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Anti-diabetic effects of stem bark extract and fractions of *Terminalia catappa* Linn. (Combretaceae)

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ABSTRACT

The pharmacognostic, toxicity and anti-diabetic profiles of stem bark of *Terminalia catappa* were evaluated. The plant material was subjected to macroscopy, microscopy and proximate analyses using standard methods. The pulverized sample was extracted using soxhlet apparatus. The methanol extract (CE) was then fractionated into n-hexane fraction (HF), ethyl acetate fraction (EF), n-butanol fraction (BF) and water fraction (WF); the crude extract and the fractions were subjected to phytochemical analysis and screened for toxicity and antidiabetic properties using streptozotocin, alloxan and alloxan-nicotinamide induced diabetic Wistar rats. The chromatographic fingerprints of both the methanol extract and butanol fraction were obtained. The retardation factors (R_f-values) of the individual spots were calculated. The microscopic examination revealed some phloem parenchyma with pits, pheloderm, fibres, parenchyma cells, sclereids, cork cells and calcium oxalate crystals. Phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, saponin, steroids, cardiac glycosides, carbohydrates, proteins and anthraquinones. Acute toxicity test on the crude extract established that at 5000 mg/kg, no toxicity was recorded; the extract and fractions of *Terminalia catappa* slightly increased WBC concentrations. The crude extract and the fractions administered for 7 days significantly ($p < 0.01$) reduced the blood glucose levels when compared with control. The potency of the extract and fractions in relation to blood glucose concentration increased in the order WF > BF > CE > EF > HF; with the WF and BF at 500 mg/kg exhibiting fasting blood glucose reduction comparable to that obtained with 500 mg/kg metformin. The results showed that stem bark of *Terminalia catappa* possess anti-diabetic activity.

Keywords: Diabetes, *Terminalia catappa*, streptozotocin, alloxan, alloxan-nicotinamide.

INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, has been one of the most devastating diseases known to man [1]. It is a major endocrine disorder which is on the rise [2-5]. It is characterized by disordered metabolism and inappropriate or chronic high blood sugar level (hyperglycaemia), increased thirst or more water ingestion (polydipsia), frequent urination, glucose in urine (glycosuria), increased volume of urine due to osmotic effect of glucose (polyuria), increased appetite (polyphagia), blurring of vision and weight loss [6]. As a chronic disease of carbohydrate, fat and protein metabolism, it has serious biochemical, physiological and anatomical consequences which are all tied to manifest high blood glucose. Also, DM is a disease in which the body does not produce or properly use insulin [7]. Insulin is a hormone that is needed to convert sugars, starches and other foods into energy needed for daily life. The cause of diabetes continues to be a mystery, although both genetic (inheritance) and environmental (diet and lifestyle) factors appear to play some roles [8, 6]. Diabetics suffer from non-atheromatous degeneration of arterioles and capillaries, especially in the kidney and retina, leading to renal failure and blindness respectively [9] which accounts for elevated mortality rate of diabetics when compared with the population as a whole [6]. The therapeutic diet, which has changed considerably over the years, focuses on complex carbohydrates, dietary fibre (particularly the soluble type), and regulated proportions of carbohydrate, protein, and fat.

The plant kingdom has long served as a prolific source of useful drugs, food and additives, flavouring agents, colourants, binders and lubricants. Most of these plants found around which are used in the treatment of different ailments or diseases are known as medicinal plants [10, 11]. The use of medicinal

plants as source of relief from illness is as old as mankind, with recorded practices going back at least 4000 years. It has been estimated that about 25 % of all prescribed medicines today are substances derived from plants. Such drugs in use today include the analgesic drug- aspirin from *Filipendula ulmaria* Linn., the antimalarial agent- quinine from *Cinchona* species Linn., the anti-hypertensive drug-reserpine from *Rauwolfia serpentina* Linn., as well as the antineoplastic alkaloids; vincristine and vimblastine from *Catharanthus roseus* Linn., artemisine from *Artemisia annua*. Hence the need to continue research into medicinal plants, especially those that are used in traditional medicine across the developing countries of Africa, Asia and South America cannot be overemphasized. Various medicinal plants have been studied using modern scientific approaches. Results from these plants have recorded potential of medicinal plants in the area of pharmacology ^[11].

The discovery of plants or herbs with hypoglycaemic activities is not limited to Africa ^[11-13]. A considerable number of Chinese medical herbs have been found to possess hypoglycaemic properties. Although, there is no herbal substitute for insulin, some herbs may help adjust blood sugar levels and manage other diabetic symptoms i.e. they have anti-diabetic effects.

Terminalia catappa is a tall, tropical, deciduous and erect tree ^[14], reaching about 15-25 m, trunk 1-1.5 m in diameter, often buttressed at the base. Whorls of nearly horizontal, slightly ascending branches spaced 1-2 m apart in tiers, or storeys up the trunk. Leaf alternates, obovate with short petioles, spirally clustered at the branch tips, dark green, leathery and glossy. Flowers slightly fetid, greenish-white, very small, with no petals but 10-12 conspicuous stamens, arranged in several slender spikes 15-25 cm long in the leaf axis. Fruit hard, to 7 cm, green-red, rounded and flattened, egg-shaped, with 2 ridges but no wings, yellow or reddish when ripe. The cylindrical, oil-containing seeds are encased in a tough, fibrous husk within a fleshy pericarp ^[14].

Extracts from different parts of the plant have proven ethno-pharmacological properties ^[15-18].

MATERIALS AND METHODS

All drugs, chemicals, solvents and reagents used for the work are of analytical grade.

Plant material

Samples of fresh stem bark of *Terminalia catappa* were collected in December, 2014 at Awka, and identified by Mr. P.O. Ugwuozor of Department of Botany, and deposited in the herbarium at the Department of Pharmacognosy and Traditional medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria; with herbarium number PCG 474/A/001.

Experimental animals

Adult Wistar rats of either sex, aged 2-3 months with body weights 100-180 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. They were housed in cages; and allowed free access to drinking water and were fed *ad libitum* a standard laboratory diet (UAC feed, Nigeria). They were acclimatized with the environment for one week, weighed and sex matched before the start of the experiment.

Preparation of plant material

Fresh stem barks of *Terminalia catappa* were collected, cleaned and air-dried at room temperature, pulverized; and the powdered sample stored in airtight container for use in standardization and extraction.

Macroscopy of the plant

The plant was visually examined. The macroscopic characters of the plant were observed and noted. The organoleptic properties such as colour, odour and taste of the plant material were also observed and noted.

Microscopic examination of powdered sample

The pulverized sample of the stem bark was boiled in chloral hydrate solution for clearing, on a Bunsen burner. 2 drops of the cleared sample was placed on a glass slide, glycerine (mountant) was added to the slide, covered with a cover slip. The glass slide was mounted and viewed under the microscope. The microscopic characters were observed and noted.

Extraction of plant material

A 1500 g of the pulverized sample was extracted in 7.5 L of methanol using Soxhlet apparatus. The extract was concentrated in a Rotary evaporator at 40°C and evaporated to dryness in a water bath at same temperature. The extract was kept in air tight containers in a refrigerator until used. The percentage (%) yield of the extract was calculated as thus:

$$\begin{aligned} \text{Initial weight of sample} &= X \\ \text{Weight of bottle} &= Y \\ \text{Weight of bottle + extract} &= Z \\ \text{Weight of extract (W)} &= (Z-Y) \end{aligned}$$

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100 \quad \text{Equation 1}$$

Fractionation of plant extract

The crude methanol extract (100 g) was subjected to fractionation by adsorbing the crude extract on silica gel and eluted in succession with 2.5 L of each solvent (N-hexane, ethyl acetate, butanol and water) to yield hexane (HF), ethyl acetate (EF), butanol and water (WF) fractions.

Phytochemical analysis

The extract and fractions were subjected to various qualitative chemical tests to know the constituents present by using standard methods as described by ^[19].

Quantitative phytochemical analysis

This was done using standard methods as edited by ^[20].

Physico-chemical analysis

This was done using standard methods as edited by ^[20].

Analytical thin layer chromatography (TLC)

This was done using standard methods as edited by ^[21].

From the chromatograms developed, the retardation factors (R_f values) of the major spots were calculated thus:

$$\text{Retention factor (Rf)} = \frac{\text{Distance moved by a solute from the origin}}{\text{Distance moved by solvent from the origin}} \quad \text{Equation 2}$$

Induction of experimental diabetes

To the overnight fasted rats, 60 mg streptozotocin (STZ), 150 mg alloxan and 100 mg nicotinamide per kg body weight respectively [22], were administered intraperitoneally After a period of 48 hr and 24 hr for streptozotocin or alloxan and alloxan-nicotinamide respectively, rats with marked hyperglycaemia (blood glucose level >150 mg/dl / 8.3 mmol/l) measured using a glucometer (ONETOUCH@Ultraeasy, USA); were used for the study.

$$\text{Percentage reduction} = \frac{00 - \text{Treatment FBGL (a)}}{\text{Hyperglycaemic FBGL (b)}} \times 100$$

Equation 3

Biochemical studies

Blood was collected in non-heparinised tubes. The blood samples were centrifuged at 4000 g for 15 min, and the (supernatant) separated collected and stored at 4°C to maintain enzyme activity. The serum separated was analysed to evaluate the liver enzymes (Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) using the method of [23, 24, 25].

Haematological studies

Blood samples collected in heparinised tubes were used to investigate White Blood Cells (WBC), Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin (Hb). The microhaematocrit methods were employed for the assay.

Acute toxicity and lethality test (LD₅₀)

The acute toxicity and lethality (LD₅₀) of the crude extract was determined in rats using OECD standard method. The methanol extract of stem barks of *Terminalia catappa* was given separately in various doses (10, 100, 1000, 2000, 3000, 4000, 5000 mg/kg) by oral route. After administration of the extract, the animals were observed for 24 h to detect changes in the behavioural responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and monitored for any mortality.

Statistical analysis

Numerical data obtained from the study were expressed as the mean values ± standard error of mean. Differences among means of control and tested groups were determined using one-way analysis of variance (ANOVA). A probability level of less than 5 % (p ≤ 0.05) was considered significant.

RESULTS

Macroscopy of plant material

The macroscopic examination of the whole plant of *Terminalia catappa* Linn., showed an upright symmetrical crown and horizontal branches arranged in tiers; sessile edible fruit and nut, tasting almost like almond; simple, broad, ovoid or obovate, leathery or glabrous, alternating veins, glossy dark green leaves clustered towards the end of branches. These yielded important characteristics of the plant under study; and can be essential in preliminary identification of the plant and are useful diagnostic characters.

Microscopic examination of plant sample

Pulverized samples of the stem bark of *Terminalia catappa* Linn. when viewed with a photomicroscope revealed the presence of phloem parenchyma with pits, phelloderm, parenchyma cells, sclereids, fibres, cork cells and calcium oxalate crystals, which may

be useful for identification and diagnostic purposes; particularly, the powdered form of *Terminalia catappa*.

Result of Extraction

Table 1: Results of fractionation process

Fraction	Weight (g)	Colour
N-hexane	2.17	Yellow
Ethyl acetate	6.00	Green
Butanol	10.00	Brown
Water	7.85	Brown

Table 2: Results of Phytochemical analysis

Fractions	CE	HF	EF	BF	WF
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Cardiac-glycosides	+	-	+	+	-
Tannins	+	-	+	+	+
Alkaloids	+	+	+	+	+
Carbohydrates	+	+	+	-	+
Proteins	+	-	+	+	+
Steroids	+	+	+	+	+
Terpenoids	+	-	-	+	+
Anthraquinones	+	-	+	+	+

Keys: - not present + present
 CE - Crude extract, HF - n-hexane fraction
 EF - Ethyl acetate fraction, BF - Butanol fraction, WF - Water fraction

Table 3: Results of Quantitative phytochemical analysis

Phytochemicals	% Composition
Alkaloids	18.80
Saponins	6.75
Tannins	23.16
Flavonoids	6.10

Table 4: Results of Physico-chemical analysis

Analytical standards	% Composition (%)
Total ash	16.14 ± 0.70
Water soluble ash	1.50 ± 0.00
Acid insoluble ash	3.00 ± 0.00
Sulphated ash	4.00 ± 0.24
Moisture content	11.50 ± 0.14
Alcohol Extractive value	4.80 ± 0.02

Values of percentage composition shown are Mean values ± Standard error of mean (SEM).

Table 5: Retardation factors (Rf-values) of Crude extract and Butanol fraction in Hexane:Ethyl acetate (9:1, 5:5, 3:7)

Extracts	Rf values in		
	Hexane:Ethylacetate (9:1)	Hexane:Ethylacetate (5:5)	Hexane:Ethylacetate (3:7)
CE	0.95, 0.98	0.33, 1.00	0.26
BF	0.22, 0.30, 0.98	0.24, 0.76, 0.79	0.23, 0.32

The TLC chromatogram of the stem bark showed characteristic spots with the retardation factors (Rf values) in different solvent systems as shown in Table 5; which could be used as markers for quality evaluation and standardization of *Terminalia catappa*.

Anti-diabetic Evaluation

Percentage reduction of FBGLs

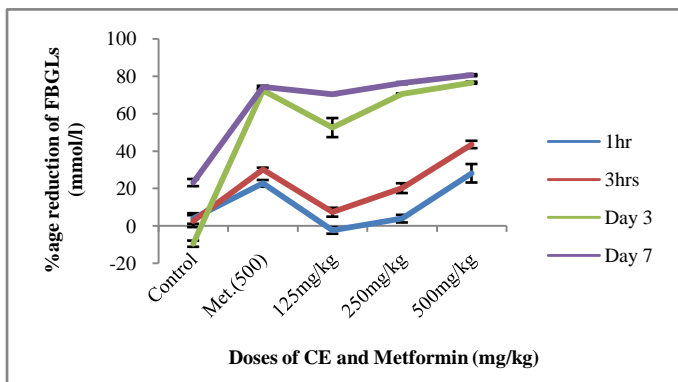


Figure 1: Percentage reductions of positive, negative control and different doses (125, 250 and 500 mg/kg) of crude extract in streptozotocin-induced diabetic rats.

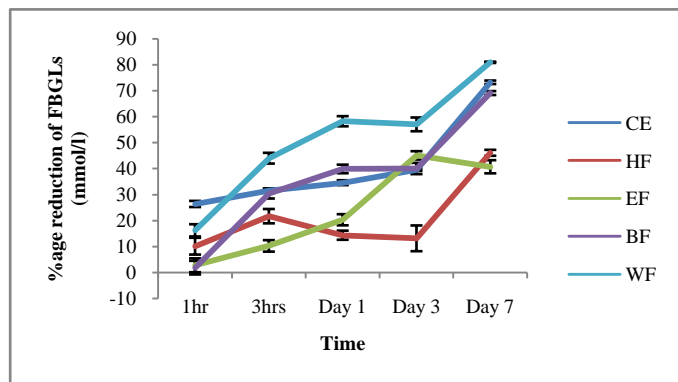


Figure 4: Percentage reductions of crude extract and the fractions in streptozotocin-induced diabetic rats
 Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction.

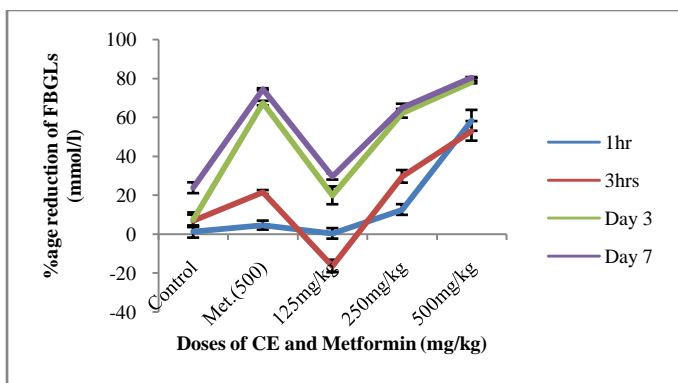


Figure 2: Percentage reductions of positive, negative control and different doses (125, 250 and 500 mg/kg) of crude extract in alloxan-induced diabetic rats

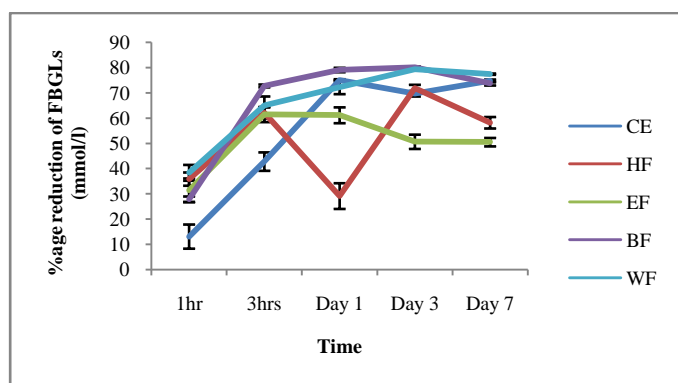


Figure 5: Percentage reductions of crude extract and the fractions in alloxan-induced diabetic rats
 Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction.

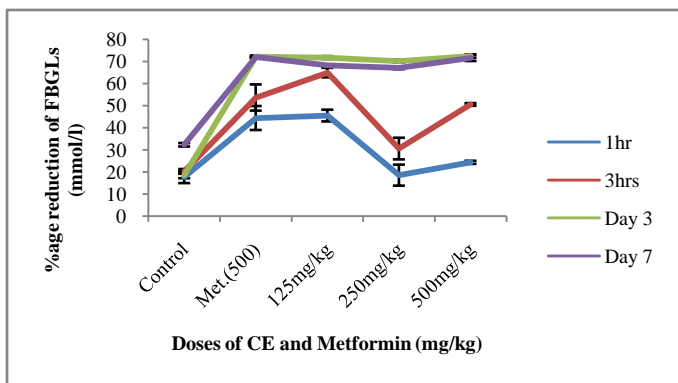


Figure 3: Percentage reductions of positive, negative control and different doses (125, 250 and 500 mg/kg) of crude extract in alloxan-nicotinamide-induced diabetic rats

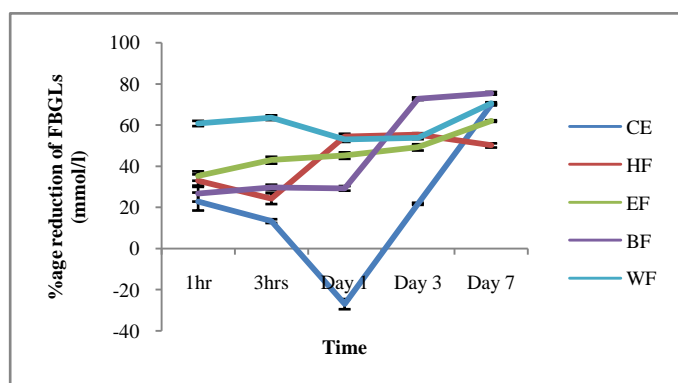


Figure 6: Percentage reductions of crude extract and the fractions in alloxan-nicotinamide-induced diabetic rats
 Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction.

Effect on biochemical studies

Aspartate aminotransferase (AST)

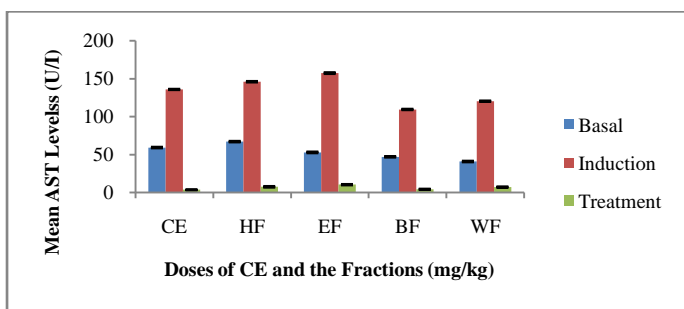
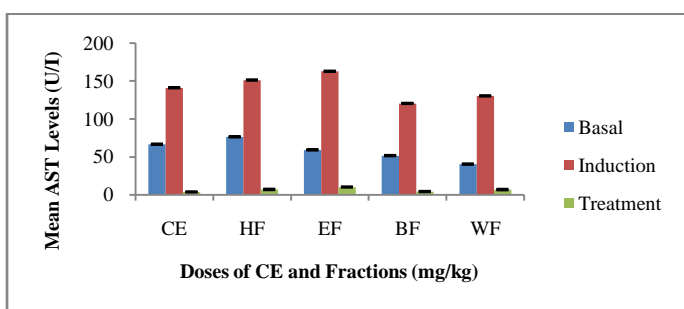


Figure 7: Effect of 500 mg/kg of crude extract and the fractions on mean AST levels in streptozotocin-induced diabetic rats



Doses of CE and Fractions (mg/kg)

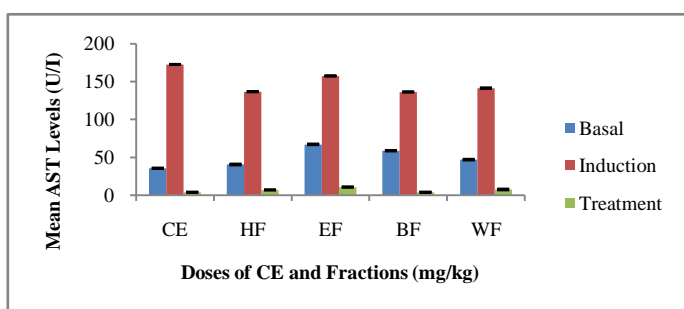


Figure 8: Effect of 500 mg/kg of crude extract and the fractions on mean AST levels in alloxan-induced diabetic rats

Figure 9: Effect of 500 mg/kg of crude extract and the fractions on mean AST levels in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction.

Alanine aminotransferase (ALT)

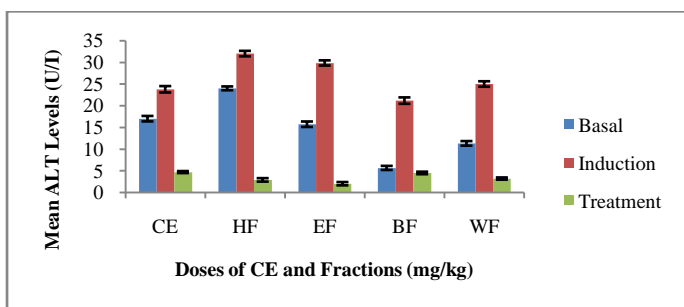


Figure 10: Effect of 500 mg/kg of crude extract and the fractions on mean ALT levels in streptozotocin-induced diabetic rats

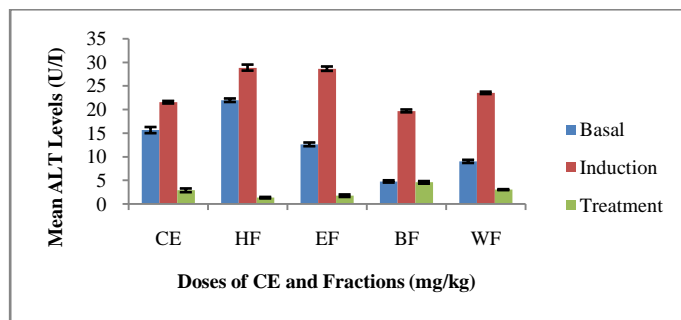


Figure 11: Effect of 500 mg/kg of crude extract and the fractions on mean ALT levels in alloxan-induced diabetic rats

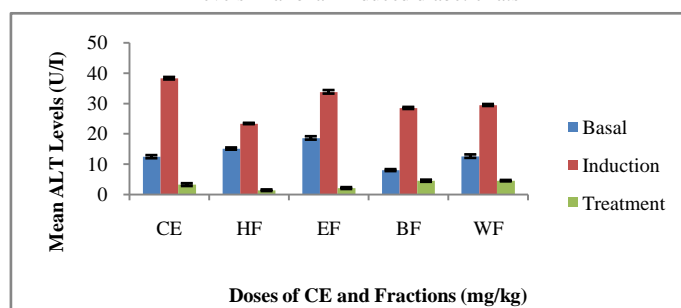


Figure 12: Effect of 500 mg/kg of crude extract and the fractions on Mean ALT levels in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

Alkaline phosphatase (ALP)

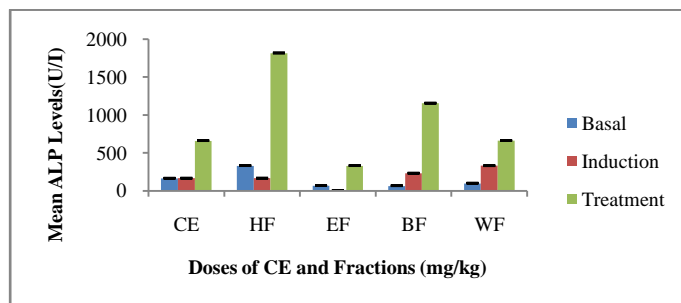


Figure 13: Effect of 500 mg/kg of crude extract and the fractions on mean ALP levels in streptozotocin-induced diabetic rats

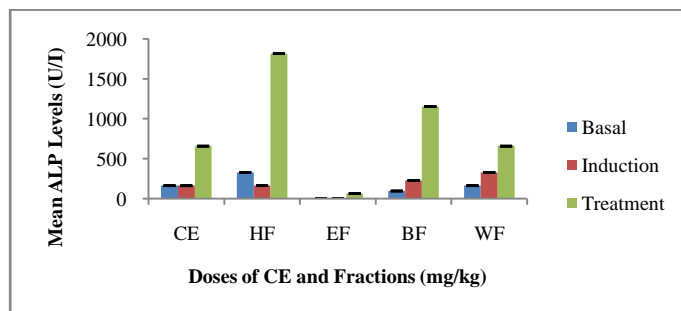


Figure 14: Effect of 500 mg/kg of crude extract and the fractions on mean ALP levels in alloxan-induced diabetic rats

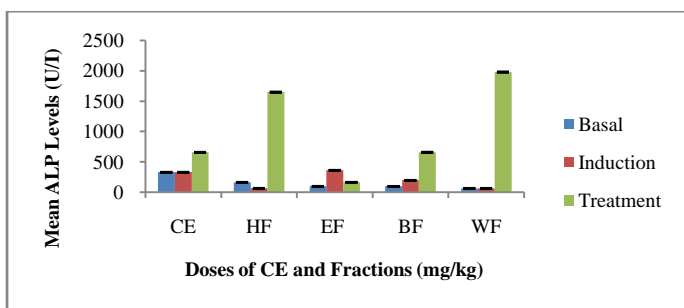


Figure 15: Effect of 500 mg/kg of crude extract and the fractions on mean ALP levels in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

Effect on haematological studies

Packed Cell Volume (PCV)

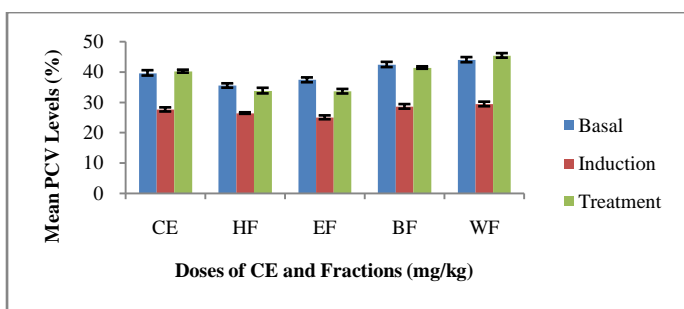


Figure 16: Effect of 500 mg/kg of crude extract and the fractions on mean PCVs in streptozotocin-induced diabetic rats

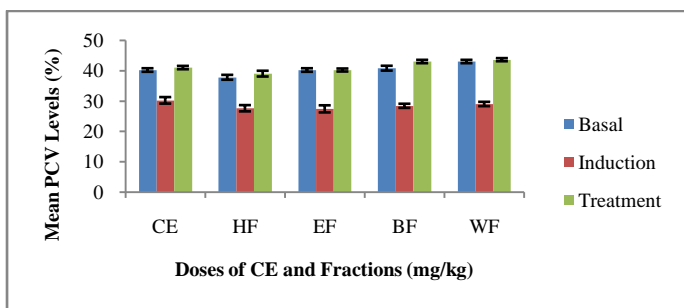


Figure 17: Effect of 500 mg/kg of crude Extract and the fractions on mean PCVs in alloxan-induced diabetic rats

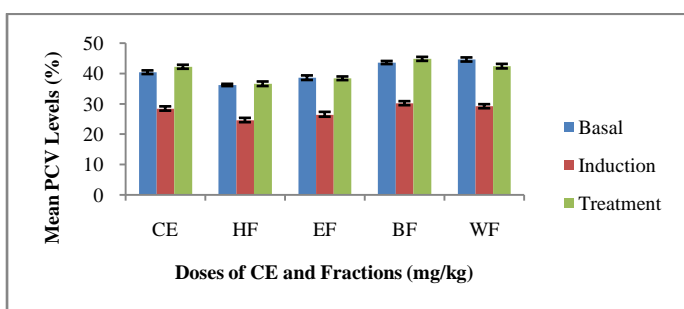


Figure 18: Effect of 500 mg/kg of crude extract and the fractions on mean PCVs in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

Haemoglobin (Hb)

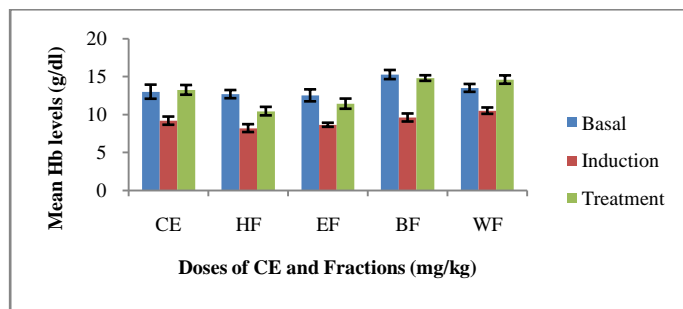


Figure 19: Effect of 500 mg/kg of crude extract and the fractions on mean Hb in streptozotocin-induced diabetic rats

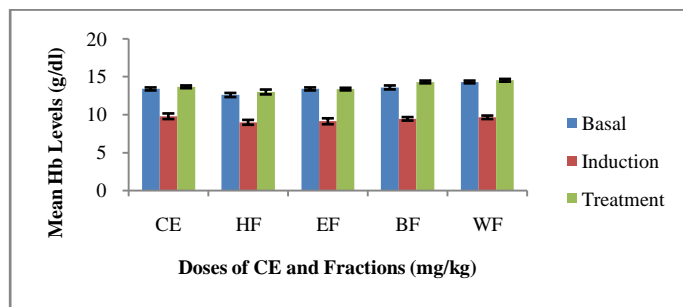


Figure 20: Effect of 500 mg/kg of crude extract and the fractions on mean Hb in alloxan-induced diabetic rats

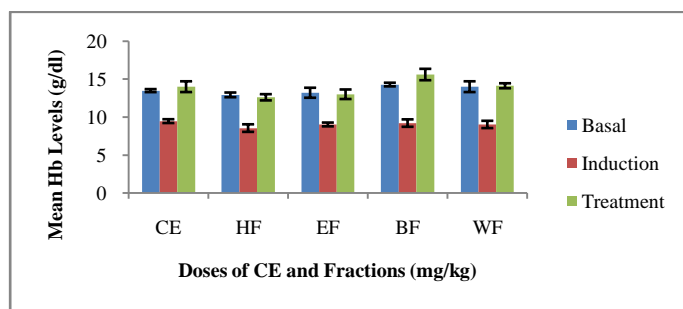


Figure 21: Effect of 500 mg/kg of crude extract and the fractions on Mean Hb in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

White Blood Cell (WBC) Count

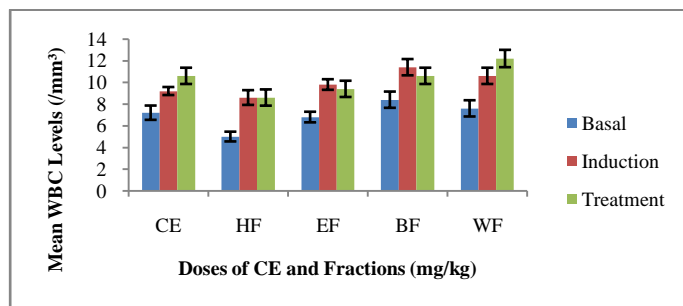


Figure 22: Effect of 500 mg/kg of crude extract and the fractions on mean WBC counts in streptozotocin-induced diabetic rats

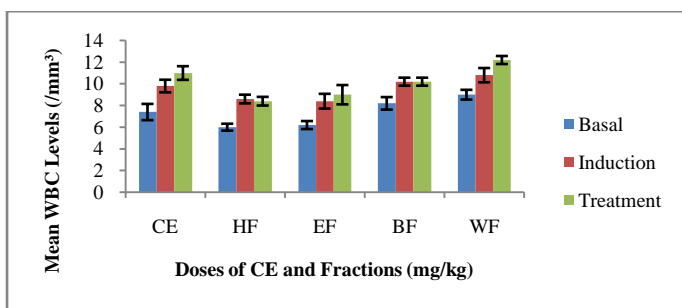


Figure 23: Effect of 500 mg/kg of crude extract and the fractions on mean WBC counts in alloxan-induced diabetic rats

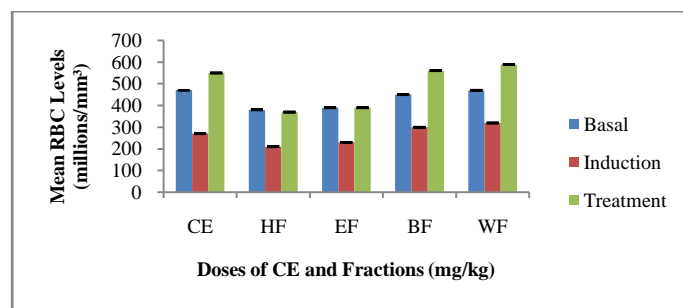


Figure 27: Effect of 500 mg/kg of crude extract and the fractions on mean RBC counts in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

Acute toxicity test

Table 6: Results of Acute toxicity (LD₅₀) test

Dose (mg/kg)	Mortality
10	0/5
100	0/5
1000	0/5
2000	0/5
3000	0/5
4000	0/5
5000	0/5

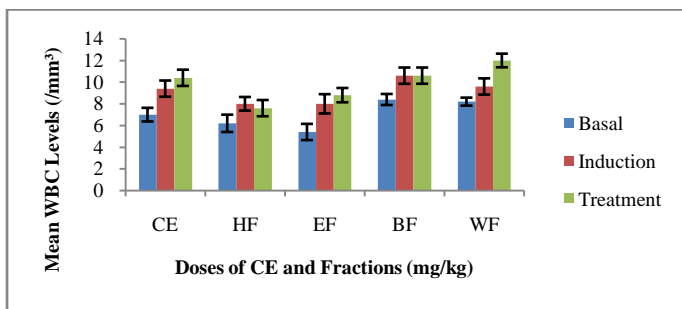


Figure 24: Effect of 500 mg/kg of crude extract and the fractions on mean WBC counts in alloxan-nicotinamide-induced diabetic rats

Key: CE – Crude extract; HF – Hexane fraction; EF – Ethylacetate fraction; BF – Butanol fraction; WF – Water fraction

Red Blood Cell (RBC) counts

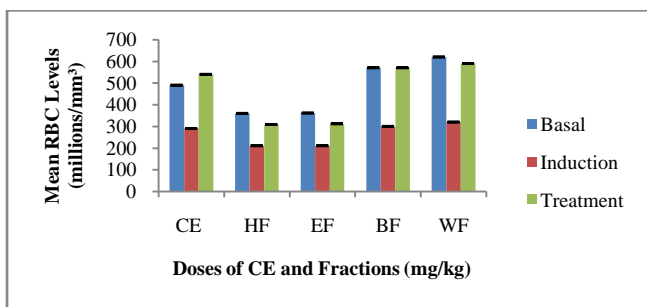


Figure 25: Effect of 500 mg/kg of crude extract and the fractions on mean RBC counts in streptozotocin-induced diabetic rats

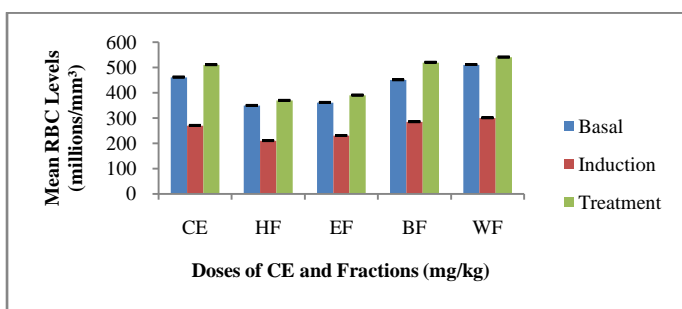


Figure 26: Effect of 500 mg/kg of crude extract and the fractions on mean RBC counts in alloxan-induced diabetic rats

DISCUSSION

Extraction: The extraction of 1500 g of pulverized plant sample yielded 510 g of a brownish crude extract (CE). Yield of natural extract is strongly dependent upon the solvent used for extraction due to the solvent polarity^[26, 27]. The crude extract, which was subjected to fractionation with solvents of increasing polarities in order to reduce the complexities of the extract, facilitated the ease of compound isolation and also give a better understanding of the polarities of not only the solvent fractions but also that of the isolated compounds. It also facilitates understanding of the most bioactive fraction polarity. The result of this experiment showed that the butanol fraction was obtained in the highest yield, while the hexane fraction has the least yield (Table 1). This is an indication that the butanol fraction contains more components compared to the other fractions.

Phytochemical analyses: The crude extract tested positive for saponins, flavonoids, cardiac glycosides, tannins, alkaloids, steroids, terpenoids, proteins, carbohydrates and anthraquinones; the n-hexane fraction gave positive reactions for alkaloids, carbohydrates, steroids, flavonoids and saponins; ethyl acetate fraction tested positive for alkaloids, tannins, flavonoids, saponins, cardiac glycosides, carbohydrates, proteins, anthraquinones and terpenoids; butanol fraction tested positive for cardiac glycosides, tannins, flavonoids, alkaloids, anthraquinones, proteins, steroids and terpenoids; while the water fraction gave positive reactions for alkaloids, flavonoids, tannins, saponins, carbohydrates, proteins, steroids, terpenoids and anthraquinones (Table 2). The presence of cardiac glycosides and tannins in the extract and fractions support the usage of *Terminalia catappa* as anti-hypertensive agent in line with^[18]; cardiac glycosides are known to reduce the effect of diabetic complications. Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo*, either to stimulate secretion or possess an insulin-like effect^[28].

In this study, the metabolites evaluated were present in certain amounts, tannin has highest concentration and flavonoids have the least concentration (Table 3). This may be due to the plant species, soil topography, harvest time as well as plant age; these factors may also affect the presence or absence of certain metabolites.

Physicochemical evaluation: The physico-chemical analysis of the pulverized stem bark of *Terminalia catappa* Linn., showed the percentage composition of the total ash, water soluble ash, sulphated ash, acid-insoluble ash, alcohol soluble extractive value and moisture content. The values of percentage composition are shown as Mean \pm standard error of mean (SEM) (Table 4). The moisture content of *Terminalia catappa* at 11.5 % is within tolerable limit of 10 % to 13 % in line with the result of [26]; this serves as a guide to processing, preservation and storage of the crude drug. Total ash in this study is 16.14 % which is a small percentage; it determines how much care that is required in the preparation of a crude drug. Acid-insoluble ash gave 3.00 % which indicates negligible amount of inorganic contaminants. Sulphated ash gave 19 % whereas water soluble ash gave 17.50 %, and is a good indicator of the water soluble salts in the drug or incorrect preparation. The alcohol extractive gave 4.80 % and indicates the presence of relatively polar constituents and non-polar constituents.

Anti-diabetic evaluations: In the light of the results (Figures 1-6), continuous treatment of all-induced diabetic rats with both metformin and the methanol extract especially at higher dosage of 500 mg/kg body weight; as well as the fractions (at 500 mg/kg) of *Terminalia catappa* stem bark for 7 days significantly ($p < 0.05$) decreased the blood glucose level in diabetic rats. This finding is in line with the result obtained by [15-17]. The percentage reduction in fasting blood glucose level showed a significant reduction with the standard drug (metformin) and 500 mg/kg extract as compared with the diabetic control group; also, the water, butanol and the crude extract. Percentage of reduction of FBGL with *Terminalia catappa* 500 mg/kg/day basal weight is greater than percentage of reduction of FBGL with metformin compared with diabetic control group, which indicates that the extract has more anti-hyperglycaemic effect to the standard drug (metformin).

Biochemical parameters: Haematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals [29-34]. The levels of AST, ALT and ALP in this study significantly increased in diabetic-induced rats in line with the result of [29]. The increase in the activities of AST, ALT and ALP may be due to the leakage of these enzymes from the liver cytosol into the blood stream, which gives an indication of the hepatotoxic effect of diabetogenic agents. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction, and muscle damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries. In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Significant return of these enzymes to basal serum value ranges following treatment with *Terminalia catappa* extract and fractions may be due to prevention of intracellular and tissue enzyme leakage resulting from cell membrane stability or cellular regeneration; though ALP significantly increased with treatment, which may be as a result of large bile duct obstruction, intrahepatic cholestasis, infiltrative diseases of the liver or increased osteoblastic activity (Figures 7- 15).

Hematological evaluation: The examination of blood has been described as a good way of accessing the health status of animals, because it plays an important role in the physiological, nutritional and pathological status of organisms [28, 35-37]. Haematological parameters provide information regarding the status of bone marrow activity and haemolysis [32]. It has been revealed in this study that haematological parameters in pre-treated group showed abnormalities which correlates with the results of [33]. This might be due to the destruction

of matured red blood cells leading to low haemoglobin count (Hb) (because of reaction of excess glucose with the haemoglobin to give rise to glycosylated haemoglobin) with decrease in red blood cell (RBC) (an indication of imbalance between its synthesis and destruction) and packed cell volume (PCV) normally being affected by LADA-induced diabetes, an indication of anaemia [24, 25, 34]. WBC slightly increased upon treatment; thus, improved the immune system in fighting foreign substances. Treatment with the extract and fractions significantly restored PCV, Hb and RBC to basal ranges when compared against induction (Figures 16- 27).

Acute toxicity evaluation: The acute toxicity of *Terminalia catappa* was investigated to determine any adverse effect that may arise as a result of a short term of (oral) administration of a single dose or multiple doses of the extracts (within 24 h period). Results did not exhibit any toxic symptoms or mortality in the test as shown in Table 6. The LD₅₀ was thus estimated to be above 5000 mg/kg, there was no toxicity; however, these findings are in line with the result of [30, 31] which suggests that the extract has low toxicity. The low toxicity obtained may have been responsible for its widespread use in different ethno-therapeutic interventions.

CONCLUSION

The standards established in this study provides useful information in regard to its identity and help to differentiate from the closely related other species of *Terminalia*; thus, aid researchers, manufacturers and individuals in selecting authentic plant material for research, drug production or as a home remedy. The results of these investigations may also be useful in the preparation of a medical monograph for this plant. The little or no toxicity observed in this study may not be unconnected with the fact that the secondary plant metabolites might only be present in very minute non-toxic levels. The present study provided evidence indicating that the methanol extract and the fractions of *Terminalia catappa* significantly reduced the levels of glucose in diabetic rats. In addition, treatment with the extract and fractions caused the recovery of certain altered biochemical and haematological parameters of diabetic animals.

Authors' Contributions:

Dr. Uduma E Osonwa, Dr. Felix A. Onyegbule and Prof. Christopher O. Ezugwu supervised the research work. Henrietta C. Nedum carried out the work as a postgraduate student. Henrietta C. Nedum, Dr. Uduma E Osonwa and Dr. Felix A. Onyegbule articulated, reviewed and structured the manuscript. All the authors approve the publication of the manuscript.

REFERENCES

1. Can A., Akev N., Ozsoy N., Bolkent S., Arda B.P., Yanardag R., Okyar A.. Effect of Aloe vera leaf gel and pulp extract on the liver in type II diabetic rats models. Biol. Pharm.Bull.2004; 27:694-698
2. Burke J.P., Williams K., Narayan K.M.V., Leibon C., Haffner S.P.. Population perspective on diabetes prevention: whom should we target for preventing weight gain. Diabetes Care.2003; 26:1999-2004
3. Petal M. and Rybezynski P.. Treatment of non-insulin dependent diabetes mellitus. Expert Opin. Investig. Drugs. 2003; 12(4):623-633
4. Wild S., Roglic G., Green A., Sicree R., King H.. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030, Diabetes Care. 2004; 27 (5); 1047—1053
5. Melmed S., Polonsky K.S., Reed M.D.P., Larsen M.D. and Kronenberg H.M. Williams Textbook of Endocrinology (12th Ed.). Philadelphia: Elsevier/Saunders. 2012; pp. 1371-1435.
6. WHO. Geneva World Health Organization Conference Definitions Diagnosis and Classification of Diabetes Mellitus and Its Complications. 1999; 146-150
7. George P., Ludvik B.. Lipids and diabetes. J Clin. Basic Cardiol. 2000; 3:159-62
8. Bain S.C., Kelly A.M., Mijovic C.H., Barnett A.H.. Genetic factors in the pathogenesis of Type 1 diabetes. Textbook of Diabetes. Oxford: Blackwell Scientific Publications.1996; pp15:1-16

9. Cudworth B..Diabetic mellitus and other metabolic disorders, Davidson Principles and Practice of Medicine 14th Edition. John Macleod ELBS.1978; 426-450
10. Olapade E.O.. Food and herbs for diabetes mellitus and hypertension. Natural Cures Series. 1995; 1, 3-6
11. Wadkar K.A, Magdum C.S., Patil S.S., Naikwade N.S.. Anti-diabetic potential and medicinal plant. Journal of Herbal Medicine and Toxicology.2008; 2 (1): 45-50
12. Onoagbe I.O., Attah V., Luther M.M.,Esekehegbe, A..Hypoglycaemic and Anti- diabetic Effect of Morindalucida and Tetracerainfora in Normal and Streptozocin- Induced Diabetic Rats. West African Journal of Biological Sciences. 1999; 1-8
13. Upendra R.M., Sreenivasulu M., Chengaiah B., Jaganmohan R.K., Madhusudhana C.C.. Herbal medicines for diabetes mellitus: A Review. Int. Journal of Pharm. Tech. Research.2010; 2 (3): 1883-1892
14. Pankaj O., Roberts E.P.. West Indian Almond Terminalia catappa Linn Combretaceae 2008; pp 273-276
15. Iwu M.M..Hypoglycaemic properties of Brideliafurruginea leaves. Fitoterapia. 1993; 54: 243-248
16. Marles R.H., Farnsworth N.R.. Antidiabetic plants and their active constituents, Phytomedicine.1995; 2: 137-187
17. Gbolade A.A., Ekor M.N., Akinlolu A.A., Ayoola M.D.. Antidiabetic activity of stem bark ethanolic extracts of Spondias mombinon alloxan-induced diabetic rats. J. Pharmaceut. Res. 2008;7, 192-195.
18. Sofowora A.. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd. 2006
19. Trease G.E. and Evans W.C. Pharmacognosy, 16th Edition, Brailliar Tiridel Can. Macmillian Publishers. 2002
20. Odoh U.E., Inya-Agha S.I., Ezugwu C.O., Ugwuoke C.E.C., Ezejiofor M., Ezea S.C.. Determination of Pharmacognostic Standards of Medicinal Plants. 1st Edition. 2011
21. Oduche A.U., Nwankwo E.C., Sama W., Ezugwu C.O.. Laboratory Manual for Professional Students. 1st Edition. 2010.
22. Bancroft J.D., Stevens A.. Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh. 1977; 16-64
23. Ravvindrana J., Vijaya R., Sachdanandam P., Shanthi P.. Toxicological, biochemical and histopathological evaluation of Tridham, a siddha medicine in wistar rats. J Biochem Tech. 2012; 4(1): 541-548.
24. Luka C.D., Saleh B., Mohammed A.. Effect of aqueous extract of Dioscorea adonitiformis on some biochemical parameters in alloxan induced diabetic rats. 2012; 3 (2) 450-453.
25. Orisakwe O.R., Afonne O.J., Chude M.A., Obi E., Dioka C.E.. Sub chronic toxicity studies of the aqueous extract of Boerhavia diffusa leaves. J. Health. Sc., 2003; 49, 444-447.
26. Anees A., Abbas F.M., Alkarkhi S.H., Lim H.K.. Extraction, Separation and identification of chemical ingredients of Elephantopus scaber L. using factorial design of experiment. Int. J. of Chemistry. 2009; Vol 1, No 1
27. Romdhane M., Gourdou C.C.. Investigation in solid-liquid extraction influence in ultrasound. J. Chem. Eng. 2002; 87, 11-19
28. Min J.G., Son K.T., Kim J.H., Park J.H.. Physiological and functional properties of Salicornia herbacea (Tungtungmadi) leaf extract. Nutraceut. Food. 2002; 7: 261-264
29. Ohaeri C.C., Eluwa M.C.. Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. Vet. Parasitol. 2011; 177: 199-202
30. Sunmonu, T.O., Oloyede, O.B.. Performance and haematological indices in rats exposed to monocrotophos contamination. Hum Exp Toxicol. 2010; 29(10): 845-50
31. Sule O.J., Elekwa I., Ayalogu E.O.. Effect of Acalypha Wilkesiana Muell Arg. on Haematological Parameters in Wistar Albino Rats. Int J Biol Med Res. 2012; 3 (1): 1234-1237.
32. Iniaghe O.M., Egharevba O., Oyewo E.B.. Effects of aqueous leaf extract of Acalypha wilkesiana on haematological parameters in male wistar albino rats. British Journal of Pharmaceutical Research. 2013; 3 (3): 456-471.
33. Mansi, K.M.S.. Effects of administration of alpha-melanocyte stimulating hormone (α -MSH) on some haematological values of alloxan induced diabetic rats. Am. J. Pharmacol. Toxicol. 2006; 1: 5-10
34. Muhammad, N.O. and Oloyede, O.B.. Haematological parameters of broilers chicks fed Aspergillus niger-fermented Terminalia catappa seed meal-based diet. Global J. Biotechnol. Biochem 2009; 4: 179-183
35. Nwanjo H.U. Studies on the effect of aqueous extract of Phyllanthus niruri on plasma glucose level and some hepatospecific markers in diabetic wistar rats. Int. J. Lab. Med. 2007; 2(2): 1-1
36. Muhammad N.O., Adeyina A.O., Peters, O.M. Nutritional evaluation of fungi treated cocoa bean shell. Nigerian J. Pure Appl. Sci. 2000; 5: 1059-64
37. Eroschencho V.P.. Atlas of histology with functional correlations, Williams and Wilkins Lippincott, USA, 9th Edn, 2000; p 12.
38. Builders M.I., Isichie C.O. and Aguiyi, J.C.. Toxicity studies of the extracts of Parkia biglobosa stem bark in rats. British Journal of Pharmaceutical Research. 2012; 2(1): 1-16
39. Saïdu Y, Nwachukwu L.S., Bilbis U.Z.F, Abbas A.Y. Toxicity studies of the crude aqueous root extract Albizziachevalieri harms in albino rats. Nigerian Journal of Basic and Applied Science. 2010; 18 (2): 308-314.

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