Cytotoxicity and Anti-Diabetic Properties of Leaves of Some Medicinal Plants collected around Zaria, Kaduna State Nigeria

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ABSTRACT: The aim of this study is to establish the anti-diabetic potential of Blighia unijugata Baker, Anthocleista djalonensis, Senna sieberiana, Senna singueana and Ficus exasperate. Blighia unijugata Baker, Anthocleista djalonensis, Senna sieberiana, Senna singueana Delile and Ficus exasperate Vahl were extracted with methanol and fractionated using petroleum ether, chloroform and ethylacetate. The phytochemical analysis, cytotoxicity and antidiabetic properties of the fractions were investigated using standard methods. Phytochemical results of the plants fractions showed various amount of alkaloids ranging from 1.2 - 4.7% with moderate amount of flavonoids, steroids, saponins and trace amount of tannins. The results of cytotoxicity using Fundulopachas gadneri showed senna singueana Delile, ethylacetate fraction and Blighia unijugata Baker-pet ether fraction being the most active (IC50=5.23). Antidiabetic results showed that most of the fractions are highly active reducing the sugar level of the induced albino rats to normal with Ficus exasperate Vahl, ethylacetate fraction and Blighia unijugata Baker-chloroform fraction showing higher activity against all the induced rats. Comparatively the two fractions showed higher activities than the standard drugs. The results of this research therefore showed Ficus exasperate Vahl, ethylacetate fraction and Blighia unijugata Baker-chloroform fraction possess great potentials as anti-diabetic agents with animals treated with Blighia unijugata Baker-chloroform fraction having better recovery because the experimental animals had steady weight gain and could therefore be a potential as anti-diabetic drugs.

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Medicinal plants have generated renewed interest amongst scientists of various fields such as agriculture, veterinary medicine, pharmacognosy, sociology, ethnobotany and anthropology (Salmeron-Manzano et al, 2020 and Marreli et al, 2021). In the last two decades, medicinal plants have received huge attention both in developed and developing countries. Overwhelming populations of the world use medicinal plants/plant based drugs for healthcare for different reasons one being in the interest of everything natural and second the exploding cost of health care (Akbar, 2020). Medicinal plants display wide-range pharmacological activities such as anti-inflammatory, antibacterial, antifungal properties and anti-diabetic activities (Ahad et al., 2021) hence their importance in human health. The medicinal plants owe this value to presence of phytochemicals which are secondary metabolites, the role of these bioactive secondary products in plants is to combat various types of abiotic and biotic stresses such as chemical defence against pathogens, predators, pathogens and allopathic agents (Naikoo et al., 2019). The medically important

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active ingredients of the medicinal plants are secondary metabolites used in medicinal and environmental sectors as well as being widely used in commercial and pharmaceutical products. Diabetes is a multifaceted chronic disease that necessitates glycemic control and ongoing medical attention in order to implement risk-reduction strategies (Association, 2022). It results in aberrations in carbohydrate, fat and protein metabolism, which arise due to defects in insulin secretion and/or action and impairs the body’s ability to process blood glucose otherwise known as blood sugar (Bos and Agyemang, 2013). It causes psychological and physical distress to patients and serious and even life-threatening complications (Yan et al., 2020), prolonged hyperglycemia affects almost every organ and system in the body, especially the eyes, kidneys, heart, feet, and nerves (Rahman et al., 2021). According to the statistics of the International Diabetes mellitus Federation, IDF 463 million people worldwide are diagnosed with diabetes mellitus in 2019, accounting for 9.3% of the adult population, and this number is expected to increase to 700 million by 2045 (Saeedi et al., 2019). In 2021, diabetes impacted 337 million individuals globally, and the worldwide prevalence has surpassed 10 %, as reported by the 10th Edition of IDF Atlas (Imitiaz et al., 2024). Oral antidiabetic medications like biguanides, sulfonylureas, thiazolidinediones, and digestive enzyme inhibitors are the first-line treatment for managing diabetes, with insulin acting as a last desperate measure (Mohamed, 2021). The development of a safer, more affordable, and less toxic medication is required due to the common side effects of synthetic antidiabetics, which include hepatic dysfunction, fluctuation in body weight, vision problems, peripheral arterial disease, and gastrointestinal distress (Asghar, 2018; Marín-Peñaálver, 2016). Due to their efficient, secure, and sustained mode of operation, medicinal plants have attracted the interest of researchers worldwide in the pursuit of finding a remedy for diabetes (Rahman, 2022). These medicinal plants contain biologically active compounds possessing valuable therapeutic value (Mohamed, 2021; Abid et al., 2022). Therefore, identifying drugs with fewer side effects and better efficacy is essential and justified by the high incidence, increasing prevalence of type II diabetes, limitations and side effects of current hypoglycemic drugs.

The aim of this study is to establish the anti-diabetic potential of Blighia unijugata Baker, Anthocleista djalonensis A. Chev, Senna sieberiana D. C, Senna singueana Delile and Ficus exasperate Vahl medicinal plants.

MATERIALS AND METHODS

Materials: The materials used include: Round bottom flask (1000 cm³). The reagents and chemicals include: Petroleum ether (95.5 % purity), Chloroform (96 % purity), Ethyl acetate 96 % purity), Methanol (97.0 % purity), Acetic anhydride (C₄H₆O₄), Lead Ethanoate, Sodium picroate paper, Magnesium metal, Wagner’s reagent, Meyer’s reagent, Fehling’s solution, Distilled,Sodium Hydroxide, Methanolic Acid, Iron (iii) chloride, Silver nitrate, Potassium hydroxide, Iron (II) chloride, Hydrochloric acid, Lead ethanoate, Atropine, Vanillin, Catechin, Quercetin, Phosphomolybdate, phosphotungstate, Sodium carconate, Gallic Acid, β-sitosterol and Liebermann-Burchard reagent.

Medicinal Plants Collection and Authentication: The plants Blighia unijugata Baker were collected from the Ahmadu Bello University botanical garden, voucher number 327. Anthocleista djalonensis was collected at Anara forest, Zaria, voucher number 798. Senna sieberiana D. C was collected at Sakaru village, Zaria, voucher 1986. Senna singueana Delile was collected from Tashar Fulani, old Jos road, Zaria, voucher number 864 and Ficus exasperate Vahl was collected at Kufena, Zaria, voucher 1126. All plants were identified and authenticated at Ahmadu Bello University botanical garden by U. S. Gall (Ethnobotanist).

Sample Preparation: The freshly collected plants leaves were separately cut into chips, and air-dried in the laboratory, grinded into powder using mortar and pestle, weighed and stored in polythene bags until needed (Imitiaz et al, 2024).

Extraction and Fractionation of Plant Material Extract: A portion (150 g) of the pulverized plant leaves was percolated in 500 cm³ of methanol in 1000 cm³ conical flask for two weeks and fractionated using solvents petroleum ether, chloroform and ethyl acetate. The extracts were separately filtered and concentrated using a rotary evaporator at 40 °C. The marc was re-percolated with recovered solvents for one week and the extracts were drained, filtered and combined with previous residue and concentrated using a rotary evaporator. The methanol extract (20 g) was suspended in water (200 ml), and partitioned with pet ether, chloroform (CHCl₃) and ethyl acetate (EtOAc).The yield was respectively cooled, weighed and stored in the refrigerator until needed (Imitiaz et al, 2024).

Quantitative Phytochemicals Analyses: Phytochemicals compounds alkaloids, saponins, flavonoids, tannins and steroids were determined using standard
Determination of Total Alkaloid Content: To a portion (1 cm³) of the extract were added with 5 cm³ of phosphate buffer (pH 4.7) and 5 cm³ Bromo Crystal Green (BCG) solution were added and the mixture was shaken with 4 cm³ of Chloroform. The extract was collected in a 10 cm³ volumetric flask and diluted to make the final volume with Chloroform. The blank was prepared as above but without the extract and the absorbance of the complex in chloroform was measured at 470 nm against the blank. Atropine was used as a standard and the results of the assay were compared with Atropine equivalents.

A portion, 5 cm³ of pH 4.7 phosphate buffer and 5 cm³ of BCG solution were mixed and then extracted with 5 cm³ of chloroform. The extract was collected in 10 cm³ volumetric flasks and then the volume adjusted to the mark with chloroform. This served as the blank solution. A standard curve of absorbance against concentration of Atropine was plotted and used for estimation of the Atropine equivalence (AE) of test sample. The total Atropine content in the test sample was computed in mg/g using equation 1:

\[
\text{Total alkaloid} \left( \frac{W}{W} \right) = \frac{AE \times V}{W} \times 100 \quad (1)
\]

Where, \( AE \) = the concentration of quercetin established from the calibration curve (mg/cm³); \( V \) = the volume of extract (cm³); \( M \) = weight of plant extract (gm)

Determination of Total Tannins Content: The Acidified Vanillin method of evaluation of tannin content was adopted for the estimation of the amount of tannin in test sample and expressed as mg catechin Equivalent per gram of plant extract as described by Ferreira, et al., 1999. A clean test tube was wrapped with aluminium foil and 500 μL of methanol was placed in it, along with 3.0 cm³ of vanillin reagent and 1.5 cm³ of concentrated hydrochloric acid and mixed thoroughly. This served as blank solution. The test sample solution was prepared by dissolving 10 mg of the sample in 10 cm³ of methanol to give a concentration of 1 mg/cm³. Cleaned test tubes (21) were wrapped with aluminium foils. To each of the tubes, 0.5 cm³ samples were pipetted and the various concentrations of the standard catechin solutions (3.0 cm³ of vanillin reagent) followed by 1.5 cm³ of concentrated hydrochloric acid were added and mixed thoroughly. The reaction mixtures were allowed to stand for 15 minutes at room temperature and the absorbance of the test samples and standards was measured at 500 nm using UV/VIS spectrophotometer against the blank. Standard calibration curve for the catechin standards was plotted and the graph was used to determine the Catechin equivalence of the test samples. The Total Tannin Content of the plant was calculated using equation 2:

\[
\text{Total Tannin content} \left( \frac{W}{W} \right) = \frac{CE \times V}{W} \times 100 \quad (2)
\]

Where, \( CE \) = Catechin equivalents (mg/ cm³), \( V \) = Volume of test sample (cm³), \( W \) = Sample weight (g)

Determination of Flavonoids Content: The total flavonoid content of the plant extracts was determined using Aluminium Chloride colorimetric method. Quercetin was used as standard and the flavonoid content of the extracts was expressed in mg of quercetin equivalent /gram of dried extract (Kumar, et al., 2014). To a portion (1 cm³) of the plant extract taken in a test tube, 2 cm³ of 5 % NaNO₂ and 3 cm³ of AlCl₃ (10 %) were added after 5 minutes. The reaction mixture was treated with 2 cm³ of 1 M NaOH in another 5 minutes and the reaction mixture was made up to 10 cm³ with water and the absorbance measured at 510 nm. The samples were prepared in triplicate and analysed, the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The Flavonoids content in the extracts was expressed in terms of Quercetin equivalents. A standard curve of absorbance against quercetin concentration was plotted, and used for estimation of the quercetin equivalence (QE) of test samples. The total flavonoid content in each of the extracts in mg/g plant extract was calculated using equation 3:

\[
\text{Total Flavonoid content} \left( \frac{W}{W} \right) = \frac{QE \times V}{W} \times 100 \quad (3)
\]

Where, \( QE \) = the concentration of quercetin established from the calibration curve, mg/ cm³; \( V \) = the volume of extract (cm³); \( M \) = weight of plant extract (gm)

Determination of Total Phenolic Content: Total phenolic content of samples was determined employing the method involving the use of Folin-Ciocalteu reagent (FCR) as oxidizing agent with...
Gallic acid as standard. Preparation of blank solution: In a 20 cm$^3$ volumetric flask 1.5 cm$^3$ Folin Ciocalteu reagent, 1 cm$^3$ distilled water and 4 cm$^3$.20 % sodium carbonate were mixed to give the blank solution. A portion (1 cm$^3$) of the test sample solution was placed in various test tubes and different concentrations of the standard gallic acid was added in the test tubes. To each of the test tubes 1 cm$^3$ of distilled water and 1.5 cm$^3$ of Folin Ciocalteu’s reagent were added, the mixture was covered with aluminium foil and incubated at room temperature for 5 minutes. A portion, 4 cm$^3$ of 20 % (w/w) Na$_2$CO$_3$ was added to each of the test tube, the mixtures were agitated and placed in a water bath at a temperature of 40 °C for 30 minutes. The test tubes were placed in ice water to quench the reaction. The absorbances of the test samples and standards were determined at 765 nm against the blank using UV/VIS spectrophotometer. The total phenol content in the test sample was calculated in mg/g using equation 4:

$$Total\ Phenols\ content \left( \frac{mg}{cm^3} \right) = \frac{GAE * V}{W} \times 100 \quad (4)$$

Where, GAE = Gallic acid equivalents (mg/cm$^3$), V = Volume of test sample (cm$^3$), W = Sample extract (g)

**Determination of Total Steroids Content:** A portion (20 mg) of each test sample was suspended in chloroform, covered and heated at 60 °C for 30 minutes in water bath with shaking. The suspension was filtered. The resultant marc was then extracted with 20 cm$^3$ of chloroform and filtered. The volume of the combined filtrate was adjusted to 50 cm$^3$ with same solvent (chloroform). To 10 cm$^3$ volumetric flasks, 5 cm$^3$ of the combined filtrate was transferred and 2 cm$^3$ of Liebermann-Burchard (LB) reagent was added. The volume was adjusted to 10 cm$^3$ mark with chloroform. The absorbance was measured using a spectrophotometer UV visible 5 min after the addition of the reagent LB at 625 nm wavelength. In 10 cm$^3$ volumetric flask, 5 cm$^3$ of chloroform, 2 cm$^3$ of LB were added and the volume adjusted to the 10 cm$^3$ mark with chloroform to give the blank solution. It was allowed to stand for 5 minutes and the absorbance measured at 625 nm.

**Cytotoxicity (Fundulopanchax gardneri Fish) Test**

**Fundulopanchax gadneri Test:** Brine Shrimp Lethality Test (BST) procedure was used on locally available Fundulopanchax gadneri killifish with a view to achieving the same result as adopted by (Ado et al., 2014).

**Anti-Diabetic Activity**

**Induction of Type II Diabetes:** Rats, albino wistar rats weighing 150-200 g (aged between 6-8 weeks) were selected for the study and marked for the purpose of individual identification. All the animals were allowed to acclimatize and were fed with standard pellet diet (SPD) and water ad libitum and maintained on a 12 hour light and 12-hour dark cycle prior to dietary manipulation for one week. Subsequently, the animals were divided into two groups (A and B). Group A were fed with standard pellet diet and group B were fed with Laboratory prepared high-fat diet, HFD (60.3 % fat, 18.4 % protein, and 21.3 % carbohydrate as percentage total kcal) ad libitum for initial 8 weeks then the high-fat diet was replaced with normal standard pellet diet and the animals were given a single intraperitoneal injection of a low dose of streptozocin, STZ (45 mg/kg body weight; dissolved in 0.1M citrate buffer pH 4.5) while the group A (control) animals were injected with citrate buffer equal volume with STZ. Parameters such as fasting blood glucose and weight were measured using ACCU-CHECK compact plus glucometer after three days of STZ administration. Rats showing fasting glucose levels ≥ 199.8 mg/dl (11.1 mmol/lit) according to modified (Acho et al., 2024) method were considered as type II diabetic and used in the study.

**Administration of Extract and Standard Drugs:** The treated groups were administered with the extracts orally at appropriate doses (500 mg/kg and 250 mg/kg 24 daily, in the morning hours, for 2 weeks using the Cannula feeding tube. The untreated groups (diabetic control and normal control) were also administered with distilled water ad libitum through the same route for 2 weeks. The rats in the metformin group received 100 mg/kg body weight metformin.

**Collection of Blood Sample/Fasting blood Sugar Test:** Fasting blood glucose was measured using ACCU-CHECK compact plus glucometer (Roche, France) after interval of three days of STZ administration. Blood samples were collected from tail vein of rats by pricking for the estimation of blood glucose by accu-check meter, rats showing fasting glucose levels ≥ 199.8 mg/dl (11.1 mmol/lit) are said to be diabetic. Glucose level was checked on day 0, 3, 7, 11 and 14th day after treatment commenced.

**Body weight Measurement:** The body weight of the rats was measured using a digital weighing balance. Each of the rats was placed on the bowl of the weighing balance and the exact weight was taken. The body weight was taken before the induction of diabetes and 24 hr after commencement of treatment.
for 14 days with AD-C concentration 250 mg/kg and 500 mg/kg. This treatment was applied to all groups with fractions BU-C, BU-E, BU-P, FE-C, FE-E, FE-P, SQ-E and SQ-P.

RESULTS AND DISCUSSION
Phytochemical Analyses: Table 1 is the results of quantitative phytochemical analysis of plants fractions used in the present study. The plant fractions analysed contains a high amount of alkaloids with SQ-C with the highest amount of 34.7% while AD-C, SS-P and SS-C having the least amount of 1.2%. All plant fractions contain an insignificant amount of tannins, this may be that they contain hydrolysable tannins and not condensed tannins as the method used was for quantifying condensed tannins. Flavonoids are present in all the plant fractions except SS-E which was not significant; BU-P had the highest amount of flavonoids of 16.3% and AD-C with the least amount of 0.1%. All the plant fractions had high amount of phenols with SQ-C having the highest amount of 47.9% and SS-P had the least amount of 7.7%. Steroids are present in trace amount with AD-C, SS-P and SS-E having no appreciable amount and FE-C having a moderate amount of 17.5%.

Table 1: Quantitative Phytochemical Analysis

<table>
<thead>
<tr>
<th>PLANT FRACTIONS</th>
<th>ALKALOIDS (w/w) %</th>
<th>TANNINS (w/w) %</th>
<th>FLAVONOIDS (w/w) %</th>
<th>PHENOLS (w/w) %</th>
<th>STEROIDS (w/w) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU-P</td>
<td>19.2</td>
<td>-</td>
<td>16.3</td>
<td>14.9</td>
<td>0.8</td>
</tr>
<tr>
<td>BU-C</td>
<td>21.4</td>
<td>-</td>
<td>14.9</td>
<td>10.1</td>
<td>0.7</td>
</tr>
<tr>
<td>BU-E</td>
<td>21.2</td>
<td>-</td>
<td>10.1</td>
<td>28.0</td>
<td>1.1</td>
</tr>
<tr>
<td>AD-P</td>
<td>21.1</td>
<td>-</td>
<td>13.5</td>
<td>9.8</td>
<td>0.6</td>
</tr>
<tr>
<td>AD-C</td>
<td>1.2</td>
<td>-</td>
<td>0.1</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>AD-E</td>
<td>15.8</td>
<td>-</td>
<td>10.0</td>
<td>39.5</td>
<td>1.1</td>
</tr>
<tr>
<td>SQ-P</td>
<td>11.3</td>
<td>-</td>
<td>14.6</td>
<td>36.5</td>
<td>0.6</td>
</tr>
<tr>
<td>SQ-C</td>
<td>34.7</td>
<td>-</td>
<td>17.0</td>
<td>47.9</td>
<td>0.3</td>
</tr>
<tr>
<td>SQ-E</td>
<td>8.3</td>
<td>-</td>
<td>5.8</td>
<td>33.3</td>
<td>0.3</td>
</tr>
<tr>
<td>SS-P</td>
<td>1.2</td>
<td>-</td>
<td>8.6</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>SS-C</td>
<td>1.2</td>
<td>-</td>
<td>8.8</td>
<td>9.0</td>
<td>1.6</td>
</tr>
<tr>
<td>SS-E</td>
<td>10.3</td>
<td>-</td>
<td>-</td>
<td>12.3</td>
<td>-</td>
</tr>
<tr>
<td>FE-P</td>
<td>10.0</td>
<td>-</td>
<td>14.4</td>
<td>20.3</td>
<td>0.2</td>
</tr>
<tr>
<td>FE-C</td>
<td>1.5</td>
<td>-</td>
<td>2.5</td>
<td>33.0</td>
<td>17.5</td>
</tr>
<tr>
<td>FE-E</td>
<td>1.6</td>
<td>-</td>
<td>8.5</td>
<td>26.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

KEY: BU-P(Blishia Unijuagata petroleum ether fraction); BU-C(Blishia Unijuagata chloroform extract); BU-E(Blishia Unijuagata Ethylacetate fraction); AD-P(Anthocelesta Djalonensis petroleum ether fraction); AD-C(Anthocelesta Djalonensis chloroform fraction); AD-E(Anthocelesta Djalonensis ethylacetate fraction); SQ-P(Senna Sinqueana petroleum ether fraction); SQ-C(Senna Sinqueana chloroform fraction); SQ-E(Senna Sinqueana ethyl acetate fraction); SSD-P(Senna siebriana petroleum ether fraction); SSD-C(Senna siebriana chloroform fraction); SSD-E(Senna siebriana ethylacetate fraction); FE-P(Ficus exasperate petroleum ether fraction); FE-C(Ficus exasperate chloroform fraction) and FE-E(Ficus exasperate ethylacetate fraction)

Toxicity Test: Table 2 result of cytotoxicity assay of contains the results of cytotoxicity assay of the plants extracts. Most of the extracts have high cytotoxic activities range of 5.2663/0.1238 and 41.0880/26.0345 ug/cm². Fraction BU-P and SQ-E have the highest activity of 5.2663/0.1238 ug/cm³. The lowest number is attributed to the highest activity because it is the least amount of extract (5.2663/0.1238) required to be active while the lowest activity is attributed to the high number because it is the least amount of that extract (41.0880/26.0345) required to have an activity for that extract. SS-E is non-convergent this could be as a result of the concentration of the extract. Other extracts that have high activities include (BU-P, BU-C, BU-E, AD-C, SQ-P, SQ-E, FE-P and FE-C) were continued with for acute toxicity test and further anti-diabetic activity.

Cytotoxicity is used to quantify the biological activities of components by determining its capacity for producing an expected biological activity (Indrayanto et al, 2021) and it is widely used for screening of cytotoxic compounds and potency in natural products (Lim et al, 2021.).

Table 2 Results of Fundulopanchus gardneri Test (Cytotoxicity).

<table>
<thead>
<tr>
<th>Plant Fractions</th>
<th>FGTLC50 (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU-P</td>
<td>8.4095/13.0123</td>
</tr>
<tr>
<td>BU-C</td>
<td>9.784/1.701425</td>
</tr>
<tr>
<td>BU-E</td>
<td>11.1256/2.86183</td>
</tr>
<tr>
<td>AD-P</td>
<td>41.0680/26.0345</td>
</tr>
<tr>
<td>AD-C</td>
<td>9.9358/22.1504</td>
</tr>
<tr>
<td>AD-E</td>
<td>41.0080/26.0345</td>
</tr>
<tr>
<td>SQ-P</td>
<td>10.1447/4.9921</td>
</tr>
<tr>
<td>SQ-C</td>
<td>34.882/27.1376</td>
</tr>
<tr>
<td>SQ-E</td>
<td>5.2663/0.1238</td>
</tr>
<tr>
<td>SS-P</td>
<td>34.1551/26.0476</td>
</tr>
<tr>
<td>SS-C</td>
<td>34.1551/26.0476</td>
</tr>
<tr>
<td>SS-E</td>
<td>5.2663/0.1238</td>
</tr>
<tr>
<td>FE-P</td>
<td>11.2945/29.624</td>
</tr>
</tbody>
</table>

Glucose Level Changes after Administration of Plant Fractions: Repeated measure ANOVA was used to assess the effectiveness of different treatments among induced diabetic rats. Type II diabetic induced species by examining fasting blood glucose and weight levels

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changes across time. Data are presented as mean values ± Standard Error of the Mean (SEM). The P values, < 0.05 were considered significant. The results from the repeated measure ANOVA revealed there was significant differences between the treatment groups in the change of glucose level measure from baseline to 2 weeks (Fig. 1) p < 0.05, total average glucose level range from 25.01 ± 8.06 (mmol/l) for the baseline and decreases drastically after intervention and follow-up to 14.28 ± 8.42 (mmol/l). At the baseline (0 Day), the average ± SEM lowest glucose level recorded was 17.43 ± 4.8 (mmol/l) and the highest was 34.83 ± 11.8 (mmol/l). Similarly after intervention the lowest average glucose level observed was 9.7 ± 8.09 (mmol/l) associated with BU-E 250 mg and the highest was 40.9 ±7.1 (mmol/l) ascribed to FE-C 250 mg. Correspondingly, it was observed during follow-up (2nd week) that fraction BU-C 250 mg with 3.16 ± 1.05 had the lowest glucose level while the highest glucose level was ascribed to FE-C 250mg with mean and standard error of 27.63 ± 1.65 (mmol/l).

Generally, in assessing the effectiveness of different treatments after follow-up, it was observed that fractions BU-C 250mg, BU-C 500 mg and Metformin with low glucose levels of 3.16 ± 1.05b, 3.26 ± 0.44b and 3.33 ± 65.69b (mmol/l) respectively in Fig. 1.

Weight loss which is one of the clinical features of diabetes mellitus due to generation of adipocytes and muscle tissues to make up for the energy loss as a result of frequent urination, loss of appetite and water intake,, and over conversion of glycogen to glucose(Samadi-Noshahr et al, 2021). In this experiment, the mean change in Weight in the baseline ranges from 115.5 ± 5.7 g to 200.3 ± 2.9 g. Correspondingly, considering the outcomes significantly affected by the treatment, it was observed that there is difference in the average weight of subjects from baseline (Fig. 2), after treatment and

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follow-up period and the difference in weight was statistically significant with P. value < 0.05. For the follow-up group there was decrease in average weight recorded for Metformin from the baseline of 156.5 ± 2.24 to 130 ± 14.9 in the 14th day of treatment, SQ-E did not effectively reduce glucose level but has an change in weight at concentration of 50mg from the baseline from 115.5 ± 5.75 to 124 ± 19.1 in the first week of treatment to increase to 130 ± 14.9 in the second week and at 25mg concentration there was increase in weight from baseline from 185 ± 12.57 decrease in first week to 184.75 ± 8.51 and increased to 198.3 ± 11.1. FE-E which effectively reduced glucose level has minimal increase in weight at 500 mg from from baseline at 166 ± 27.31 to 175.4 ± 5.69 in the first week of treatment to 174.75 ± 28.1 in the second week of treatment and at 250 mg concentration from baseline of 180.33 ± 11 to 182.67 ± 5.69 to 186.33 ± 7.2 in the second week. BU-C which effectively increased the weight at 500 mg concentration from baseline of 141.6 ± 11.7 to 140.2 ± 9.42 in the second week of treatment. BU-C was which effectively reduced glucose level and shows improvement in weight was used to further the research.

**Conclusion:** Quantitative phytochemical analysis of all the fractions revealed the presence of alkaloids, tannins, flavonoids and steroids. FG TEST was adopted for cytotoxicity test the plant fractions are found to be generally active, the result of cytotoxicity assay of the plants extracts with most of the extract having high activity, extracts having high activity was continued with for acute toxicity test and further antidiabetic activity. In this study, all plants fractions in the study had significant anti-diabetic activity with some having better activity than the standard drug. *Blighia Unijugata* Baker has the highest activity restoring normal diabetic rats to normal sugar level and weight. *Blighia unijugata* Baker chloroform extracts possess great potentials of antidiabetic agents and could therefore be a potential precursor in drugs used for the treatment of diabetics. *Ficus exasperata* extracts also has potential in diabetes treatment but the weight gain is much lower than *Blighia unijugata*.

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