

## Hypoglycemic Effect of *Anthocleista djalonensis* and *Blighia unijugata*

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### ABSTRACT

*Anthocleista Djalonensis* A Chev and *Blighia unijugata* Baker were collected and fractionated using petroleum ether, chloroform and ethylacetate. Spectroscopic quantitative phytochemical analysis was carried out on the fractions and they were found to contain alkaloids, flavonoids, phenols and steroids while tannins were found in significant quantity. Cytotoxicity was carried out on the fractions the fractions and they were found to be active except AD-P, 41.0680/26.0345  $\mu\text{g}/\text{cm}^3$  and AD-E, 41.0080/26.0345  $\mu\text{g}/\text{cm}^3$  while BU-P was found to be the most active at 8.4095/15.0123  $\mu\text{g}/\text{cm}^3$ . Experimental animals were induced with diabetes using streptozotocin and treated with the active fractions, BU-C 250mg dosage was found to have reduced glucose sugar level from 21.68  $\pm$ 1.54 mmol/l in the first week to 3.16  $\pm$ 1.05<sup>b</sup> mmol/l while BU-C 500mg dosage was found to increase weight from 112.8  $\pm$ 53.2mg to in the first week to 140.2  $\pm$ 9.4<sup>b</sup>g in the second week of intervention. The presence of phytochemicals, non-toxicity and being able to reduce glucose sugar in experimental animals by *Blighia unijugata* Baker chloroform fraction of makes a potential plant for treatment of diabetes.

**Key Words:** *Anthocleista Djalonensis* A Chev, *Blighia unijugata* Baker, Fractionisation.

### INTRODUCTION

Plants with medicinal values are well known to man as indispensable materials in drug discovery and are utilized by man for various purposes from the inception of human history.<sup>1</sup> Medicinal plants contain some organic compounds which produce definite physiological action on the human body and their bioactive substances include tannins, alkaloids, carbohydrate, terpenoids, steroids

and flavonoids.<sup>2</sup> Medicinal plants are of great importance to the health individuals and communities.<sup>2</sup> The economic importance of medical plants now draws the attention of various world bodies, in particular the World Health Organization (WHO). Since WHO supports and encourages the introduction of traditional medicine resources into health systems around the world, the use of

medicinal plants has shown a marked increase. For this reason, interest through ethnobotanical studies enables the development of contemporary drugs and treatments as well as for plant conservation.<sup>3</sup> Many ethnobotanical studies around the world report the use of herbal plants for the healing process, which has been in use for several generations in their respective societies.<sup>4</sup>

#### ***Anthocleista Djalensis* A. Chev**

*Anthocleista djalensis* (A. *djalensis*) A. Chev commonly known as Cabbage tree, called “Kwari” in Hausa, “Ewe” by the Yoruba people of South-West of Nigeria belong to the family Loganiaceae is a medium-sized tree of the West tropical Africa, 30-45 feet high with blunt spines on the branch, pale grey trunk and widespreading crown.<sup>5</sup> The stem, root, bark and leaves of *A. djalensis* are used to treat malaria, jaundice, diabetes and abscesses.<sup>5</sup>

#### ***Blighia Unijugata* Baker**

*B. unijugata* Baker commonly known as ‘Akee Nut’ or “Gwanja Kusa” in Hausa, “Akoko-Isin” in Yoruba, belong to the Sapindaceae family. Members of this family have been widely studied for their pharmacological activities. Insecticidal, antioxidant, anti-inflammatory and anti-

diabetic properties are the pharmacological activities most commonly ascribed to this family. *B. unijugata* Baker is a tree indigenous to the forests of West Tropical Africa. It is usually small but sometimes attains 35 meters in height. It is planted for shade and is attractive in appearance, having red or pinkish-yellow fruit. Leaflets of *B. unijugata* Baker can be differentiated from other species by tufts of hairs in the axils of lateral veins and its fruits are up to 3 cm long (at least 4 cm long in the other species). Wood is used in building construction and the pulp is used as an enema. *B. unijugata* Baker is used in the treatment of fever, kidney pain and stiffness, leprosy, eyes aches, coughing, headaches, rheumatism, nausea and vomiting, dizziness, high blood pressure and also as a vegetable.<sup>6,7</sup>

## **MATERIALS AND METHODS**

### ***Plant Collection and Identification***

The sample of *Anthocleista Djalensis* A Chev (voucher number 798) and *Blighia unijugata* Baker (voucher number 327) plant leaves was collected at Ahmadu Bello University botanical garden Plants were identified and authenticated at Kaduna State

University, Biological Science Department,  
Kaduna by U. S. Galla.

### ***Sample preparation and Extraction***

The freshly collected plants leaves was separately cut into chips, and air-dried in the laboratory, grounded into powder using mortar and pestle, weighed and stored in polythene bags until needed.<sup>8</sup>

A portion (150g) of the ground plant leaves was percolated in 500 cm<sup>3</sup> of methanol for two weeks and successively fractionated in petroleum ether, chloroform and ethyl acetate. The extracts were separately filtered and concentrated using a rotary evaporator at 45 °C. The marc was re-percolated with the recovered solvents for one week. The filtrates were drained, filtered, combined with respective ones each and concentrated using a rotary evaporator. Each extract was cooled, weighed and stored in the refrigerator until needed.<sup>8</sup>

### ***Chemicals and reagents***

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

### ***Spectrophotometric Quantitative Phytochemical Analysis***

Phytochemicals in chloroform fractions were determined, UV-Visible Spectrophotometer

was employed for the quantitative estimation of phytochemical compounds from the plant fraction.

### ***Determination of Total Alkaloid Content***

To a portion (1 cm<sup>3</sup>) of the extract was added with 5 cm<sup>3</sup> of phosphate buffer (pH 4.7) and 5 cm<sup>3</sup> Bromo Crystal Green (BCG) solution and the mixture was shaken with 4 cm<sup>3</sup> of Chloroform. The extract was collected in a 10 cm<sup>3</sup> volumetric flask and is diluted to make up 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.<sup>9</sup>

Blank solution: A portion, 5 cm<sup>3</sup> of pH 4.7 phosphate buffer and 5 cm<sup>3</sup> of BCG solution was mixed and then extracted with 5 cm<sup>3</sup> of chloroform. Extract was collected in 10 cm<sup>3</sup> volumetric flasks and then adjusted the volume to the mark with chloroform.

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 equation:

Total Alkaloid Content (% w/w)

$$= \frac{AE \times V \times 100}{W}$$

Where,

AE = the concentration of quercetin established from the calibration curve, mg/ml

V = the volume of extract (cm<sup>3</sup>)

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.<sup>10, 11</sup>

Blank solution: A clean test tube was wrapped with aluminum foil and 500 µL of methanol was placed in it, along with 3.0 cm<sup>3</sup> of vanillin reagent and 1.5 cm<sup>3</sup> of concentrated hydrochloric acid and mixed thoroughly.

Test sample solution: 10mg of the test sample was dissolved in 10 cm<sup>3</sup> of methanol to 1mg/cm<sup>3</sup> concentration.

Cleaned test tubes (21) were wrapped with aluminum foils. To each of the tubes, 0.5 cm<sup>3</sup> was pipetted with the test sample solutions and the various concentrations of the standard catechin solutions. 3.0 cm<sup>3</sup> of vanillin reagent, followed by 1.5 cm<sup>3</sup> 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 can therefore be calculated equation:

$$\begin{aligned} \text{Total Tannin Content \% (w/w)} \\ = \frac{CE \times V \times 100}{W} \end{aligned}$$

Where,

CE = Catechin equivalents (mg/ cm<sup>3</sup>),

V = Volume of test sample (cm<sup>3</sup>),

W = Sample weight (g)

#### ***Determination of Flavonoids Content***

The total flavonoid content of the plant extracts was determined using Aluminum Chloride colorimetric method. Quercetin was used as standard, and the flavonoid content of the extracts was expressed as mg of quercetin equivalent /gram of dried extract.<sup>12</sup>

To a portion (1 cm<sup>3</sup>) of the plant extract was taken in a test tube which is added with 2 cm<sup>3</sup> of 5% NaNO<sub>2</sub> and 3 cm<sup>3</sup> of AlCl<sub>3</sub> (10%) was added to this after 5 minutes, the reaction mixture was treated with 2 cm<sup>3</sup> of 1 M NaOH

in another 5 minutes and the reaction mixture was made up to 10 cm<sup>3</sup> with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and 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 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 as mg/g plant extract was calculated using equation 3.4:

Total Flavonoid Content (% w/w)

$$= \frac{QE \times V \times 100}{W}$$

Where,

QE = the concentration of quercetin established from the calibration curve, mg/ml

V = the volume of extract (cm<sup>3</sup>)

M = weight of plant extract (gm)

#### ***Estimation 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 and with Gallic acid as standard.

Preparation of blank solution: In a 20 cm<sup>3</sup> volumetric flask 1.5cm<sup>3</sup> FolinCiocalteu reagent, 1 cm<sup>3</sup> distilled water and 4 ml 20% sodium carbonate was mixed.

Experimental procedure for the phenolic content determination

A portion (1 cm<sup>3</sup>) of test sample solution was placed in various test tubes and different concentrations of the standard gallic acid were added in the test tubes. To each of the test tubes 1 cm<sup>3</sup> of distilled water and 1.5 cm<sup>3</sup>FolinCiocalteu's reagent was added, the mixture was covered with aluminum foil and incubated at room temperature for 5 minutes. A portion of 4 cm<sup>3</sup> of 20% (w/w) Na<sub>2</sub>CO<sub>3</sub> 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 absorbance of the test samples and standards at 765 nm against the blank using UV/Vis spectrophotometer. The total phenol content in the test sample was calculated as mg/g as in equation:

Total Phenols Content (% w/w)

$$= \frac{GAE \times V \times 100}{W}$$

Where,

GAE = Gallic acid equivalents (mg/ cm<sup>3</sup>),

V = Volume of test sample (cm<sup>3</sup>),

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<sup>3</sup> of chloroform and filtered. The volume of the combined filtrate was adjusted to 50 cm<sup>3</sup> with same solvent (chloroform).

To 10 cm<sup>3</sup> volumetric flasks 5 cm<sup>3</sup> of combined filtrate was transferred and 2 cm<sup>3</sup> of Liebermann-Burchard (LB) reagent was added. The volume was adjusted with chloroform. The absorbance was measured using a spectrophotometer UV visible 5 min after the addition of the reagent LB at 625 nm wavelength.

#### ***Cytotoxicity (Fundulopanchax Gadneri (FG) 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.<sup>13</sup>

A portion (0.5 g) of each extract was dissolved in 5 cm<sup>3</sup> of solvent of extraction to give a stock solution of 100,000 µg/cm<sup>3</sup>. A serial dilution was made by taking 0.5, 0.05

and 0.005 cm<sup>3</sup> of the stock solution respectively in to 50 cm<sup>3</sup> beaker and the solution was allowed to evaporate. Two drops of dimethylsulfoxide, DMSO, 25 cm<sup>3</sup> of water and 10 (five day old) *Fundulopanchax gadneri* killifish was added to each beaker and the volume was adjusted to 50 cm<sup>3</sup> to give approximate concentrations of 1000, 100 and 10 µg/cm<sup>3</sup> respectively. Each test dosage was carried out in triplicate to give a total of 30 *Fundulopanchax gadneri* killifish per test concentration. These was kept on the bench at ambient temperature for 24hrs. Each beaker was examined and the number of surviving *F. gadneri* fingerlings was determined and recorded. A control consisting of 10 *F. gadneri*, 2 drops of DMSO and water was similarly set up. The concentration that kills 50 % of the test organisms (LC50) was be computed using Finney probit analysis programme.<sup>14</sup>

#### ***ANTIDIABETIC ACTIVITY***

##### ***Experimental Animals***

Albino (Wister strain) rats weighing between 150 – 180g (7-10 weeks old) were used for the present study. All the animals were housed and maintained in a controlled environment comprising standard conditions of temperature, humidity and a 12-hour light



and 12-hour dark cycle. Animals are allowed free access to food and water *ad libitum*.

Studies (Antidiabetic assessment was performed after the approval of the NDA animal ethical committee in accordance with institutional ethical guidance).

### ***Induction of Type II Diabetes***

Rats weighing 150-200g (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 12hour 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 streptozotocin, STZ (45mg/kg body weight; dissolved in 0.1M citrate buffer p<sup>H</sup> 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  $\geq 199.8$  mg/dl (11.1 mmol/lit) as modified.<sup>15</sup> method and were considered as type II diabetic and used in the study.

Glucose levels of diabetic rats were checked before commencement of treatment so that animals could be homogenously and randomly distributed between the groups.

### ***Administration of Extract and Standard Drugs***

The treated groups were administered with the extracts orally at appropriate doses (500mg/kg and 250mg/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 *adlibitum* through the same route for 2 weeks. The rats in the metformin group receive 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  $\geq$  199.8 mg/dl (11.1 mmol/lit) are said to be diabetic.

Glucose level was checked on day 0, 3, 7, 11 and 14<sup>th</sup> 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 500mg/kg.

## RESULTS AND DISCUSSION

### *Extraction and Fractionation of Leaves*

Fractions of the leaves of *Anthocleista Djalonenensis* A Chev and *Blighia unijugata* Baker used for analyses were coded as: AD-P (*Anthocleista Djalonenensis* petroleum ether fraction), AD-C (*Anthocleista Djalonenensis* chloroform fraction), AD-E (*Anthocleista Djalonenensis* ethylacetate fraction), BU-P (*Blighia Unijugata* petroleum ether fraction), BU-C (*Blighia Unijugata* chloroform extract), and BU-E (*Blighia Unijugata* Ethylacetate fraction).

### *Results of Quantitative Phytochemical Analysis*

Table 1: Results of quantitative phytochemical analysis of fractions of plant leaves.

PLANT FRACTIONS	ALKALOIDS (w/w) %	TANNINS (w/w) %	FLAVONOIDS (w/w) %	PHENOLS (w/w) %	STEROIDS (w/w) %
AD-P	21.1	-	13.5	9.8	0.6
AD-C	1.2	-	0.1	8.0	-
AD-E	15.8	-	10.0	39.5	1.1
BU-P	19.2	-	16.3	14.9	0.8
BU-C	21.4	-	14.9	10.1	0.7
BU-E	21.2	-	10.1	28.0	1.1

Fractions of both plants (*Anthocleista Djalonenensis* and *Blighia Unijugata*) have high amount of alkaloids with BU-C having

the least amount of alkaloids, 21.4 %. Tannins in all fractions is in significant amount, this may that they contain



hydrolysable tannins and not condensed tannins as there are traces of tannins in the phytochemical screening which was not quantified. Flavonoids are present in all the plant fractions with AD-C having the least amount, 0.1 %. All the fractions have high % of phenols with AD-E having the highest, 39.5% and AD-C having 8.0%. Steroids are present in trace amount with AD-P, BU-C, BU-P and BU-C while AD-C only have a significant amount.

Alkaloids are known to have antibacterial and antifungal<sup>16</sup> and hypoglycemic activity<sup>17</sup>.

Tannins are widely reported to possess antimicrobial and antioxidant activities<sup>18</sup>, it also stimulates glucose transport and inhibits adipocyte differentiation<sup>19</sup>. Flavonoids, one of the secondary metabolites in the plant and the most active fraction reported to stimulate the secretion of insulin (Hong et al., 2013, 2008). The presence of these phytochemicals in these medical plant fractions produces some biological activities responsible for their potential use as drugs.

Table 2: Results of Cytotoxicity (FGT Test)

PLANT FRACTIONS	FGT LC50 ( $\mu\text{g}/\text{cm}^3$ )
AD-P	41.0680/26.0345
AD-C	9.9358/22.1504
AD-E	41.0080/26.0345
BU-P	8.4095/15.0123
BU-C	9.7844/1.7014125
BU-E	11.1256/2.86183

Cytotoxicity bioassay is widely used for screening of cytotoxic compounds and potency in natural products.<sup>20</sup>

Result of cytotoxicity assay of the plants fraction with most of the extract having high activity between 8.4095/15.0123 and

41.0880/26.0345. With BU-P and BU-C having the highest activity of 8.4095/15.0123 and 9.7844/1.7014125 respectively while AD-P and AD-E have the lowest activity of 41.0680/26.0345 and 41.0080/26.0345 respectively.

Extracts having high activity (AD-C BU-P, BU-C and BU-E) was continued with for further anti-diabetic activity.

**Results Of Activity of Plant Fractions On Diabetic Albino Rats : Glucose Level Changes after Administration of Plant Fractions.**

Data are presented as mean values  $\pm$  Standard error of the mean (SEM). The P values,  $< 0.05$  were Considered significant.

Repeated measure ANOVA was used to assess the effectiveness of different treatments among Type II diabetic induced species by examining fasting blood glucose levels changes across time.

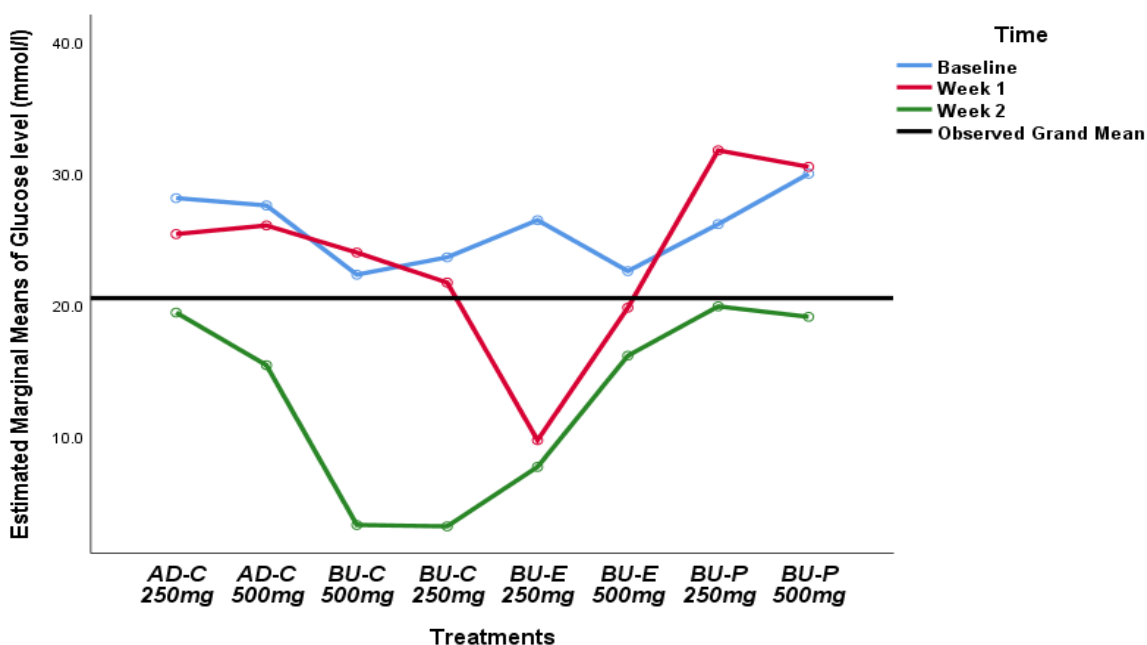


FIG. 1 Estimated Marginal means of glucose level of various treatments and time

The result 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)  $p < 0.05$ . At the baseline (0 Day), the average  $\pm$ SEM lowest glucose level recorded was  $22.28 \pm 2.6$  (mmol/l) and the highest was  $29.95 \pm 10.65$

(mmol/l). Similarly after intervention the lowest average glucose level observed was 9.7 ±8.09 (mmol/l) associated with BU-E 250mg and the highest was 31.73 ±1.52 (mmol/l) ascribed with BU-P 250mg. Correspondingly, it was observed during follow-up (2<sup>nd</sup> week) BU-C 250mg with 3.16 ±1.05 had lowest glucose level while the highest glucose level was obtained at BU-P

250mg with mean and standard error of 19.88 ±11.46<sup>a</sup> (mmol/l). Generally, in assessing the effectiveness of different treatments after follow-up it was observed that BU-C 250mg, BU-C 500mg were more efficacious among the treatments as shown in Table 3. Results  
Body Glucose level Changes After Administration of Plant Fractions

Table 3: Mean and Standard error of Glucose level from baseline to 14days.

Treatments	Baseline(mmol/l)	After Intervention(mmol/l)	
		1 <sup>st</sup> week	Follow-up(mmol/l) 2 <sup>nd</sup> week
AD-C 250mg	28.1 ±4.29	25.37 ±1.56	19.4 ±0.92 <sup>a</sup>
AD-C 500mg	27.54 ±10.3	26.02 ±2.32	15.4 ±3.92 <sup>a</sup>
BU-C 500mg	22.28 ±2.26	23.96 ±2.38	3.26 ±0.44 <sup>b</sup>
BU-C 250mg	23.6 ±3.44	21.68 ±1.54	3.16 ±1.05 <sup>b</sup>
BU-E 250mg	26.43 ±3.25	9.7 ±8.09	7.68 ±3.74 <sup>c</sup>
BU-E 500mg	22.55 ±8.7	19.78 ±10.56	16.13 ±9.47 <sup>a</sup>
BU-P 250mg	26.13 ±2.46	31.73 ±1.52	19.88 ±11.46 <sup>a</sup>
BU-P 500mg	29.95 ±10.65	30.48 ±1.32	19.08 ±3.2 <sup>a</sup>

All values are Mean ±SEM. Means in the same row/column with different superscript letters are significantly different,  $P < 0.05$  (repeated-measures ANOVA).

Table 4: Mean and Standard error Weight (kg) from baseline to 14days.

Treatments	Baseline(Kg)	After intervention(Kg)	
		Week1	Follow-up(Kg) Week 2 <sup>nd</sup>
AD-C 250mg	176.7 ±29	179.7 ±13.2	171.3 ±13.7 <sup>a</sup>
AD-C 500mg	151.8 ±22.6	143.6 ±16.3	144.2 ±12.4 <sup>b</sup>
BU-C 500mg	141.6 ±11.7	112.8 ±53.2	140.2 ±9.4 <sup>b</sup>

BU-C 250mg	173.4 ±31.5	151.4 ±22.3	156.4 ±18.2 <sup>c</sup>
BU-E 250mg	170.5 ±44.2	156 ±29.6	144.3 ±15.4 <sup>b</sup>
BU-E 500mg	154.8 ±20	156.3 ±37.3	149 ±28 <sup>b</sup>
BU-P 250mg	179.5 ±56.5	159 ±44	155 ±26.8 <sup>c</sup>
BU-P 500mg	154.8 ±28.7	122.3 ±28.5	140 ±22.9 <sup>b</sup>

All values are Mean ±SEM. Means in the same row/column with different superscript letters are significantly different,  $P < 0.05$  (repeated-measures ANOVA).

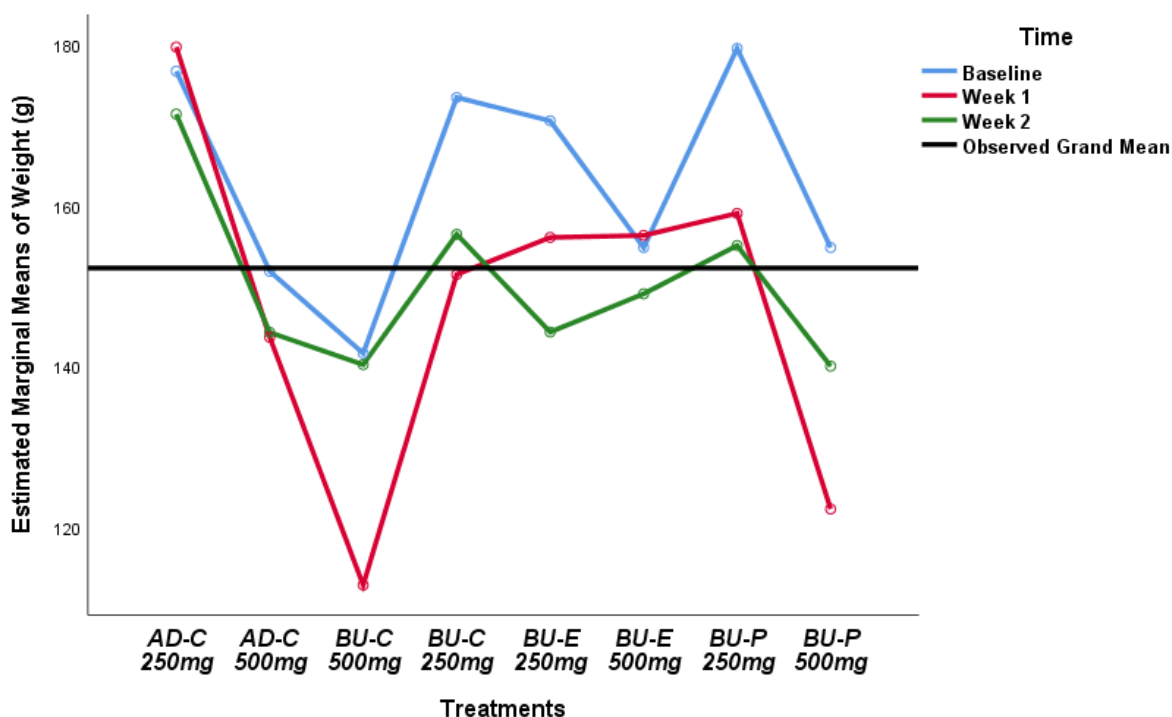


FIG. 2: Estimated Marginal means of Weight of various treatments and time.

Weight loss which is one of the clinical features of diabetes mellitus maybe due to generation of Adipocytes and muscle tissues to make up for the energy loss as a result of frequent urination and

Over conversion of glycogen to glucose. Weight loss is a very serious issue in the management of Diabetes mellitus.<sup>21</sup>

In this experiment, the mean change in Weight in the baseline ranges from 141.6 ±11.7 to 179.5 ±56.5g. Correspondingly,

considering the outcomes significantly affected by the intervention, it was observed that there is difference in the average weight of subjects from baseline, after intervention and follow-up period and the difference in weight was statistically significant with  $P$ .value  $< 0.05$ . In Fig 2, for the follow-up group there was increase in average weight recorded for all intervention except AD-C 250mg from  $179.7 \pm 13.2$  to  $171.3 \pm 13.7^a$  and BU-P  $159 \pm 44$  to  $155 \pm 26.8^c$ , while the highest average weight recorded was at  $140.2 \pm 9.4^b$ g of BU-C.

The repeated-measures ANOVA model for weight revealed significant effects for principal effects of the treatments (Fig 2), indicating actual effectiveness of the intervention on weight change from the baseline to 14 days of intervention,  $p < 0.05$ .

## CONCLUSION

Fractions of *Anthocleista Djalensis* and *Blighia Unijugata* are found to contain considerable amount of phytochemicals (Alkaloids, tannins, flavonoids, phenols and steroids) which is useful in treating diseases. Cytotoxicity of both shows they are non-toxic with BU-P being the most active,  $8.4095/15.0123 \mu\text{g}/\text{cm}^3$ .

After inducing experimental animals, albino rats with diabetes and treating them with plant fractions there was significant decrease in glucose level with BU-C 250mg. Similarly after intervention the lowest average glucose level observed was  $9.7 \pm 8.09$  (mmol/l) associated with BU-E 250mg and the highest was  $31.73 \pm 1.52$  (mmol/l) ascribed with BU-P 250mg. Correspondingly, it was observed during follow-up (2<sup>nd</sup> week) BU-C 250mg with  $3.16 \pm 1.05$  had lowest glucose level. Weight gain due to an increase in adipose tissue in animals was found to be highest in BU-C at  $140.2 \pm 9.4^b$ g in the second week of intervention.

BU-C is found to reduce glucose level and significant weight gain in experimental animals in this experiment better than the fractions experimented with.

Chloroform fraction of *blighia unijugata* has a potential to be used for diabetes treatment/intervention.

## REFERENCES

1. Farns, N.W (2008). The role of enthopharmacology in drug development Bio active compounds from plant. John Wiley & Sons.
2. Edeoga, H.O., Okwu, D. O and Mbaebie, B.O (2005). *African Journal of Biothchnology*: 4(7), 685.

3. Calzada F and Bautista E (2020). Plants used for the treatment of diarrhoea from Mexican flora with amoebicidal and giadicial activity, and their phytochemical constituents. *J. Ethnopharmacology* 2020; 253: 112676.
4. Sugarmo, V. B., Silambanan, S. K and Krithika (2015). Osazones of the Uncommonly Encountered Reducing Sugars. *International Journal of Interdisciplinary Studies.*, 2(9): 24-29.
5. Dalziel, J.M (1954). The useful plants of West Tropical Africa. London: Crown Agents for Overseas Colonies. Pp 15-17.
6. Burkill H. M (2000). Useful plants of West Tropical Africa. Families S-Z., 2nd Edition, *Royal Botanical Garden Kews* (5), 6–34, 586-593.
7. Obeng, E.A., *Blighia unijugata* Baker (2010). Record from PROTA4U. *Lemmens PROTA (Plant Resource Tropical Africa. Ressources végétalesl’Afrique Trop. Wageningen, Netherlands.*
8. Garba, S. and Salihu L. (2009). A *Phyosemion Gardneri* Test (AGT) for Cytotoxicity. *Nig. Journal of Scientific Research*, 2 (3):56-57.
9. Trease G.E and Evans W.C (1989). “A textbook of pharmacology”13<sup>th</sup> Edition, Bailliere Tindall Ltd. London, pp.134 and pp 683-684.
10. Broadhurst, R. B and Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture*, 29(9), 788–794.
11. Ferreira, D., Nel, R. J. J and Bekker, R. (1999). Condensed Tannins. In *Comprehensive Natural Products Chemistry* (pp. 747–797).
12. Kumar A, Bharti SK and Kumar A (2017). Therapeutic molecules against type 2 diabetes: what we have and what are we expecting? *Pharmacology*; 69: 959–970.
13. Ado K, Garba, S and Salihu L (2014) *Fundulopanchax gardneri* Test: A Convenient Method of Bioassay for active constituents of natural products. *Natural Product Chemistry and Research: 2*(4)
14. Finney, D. J (1971) Probit analysis (3rd Ed). Cambridge University Press 60:76-80.
15. Zhang, J., Yao, W., Dong, C., Yang, C and Ren Q (2015). Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. *Psytopharmacology Journal*. 232(23). Pp 4325-4335.
16. Jindal, A. and P. Kumar (2012). Antimicrobial activity of alkaloids of *Tridax procumbens* L. Against human pathogens. *International Journal of Pharmaceutical Science Research*, 3(9): 3481 – 3485.
17. Shukla, A., P. Bogoniya and B. Srivastava (2012). Hypoglycemic activity of *Lepidium sativum* Linn seeds total alkaloid on alloxan-induced diabetic rats. *Research*



- Journal of Medicinal Plants*; **6(8)**: 587 – 596.
- Rievere, C., V.J.H. Nguyen, L. Pieters, B. Dejaegher, Y.V. Heyden, C.V. Minh, et al., (2009). Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. *Phytochemistry*. **70**: 86 – 94.
18. Gupta S and Abu-Ghannam N (2011). Bioactive potential and possible health effects of edible brown seaweeds, *Trends in Food Science Technology*, **22 (6)**:315.
19. Cow-chin, J. R., Dinis T.C., Cunha A.P and Almeda L.M. (1994). Antioxidant activities of some extracts of *Thymus*. *Free radical Research*. 26(4): 469-478.
20. Zink, T. and Chaffin, J. (1998). Herbal “health” products: what family physician need to know. *American Family Physician* **58**: 1133- 40.