wjpmr, 2024, 10(3), 15-28

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ANTIDIABETIC EFFECT OF *ANDROGRAPHIS PANICULATA* LEAVES; HEMATOLOGY AND SERUM BIOCHEMISTRY; GCMS AND MOLECULAR DOCKING STUDY IN RATS

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Article Received on 03/01/2024 **Article Revised on 23/01/2024** **Article Accepted on 13/02/2024**

ABSTRACT

Anti-diabetic effect of *Andrographis paniculata* leaves and serum biochemistry were studied in rats. Compounds in *A. Paniculata* were also analysed using GCMS and molecular docking study. Thirty (30) rats were used for the research and were grouped into six (6) of five (5) rats each. Group 1 was the normal control group and was administered distilled water. Groups 2 was diabetic non- treated. Groups 3, 4 and 5 were the treatment groups which received 500, 1000 and 1500 mg/kg body weight of the extract *A. paniculata* respectively. Group 6 was the positive Glibenclamide know drug. The rats were dosed for 14 days, thereafter were sacrificed by cardiac puncture and blood collected for analysis. All results in treatment groups were compared with the untreated diabetic group at statistical confidence of 95% ($p<0.05$). The normal control group saved as reference point. There was progressive recovery from diabetes induced rats as the blood glucose level reduced to normal range. Hb, PCV and RBC values were restored to normal. AST, ALT and ALP were within normal range indicating liver safety. Urea, creatinine and bilirubin were not affected showing kidney safety. Gas chromatogram showed 13 peaks indicating 13 phytocompounds present in *A. Paniculata* with OLA, HDA and MDH as the most abundant compounds. Molecular interaction and docking score of selected *A. paniculata* phytocompounds with α-amylase (1B2Y) complexes shows that 1B2Y-OLA, 1B2Y-HDA and 1B2Y-MHDcomplexes gave a docking score of -4.2 Kcal/mol, -4.5 Kcal/mol and -4.5 Kcal/mol respectively. The negative binding energy (exergonic) value suggests that the docking process was successful. Drug-likeness prediction showed 1 violation out of 5, for the compounds of *A. paniculata*. This suggests that all the docked compounds met the requirements of Lipinski Rule of 5 (RO5). These results suggest that *A. paniculata* compounds will be good candidates for drug development process.

INTRODUCTION

Plants play important role in prevention and treatment of diseases. (Bachrach 2012). They have reduced side effects compared to conventional drugs. Plants are sources of drugs and novel lead compound which are essential part of organic chemistry and biochemistry. (Atanasov *et al.,* 2015) Plants have been known to act as an adjunct therapy since time immemorial. (Dai *et al.,* 2019). Diabetes mellitus is a metabolic disease that occurs due to high blood glucose level, caused by insulin deficiency(type 1 DM) or quite often combined with resistance (type 2 DM). (Rang *et al.,* 2003) It is a common endocrine disorder caused by insulin deficiency leading to elevated blood sugar level. (Guyton 2006) Causes include failure of β-cell of islets of Langerhans of the pancreas to produce insulin. (Lenzen 2008, Ezeja *et al.,* 2015). It could be controlled by regulation of dietary sugar intake, (Bantle *et al.,* 2006) or byinsulin therapyusing sulfonylurea drugs orwhole dietary modification. (Papachristoforou *et al.,* 2020*).* Risk factors of diabetes include cardiovascular diseases such as hypertension, heart failure, nephropathy, (Bantle *et al* 2006 and Papachristoforou *et al.,* 2020). Diabetes retinopathy which is a leading cause of blindness is closely linked to elevation in blood glucose and hyperlipidemia seen in people with uncontrolled diabetes. (ADS 2004). Diabetic foot ulcers has been associated with diabetic patients, causing ulceration,

infection and eventually the need for amputation. (ADS 2004). *Andrographis paniculata* wall (family Acanthaceae) is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria for centuries in Asia, America and African continents. The plant has bitter taste hence the name king of bitters. It possesses several phytochemical constituents with unique and interesting properties. Diterpenes, flavonoids, xanthones, noriridoides and other miscellaneous compounds have been isolated from the plant. Extract and pure compounds of the plant have been reported for their antimicrobial, cytotoxicity, anti-protozoan, anti-inflammatory, anti-oxidant, immune-stimulant, anti-diabetic, anti-infective, anti-angiogenic, hepato-renal protective, sex hormone/ sexual function modulation, liver enzymes modulation, insecticidal and toxicity activities. It is also used for treatment of infectious diseases such as; fever, tonic for gastric problem, liver problems, detoxification, Carminative. It is also native to India and Srilanka. (Jarukamjourn *et al*., 2008). Its pharmacological properties include anti-inflammatory, Anti-cancer, Immunomodulation, Anti HIV, Anti diabetic, (Akbar, 2011), Anti spasmodic, Anti- infective and anti- oxidant. (Okhuarobo *et al*., 2014; Kishore *et al*., 2017). The compounds in *Andrographis paniculata* were separated using Gas Chromatography Mass Spectrometry Igwe *et al* (2015). 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). This research is therefore designed to check the antidiabetic potentials, serum biochemistry, haematology and thereafter dock the most abundant compound separated in *A. paniculata* with α-amylase (1B2Y) complexes to establish its binding energy and drug likeness prediction. Figure 1 shows the picture of *A. paniculata*.

Fig. 1: Pictures of *Andrographis paniculata* showing leaves and flower.

MATERIALS AND METHODS

Plant Materials

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

Preparation of Plant Extract

The identified leaves of *A.paniculata*, 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 (35g) 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 500, 1000 and 1500 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 done 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).

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

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

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

(Lewis *et al* 2006, Hoffbrand and Moss 2011, Igwe *et al* 2019).

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 analysed 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.*

Experimental Diabetes Induction

The method of Lenzen (2008) was adopted. The animals were fasted for 16–18 hours with free access to water before the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of Alloxan Monohydrate (Sigma St Louis, M.O., USA) dissolved in 0.9%V/V normal saline solution at a dose of 160 mg/kg body weight (Katsumat *et al.*, 1999). The diabetes was assessed in alloxan induced rats by determining the blood glucose concentration using one touch glucometer and Accu-check strips at day 1 and day 3 after injection of alloxan. The rats that recorded elevated blood glucose level above 240 g/dL were considered diabetic and were selected for the study.

Blood Glucose Levels determination

The modified procedure of Beach and Turner, (1958) based on the glucose oxidase principle was adopted in the determination of blood glucose level of the experimental rats. The blood samples were collected by cutting the tip of the tail artery of the rats, and a drop allowed to touch the sensor part of one touch glucometer strips. The values obtained were recorded in mg/dl. The blood glucose levels were sampled at intervals of day 1, day 3 and day 7 of treatment, respectively.

Experimental Design

Thirty (30) rats were used for the research, they were grouped into six (6) of five (5) rats each. Group 1 was the normal control group and was administered distilled water. Groups 2 was diabetic non- treated. Groups 3, 4 and 5 were the treatment groups which received 500, 1000 and 1500 mg/kg body weight of the extract *A. paniculata* respectively. Group 6 was the positive control with known drug Glibenclamide. The rats were dosed for 14 days, thereafter were sacrificed by cardiac puncture and blood collected for analysis. All results in treatment groups were compared with the untreated diabetic group at statistical confidence of 95% ($p<0.05$). The normal control group saved as reference point.

GC-MS analysis

The 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 and 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 phytcompounds in *A. paniculata* by 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 α-amylase and prominent compounds

Alpha-amylase (PDB ID: 1B2Y) (Figure 2) was obtained from the RCSB Protein Databank. Water molecules and the substrate ligand (**acarbose**) were removed using Molecular molegro viewer software. The PDB of three most abundant compounds (n-hexadecanoic acid; (Z)-Methyl heptadec-9-enoate and oleic acid with the highest concentrations were downloaded from PubChem. They have been abbreviated as HDA, MHD, and OLA respectively.

***In Silico* Studies**

Docking protocol

The molecule 1B2Y was loaded to PYRX software (Dallakyan and Olsonn, 2015). The ligands were loaded

using Open Babel Software embedded in PYRX (Dallakyan and Olsonn, 2015). The energies of the ligands were minimized and converted to PDBQT format. The docking was finally completed using the Vina Wizard. The interactions were visualized using PLIP server (Adasme *et al*., 2021).

Molecular Dynamics

The protein flexibility of the ligand–protein complexes was evaluated by CABS-flex 2.0 server (Kuriata *et al*., 2018; Jamroz *et al*., 2013) and presented with Root Mean Square Fluctuation (RMSF).

The structure of human pancreatic alpha-amylase in complex with the carbohydrate inhibitor acarbose (1B2Y) is presented in Figure 2.

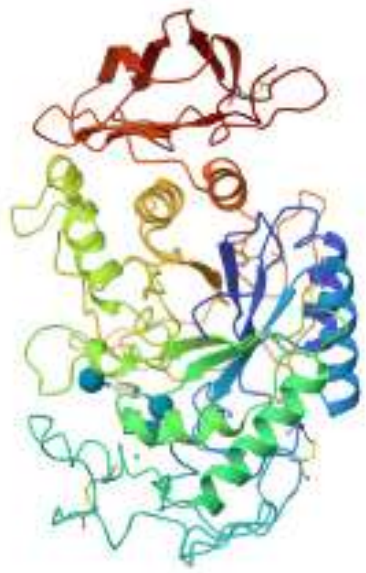


Figure 2: Structure of human pancreatic alpha-amylase in complex with the carbohydrate inhibitor acarbose (1B2Y).

RESULTS

The result as presented inTable 1 showed the percentage reduction of *A. paniculata* extract on blood glucose concentration (mg/dl) of alloxan induced diabetic rats after seven (7) days of treatment.

Table 1: Percentage reduction of leaves of *A. paniculata* mean fasting on blood glucose concentration (mg/dl) of alloxan induced diabetic Wistar rats after day seven of treatment.

Treatment groups	Value before induction	Day 0	Day 1	Day 3	Day 7	Percentage reduction after Day 7 (%)
5 mg/kg Gilbenclamide	60.60±2.24	299.60±16.85	289.60±16.85	235.20±5.47^{b}	81.80±1.52^{c}	72.83±2.07^{a}
Diabetic group	60.80±1.39	299.00±10.25	315.17±3.16	398.00±25.05^{a}	496.80±4.74^{a}	0.00±0.00^{c}
500 mg/kg leaf extract of ***A. paniculata***	64.20±1.11	313.20±2.87	288.18±9.08	252.00±17.07^{b}	145.00±11.33^{b}	53.56±4.10^{b}
1000 mg/kg leaf extract of ***A. paniculata***	62.60±1.02	301.80±12.71	280.11±10.20	219.00±5.07^{b}	122.40±3.18^{b}	55.67±2.81^{b}
1500 mg/kg leaf extract of ***A. paniculata***	62.00±0.57	311.50±10.23	298.08±10.42	209.00±15.03^{b}	93.00±7.68^{c}	70.25±1.86^{a}

Note: Values are presented as mean ± S.E.M. Different superscripts represent significant differences at $p<0.05$.

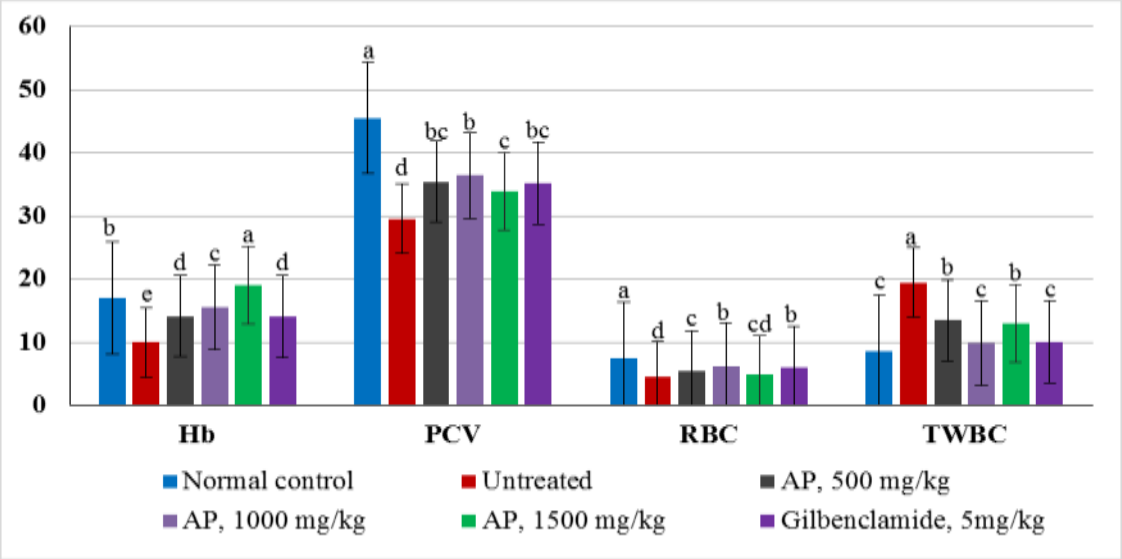


Fig. 3: Effect of extract of *A. paniculata* leaf on haematology parameters of alloxan induced diabetic Wistar rats.

Values are presented as mean ± SEM (Standard Error of Mean). Means with different superscriptacross treatment groups are significantly different at p<0.05. **Hb:** Haemoglobin; **PCV:** Packed Cell Volume; **RBC:** Red Blood Cell; **TWBC:** Total White Blood Cell; **AP:** *Andrographis paniculata*

Hb (g/dL) Normal control:[16.98±0.31^{b}]Untreated: [9.96±0.29^{e}]500mg/kg:[4.16±0.14^{d}]1000mg/kg [15.60±0.18^{c}] 1500mg/kg [19.04±0.25^{a}]Glibeclamide [14.16±0.35^{d}]

PCV (%) Normal control:[45.55±0.68^{a}]Untreated: [29.60±0.50^{d}]500mg/kg:[35.40±0.51^{bc}]1000mg/kg [36.40±0.51^{b}] 1500mg/kg [33.90±0.24^{c}]Glibeclamide [35.20±0.58^{bc}]

RBC ($\times 10^{6}mm^{3}$) Normal control:[7.55±0.20^{a}]Untreated: [4.54±0.07^{d}]500mg/kg:[5.39±0.09^{c}]1000mg/kg [6.28±0.13^{b}] 1500mg/kg [4.94±0.26^{cd}]Glibeclamide [5.94±0.25^{b}]

TWBC ($\times 10^{3}mm^{3}$) Normal control:[8.65±0.32^{c}]Untreated: [19.48±0.32^{a}]500mg/kg:[13.47±0.94^{b}]1000mg/kg [9.92±0.23^{c}] 1500mg/kg [13.00±0.31^{b}]Glibeclamide [9.99±0.38^{c}]

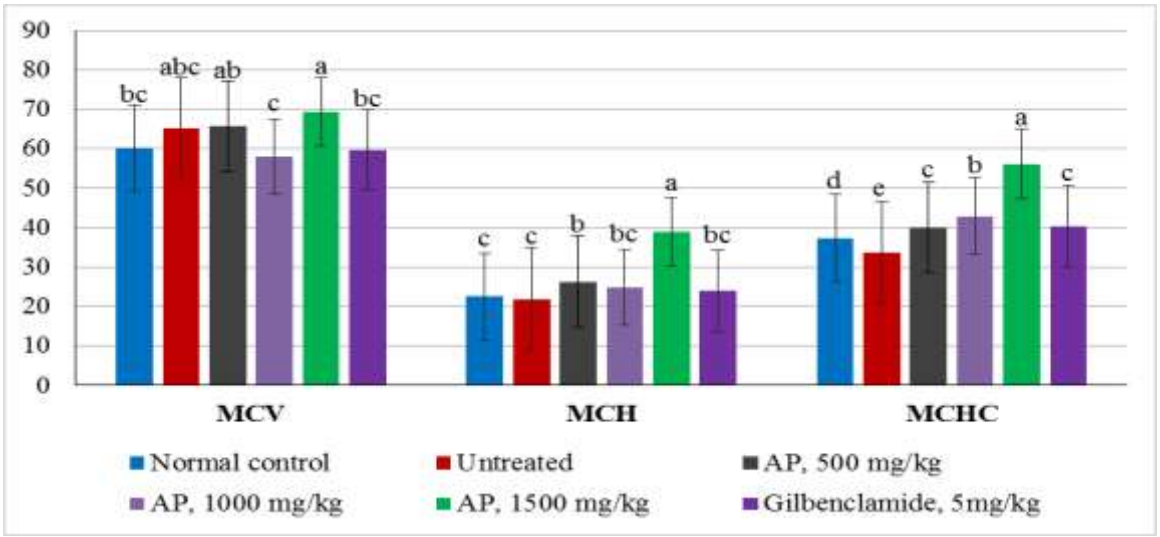


Fig. 4: Effect of *A. paniculata* leaf extract on haemoglobin morphologic charateristics in alloxan induced diabeticWistar rats.

Values are presented as mean ± SEM (Standard Error of Mean). Means with different superscriptacross treatment groups are significantly different at p<0.05. **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Haemoglobin and **MCHC:** Mean Corpuscular Haemoglobin Concentration:

MCV (fl) Normal control:[60.22±0.92^{bc}]Untreated: [65.19±1.96^{abc}]500mg/kg:[65.71±1.25^{ab}]1000mg/kg [58.03±1.79^{c}]1500mg/kg [69.29±3.59^{a}]Glibeclamide [59.76±3.06^{bc}]

MCH(pg) Normal control:[22.50±0.35^{c}]Untreated: [21.91±0.69^{c}]500mg/kg:[26.28±0.49^{b}]1000mg/kg

[24.83±0.26^{bc}]1500mg/kg [38.91±2.01^{a}]Glibeclamide [23.95±0.82^{bc}]

MCHC(g/dL) Normal control:[37.36±0.28^{d}]Untreated: [33.69±1.19^{e}]500mg/kg:[40.02±0.59^{c}]1000mg/kg [42.90±0.96^{b}]1500mg/kg [56.16±0.52^{a}]Glibeclamide [40.23±0.81^{c}]

The result of the MCV, MCH and MCHC (Fig 4) showed no morphological change because all values were within normal reference range thus normocytic normochromic state of red blood cells.

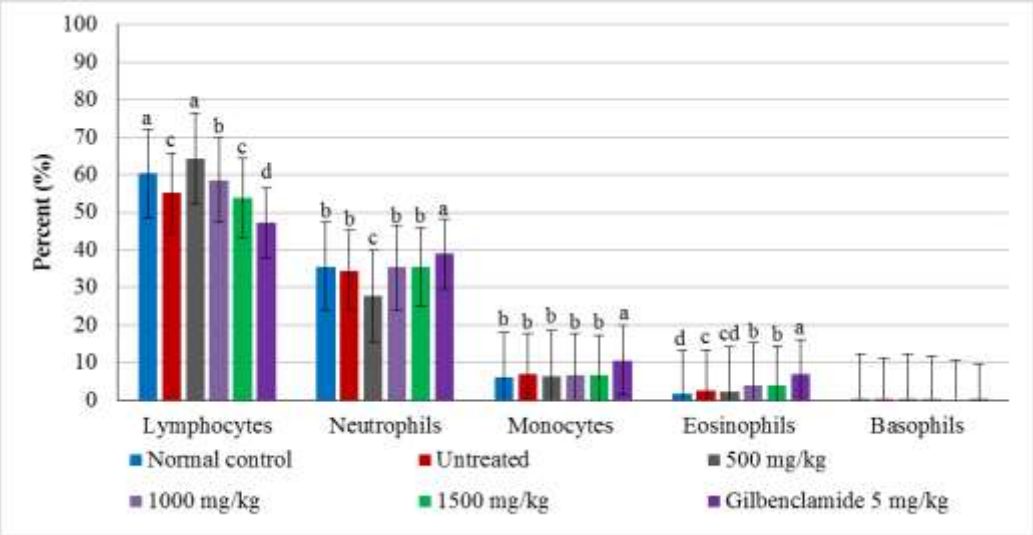


Fig. 5: Effect extract of *A. paniculata* on differential leukocyte count on alloxan induced diabetic Wistar rats.

Values are presented as mean ± SEM (Standard Error of Mean). Means with different superscriptacross treatment groups are significantly different at p<0.05.

Lymphocytes(%) Normal control: [60.40±1.02^{b}]Untreated: [55.20±10.73^{c}]500mg/kg:[64.40±1.43^{a}]1000mg/kg [58.60±1.60^{b}]1500mg/kg [53.80±0.58^{c}]Glibeclamide [47.20±0.86^{d}]

Neutrophils(%) Normalcontrol:[35.60±1.20^{b}] Untreated:[34.40±0.67^{b}]500mg/kg:[27.80±1.59^{c}]1000mg/kg [35.40±0.74^{b}]1500mg/kg [35.40±0.50^{b}]Glibeclamide [39.00±1.04^{a}]

Monocytes (%) Normal control:[6.20±0.37^{b}]Untreated: [7.00±0.31^{b}]500mg/kg:[6.40±0.51^{b}]1000mg/kg [6.60±0.24^{b}] 1500mg/kg [6.60±0.50^{b}]Glibeclamide [10.60±0.74^{a}]

Eosinophils (%) Normalcontrol:[1.60±0.24^{d}]Untreated:[2.60±0.25^{c}]500mg/kg:[2.20±0.20^{cd}]1000mg/kg [4.00±0.31^{b}] 1500mg/kg [3.80±0.37^{b}]Glibeclamide [6.80±0.37^{a}]

Basophils (%) Normal control:[0.40±0.24]Untreated: [0.40±0.24]500mg/kg:[0.20±0.20]1000mg/kg [0.40±0.21] 1500mg/kg [0.00±0.00]Glibeclamide [0.20±0.20]

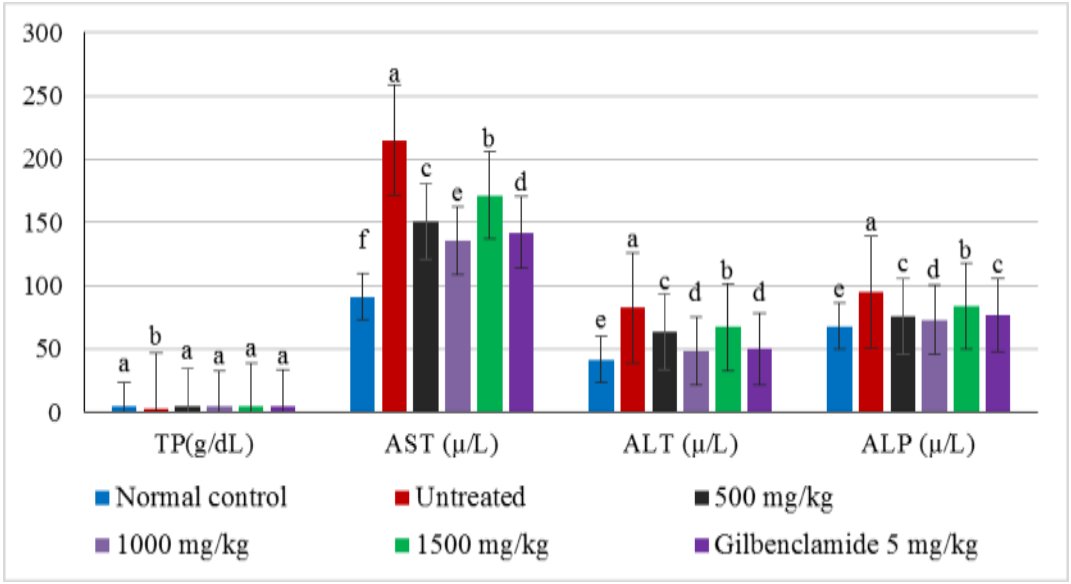


Fig. 6: Effect of graded doses of ethanol extract of *A. paniculata* plant on liver and biomarkers of alloxan induced diabetic Wistar rats.

Values are presented as mean ± SEM (Standard Error of Mean). Means with different superscriptacross treatment groups are significantly different at p<0.05. **TP:** Total protein; **AST:** Aspartate Transaminase, **ALT:** Alanine Aminotransferase; **ALP:** Alkaline Phosphatase.
TP(g/dL) Normal control:[5.07±0.09^{a}]Untreated: [3.16±0.09^{b}]500mg/kg:[4.74±0.02^{a}]1000mg/kg [4.69±0.07^{a}] 1500mg/kg [4.69±0.07^{a}]Glibeclamide [5.06±0.20^{a}]
AST (µ/L) Normal control:[91.41±1.30^{f}]Untreated: [214.94±0.83^{a}]500mg/kg:[150.61±0.28^{c}]1000mg/kg [135.51±3.04^{e}] 1500mg/kg [171.44±2.37^{b}]Glibeclamide [142.03±0.56^{d}]
ALT (µ/L) Normal control:[41.58±0.84^{e}]Untreated: [82.60±0.55^{a}]500mg/kg:[63.61±1.09^{c}]1000mg/kg [48.51±2.11^{d}] 1500mg/kg [67.32±0.45^{b}]Glibeclamide [76.54±0.61^{c}]
ALP (µ/L) Normal control:[68.08±0.65^{e}]Untreated: [95.09±0.93^{a}]500mg/kg:[75.66±0.73^{c}]1000mg/kg [48.51±2.11^{d}] 1500mg/kg [83.86±0.97^{b}]Glibeclamide [76.54±0.61^{c}]

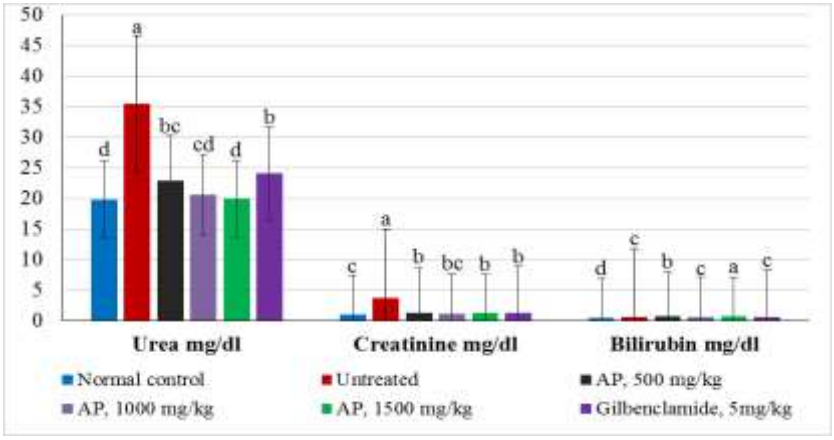


Fig. 7: Effect extract of *A. paniculata* on kidney biomarkers of alloxan induced diabetic Wistar rats.

Urea (mg/dL) Normal control: [19.82±0.29^{d}]Untreated: [35.48±0.50^{a}]500mg/kg: [22.91±0.92^{bc}]1000mg/kg [20.58±1.13^{cd}]1500mg/kg [19.89±1.57^{d}]Glibeclamide [24.08±0.37^{b}]
Creatinine (mg/dL) Normal control: [1.02±0.03^{c}] Untreated: [3.74±0.12^{a}] 500mg/kg: [1.26±0.01^{b}] 1000mg/kg [1.14±0.02^{bc}] 1500mg/kg [1.23±0.01^{b}] Glibeclamide [1.22±0.03^{b}]
Bilirubin (mg/dL) Normal control: [0.50±0.01^{d}] Untreated: [0.56±0.00^{c}]500mg/kg: [0.70±0.03^{b}] 1000mg/kg [0.58±0.02^{c}] 1500mg/kg [0.76±0.01^{a}] Glibeclamide [0.60±0.01^{c}]

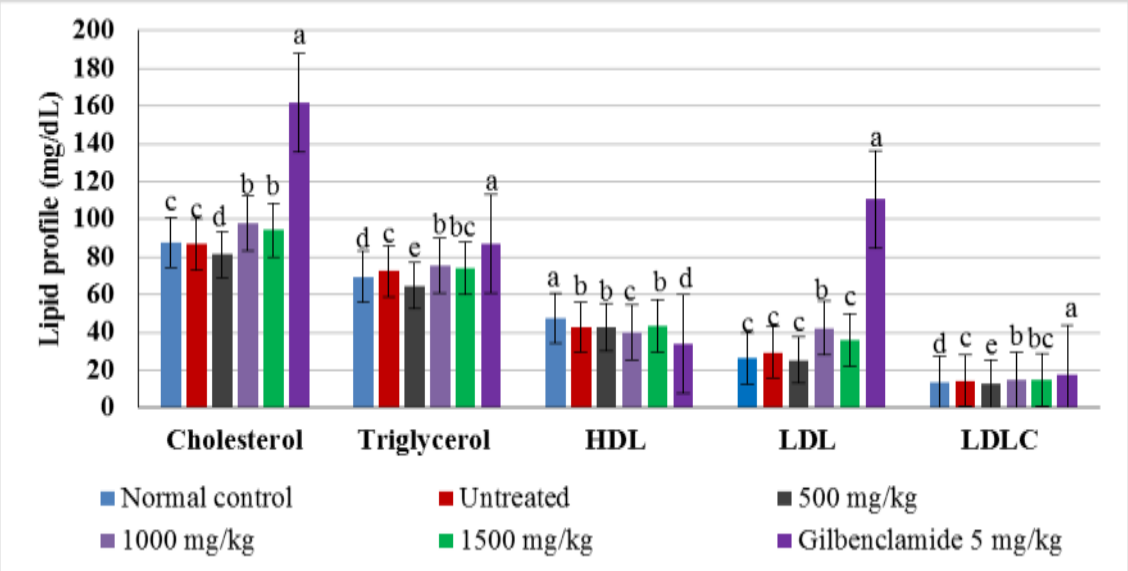


Fig. 8: Effect of extract of *A. paniculata* on lipid profile of alloxan induced diabetic Wistar rats.

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups showed significant (p<0.05) differences. **HDL:** High-density lipoprotein; **LDL:** Low-density lipoprotein; **LDLC:** Low-density lipoprotein cholesterol
Cholesterol(mg/dL) Normal control: [87.64±4.03^{c}] Untreated: [87.64±4.03^{c}]500mg/kg: [81.29±0.74^{d}]

1000mg/kg [97.72±0.41[b]]1500mg/kg [94.18±1.20[b]] Glibeclamide [161.91±0.78[a]]

Triglycerol (mg/dL) Normal control: [69.42±0.63[d]] Untreated: [72.50±0.86[c]]500mg/kg: [64.91±0.84[e]] 1000mg/kg [75.51±0.70[b]] 1500mg/kg [73.92±0.58[bc]] Glibeclamide [87.34±0.70[a]]

HDL (mg/dL) Normal control: [69.42±0.63[d]]Untreated: [72.50±0.86[c]]500mg/kg: [64.91±0.84[e]]1000mg/kg [75.51±0.70[b]] 1500mg/kg [73.92±0.58[bc]]Glibeclamide [87.34±0.70[a]]

LDL (mg/dL) Normal control: [26.18±4.29[c]]Untreated: [29.44±0.48[c]]500mg/kg: [25.41±1.09[c]]1000mg/kg [42.45±0.70[b]]1500mg/kg [35.89±0.68[c]]Glibeclamide [110.50±0.75[a]]

LDLC (mg/dL) Normal control: [13.88±0.12[d]]Untreated: [14.50±0.17[c]]500mg/kg: [12.98±0.16[e]]1000mg/kg [14.78±0.11[bc]]1500mg/kg [14.78±0.11[bc]]Glibeclamide [17.46±0.14[a]]

The Gas chromatogram of ethanol extract of *A. paniculata* revealed a total of 13 peaks corresponding to the bioactive compounds. Figure 9 depicts the gas chromatogram. The compounds have been listed in Table 2. Structures of three most abundant compounds identified from GC-MS of ethanol extract of *A. paniculata* are presented in Figure 10. Table 3 shows the docking score (Kcal/mol) and interactions of *A. paniculata* phytocompounds/1B2Y complexes. PLIP molecular docking interactions of 1B2Y with *A. paniculata* phytocompounds and MET (standard drug) are presented in Figure 11. The RMSF of 1B2Y and the docked complexes are presented in Figure 12.

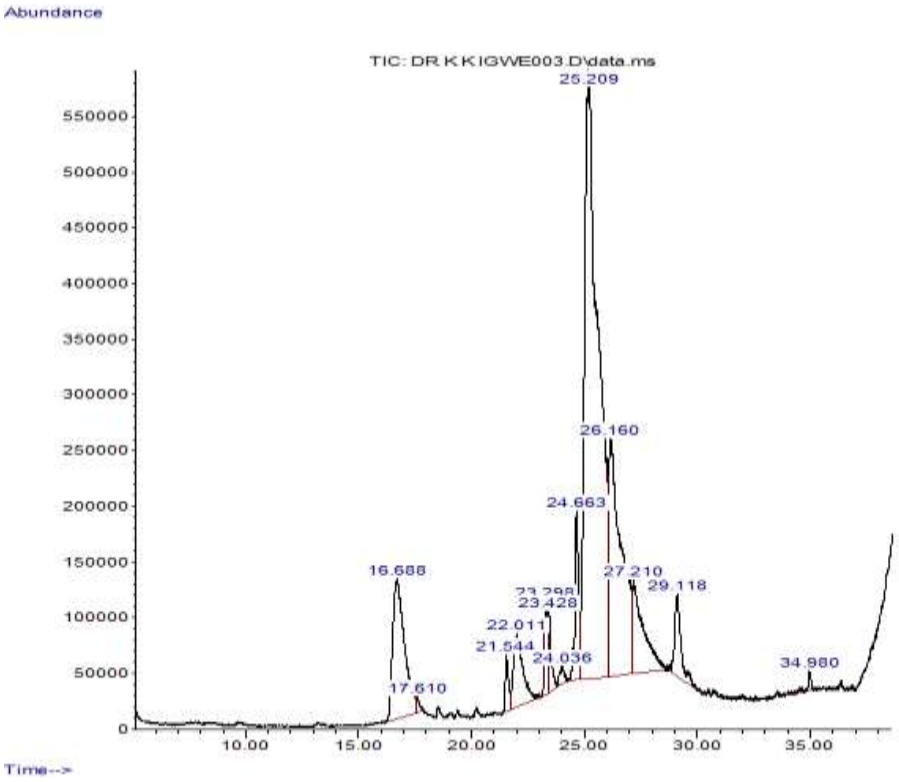


Figure 9: Gas chromatogram of ethanol extract of *A. paniculata.*

Table 2: Identified compounds from the GC-MS of ethanol extract of *A. paniculata.*

S/No	RT (mins)	Composition (%)	Library/ID
1	16.688	9.6836	delta.-Lindane
2	17.6102	0.2873	alpha.-Lindane
3	21.5436	1.0674	Hexadecanoic acid, ethyl ester
4	22.0108	4.0685	n-Hexadecanoic acid
5	23.2978	1.5513	9,12-Octadecadienoic acid, methyl ester
6	23.4276	1.5652	9,17-Octadecadienal, (Z)-
7	24.0362	0.5078	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-
8	24.6633	3.6225	Ethyl Oleate
9	25.2093	49.7185	Oleic Acid
10	26.1595	18.4608	Oleic Acid
11	27.2096	5.9747	(Z)-Methyl heptadec-9-enoate
12	29.118	3.143	9,17-Octadecadienal, (Z)-
13	34.98	0.3493	cis-2,6-Dimethyl-2,6-octadiene

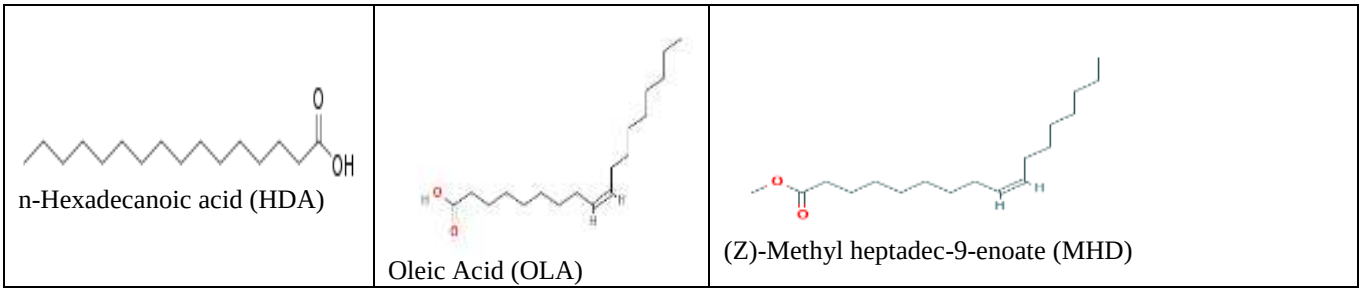


Figure 10: Structures of three most abundant compounds isolated from GC-MS of ethanol extract of *A. paniculata.*

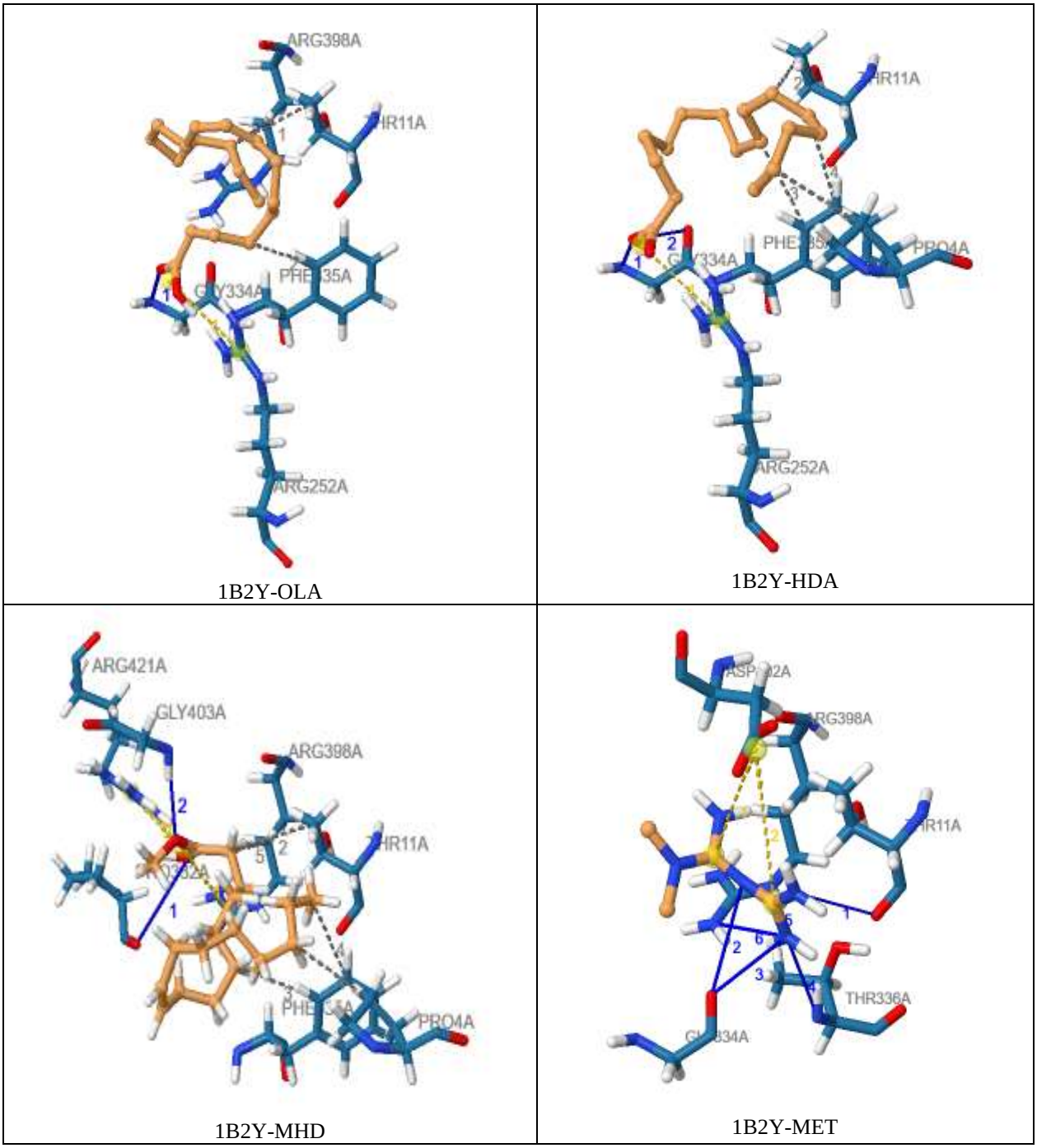
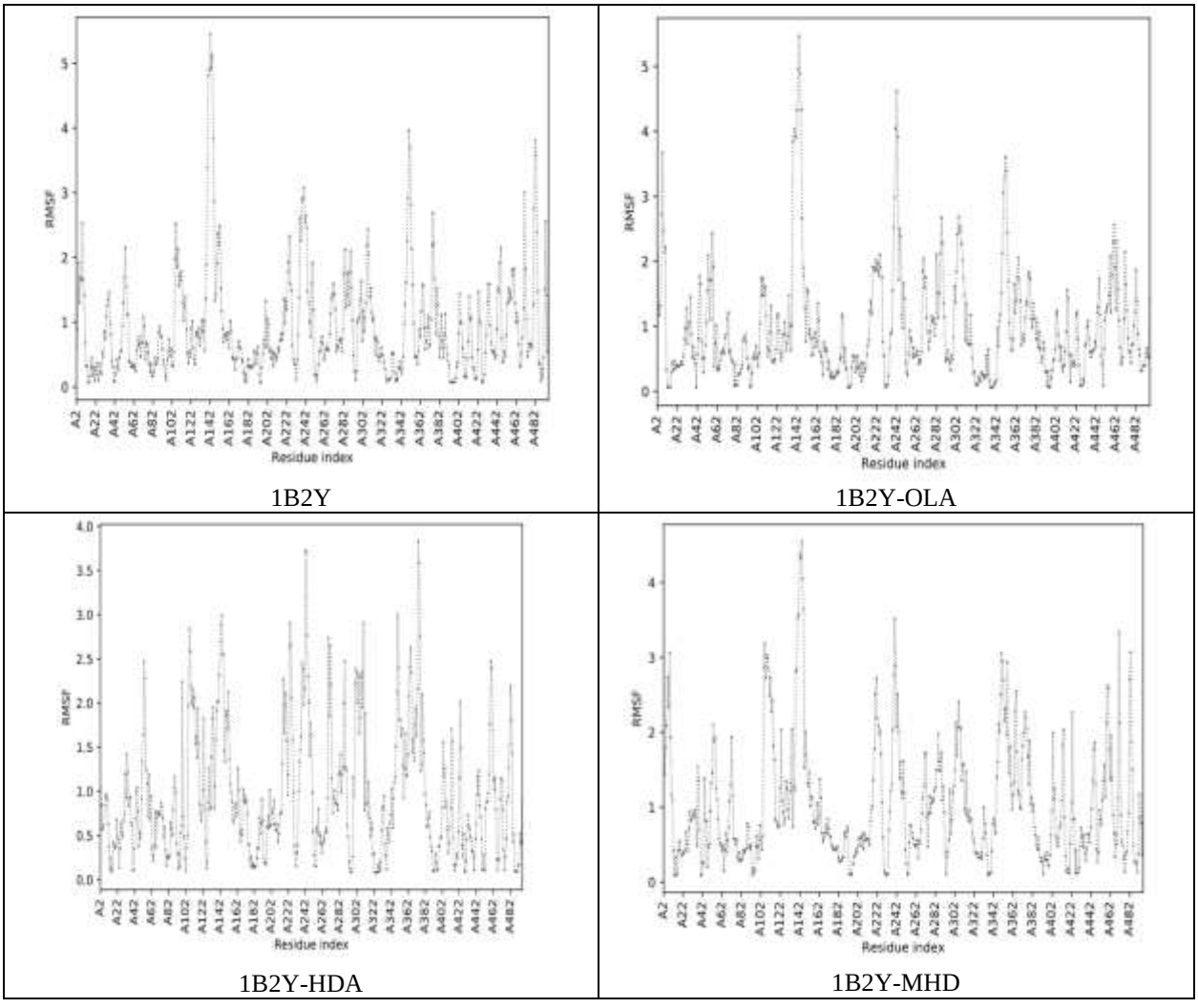


Figure 11: The molecular interactions of α-amylase (1B2Y) and the phytocompounds.

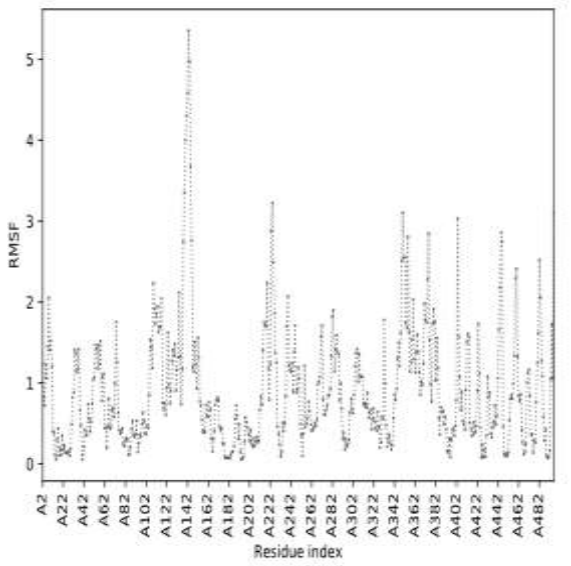
Table 3: Docking score (Kcal/mol) of selected *A. paniculata* phytocompounds/α-amylase (1B2Y) complex.

Compound	Binding Energy (Kcal/mol)	Hydrophobic Interactions	Hydrogen Bonds	Salt Bridges
1B2Y-OLA	-4.2	THR 11A, PHE 335A, ARG 398A	GLY 334A	ARG 252A
1B2Y-HDA	-4.5	PRO 4A, THR 11A, PHE 335A, PHE 335A	GLY 334A, GLY 334A	ARG 252A
1B2Y-MHD	-4.5	PRO 4A, THR 11A, PHE 335A, PHE 335A, ARG 398A	PRO 332A, GLY 403A	ARG 398A, ARG 421A
1B2Y-MET (Standard drug)	-4.5	-	THR 11A, GLY 334A, GLY 334A, THR 336A, ARG 398A, ARG 398A	ASP 402A, ASP 402A



1B2Y 1B2Y-OLA

1B2Y-HDA 1B2Y-MHD



1B2Y-MET

Figure 12: RMSF of 1B2Y and the docked complexes.

The docking score of selected *A. paniculata* phytocompounds/α-amylase (1B2Y) complex are depicted in Table 3. The 1B2Y-OLA complex gave a docking score of -4.2 Kcal/mol. The negative binding energy value suggested that the docking process was successful. Hydrophobic interactions were observed with amino acids THR 11A, PHE 335A, and ARG 398A. Hydrogen bond was observed with amino acid GLY 334A. Salt bridge occurred through ARG 252A. The 1B2Y-HDA complex gave a docking score of-4.5 Kcal/mol. The negative binding energy value suggested successful docking process. Hydrophobic interactions were observed with amino acids PRO 4A, THR 11A, PHE 335A and PHE 335A. Hydrogen bonds were observed with amino acids GLY 334A and GLY 334A Salt bridge occurred through ARG 252A.The 1B2Y-MHD complex gave a docking score of -4.5 Kcal/mol. The negative binding energy value suggested successful docking process. Hydrophobic interactions were observed with amino acids PRO 4A, THR 11A, PHE 335A, PHE 335A and ARG 398A. Hydrogen bonds were observed with amino acids PRO 332A and GLY 403A. Salt bridge occurred through ARG 398A and ARG 421A. The 1B2Y-MET complex gave a docking score of -4.5 Kcal/mol. The negative binding energy value suggested successful docking process. Hydrophobic interactions were not observed. Hydrogen bonds were observed with amino acids THR 11A, GLY 334A, GLY 334A, THR 336A, ARG 398A and ARG 398A. Salt bridge occurred through ASP 402A.

The RMSF was analysed during molecular dynamics simulation Figure 12. The RMSF showed a very similar fluctuation pattern between the free protein and the protein in complex with the ligands, especially with the OLA and MET compounds. However, the RMSF calculation revealed that the complex with the HDA and MHD ligands showed lower fluctuations at residue A142 compared to the protein. These low fluctuations suggested that the complexes were very stable in the physiological conditions.

Table 4: Drug-likeness prediction of *A. paniculata* phytocompounds.

Compounds	Mol. weight (g/mol.)	HB Acceptor	HB Donor	Lipophilicity LogP	Molar refractivity	No. of violations
HDA	256.42	2	1	4.19	80.80	1 MLOGP>4.15
MHD	318.75	2	0	4.67	89.92	1 MLOGP>4.15
OLA	282.46	2	1	4.57	89.94	1 MLOGP>4.15

DISCUSSION

Diabetes mellitus is a prevalent problem of a large percentage of the world. The application of plant metabolites for the management of diabetes has been studied extensively. (Russell *et al.,* 2008) Many medicinal plants are reported to have the potential to improve the management of diabetes in humans and some animals. (Bindu et al., 2018) *A. paniculata,* a medicinal plant typically used as an anti-inflammatory and anti-microbial agent has been studied for its possible impact on the management of Diabetes Mellitus in humans using rats as model. (Nugroho *et al.,* 2012; Dai *et al.,* 2019).

Our findings are in concordance with (Yu et al., 2003 Akhtar *et al.*, 2016; Chen et al., 2020; Jaiyesimi *et al.*,

2020; Wediasari *et al.,* 2020) that *A. paniculata* lowered blood glucose levels of rats in a dose-dependent manner. Furthermore, *A. paniculata* extract was reported to reduce hyperglycemia by inhibiting β-cell dysfunction in alloxan- induced diabetic rats. (Jaiyesimi et al., 2020).

The result as presented in Table 1 showed the percentage reduction of *A. paniculata* extract on blood glucose concentration (mg/dl) of alloxan induced diabetic rats after seven (7) days of treatment. At 500 mg/kg, 1000 mg/kg and 1500 mg/kg showed glucose percentage reduction of 53%, 55% and 70% respectively compared to standard drug glibenclamide with72%.

Effect of extract of *A. paniculata* leaf on haematology parameters of alloxan induced diabetic inWistar rats was shown in Fig 3. All haematological parameters showed recovery from anaemia which resulted from alloxan induction. The extract of *A. paniculata* leaf exhibited some anti anaemic properties.

Haemoglobin morphologic charateristics in alloxan induced diabeticWistar rats MCV, MCH and MCHC were restored to normal showing normocytic normochromic state in Fig 4. Effect extract of *A. paniculata* on differential leukocyte count on alloxan induced diabetic Wistar rats showed mild lymphocytopaenia, neutropaenia, monocytopaenia and eosinopaenia indicating a stress leucogram Fig 5. Liver and kidney biomarkers of alloxan induced diabetic Wistar rats showed no elevation above normal reference range indication safty of these organs Fig 6 and Fig 7.

The extract of *A. paniculata* reduced the levels of blood glucose, triglyceride, and lipid profile compared to control. However, no changes were observed in serum cholesterol. Gilbenclamide also showed similar effects on these parameters. Our finding is in aggreement with the work of Nugroho *et al.,* (2012).

There was progressive recovery from diabetes induced rats as the blood glucose level reduced to normal range. Hb, PCV and RBC values were restored to normal. AST, ALT and ALP were within normal range indicating liver safety. Urea, creatinine and bilirubin were not affected showing kidney safety. Gas chromatogram showed 13 peaks indicating 13 phytocompounds present in *A. Paniculata* with OLA, HDA and MDH as the most abundant compounds Table 2. Molecular interaction and docking score ofselected *A. paniculata* phytocompounds with α-amylase (1B2Y) complexes shows that 1B2Y-OLA, 1B2Y-HDA and 1B2Y-MHDcomplexes gave a docking score of -4.2, Kcal/mol, -4.5 Kcal/mol and -4.5 Kcal/mol respectively. The negative binding energy (exergonic) value suggests that the docking process was successful. Drug-likeness prediction showed 1 violation out of 5, for the compounds of *A. paniculata*,Table 4 This suggests that all the docked compounds met the requirements of Lipinski Rule of 5 (RO5). These results suggest that *A. paniculata* compounds will be good candidates for drug development process.

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.

The Lipinski Rule of 5 (RO5) is a thumb's rule developed by Lipinski (Lipinski *et al.,* 2012) for determining whether a compound with a particular bioactivity has physical and chemical characteristics that are expected to be an orally active medication. The Drug-likeness prediction (Table 4) showed 1 violation out of 5, for the compounds of *A. paniculata*. These suggests that all the docked compounds from *A. paniculata* met the requirements of RO5. These results suggests that *A. paniculata* compounds will be a good candidate for drug development process (Otuokere *et al*., 2022; Nwankwo *et al.,* 2022 ; Igwe *et al.,* 2020).

CONCLUSION

The extract of *A. paniculata* controled diabetes in a dose dependant manner. Haematological parameters were restored. Serum enzymes were within normal reference range showing safty of liver and kidney. Gas chromatogram indicated 13 phytocompounds present in *A. paniculata* with OLA, HDA and MDH as the most abundant compounds (Fig 10). Molecular interaction and docking score of selected *A. paniculata* phytocompounds with α-amylase (1B2Y) complexes shows that 1B2Y-OLA, 1B2Y-HDA and 1B2Y-MHD complexes gave a docking score of -4.2, Kcal/mol, -4.5 Kcal/mol and -4.5 Kcal/mol respectively ssuggesting success. Druglikeness prediction showed 1 violation out of 5, suggests that all the docked compounds met the requirements of Lipinski Rule of 5 (RO5). Therefore *A. paniculata* compounds will be good candidates for drug development.

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