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Research Article
Evaluation of Anti-hyperglycemic Effect of Sub-fractions Derived from Ethanol-aqueous Fraction of *Balanites aegyptiaca* Leaves in Streptozotocin-induced Diabetic Rats

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Abstract

Background and Objective: Previous studies showed both leaves and stem-bark of *Balanites aegyptiaca* possess anti-diabetic properties however, extract from the leaves seem to be more effective. In comparison of activity between fractionated ethanol extracts; aqueous fraction (ALF) and ethyl acetate fraction (ELF) of the plant leaves, it was found that the aqueous fraction was highly effective over the ethyl acetate fraction. Guided by this, the present study subjected ALF to bio-guided fractionation where sub-fractions were validated in STZ-induced diabetic rats with the aim to ascertain whether anti-diabetic components presence could be further separated. **Materials and Methods:** Ethanol-aqueous fraction (ALF) of *Balanites aegyptiaca* leaves was fractionated by Gel Filtration Chromatograph using silica gel packed in a chromatographic column. The ALF was dissolved in distilled water and then applied unto the gel packed in a chromatographic column and eluted with solvent system of increasing polarity; diethyl ether, diethyl ether/chloroform (1:1 v/v), chloroform and methanol. The eluted sub-fractions namely: L1, L2, L3 and L4 were kept at room temperature (25°C) for the solvents to be evaporated and sub-fractions subjected to thin layer chromatography using silica gel G-coated plate. Diabetic rat groups were administered to various sub-fractions while positive control rats received metformin, normal and negative control rat groups received distilled water. **Results:** The yields, total phenolics and flavonoids content of sub-fractions obtained from the column fractionation of ethanol-aqueous fraction of *Balanites aegyptiaca* leaves showed methanol sub-fraction (L4) with highest percent yield and total phenolics content whereas diethyl ether/chloroform leaves sub-fraction (L2) was high in flavonoids content. Significant anti-hyper-glycemic effect were recorded from diabetic rat groups that received metformin and methanol leaves sub-fraction (L4) as indicative by a significant decrease in the trend of their weekly fasting blood glucose levels and values of area under fasting blood glucose curve (AUC). Diabetic rat groups that received L4 showed a significant reverse in serum lipid levels but increase in body weight comparable with diabetic rat groups treated with other sub-fractions. Water and feed intake of all diabetic treated rats were significantly different when compared with the untreated diabetic and normal control rats. **Conclusion:** The study suggested that bio-active compound(s) of *Balanites aegyptiaca* leaves is/are polar in nature and could be carefully isolated by bio-guided fractionation.

Key words: Antihyperglycemic, sub-fractions, leaves, *Balanites aegyptiaca*, diabetic rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders. It is one of the key health problem, affecting millions of people worldwide with high frequency rate and is projected to reach 592 million people in the year[1] 2035. There are several types of diabetes mellitus, but the two most common types are Type 1 and 2 diabetes mellitus. Type 1 diabetes mellitus is caused by an auto-immune response leading to a breakdown of insulin producing cells[2]. The pathogenesis of Type 2 diabetes mellitus differs from Type 1 diabetes mellitus, two key features are important; insulin resistance and beta-cell failure which resulted from a progressive insulin secretory defect on the background of insulin resistance[3].

Medicinal plants have a long history of traditional uses in the management of diabetes mellitus and have been found to improve diabetic control with reduced side effects compared to synthetic ones[4,5] and this have since be supported by the WHO[6].

Balanites aegyptiaca is one of the medicinal plant used by traditional medicine practitioners in the management of diabetes mellitus[7]. Its fruits and seeds were scientifically reported as useful source of hypo-glycemic remedy for management of diabetes mellitus[8-12]. The fruit extract was reported to have stimulated insulin secretion[8,13], intestinal α-amylase activity[9] and increased muscle basal glucose uptake[14] to lower blood glucose level. Bio-active compounds like vanillic and syringic acid was reported from the fruit[11]. The seed extract was reported to have exerted anti-hyperglycemic effect by ameliorating beta-cell dysfunction[10] while Shafik *et al.*[12] suggested anti-oxidant activity where isorhamnetin 3-rutinoside, 3-robinobioside, 3-0-glucoside, 3-0-galactoside, 3,7-diglucoside, quercetin-3-glucoside, 3-rutinoside, aglycones quercetin and isorhamnetin were reported to be the likely active compounds[12].

Research have shown that the leaves and stem-bark of *Balanites aegyptiaca* are used by the traditional medicine practitioners in the management of diabetes mellitus in northern Nigeria[7]. This claimed has been authenticated by author's previous studies where extracts from the plant leaves and stem-bark were elucidated in STZ-induced diabetic rats[15,16]. Literature review from the previous studies, showed both leaves and stem-bark of *Balanites aegyptiaca* possessing anti-diabetic properties however, extract from the leaves seem to be more effective. Findings from these studies has drawn the attention to the plant leaves as a part with potential anti-diabetic components. In comparison of activity between fractionated ethanol extracts; aqueous fraction (ALF) and ethyl acetate fraction (ELF) of the plant leaves, it was found that the aqueous fraction was highly effective over the ethyl acetate fraction. Findings from the previous study[15] suggest that bio-active component(s) in the leaves can be isolated as a single entity with less or no synergetic influence on its activity from the pool of other existing compounds. This finding calls for isolation of the active component (s) in the plant leaves.

Guided by this, the present study subjected ethanol-aqueous fraction (ALF) that was found highly effective from the previous study[15] to bio-guided fractionation where sub-fractions were validated in STZ-induced diabetic rats with the aim to ascertain whether anti-diabetic components presence could be further separated. Results from the study showed that the highest activity was recorded by the methanol sub-fraction (L4) indicating that active components from pool of compounds in *Balanites aegyptiaca* leaves can be pull-out by a stepwise fractionation techniques as evidence by a spot shown on the TLC chromatogram. The study therefore, reported that the likely active components of *Balanites aegyptiaca* leaves can be isolated by bio-guided fractionation following elution with polar solvents.

MATERIALS AND METHODS

Plant collection and identification: *Balanites aegyptiaca* leaves were collected from Gubi village (latitude 10°45'N and longitude 9°82'E) in Bauchi LGA, Bauchi State and was identified at the Herbarium Unit, Department of Biological Science, Ahmadu Bello University Zaria. A specimen voucher No. 900175 was deposited.

Chemicals: All chemicals and reagents used were of analytical grade and obtained from Sigma Aldrich, USA and BDH Ltd., Poole, England. These include solvents; ethyl acetate, ethanol, diethyl ether, chloroform and methanol. Chemicals include; silica gel (60-200 mesh size), Metformin (Hovid, Malaysia) and Streptozotocin (Adooq Bioscience, LLC, United States). Reagent kit; Cholesterol assay kit, Triglycerides assay kit, HDL-cholesterol assay kit were of Agappe Diagnostics Switzerland GmbH.

Experimental animals: A total of 35 male Wistar albino rats were used in the study. The rats were obtained from the Animal House, University of Jos, Plateau state and kept in clean cages with 12/12 h light/dark photoperiod. Water and feed 'growers mash' (Vital feeds, Jos) were supplied *ad libitum*. The rats were allowed to grow weighing between 180-230 g before used. Experimental protocol was in conformity with national and international laws and guidelines for care and use of laboratory animals as in 'Principle of Laboratory Animal Care'[17].

Plant sample extraction and column fractionation: The ethanol-aqueous fraction (ALF) of *Balanites aegyptiaca* leaves was obtained from author's previous study[15] following extraction procedure described by Jung e*t al.*[18] and Govorko *et al.*[19] while column fractionation was carried out on silica gel (60-200 Mesh) packed in a chromatographic column and eluted with solvents of increasing polarity; diethyl either, chloroform and methanol. Briefly, the column was packed with silica gel by stocking the lower part of the glass column with glass wool. Exactly, 75 g of silica gel was dissolved in 180 mL of absolute chloroform to make the slurry. The chromatographic column was packed with silica gel and allowed free flow of the solvent into a conical flask. The set up was in order as the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed to stand for 24 h to stabilize, the clear solvent at the top of the silica gel was drained down the silica gel meniscus.

Exact 5.0 g of ALF was dissolved in 5.0 mL of distilled water and then applied unto the chromatographic column and eluted with solvent system of increasing polarity: diethyl ether, diethyl ether/chloroform (1:1 v/v), chloroform and methanol. Exact 400 mL of each solvent was poured into the column each time using syringe. The eluted fractions were collected in aliquots of 40 mL in test tubes. The content in each tube was allowed to evaporate at room temperature (25°C) to solid form. The whole procedure was repeated until about 150 g of the ALF was used.

Thin layer chromatography (TLC): Thin layer chromatography of the sub-fractions was performed on silica gel G-coated plates (0.25 mm for analytical). Exactly, 50 µL (100 mg sub-fraction dissolved in 1.0 mL distilled water) each of the diethyl ether, diethyl ether/chloroform, chloroform and methanol sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves was separately applied 1 cm above the lower edge of the thin layer chromatograph slide and dried. It was immersed to a depth of 1 cm in the solvents system comprised of butanol, acetic acid and water in the ratio of 6:3:1. Compounds were visualized under UV light (254 nm).

Study design: Anti-hyperglycemic effect of sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves were assessed in streptozotocin-induced diabetic rats. Each sub-fraction at a dose of 400 mg kg^{-1} b.wt., as done in author's previous study Mhya *et al.*[15] was administered orally to rats using oral gastric tube. Rats were randomly allocated into 7 groups of 5 rats each as follows:

Group A: Diabetic+diethyl ether leaves sub-fraction (L1)
Group B: Diabetic+diethyl ether/chloroform leaves sub-fraction (L2)
Group C: Diabetic+chloroform leaves sub-fraction (L3)
Group D: Diabetic+methanol leaves sub-fraction (L4)
Group E: Diabetic+metformin (200 mg kg^{-1} b.wt.,)[20]
Group F: Diabetic control
Group G: Normal control

Feed and water intake estimations: During the experiment, food and water intake were recorded daily per each group. Feed was weighed using a weighing scale to ascertain the quantity given and the remnants in each rat groups after 24 h. The volume of water given and after 24 h was measured using a measuring cylinder (1000 mL).

Animal body weight estimations: Animal weight was determined weekly by weighing the rats using a weighing scale. The rats were weighed after an overnight fast by properly placing the rats in the weighing pan of the weighing scale thereafter the weights were recorded.

Determination of blood glucose levels: Blood glucose was determined by method of Beach and Turner[21] using glucometer by collecting blood sample from an overnight fast rats through their tail vein. Total area under fasting blood glucose curve was determined by the formula of Tai[22].

Determination of serum lipid profile: Serum triglyceride (TG) was determined by the method of Fossati and Prencipe[23]. Serum total cholesterol was determined spectrophotometrically according to the method of Roeschlau *et al.*[24] while high density lipoprotein Cholesterol (HDL-C) was measured by the method of Lopes-Virella *et al.*[25]. Agappe assay kits were used and procedures were according to the manufacturer's instruction. LDL-cholesterol and VLDL-cholesterol (VLDL-C) were determined by the equation described by Marchell[26]:

$$\text{LDL-cholesterol concentration (mg dL}^{-1}) = \frac{\text{TC-(HDL-C+Triglycerides)}}{5}$$

and:

$$\text{VLDL-cholesterol concentration (mg dL}^{-1}) = \frac{\text{Triglycerides}}{5}$$

Statistical analysis: Data from the experiments were expressed as Mean±standard deviation (SD). Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan's multiple range test (DMRT)[27]. Significant difference was accepted at $p<0.05$.

RESULTS

Yields, phenolics and flavonoids content of sub-fractions of *Balanites aegyptiaca* leaves: Yields, total phenolics and flavonoids content of sub-fractions obtained from the column fractionation of ethanol-aqueous fraction of *Balanites aegyptiaca* leaves was shown in Table 1. The leaves sub-fractions varied in yield and the content of both phenolics and flavonoids. Methanol sub-fraction (L4) had the highest percent yield and total phenolics whereas diethyl ether/chloroform leaves sub-fraction (L2) recorded the highest flavonoids content as shown in Table 1.

TLC analysis of sub-fractions of *Balanites aegyptiaca* leaves: The TLC analysis of sub-fractions obtained from the column fractionation of ethanol-aqueous fraction of *Balanites aegyptiaca* leaves using solvent system comprised of butanol, acetic acid and water in a ratio of 6:3:1. The TLC chromatogram was presented in Plate 1.

Anti-hyperglycemic effect of sub-fractions in diabetic rats: The result of anti-hyperglycemic effect of various sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves in streptozotocin-induced diabetic rats was presented in Fig. 1. The study recorded high levels of fasting blood glucose in the diabetic treated rats at the early period of sub-fractions administration but as the treatment advances the fasting blood glucose levels reduced significantly in Fig. 2. The significant anti-hyperglycemic effect were recorded in diabetic rats groups that received metformin and methanol leaves sub-fraction (L4) as indicative by the significant decrease in the trend of their weekly fasting blood glucose levels and values of area under fasting blood glucose curve (AUC).

Effect of sub-fractions on serum lipid profile in diabetic rats: There was significant ($p<0.05$) reversal effect in serum cholesterol, TG, HDL, LDL levels in the diabetic rats groups treated with diethyl ether, diethyl ether/chloroform, chloroform and methanol sub-fractions (Table 2). Among the sub-fractions used; methanol leaves sub-fraction (L4) was more potent in lowering cholesterol from 205.17±6.09-110.00±12.75 mg dL^{-1} and TG from 271.96±9.06-118.31±11.9 mg dL^{-1} in a close relation to Metformin, the standard anti-diabetic drug; cholesterol was lowered to 106.21±15.35 mg dL^{-1} and TG to 103.55±10.09 mg dL^{-1}.

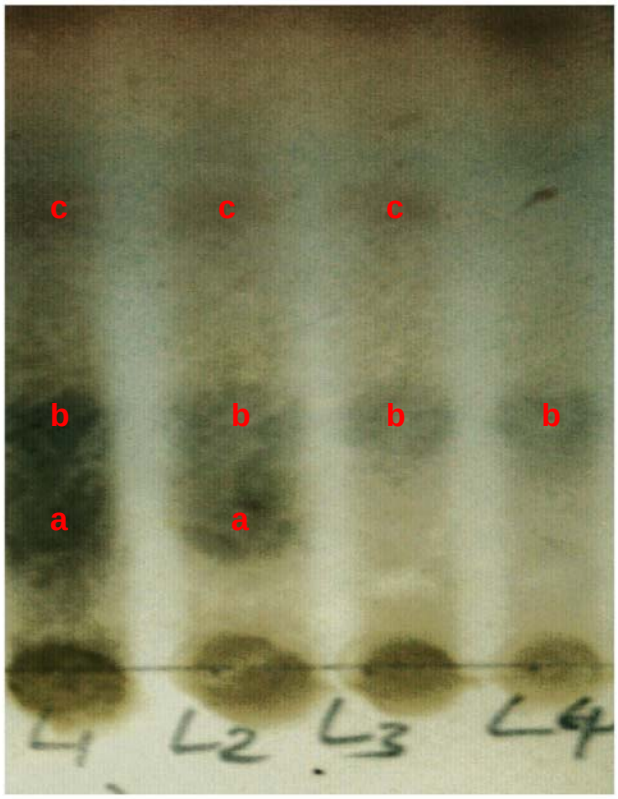

Plate 1: TLC chromatogram of sub-fractions following column fractionation of *Balanites aegyptiaca* leaves
Letter a, b and c designated spots of various component in the extract sub-fractions. L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction

Table 1: Yield, total phenolics and flavonoids contents of sub-fractions of *Balanites aegyptiaca* leaves

	Sub-fractions			
Contents	L1	L2	L3	L4
Yield (g/150 g)	15.0 (10.0%)	13.2 (8.8%)	17.7 (11.8%)	19.8 (13.2%)
Phenolics (mg GAE^{-1} g^{-1}) $\times 10^{-2}$	5.54±0.29^{bc}	4.76±0.45^{b}	3.21±0.29^{a}	6.32±0.94^{bc}
Flavonoids (mg QE^{-1} g^{-1}) $\times 10^{-2}$	0.10±0.00^{a}	0.31±0.06^{bc}	0.17±0.01^{b}	0.23±0.00^{b}

Values are Mean±SD of triplicate determinations. Values with different superscript across the rows are significantly different ($p<0.05$), L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction

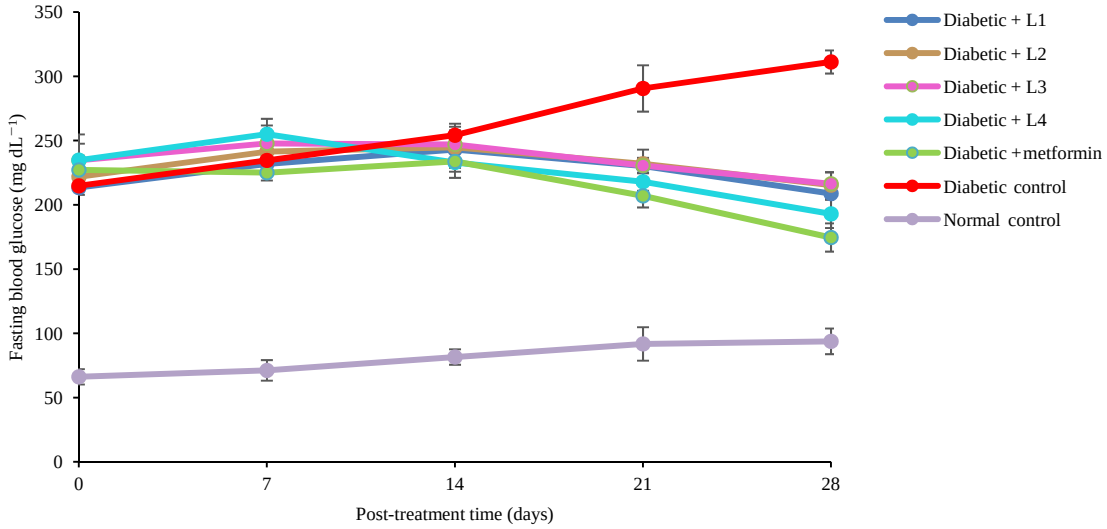

Fig. 1: Anti-hyperglycemic effect of sub-fractions of *Balanites aegyptiaca* leaves in streptozotocin-induced diabetic rats
L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction

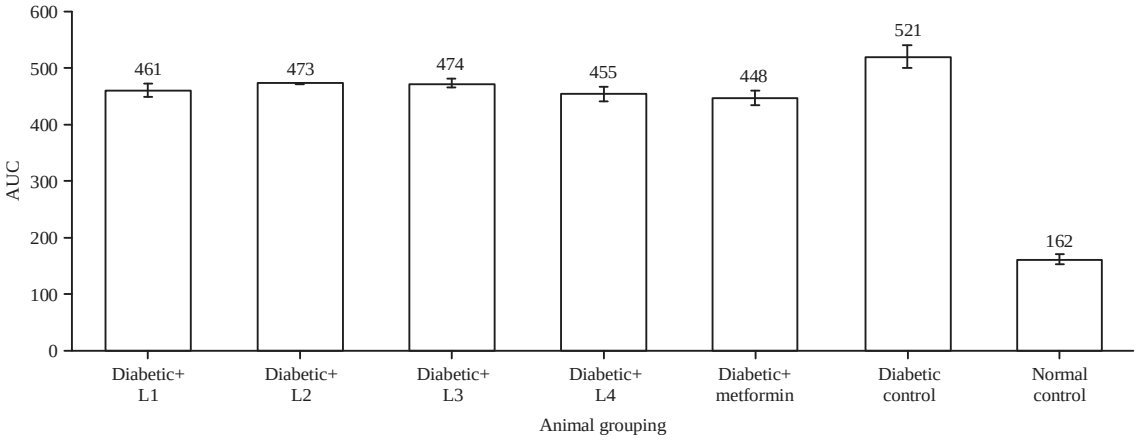

Fig. 2: Total area under fasting blood glucose curve of streptozotocin-induced diabetic rats treated with sub-fractions of *Balanites aegyptiaca* leaves and non-diabetic rats
L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leave sub-fraction

Table 2: Effect of sub-fractions of *Balanites aegyptiaca* leaves on serum lipid profile in diabetic rats

	Animal grouping						
Effects of sub-fraction	Diabetic+L1	Diabetic+L2	Diabetic+L3	Diabetic+L4	Diabetic+metformin	Diabetic control	Normal control
Cholesterol (mg dL^{-1})	190.69±11.48^{bcd}	197.24±5.26^{bcd}	162.07±14.52^{bc}	110.00±12.75^{b}	106.21±15.35^{b}	205.17±6.09^{bcd}	81.38±12.03^{a}
Triglyceride (mg dL^{-1})	197.20±8.39^{bcd}	199.81±25.09^{bcd}	142.99±42.37^{bc}	118.31±11.9^{b}	103.55±10.09^{ab}	271.96±9.06^{bcde}	80.75±11.60^{a}
HDL-C (mg dL^{-1})	54.78±3.44^{b}	50.54±1.33^{b}	55.96±1.58^{b}	68.97±4.30^{bc}	76.36±12.03^{bcd}	41.58±7.65^{a}	84.63±7.43^{bcd}
LDL-C (mg dL^{-1})	140.30±10.12^{bcd}	147.17±7.99^{bcd}	122.28±8.90^{bc}	72.54±14.82^{b}	70.23±12.30^{b}	142.46±4.59^{bcd}	48.30±13.79^{a}
VLDL (mg dL^{-1})	39.44±1.45^{bcd}	39.96±5.02^{bcd}	28.60±8.47^{bc}	23.66±2.39^{b}	20.71±2.02^{ab}	54.39±1.81^{bcde}	16.15±2.32^{a}

Values are Mean±SD of 5 determinations. Values with different superscript across the rows are significantly different ($p<0.05$), L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

Effect of sub-fractions on body weight of diabetic rats: The body weight of STZ-induced diabetic untreated and treated rats with sub-fractions derived from ethanol-aqueous fraction of *Balanites aegyptiaca* leaves were presented in Fig. 3. Body weight of diabetic untreated rats decreased gradually and continued throughout the experimental

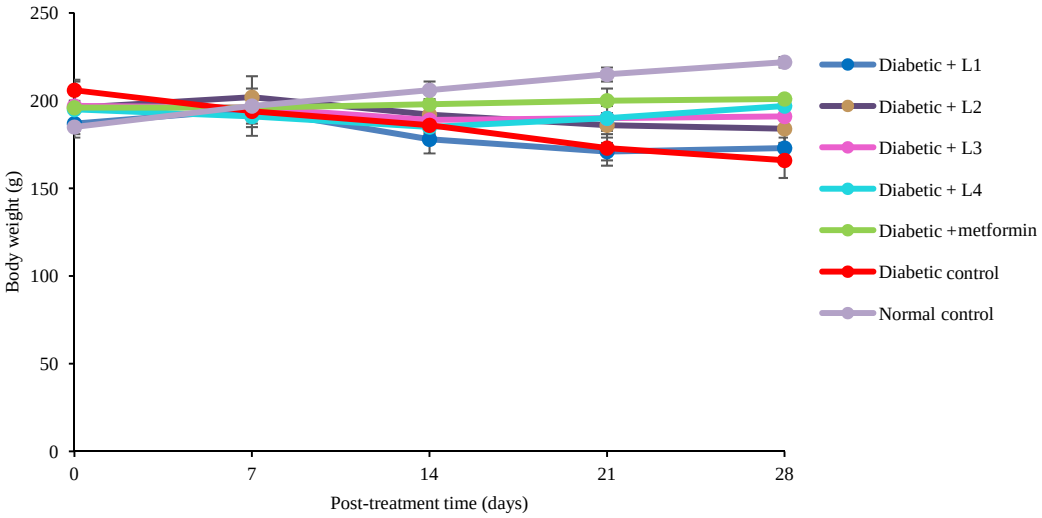

Fig. 3: Effect of sub-fractions of *Balanites aegyptiaca* leaves on body weight of streptozotocin-induced diabetic rats
L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction

Table 3: Effect of sub-fractions of *Balanites aegyptiaca* leaves on water and feed intake in streptozotocin-induced diabetic rats

	Animal grouping						
Effects of sub-fraction	Diabetic+L1	Diabetic+L2	Diabetic+L3	Diabetic+L4	Diabetic+metformin	Diabetic control	Normal control
Feed (g day^{-1} rat^{-1})	27.71±3.36^{bcde}	25.40±2.31^{bc}	25.08±1.78^{bc}	25.59±1.85^{bcd}	23.82±1.54^{b}	29.25±5.87^{bcdef}	21.78±1.48^{a}
Water (mL day^{-1} rat^{-1})	27.07±3.86^{b}	29.61±4.06^{bcd}	28.01±2.23^{bc}	27.01±2.45^{b}	27.73±2.97^{bc}	32.64±5.19^{bcde}	23.44±1.78^{a}

Values are Mean±SD of 28 determinations. Values with different superscript across the rows are significantly different ($p<0.05$), L1: Diethyl ether leaves sub-fraction, L2: Diethyl ether/chloroform leaves sub-fraction, L3: Chloroform leaves sub-fraction, L4: Methanol leaves sub-fraction

period while that of the treated diabetic rat groups decreased at the initial of the experiment but later there was gain in body weight by all diabetic rats treated groups.

Effect of sub-fractions on water and feed intake of diabetic rats: Feed and water intake of STZ-induced diabetic treated, diabetic untreated and non-diabetic rats groups were presented in Table 3. There were significant ($p<0.05$) differences observed in the feed and water intake of STZ-induced diabetic rats treated with sub-fractions and diabetic untreated and normal control rats.

DISCUSSION

Balanites aegyptiaca has been reported as anti-diabetic medicinal plant which has been studied on both diabetic mice and rats[14,28] where the fruit and seed were reported as useful source of hypo-glycemic remedy for the management of diabetes mellitus[8,10,29]. Compounds like saponins[30], rutin[14] as well as vanillic and syringic acids[11] were postulated as the active compounds in the plant fruit extract while isorhamnetin 3-rutinoside, 3-robinobioside, quercetin-3-glucoside, aglycones quercetin was reported in the seed kernel[12].

Balanites aegyptiaca leaves is used by traditional medicine practitioners in the management of diabetes mellitus and some ailments[5,7]. In a previous study, it was found that the plant leaves possess anti-diabetic properties as evidence by lowering fasting blood glucose in diabetic rats treated with extract of the plant leaves. It was also reported that the crude leaves extract of *Balanites aegyptiaca* exert antidiabetic effect in type II diabetic patients[16]. In an effort to isolate bioactive compound(s) from the *Balanites aegyptica* leaves, bioassay of the leaves extract based on the report from author's previous[15] work was carried out and sub-fractions evaluated in streptozotocin induced diabetic rats where the study found that the sub-fractions particularly the methanolic sub-fraction (L4) exhibit anti-hyperglycemic activity in the STZ diabetic rats.

It had been reported that *Balanites aegyptiaca* leaves extract contain a variety of biologically active compounds[29,31,32]. Presence of flavonoids and phenolics in the sub-fractions of *Balanites aegyptiaca* leaves extract

suggested their usefulness in disease prevention. Differences in yields of the sub-fractions of *Balanites aegyptiaca* leaves extract may be due to differences in solubilities and polarities of the extracted compounds. High phenolic content in the methanolic subfraction (L4) at test to the fact that polar solvents promoted dissolution of phenolics[33].

It was a known fact that level of cholesterol in diabetes mellitus increased as was recorded in this study. However, in STZ induced diabetic treated rats with sub-fractions of *Balanites aegyptiaca* leaves a significant reduction of the cholesterol level in serum was recorded possibly by decreasing cholesterol absorption from the intestine as suggested by Aderibigbe *et al.*[34] or inhibition of HMG-CoA reductase activity as done by some medicinal plants extract since study has postulated increased HMG-CoA reductase activity in diabetic state[35]. Hyper-triglyceridemia was characterized in diabetic state as a consequence of uninhibited actions of pancreatic lipase[36]. The anti-hypertriglyceridemic effect observed in the diabetic treated rats may be assumed that administration of sub-fractions of *Balanites aegyptiaca* leaves inhibited the pancreatic lipase activity as suggested by some studies[37,38].

Type 1 diabetes mellitus was characterized by severe loss of body weight that may result from relative or absolute deficiency of insulin due to defective β- cells[39]. Loss of weight in STZ-induced diabetic rats might be accompanied by increased breakdown of muscle proteins as was suggested by Bastaki[39]. The plant leaves sub-fractions might have prevented breakdown of muscle proteins hence prevent body weight loss. Increased food and water intake by diabetic rats may be due to chronic reduction in glucose utilization by the cells as evidence from a considerable loss of glucose accompanied with large volume of water[40]. Continuous loss of large volume of water in the diabetic rats might have stimulated thirst mechanism, hence, the increased fluid intake by these animals while defects in glucose utilization might have increased the demand for food as suggested by Raju and Raju[40] and which might have been minimized following administration of the leaves sub-fractions in the diabetic rats.

CONCLUSION

The study suggests that anti-diabetic bioactive compound(s) of *Balanites aegyptiaca* leaves is/are polar in nature and could be carefully isolated by bio-guided study.

SIGNIFICANCE STATEMENT

The study discover that careful and continuous bioassay fractionation of the *Balanites aegyptiaca* leaves could lead to further separation of active component that could be used as pharmaceutical entity or as simple dietary adjunct to existing therapies.

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