

Haematological Effects of Methanol and n-Hexane Seed Extracts of *Hunteria umbellata* (abeere) on Alloxan Induced Diabetic Wistar Rats

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ABSTRACT

There is growing research studies in identifying local medicinal plants with potentials in replacing the orthodox medicines and synthetic drugs that are now expensive, less effective with side effects when used in treatments of chronic diseases like diabetes mellitus. The chemical compositions, and effects of Methanol and n-Hexane extracts of *Hunteria umbellata* (HU) seeds (folkloric remedies for diabetes, obesity, anaemia etc.) on haematological parameters of alloxan induced diabetic wistar rats, were investigated. Thirty albino rats were divided into six groups- (1) Diabetic rats without treatment (Negative control); (2) Diabetic rats, treated with glibenclamide; (3) Diabetic rats treated with 250mg/kg body weight of methanol extract of HU seeds; (4) Diabetic rats treated with 500mg/kg body weight of methanol extract of HU seeds; (5) Diabetic rats treated with 250mg/kg body weight of n-hexane extract of HU seeds; (6) Diabetic rats treated with 500mg/kg body weight of n-hexane extract of HU seeds. Treatments of the induced diabetic rodents lasted for 14 days before they were sacrificed and blood was collected for biochemical evaluation. From the results, significant ($P \leq 0.05$) high white blood cells, platelets and mean corpuscular volume were observed in group 2. Reducing sugars (in high amounts), flavonoids, tannin, alkaloids, saponins and 14 important compounds were found present in the HU seed extracts. The acute toxicity study of the extracts showed no death at doses of up to 500 mg/kg body weight after 24 h and for 7 days of observation. Treatments with glibenclamide, and extracts of HU seeds at various doses significantly increased ($P < 0.05$) the red blood cells (RBC), packed cell volume and hemoglobin but significantly ($P < 0.05$) reduced the white blood cells and platelets of the Haematological parameters. Thus, Methanol and n-Hexane extracts of HU seeds possess anti-hyperglycemic effects on the haematology due to their high contents of phytochemicals.

Keywords: Diabetic treatment, *Hunteria umbellata*, phytochemicals, haematology, acute toxicity.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (WHO, 2016; Kumar *et al.*, 2021). It is one of the fastest-growing diseases of the 21st century, with a disproportionate burden on low and middle-income countries (Pragya, 2015; IDF, 2021). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Kesavadev *et al.*, 2017). Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the beta-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The blood is a vital fluid, which contains the red blood cells (RBC), white blood cells (WBC) and platelets suspended in the serum in homeostatic concentrations. The blood is important for pulmonary and tissue respiration, as a medium of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion (Adebayo *et al.*, 2005), nutritional and immunological processes, as well as homeostatic responses (Oze, 1992).

Over the years, diabetes mellitus has been the problem of the world, and the disease is ranked one of the top killers in the world (Kesavadev *et al.*, 2017). It is the major cause of heart failure, stroke, sexual dysfunction, nephropathy, retinopathy, vascular dysfunction, hypertension, coronary artery disease, hearing impairment, different forms of cancer, and non-healing of wounds leading to skin complications, amputation of limbs and feet (Forbes *et al.*, 2013; Padhi *et al.*, 2020; Kumar *et al.*, 2021). The global prevalence of diabetes mellitus estimated at 2.8% in 2000 (171 million people) is expected to rise to 9.9% (552 million) of adult population in year 2030 if no urgent efforts are directed at stemming the tide of this global public health burden (Whiting *et al.*, 2011; Padhi *et al.*, 2020). It is also predicted that by 2030 India, China and United States will have the largest number of diabetes sufferers (Pragya, 2015). In 2016, Nigeria had the highest number of people with diabetes with 3.9 million and there has been a progressive increase since then (Adeleye, 2021). Today, the average household in Nigeria has a diabetes scare and that craves for urgent effective prevention, treatment and management in order to avert crash of health and socio-economic development in the country.

However, many synthetic drugs have been developed for treatment of diabetes mellitus, but still they have not provided a complete cure, and worst still, long-term use of these expensive synthetic drugs causes severe side effects (Pragya, 2015; Kumar *et al.*, 2021). Thus, revolutionized research studies are on top gear to provide alternative remedies that are effective, non-toxic and affordable for treatment of diabetics. In the history of man, traditional treatments have been an extremely valued sources of medicines. Of course, World Health Organization (WHO) has listed a total of 21,000 plants, as valid sources of potent medicines in the world (Kumar *et al.*, 2021). Among them, more than 400 plants are available for the treatment of diabetes due to their composition of bioactive compounds. Unfortunately, only a small number of these plants have undergone scientific and medical evaluation to assess their efficacy and this has limited their wide utilization in formulation of drugs. The traditional medicinal plants are used due to the high cost of orthodox drugs and low potency of some orthodox drugs.

Hunteria umbellata is a good source of bioactive compounds, carbohydrate, protein and minerals (Adeneye and Adeyemi, 2009; Adeneye *et al.*, 2012). The fruit is yellow in colour, smooth, about 5–25cm and consists of two separates globose mericaps (3–6 cm long), 8 – 25 seeds embedded in a gelatinous pulp. In African folk medicine, different parts of *Hunteria umbellata* plant are highly valued in the local treatment of gastric ulcers, liver diseases, diabetes mellitus and obesity (Boone, 2006; Adeneye *et al.*, 2012a). In Germany, extracts of *Hunteria umbellata* are used for reducing the heart rate, as an aphrodisiac, to decrease blood pressure and reduce blood lipids (Boone, 2006; Ahajumobi and Anderson, 2022). Among the Southwest Nigerians, the plant is reputed for its anti-helminthic, anti-inflammatory, uterotonic and antidiabetic activities (Falodun *et al.*, 2006). Cold decoction made from the plant seeds is also reputed for the management of obesity and hyperlipidaemia among the Yoruba herbalists in Nigeria (Adeneye and Adeyemi, 2009; Ogunlana *et al.*, 2021; Ahajumobi and Anderson, 2022). Also, extracts of the seeds of the plant have been reported to be very active against micro-organisms such as *Escherichia coli*, *Proteus spp.* and *Staphylococcus aureus* (Adeneye *et al.*, 2012a). *Hunteria umbellata* seeds extract contains secondary metabolite like tannins, cardia glycosides, flavonoids, saponins, alkaloids, anthraquinones, polyphenols and terpenoids (Igbe *et al.*, 2009; Adeneye *et al.*, 2012; Oboh *et al.*, 2018). The extract reduces the blood glucose levels, cholesterol levels and triglycerides (Adeneye *et al.*, 2011; Adeneye and Crooks, 2015). *Hunteria umbellata* (HU) significantly elevates red blood cell, Packed cell volume, and Hemoglobin levels (Ogunlana *et al.*, 2021; Ahajumobi and Anderson, 2022), which strongly suggests that HU could be useful in the management of anemia.

The aim of this study was to assess the haematological effects of Methanol and n-Hexane extracts of *Hunteria umbellata* seeds on diabetes mellitus using alloxan induced diabetic wistar rats as test animals.

MATERIALS AND METHODS

Materials

Procurement of Sample Material:

The fresh fruits of *Hunteria umbellata* (HU) were purchased from Aba in Aba North local government area of Abia State. They were botanically authenticated at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria.

Methods

Preparation of Sample Material:

The fruits were washed with distilled water and peeled to obtain the seeds. The *Hunteria umbellata* (HU) seeds were rinsed in clean tap water and air dried in an oven (Model, DHG 9101.1 USA) at 25°C for 7 weeks. The dried seeds were pulverized into powder using an electric blender (Model serial NO. 4745, Christy and Noris Ltd. England) and stored in a dry container at room temperature.

Preparation of Methanol *Hunteria umbellata* (HU) Seed Extract:

Hunteria umbellata (HU) seed powder (841g) was poured into the material chamber of the soxhlet extractor. Then extraction using methanol (solvent) of 1600 mL at a temperature of 60°C took place for 48 h. The solvent (methanol) in the *Hunteria umbellata* (HU) seed extract was recovered by evaporating at 40°C using a rotary evaporator to obtain a crude extract. The

Hunteria umbellata (HU) seed extract was packaged in a sealed zip lock and stored in refrigerator for further analysis.

Preparation of n-Hexane *Hunteria umbellata* (HU) Seed Extract:

Hunteria umbellata (HU) seed powder (841g) was poured into the material chamber of the Soxhlet extractor. Then extraction using n-hexane (solvent) of 1600 mL at a temperature of 60°C took place for 48 h. The solvent (n-hexane) in the *Hunteria umbellata* (HU) seed extract was recovered by evaporating at 40°C using a rotary evaporator to obtain a crude extract. The *Hunteria umbellata* (HU) seed extract was packaged in a sealed zip lock and stored in refrigerator for further analysis.

Determination of Phytochemicals in the HU Seed Extracts:

The phytochemical screening was done on the methanol and n-hexane HU seed extracts using GCMS-QP 2010 SHIMADZU instrument (Trease and Evans, 1989; Shale *et al.*, 1999; Evans *et al.*, 2000; Abate, 2002; Sawadogo *et al.*, 2006; Agim *et al.*, 2017). The constituents tested include: Alkaloids, Tanins, saponins, phenolic compounds, cardiac glycoside, flavonoids and steroids. The HU seed extract (3 g) was dissolved in aqueous solvent of methanol. To analyze the sample, the column oven temperature and Injector temperature were set at 800°C and 200°C respectively. The flow control mode was maintained in linear velocity with a split injection mode split ratio of 20. The column flow was 1.46 mL/min with a helium carrier gas of 99.9995% purity. The column oven temperature program was set as follows: The temperature was set at 80°C with 2 minutes hold time by the rate of 10. The temperature was 300°C with 10 minutes hold time. The column at 5 minutes was used with a length of 30 mL and diameter of 0.25 mm and its film thickness was 0.25 µm. The ion source temperature for MS condition was 200°C and interface temperature was 240°C. Starting m/z (Mass to charge) ratio was 40 and ending with m/z ratio of 700. (40-700 m/z).

Identification of the Phytochemical Constituents:

The unknown phytochemical components present in the extracts, were identified by comparing their individual mass spectral peak values with the data base of National Institute of Standard and Technology (NIST) which holds about 62,000 patterns. Then, the phytochemical was identified based on the hits returned after comparing the unknown peak value and the chromatogram from GCMS against the known chromatogram, peak value from the NIST library data base. Subsequently, the detail about their molecular formula, molecular weight, structures was obtained.

Animal for the Experiment:

Sixty-six sexually mature male Wistar rats (10-12 weeks old; weights-150-153g) obtained from the laboratory animal house of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria, were used for the experiment. Thirty of the rats were assigned to six groups of five rats each, in metabolic cages equipped to separate faeces and urine. The rats had exactly 12 h of light and 12 h of darkness in a day. National Research Council guidelines on the care and use of laboratory animals (National Research Council, 2010) were strictly adhered to. The rats had free access to food (Standard pellet diet, from Vital feed Ltd. Aba, Abia State, Nigeria) and water at will for an acclimatization period of 21 days prior to experiment. Baseline blood glucose and body weights of the rats were taken after acclimatization period. The study was approved by Animal Experimentation Ethics Committee

of Michael Okpara University of Agriculture, Umudike, Nigeria. The seven groups of the rodents (5 rats in each group) were obtained on the basis that the difference in mean body weight of each group did not exceed 5g (AOAC, 1995). The rats were housed in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike. The animals were acclimatized for a period of 21 days prior to the experiment. Rats were allowed free access to water throughout the study and were maintained on a 12 h light: 12 h dark phase schedule.

Experimental Design

The studies on diabetes mellitus were carried out using the Completely Randomized Design (CRD). Thirty rats were randomly assigned to six groups of five rats each based on treatments for them. There were six treatments, each replicated five times. The rats were the replicates while the different diets/treatments differentiated the groups. Then, for the Acute Toxicity Test (LD₅₀), thirty-six rats (nine rats in phase one and nine rats in phase two for Methanol HU seed extract; nine rats in phase one and nine rats in phase two for n-Hexane HU seed extract) were used for the experiment

Induction of Diabetes Mellitus

The rats were fasted for 17 hours before induction of diabetes mellitus. Diabetes mellitus was induced by a single intraperitoneal injection of 160 mg/kg body weight of Alloxan monohydrate (Pari and Venkateswaran, 2002). Alloxan was dissolved in 0.9% normal saline as vehicle. Therapeutic measures were adopted to ensure the survival of the rats by administration of glucose to tide over hypoglycemic phase. The blood glucose levels were assessed on days 3 and 6 following administration of alloxan. The blood samples were obtained by sequential snipping of the tail. Animals with blood glucose level greater than 200 mg/dl were considered diabetic after 2 days of induction of alloxan and were used for the experiment. All animals were allowed free access to water and pellet diet.

Experimental Groups of the Animals for the Diabetes Mellitus studies

The six groups of the rats were treated as follows:

1. Group 1 (Negative control- diabetic rats without treatment)- allowed on animal feed and water *ad libitum*;
2. Group 2 (Positive control- diabetic rats)- were given oral hypoglycemic drug (Glibenclimide at 500 mg/kg body weight) and allowed on animal feed and water *ad libitum*;
3. Group 3 (Test group- diabetic rats)- rats were orally administered 250mg/kg body weight of methanol HU seed extract and allowed on animal feed and water *ad libitum*;
4. Group 4 (Test group- diabetic rats)- rats were orally administered 500mg/kg body weight of methanol HU seed extract and allowed on animal feed and water *ad libitum*;
5. Group 5 (Test group-diabetic rats)- rats were orally administered 250mg/kg body weight of n- hexane HU seed extract and allowed on animal feed and water *ad libitum*;
6. Group 6 (Test group- diabetic rats)- rats were orally administered 500mg/kg body weight of n- hexane HU seed extract and allowed on animal feed and water *ad libitu*

Note: The rats were all sacrificed after 14 days of the study for biochemical analysis.

Composition of the Animal Feed

The composition of rodent pelleted chow was 50% as carbohydrate, 21% as protein, 5% as fat, 15% as moisture and 9% mineral. The diets were formulated using AIN-93G (American Institute of Nutrition) method (National Research Council, 1995).

Biological Studies

Acute Toxicity Test LD₅₀:

Acute toxicity tests for the methanol and n-hexane seed extracts were done according to the method of Lorke (1983) as follows:

Phase 1:

In this phase, nine test animals were divided into three groups of three animals each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg body weight) of test substance respectively. The animals were placed under observation for 24 hours to monitor their behaviors as well as if mortality will occur.

Phase 2:

In this phase, nine test animals were distributed into three groups of three animals each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg body weight) of test substance and then observed for 24 hours for behavioral change as well as mortality.

LD₅₀ was calculated by the formula;

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where; D₀ is the highest dose that gave no mortality

D₁₀₀ is the lowest dose that produced mortality

Blood Sample Collection

At the 14th day, the animals were fasted overnight, anaesthetized with chloroform and sacrificed. Blood was collected through sharp cardiac puncture. Blood samples were collected into EDTA containers (whole blood for hematological analysis).

Determination of Blood Glucose Concentration (mg/dl)

The one touch glucose monitoring meter and test strips were used as described in the operational manual. A drop of whole blood (0.04 mL) was placed on a strip inserted into the glucometer. The glucometer automatically separated serum from blood cells and determined the blood glucose, which was displayed on the glucometer and the reading taken.

Determination of Hematological Parameters

Hematological indices were determined by standard method as described by Ochei and Kolhatkar (2008). The indices examined were Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC) and Platelets. The standard procedures used are described below.

Packed Cell Volume (PCV)

This was determined using standard haematological procedure as described by Ochei and Kolhatkar (2008). Well mixed anticoagulated blood (0.5 ml) was aspirated into a capillary tube with one end sealed with plasticin. The tube was spun in a haematocrit centrifuge for 5 minutes and then read off in a PCV reader. The values obtained were recorded as the PCV values.

Red Blood Cells

This was determined using standard haematological procedure as described by Ochei and Kolhatkar (2008). Well mixed anticoagulated blood was diluted 1: 20 with 10% Na₂CO₃ solution. The mixture (0.5ml) was loaded into an improved Neubauer counting chamber. Appropriate squares (5 significant squares) were counted and added up to determine the total red cell count.

White Blood Cells (Total White Blood Cell Count)

This was determined using standard haematological procedure as described by Ochei and Kolhatkar (2008). Well mixed anticoagulated blood was diluted 1: 20 with Turk solution (2% Glacial acetic acid) in a test tube. This was loaded into an improved Neubauer counting chamber. Appropriate squares (4 significant squares) were counted, added up and divided by 2 to determine the total white cell count.

Platelets

This was determined using the method described by Cheesbrough (2000). The blood sample was diluted 1: 20 with 2% ammonium oxalate. The diluted sample was loaded into the Neubauer counting chamber with the aid of a pasteur pipette. The platelets were counted from appropriate five squares on the chamber under a microscope and summed up to obtain the platelet values.

Mean Corpuscular Volume (MCV)

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by calculation.

$$\text{MCV (f/L)} = \frac{\text{PCV (decimal fraction)} \times 1000}{\text{RBC } (\times 10^{12}/\text{L})}$$

Where MCV = Mean Corpuscular Volume

PCV = Packed Cell Volume

RBC = Red Blood Cells

$$\text{MCHC (g/L)} = \frac{\text{whole blood hemoglobin concentration (g/L)}}{\text{PCV (decimal fraction)}}$$

Where MCHC = Mean Corpuscular Hemoglobin Concentration

Statistical Analysis

Data obtained in this study were expressed as means of triplicate values. Then, the phytochemical data were subjected to statistical analysis. Analysis of variance (ANOVA) were

determined using SPSS Version 21.0 and the differences between the mean values were evaluated at $p < 0.05$ using Duncan's multiple range test.

RESULTS

Table 1: The Median Lethal Dose (LD₅₀) of the methanol HU seed extract

Dose mg/kg	D/T	Physical Observation
Phase 1		
10	0/3	Scratching of the mouth within a minute
100	0/3	Scratching of the mouth and calmness after a while
1000	0/3	Calmness and slow movement
Phase 2		
1600	0/3	Scratching of the mouth and a little restlessness
2900	3/3	Restlessness, Agitation, Climbing on other rats and death within 1hr
5000	3/3	Restlessness, Agitation and death within 1hr

D = Number of deaths; Number of albino rats used per experiment = 3

The acute toxicity test for the methanol HU seed extract shown in Table 1 revealed that in phase 1 of the study, no death of animal occurred at a dose of up to 1000 mg/kg body weight and there were no observable signs of toxicity. In phase 2, treatment of the animals with doses from 2900 to 5000 mg/kg body weight caused the death of all three animals with signs of toxicity.

Table 2: The Median Lethal Dose (LD₅₀) of the N-hexane HU seed extract

Dose mg/kg	D/T	Physical Observation
Phase 1		
10	0/3	Scratching of the mouth and calmness within a minute
100	0/3	Scratching of the mouth and calmness after a while
1000	0/3	Calmness and slow movement
Phase 2		
1600	0/3	Calmness and slow movement
2900	0/3	Calmness and slow movement
5000	0/3	Calmness and slow movement

D = Number of deaths = 0; T = Number of albino rats used for the experiment = 3

The acute toxicity test of the n-hexane HU seed extracts as shown in Table 2, revealed that in phase 1 of the study, no death of a rat occurred at a dose of up to 1000 mg/kg body weight, and there were no observable signs of toxicity. In phase 2, treatment of the animals with doses of up to 5000 mg/kg body weight did not cause any death or produce any signs of toxicity.

Table 3: Results for GC-MS Profiling of Methanol HU Seed Extract

Compound/ species	probabilit y	Retentio n time	Molecular formula	Molecula r weight	Compound Nature	Activity
Benzenemethanol, $\alpha\alpha$ - Dimethyl	48.2%	3.849	C ₉ H ₁₂ O	136	Colourless aromatic alcohol.	Acts as a solvent, an antioxidant and a fragrance.

Spiro-9,9'-bis(2-hydroxyfluorene)	29.9%	3.913	C ₂₅ H ₁₆ O ₂	348	Extremely weak basic (essentially neutral)	Potentially toxic compound.
Vismiaquinone	44.8%	5.013	C ₂₁ H ₂₀ O ₅	352	-	-
Dichloroacetaldehyde	98.9%	7.550	C ₂ H ₂ Cl ₂ O	112	Colourless organochlorine compound	Anti-fungal activity
Trichloromethane	96.9%	8.022	C HCl ₃	118	Colourless, volatile liquid with an ether-like odour.	Acute chloroform toxicity results in impaired liver function, cardiac arrhythmia, nausea and central nervous system dysfunction.
1,3-Diphenyl-2,4,5-trioximidazolidine	15.6%	8.569	C ₁₅ H ₁₀ N ₂ O ₃	266	-	-
2-Norpinanol, 3,6,6-trimethyl	8.78%	9.651	C ₁₀ H ₁₈ O	154	-	-
Nonanamide, N'[(3-methoxy-4[(trimethylsilyl)oxy]phenyl)methyl]-8-methyl	29.5%	10.012	C ₂₁ H ₃₇ NO ₃ Si	379	-	Harmful by ingestion
n-Hexadecanoic acid	94.5%	13.906	C ₁₆ H ₃₂ O ₂	256	Saturated fatty acid	Anti-bacterial activity
5,5'-Di(benzyloxycarbonyl)-3,3',4,4'-tetramethyl-2,2'-dipyrrylmethane	54.5%	15.826	C ₂₉ H ₃₀ N ₂ O ₄	470	-	Synthesis of anti-oxidant
Succinic acid, 2-pentyl 2,2,3,3-tetrafluoropropyl ester	98.0%	16.746	C ₁₂ H ₁₈ F ₄ O ₄	302	Alpha, omega, dicarboxylic acid	Atheimic activity
Glycerol tricaprylate	97.9%	17.398	C ₂₇ H ₅₀ O ₆	470	-	Fragrant ingredient
Vanadium, (pentamethylcyclopentadienyl) (Tetraphenylcyclobutadiene	93.6%	18.527	C ₃₈ H ₃₅ V	542	Hard, silvery, grey, malleable transition metal	It is used for pre-diabetes and diabetes
Acetic acid, 4,4,6a,8a,11,12,14b-heptamethyl-13-oxodocosahydricen-3-ly ester	98.9%	18.638	C ₃₁ H ₅₀ O ₃	470	Carboxylic acid, clear, colorless organic fluid	Anti-bacterial

Key: (-) Not present

The GC-MS spectra analysis in Table 3 revealed the presence of different compounds present in the methanol HU seed extract, as well as their retention time, molecular formula, molecular weight, compound nature and their different activities.

Table 4: Results for GC-MS Profiling of N-Hexane HU Seed Extract

Compound/species	Probability	Retention time	Molecular formulae	Molecular weight	Compound Nature	Activity
1 α -Hydroxyallobetulane	97.2%	5.455	C ₅₀ H ₅₀ O ₂	442	Cyclic dimeric esters	Anti-fungal activities
Benzoic acid, 2,4-dimethoxy-6-[(4-methoxyphenyl)]	95.1%	6.133	C ₁₉ H ₂₀ O ₆	344	Aromatic carboxylic acid	Anti-microbial activity
Benzenepropanoic acid	97.4%	6.776	C ₉ H ₈ BrNO ₄	273	-	-
5 α -Cholestane-3 β ,4 β ,6 α , 7 α ,8 β , 15 α ,16 β ,26-octol	41.8%	7.027	C ₂₇ H ₄₈ O ₈	500	Esterases that lyses choline based esters	Neurotransmitters toxic by ingestion
5,9,23-Tricontatrienoic acid, methyl ester		11.101	C ₃₁ H ₅₆ O ₂	460	-	-
Octadecanoic acid, 10,11,13,14-tetrakis[(trimethylsilyl)oxy]	93.0%	17.759	C ₃₁ H ₇₀ O ₆ Si ₄	650	Esters of fatty acid	Anti-bacterial activity
Nonanamide, 5-hydroxy-5-methyl-2-(2-methylpropyl)-N-benzyl-	91.6%	18.387	C ₂₁ H ₃₅ NO ₂	333	-	-
D-Ribose	90.1%	18.725	C ₅ H ₁₀ O ₅	150	Water soluble pentose sugar	Coding, decoding, regulation and expression of gene
1(3H)-Isobenzofuranone, 3,3'-(4-hydroxy-1,3-phenylene)bis[3-(4-hydroxyphenyl)]	81.3%	20.017	C ₃₄ H ₂₂ O ₇	542	Natural oil	Anti-oxidant and antimicrobial activity
Spirostan-3,11-diol diacetate(ester)	4.06%	20.314	C ₃₁ H ₄₈ O ₆	516	Steroid saponies in plant	Anti-bacterial activity

1H-Indole-3-acetic acid, 1-(tert-butyldimethylsilyl)-5-[(tert-butyldimethylsilyl)oxy]	98.4%	20.541	C ₂₈ H ₅₁ NO ₃ Si ₃	533	Naturally occurring plant hormone of the auxin class	-
Hex-5-ynoic acid, methyl ester	96.8%	23.055	C ₇ H ₁₀ O ₂	126	-	-
Sebacic acid, dodecyl pentafluorobenzyl ester	85.4%	23.940	C ₂₉ H ₄₃ F ₅ O ₄	550	Naturally occurring dicarboxylic acid derivatives of oil castol oil	-
2,4-Cyclopentadiene-1,2,3,4-tetracarboxylic acid, 5-(2-quinoliny)-, tetramethyl ester	32.7%	24.854	C ₂₂ H ₁₉ NO ₈	425	-	-

Key: (-) = Not present

The GC-MS spectra analysis in Table 4 revealed the presence of different compounds present in N-hexane seed extract of *Hunteria umbellata* as well as their retention time, molecular formula, molecular weight, compound nature and their different activities.

Table 5: Effect of methanol and n-Hexane seed extract on the haematological profile of Alloxan-induced diabetic Wistar rats

GROUP	RBC	PCV (%)	HB(g/dL)	WBC($\times 10^9$ /L)	PLT($\times 10^3$ /mL)	MCV(f/L)	MCH(p/L)	MCHC(g/dL)
1	5.99 $\pm$ 0.21 ^{ab}	39.00 $\pm$ 1.00 ^{ab}	12.90 $\pm$ 0.95 ^a	12.95 $\pm$ 1.56 ^b	131.00 $\pm$ 6.89 ^b	65.09 $\pm$ 1.06 ^{bc}	21.50 $\pm$ 0.82 ^a	33.05 $\pm$ 1.76 ^a
2	6.40 $\pm$ 0.27 ^b	41.33 $\pm$ 1.52 ^{ab}	13.83 $\pm$ 0.20 ^a	13.22 $\pm$ 2.68 ^b	118.50 $\pm$ 2.05 ^b	64.55 $\pm$ 0.41 ^{abc}	21.62 $\pm$ 0.78 ^a	33.49 $\pm$ 1.01 ^{ab}
3	6.70 $\pm$ 0.12 ^{bc}	42.33 $\pm$ 1.52 ^c	15.80 $\pm$ 0.43 ^b	9.15 $\pm$ 0.67 ^a	117.10 $\pm$ 5.77 ^b	63.17 $\pm$ 1.13 ^{ab}	23.58 $\pm$ 0.36 ^c	37.33 $\pm$ 0.75 ^c
4	5.18 $\pm$ 1.13 ^c	46.66 $\pm$ 1.15 ^d	16.46 $\pm$ 0.15 ^b	10.86 $\pm$ 0.40 ^{ab}	110.13 $\pm$ 3.23 ^b	63.72 $\pm$ 0.14 ^{abc}	22.49 $\pm$ 0.55 ^{abc}	35.30 $\pm$ 0.86 ^{bc}
5	5.19 $\pm$ 0.07 ^a	38.66 $\pm$ 2.08 ^a	13.66 $\pm$ 0.15 ^a	10.59 $\pm$ 0.63 ^a	113.63 $\pm$ 0.90 ^b	66.13 $\pm$ 2.96 ^c	23.38 $\pm$ 0.35 ^{bc}	35.41 $\pm$ 1.94 ^{bc}
6	6.40 $\pm$ 0.8 ^{ab}	38.33 $\pm$ 0.57 ^a	13.76 $\pm$ 0.35 ^a	11.12 $\pm$ 0.42 ^{ab}	116.10 $\pm$ 2.00 ^b	64.82 $\pm$ 0.55 ^{abc}	23.28 $\pm$ 0.55 ^{bc}	35.910 $\pm$ 0.56 ^c

Values represent the mean $\pm$ SD for n=3. Values in the same column bearing the same alphabets are not significantly different from each other (P<0.05).

Key: Group 1= Diabetic control (untreated rats), Group 2= Positive control (Standard drug (Glibenclamide, 150mg/kg body weight); Group 3= (methanol extract low dose, 250mg/kg

body weight), Group 4= n(methanol extract high dose, 500mg/kg body weight); Group 5=(n-hexane extract low dose 250mg/kg body weight) Group 6=(n- hexane extract high dose, 500mg/Kg body weight). Red blood cell (RBC), Pack cell volume (PCV), Haemoglobin (HB), White blood cell (WBC), Platelets (PLT), MCV, MCH, MCHC.

The result of the effect of aqueous extract of *Hunteria Umbellata* on the haematological parameters of Alloxan induced diabetic rats are shown in Table 5. The result of the methanol /n-hexane extract of *Hunteria Umbellata* had significant effect on RBC, Hb, MCHC, MCH, PCV, MCV, WBC. Group 1 (Negative control) were significantly ($P<0.05$) high in WBC, PLT, MCV while RBC, PCV, Hb, MCH, MCHC were significantly ($P<0.05$) low.

However, WBC, PLT, MCV decreased significantly in all treatment groups except WBC in group 3 (treated with 500 mg of Glibenclamide, a standard known drug) and MCV in group 6 (treated with 250 mg/kg body weight of n-hexane). Also, RBC, PCV, HB, MCH, MCHC increased significantly in all treatment groups except RBC in (group 6), PCV in group 6, and group 7. When compared to the group 2 (Diabetes or Negative control).

DISCUSSION

The qualitative phytochemical screening of Methanol and n-hexane seed extracts of *Hunteria Umbellata* indicated significant contents of secondary metabolites such as phenols, alkaloids, flavonoids, oxalate, saponins, combined and free reducing sugars, tannins, sterols and balsams. The phytochemicals exert a wide range of antidiabetic activities (Kong *et al.*, 2021; Ramirez-Alarcon *et al.*, 2021; Bahrapour *et al.*, 2023; Yedjou *et al.*, 2023). The Gas Chromatography Mass Spectrophotometry analysis of Methanol and n- hexane seed extracts of *Hunteria umbellata* revealed the presence of different compounds present, including their retention time, molecular formula, molecular weight, compound nature and their different activities.

Acute Toxicity Test LD₅₀ (Locke's Method, 1983)

Safety assessment of pharmaceuticals and foods are very crucial prior to their approval for human uses (Erhirhie *et al.*, 2018). LD₅₀ is the amount of a substance, or material given at once which causes death of 50% of a group of test animals. It is usually expressed as the amount of chemical administered (eg. mg per 100 g for smaller animals) or (mg per kg for bigger subjects) of the weight of the test animal. LD₅₀ is important for drugs, food and accidental domestic poisoning. In general, the smaller the LD₅₀ value, the more toxic the chemicals are; the larger the LD₅₀ value, the lower the toxicity (Chinedu *et al.*, 2013). Of course, extrapolation of these animal data into humans may guarantee potential hazards of these extracts to humans.

Assessment of hematological parameters can not only be used to determine the extent of deleterious effect of extract on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its product (Yakubu *et al.*, 2007).

Haemotoxicity sets in when there is elevation of these blood components beyond their reference ranges. There was a significant reduction ($P<0.05$) in WBC levels of diabetic rats treated with aqueous extract of *Hunteria umbellata* when compared with the untreated Wistar rats. The study shows that there is a significant increase ($P<0.05$) in the level of RBC, HB and PCV of group 3 and the treated groups, (250 mg/kg body weight of the methanol extract of *Hunteria umbellata*; 500 mg/kg body weight of the methanol extract of *Hunteria umbellata*)

when compared to the negative control. But there was a decrease in the 250 mg/kg body weight of n-hexane extract of *Hunteria umbellata* and 500 mg/kg body weight of n-hexane extract of *Hunteria umbellata*. This may be as a result of anemia or the onset of glycosylation process in the untreated diabetic rats. There was a significant decrease ($P<0.05$) in the PLT concentration of the diabetic rats when treated with the extracts of *Hunteria umbellata*. There was a significant reduction ($P<0.05$) in the MCV except the group 6 treated with (250 mg/kg body weight of the n- hexane extract) values.

There was a significant increase ($P<0.05$) in the MCH when treated with the extracts of *Hunteria Umbellata* (i.e. group 3- group 6) when compared to the negative control. Also, there was a significant increase ($P<0.05$) in MCHC when treated with extracts of *Hunteria Umbellata* on the diabetic rats. The results obtained shows significant values of WBC; therefore, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alters their normal physiological processes. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction (Albers, 1995; George, 2000). Therefore, the high dose (500 mg/kg body weight) of the *Hunteria umbellata* extracts were useful in reducing the platelets which in turn might be useful in reducing the cardiovascular diseases as some studies suggested various mechanisms by which flavonoid exert its antiplatelet property by lowering intracellular Ca^{2+} levels; alteration in the metabolism of cAMP, and thromboxane A₂ (Roy *et al.*, 1999; Kang *et al.*, 2001). The haemoglobin content, RBC and PCV also significantly increased stimulate erythropoietin release in the kidney which is the humoral regulators of RBC production (Degruchy, 19756; Polenakovic and Sikole, 1996). The result of the study suggested that *Hunteria Umbellata* extract studied showed positive haematological activities in rats and can be recommended on the management of anemia and immunity dependent disorders.

CONCLUSION

Methanol and n-Hexane extracts of *Hunteria umbellata* (at both 250 mg and 500 mg per kg body weight) exhibited effective positive antidiabetic properties, significant physiological and biochemical functions in treatment of diabetic wistar rats, induced with alloxan. The methanol and n-hexane seed extracts of *Hunteria umbellata* had significant composition of phytochemicals such as phenols alkaloids, flavonoids, oxalate, saponins etc. The acute toxicity test for methanol HU seed extract showed its safe nature at dose of up to 1600 mg/kg body weight; while that of n-hexane HU seed extract showed no sign of toxicity at dose of up to 5000 mg/kg body weight. Therefore, these methanol and n-Hexane extracts of *Hunteria umbellata* possess the potentials of management of diabetes mellitus in animals and humans better than the standard drug (Glibenclamide); and thus, could serve as alternatives or replacements to orthodox antidiabetic drugs that are associated with long-term side effects. Utilization of medicinal plants (such as *Hunteria umbellata*) in the management of diabetes mellitus should be encouraged in Africa.

RECOMMENDATION

Further studies should be undertaken on the effects of the mechanism of action of Methanol and n-Hexane extracts of *Hunteria Umbellata* seeds on alloxan induced diabetic male wistar rats. The results of such study can be used to verify the efficacy and potency of the results of

this present study. Attention should be drawn to proper utilization of medicinal plants in the treatment of diseases in Africa.

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