins)	Concentration (%)	Compounds
1	19.5507	0.5231	Cyclotetradecane
2	21.5546	0.4092	Hexadecanoic acid, ethyl ester
3	22.0151	1.7653	n-Hexadecanoic acid
4	23.4355	1.4296	17-Pentatriacontene
5	24.6736	0.7883	E-11-Hexadecenoic acid, ethyl ester
6	25.1322	4.9985	trans-13-Octadecenoic acid
7	25.5163	0.2836	Oleic Acid
8	25.5505	1.2668	Oleic Acid
9	29.1421	1.5387	9-Octadecenal, (Z)-
10	29.6157	0.184	cis-9-Hexadecenal

11	31.8192	0.0352	Undec-10-ynoic acid
12	31.8946	0.0157	Oleic Acid
13	32.0733	0.1221	Heptadecanoic acid, heptadecyl ester
14	32.4059	0.092	Heptafluorobutyric acid, pentadecyl ester
15	32.5603	0.0667	9,12-Octadecadienal
16	33.0992	0.6011	Oleic Acid
17	33.5644	1.5482	Oleic Acid
18	33.7686	0.7556	9,17-Octadecadienal, (Z)-
19	34.6995	11.1954	2-Methyl-Z,Z-3,13-octadecadienol
20	35.0064	13.9947	Squalene
21	35.2949	5.3517	Erucic acid
22	35.4244	4.8241	Oleic Acid
23	35.4762	3.1796	Oleic Acid
24	35.5716	3.1101	Oleic Acid
25	35.6152	0.7447	Oleic Acid
26	35.6451	1.5583	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide
27	35.6954	1.9315	n-Propyl 11-octadecenoate
28	35.7533	3.0931	n-Propyl 11-octadecenoate
29	35.8162	1.6475	13-Bromotetradecanoic acid
30	35.8588	1.0738	Oleic Acid
31	35.8917	9.2875	Erucic acid
32	36.158	2.0647	Oleic Acid
33	36.3124	3.435	Oxirane, tetradecyl-
34	36.348	17.0846	Oleic Acid

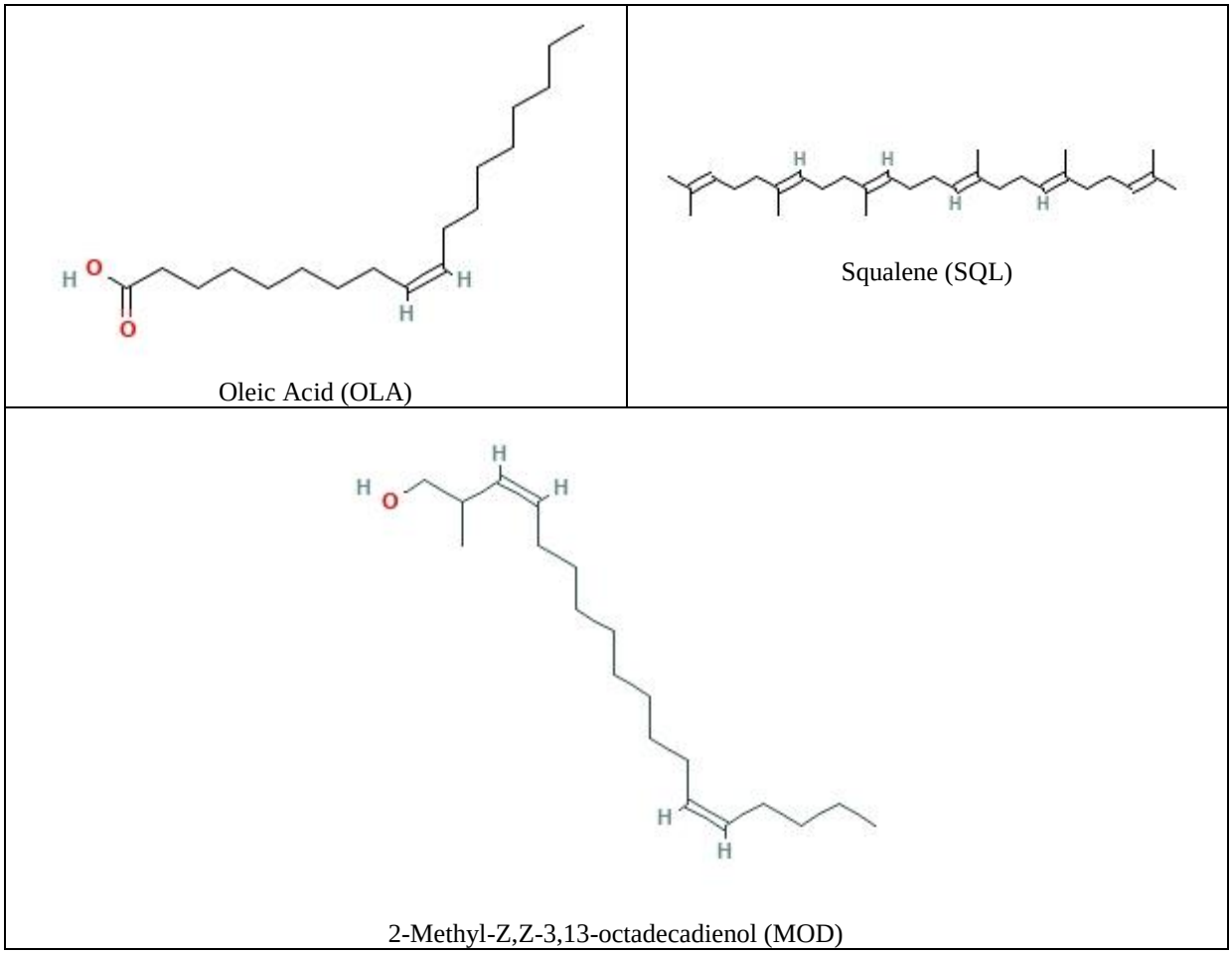


Figure 8: Structures of the three most abundant compounds identified from GC-MS of ethanol extract of *M. esculenta*.

Table 2. ADMET properties of three lead compounds in M. esculenta.

Model		**Leadcompounds**	
Absorption	**OLA**	SQL	MOD
Blood-BrainBarrier	BBB+	BBB+	BBB+
HumanIntestinalAbsorption	HIA+	HIA+	HIA+
Caco-2Permeability	Caco2+	Caco2+	Caco2+
P-glycoproteinSubstrate	Non-Substrate	Non-Substrate	Non-Substrate
P-glycoproteinInhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
	Non-inhibitor	Inhibitor	Non-inhibitor
RenalOrganicCationTransporter	Non-inhibitor	Non-inhibitor	Non-inhibitor
DistributionandMetabolism			
CYP4502C9Substrate	Non-substrate	Non-substrate	Non-substrate
CYP4502D6Substrate	Non-substrate	Non-substrate	Non-substrate
CYP4503A4Substrate	Non-substrate	Non-substrate	Non-substrate
CYP4501A2Inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor
CYP4502C9Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP4502D6Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP4502C19Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP4503A4Inhibitor	Non-Inhibitor	Non-inhibitor	Non-inhibitor
CYPInhibitoryPromiscuity	LowCYPInhibitory Promiscuity	LowCYPInhibitory Promiscuity	LowCYPInhibitory Promiscuity
ExcretionandToxicity			
HumanEther-a-go-go-RelatedGene Inhibition	Weakinhibitor	Weakinhibitor	Weakinhibitor
	Non-inhibitor	Non-inhibitor	Non-inhibitor
AMESToxicity	NonAMEStoxic	Non AMEStoxic	Non AMEStoxic
Carcinogens	Non-carcinogens	Carcinogens	Carcinogens
FishToxicity	HighFHMT	HighFHMT	HighFHMT
TetrahymenaPyriformisToxicity	HighTPT	HighTPT	HighTPT
HoneyBeeToxicity	HighHBT	HighHBT	HighHBT
Biodegradation	Readybiodegradable	Readybiodegradable	Readybiodegradable
AcuteOralToxicity	IV	III	III
Carcinogenicity(Three-class)	Non-required	Warning	Non-required
ADMETPredictedProfile(Regression)			
Absorption			
Aqueoussolubility(LogS)	-4.0398	-5.2141	-2. 1723
Caco-2Permeability(LogPapp,cm/s)	1.3956	1.3403	1.3095
Toxicity			
RatAcuteToxicity(LD_{50},mol/kg)	1.3991	1.5057	1.7380
FishToxicity(pLC50,mg/L)	1.3809	-0.6657	0.8897
TetrahymenaPyriformisToxicity(pIGC50,ug/L)	0.7121	0.9942	0.8251

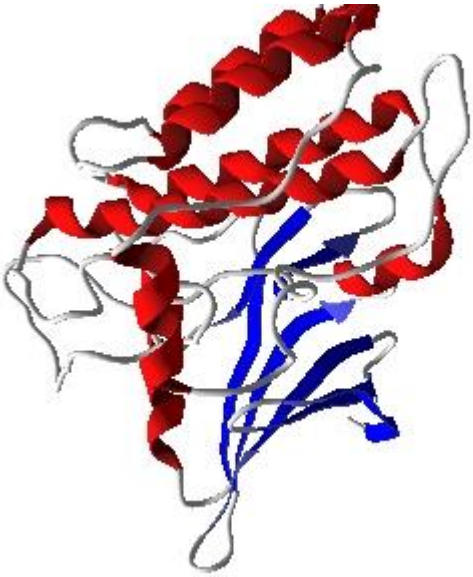
ADMET properties

The ADMET properties of the studied leads were calculated using admet SAR. Blood-Brain Barrier (BBB) penetration, Human Intestinal Absorption (HIA), Caco-2 cell permeability and Ames test were calculated.

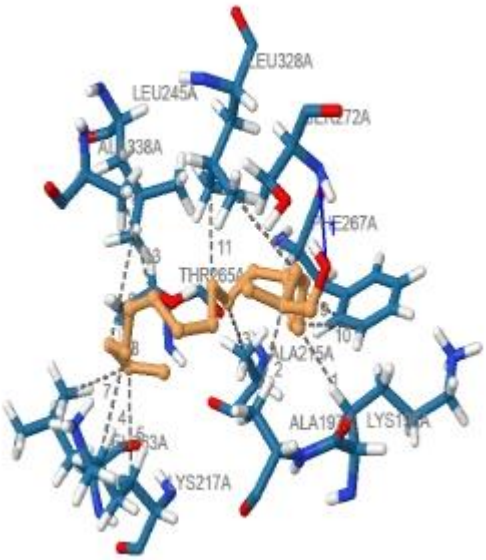
The results showed that the compounds can penetrated the Blood-BrainBarrier (BBB). It was also found that all tested compounds could be absorbed by the human intestine, and can also penetrate to Caco-2 (Table 2). Nevertheless, the tested compounds proved to be potential substrates for P glycoprotein (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 P450s aromatase catalyze the metabolism of a wide variety of compounds including xenobiotic and drugs Ghosh *et al.*, (2012). Thus, inhibition of cytochrome P450 isoforms might cause drug-drug interactions in which co-administered drugs fail to be metabolized and accumulate to toxic levels, Lynch, a n d Price, (2007)**.** Results showed that most of the cytochrome P450 isoforms cannot be inhibited by the tested compounds. Fortunately, OLA did not show any acute toxicity and carcinogens effect with respect to the Ames test data. Surprisingly, SQL and MOD were found carcinogenic (Mortelmans and Zeiger, 2000) and they need to be administered cautiously. The different compounds were classified into Category III (compounds with LD_{50} values greater than 500 mg/kg but less than 5000 mg/kg) and Category IV (compounds with LD_{50} values greater than 5000 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 and are less toxic.

Table 3: Binding energies and interactions of 2QLU with lead compounds.

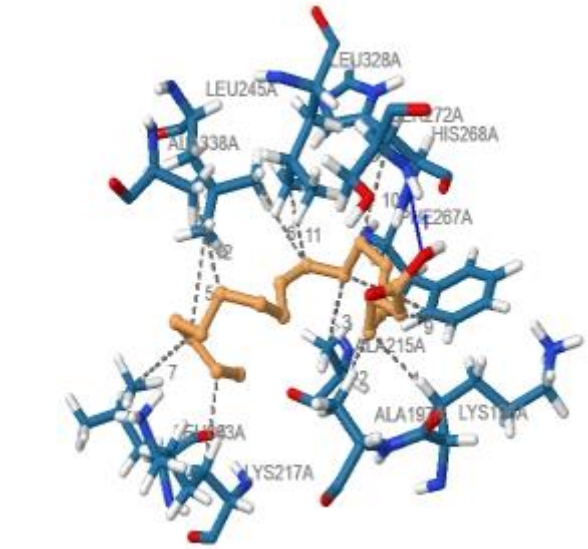
Compound	Binding Energy (Kcal/mol)	Hydrophobic Interactions	Hydrogen bonds	Salt bridge
OLA	-6.6	LYS 196A, ALA 197A, ALA 215A, LYS 217A, LEU 245A, LEU 245A, LEU 263A, PHE 267A, PHE 267A, HIS 268A, LEU 328A, ALA 338A	SER 272A	-
SQL	-8.4	LYS 196A, ALA 197A, VAL 204A, VAL 204A, LYS 217A, LEU 245A, LEU 263A, LYS 278A, LYS 325A, LEU 328A, LEU 328A, ALA 338A, LEU 412A, LEU 412A, LEU 412A	-	
MOD	-6.6	LYS196A, ALA197A, ALA 215A, LYS 217A, LYS 217A, LEU 245A, LEU 263A, THR 265A, PHE 267A, PHE 267A, LEU 328A, LEU 328A, ALA 338A	SER 272A	-
FAC (standard drug)	-6.0	VAL 369A, ILE 384A	ARG 320A, ARG 320A, ASP 321A, ASP 321A, PHE 322A, GLY 360A,THR 361A, THR 361A, TYR 364A, ALA 366A	ARG 320A



A



B



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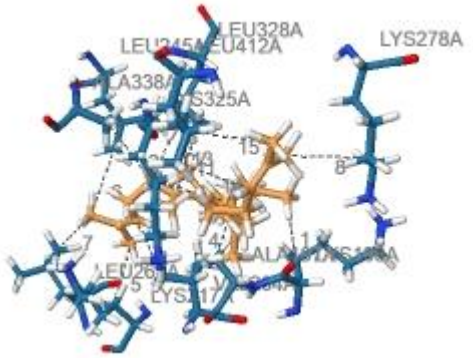

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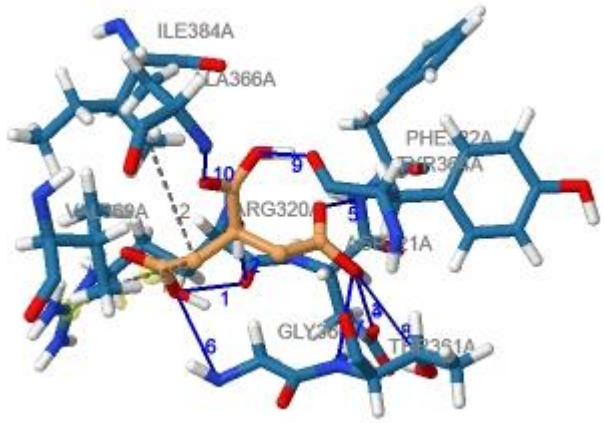

E

Figure 9: (A) Crystal structure of ActRIIB, (B)ActRIIB-MOD complex (C) ActRIIB-OLA complex (D) ActRIIB-SQL complex (E) ActRIIB-FAC complex.

The binding energies and interactions of 2QLU with lead compounds are presented in Table 3. The binding energies showed that the molecular docking studies were feasible. The lead compounds showed better binding affinity than the standard drug. In 2QLU – OLA complex, hydrophobic interactions were observed with amino acids LYS 196A, ALA 197A, ALA 215A, LYS 217A, LEU 245A, LEU 245A, LEU 263A, PHE 267A, PHE 267A, HIS 268A, LEU 328A and ALA 338A. Hydrogen bonds were observed with amino acid SER 272A. In 2QLU – SQL complex, hydrophobic interactions were observed with amino acids LYS 196A, ALA 197A, VAL 204A, VAL 204A, LYS 217A, LEU 245A, LEU 263A, LYS 278A, LYS 325A, LEU 328A, LEU 328A, ALA 338A, LEU 412A, LEU 412A and LEU 412A. In 2QLU – MOD complex, hydrophobic interactions were observed with amino acidsLYS196A, ALA197A, ALA 215A, LYS 217A, LYS 217A, LEU 245A, LEU 263A, THR 265A, PHE 267A, PHE 267A, LEU 328A, LEU 328A and ALA 338A. Hydrogen bonds were observed with amino acid SER 272A.

DISCUSSION

The result presented in Fig 2 revealed significant reduction in the Hb, PCV and RBC mean values following induction of anaemia using PHZ, while, those treated with the graded doses of *M.esculenta* restored the blood cells in a dose dependent manner compared with those untreated anaemic group. The leukogram indicated lymphopenia, neutrophilia, monocytosis and eosinophilia Fig 3. This is an indication of stress leukogram. *M.esculenta* caused stress on the leukocytes. In Fig 4 AST, ALP and ALP were within normal reference range indicating liver safty while bilirubin, urea and creatinine showed no kidney problem Fig 6. Total protein was recovered in dose dependant manner compared with untreated anaemic group Fig 5.

Gas chromatogram showed 34 picks (Fig 7) indicating 34 phytocompounds present in *M.esculenta* Table 1. The three most abundant compounds were Oleic acid, Squalene and 2-Methyl-Z,Z-3,13-octadecadienol Fig 8. These three lead compounds had good absorption,

distribution metabolism and excretion with no toxicity Table 2. Fig 9 showed (A) Crystal structure of ActRIIB, (B)ActRIIB-MOD complex (C) ActRIIB-OLA complex (D) ActRIIB-SQL complex (E) ActRIIB-FAC complex. Therefore these lead compound can be good candidate for drug production.

Anaemia is a clinical syndrome of blood characterized by decrease in the haemoglobin content in the red blood cells resulting in the marked reduction of the oxygen carrying capacity of the blood. The Activin receptor type IIB (ActRIIB) is a transmembrane receptor involved in the negative regulation of red blood cells and skeletal muscle cells. Thus the inhibition of ActRIIB receptor protein results in increase growth of red blood cells and helps to recover anaemic conditions. The lead showed promising inhibitory activities against ActRIIB receptor. Molecular docking studies, similar *in silico* inhibitory activities against ActRIIB receptor were also reported by Jie *et al*., 2017.

CONCLUSION

Extract of *M.esculenta* restored the blood cells in a dose dependent manner compared with those untreated anaemic group. The leukogram indicated lymphopenia, neutrophilia, monocytosis and eosinophilia. AST, ALP and ALP were within normal reference range indicating liver safty while bilirubin, urea and creatinine showed no kidney problem. Total protein was recovered in dose dependant manner compared with untreated anaemic group. Gas chromatogram showed 34 phytocompounds present in *M.esculenta* and the three most abundant compounds were Oleic acid, Squalene and 2-Methyl-Z,Z-3,13-octadecadienol. The lead compounds had good absorption, distribution metabolism and excretion with no toxicity and can be good candidate for drug production.

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