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PHYSIOLOGICAL EVALUATION OF THE ANTI-DIABETIC PROPERTIES OF *Hibiscus sabdariffa* ON RATS

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ABSTRACT

Effects of different quantities (75g, 100g and 125g) of *Hibiscus sabdariffa* leaf, stem and root aqueous extracts and combination of 300g *Hibiscus sabdariffa* +100g *Allium sativum*+100g *Zingiber officinale* on alloxan-induced diabetic Wister albino rats at 1ml/kg/day for17 days were investigated. Body weight and fasting blood glucose level of rats were monitored before and during the experiment. Quantitative analysis of secondary plant products of various parts of the investigated plants was also studied. Results showed that 1ml/kg/day of 75g *Hibiscus sabdariffa* aqueous extract did not cause significant ($p>0.05$) sugar reduction compared with basal values as high sugar level was still observed in alloxan-induced rats treated with 1ml/kg /day of 75 *Hibiscus sabdariffa* leaf (246.00$\pm$6.00mg/dl) and stem (207.50$\pm$3.50 mg/dl) extracts except root (196.00$\pm$1.20 mg/dl). Combination of the extracts caused significant ($P>0.05$) sugar reduction compared with any of the single parts.1ml/kg of 100g *Hibiscus sabdariffa* leaf extracts ameliorated rats' weights loss by 14.75%.1ml/kg of 125g *Hibiscus sabdariffa* leaf, stem and root extracts significantly ($p<0.05$) reduced the glucose level of diabetic treated rats by 54.08%, 58.95% and 62.44% compared with glibenclamide (22.77%). Phytochemical analysis revealed that flavonoids (0.79mg/g) and alkaloids (0.86mg/g) were significantly higher ($p<0.05$) in root than in stem and leaf of *Hibiscus sabdariffa*. The combination 300g*Hibiscus sabdariffa* + 100g *Allium sativum* 100g *Zingiber officinale* revealed significantly ($p<0.05$) higher flavonoids (0.85mg/g), saponins (0.95mg/g) alkaloids (1.81mg/g) and tannins (0.56mg/g). Combination of 300g *Hibiscus sabdariffa* +100g *Allium sativum*+100g *Zingiber officinale* produced the best hypoglycaemic effect (71.05%).

Keywords:, Anti-Diabetic, *Hibiscus sabdariffa,* Hypoglycemic, Extract, Phytochemical. Rats

INTRODUCTION

The use of plant derived products containing high concentration of dietary fibre and complex polysaccharide for the management of diabetes have been proposed. Natural products of plant origin have been found to be potential sources of novel molecules for the treatment of diabetes (Farnsworth, 1996). Considering the rate at which the vegetation is getting depleted in this part of the world, the precious knowledge of these plants and to search for more plants with anti-diabetic potential is necessary for documentation. Numerous plants are used in traditional herbal medicine for their hypoglycemic potentials, and available literatures indicate that there are more than 800 plant species showing hypoglycemic activity. There has been increasing demand for the use of plant products with anti-diabetic activity due to low cost, easy availability and lesser side effects, hence plant materials are

continuously scrutinized and explored for their effect as hypoglycemic agents. One of such plants is *Hibiscus sabdariffa l.,* a plant commonly called Roselle plant. It belongs to family Malaceae and is locally called *Isapa* by Yorubas and *Yakuwa* by Hausas. It is found in almost all the geographical zones of Nigeria (Duke *et al.,* 1984). It is cultivated for leaf, fleshy calyx, seed or fiber in all parts of the world and it is taken as a common local drink popularly known as '' ''Zobo'' in Nigeria. (Okasha., 2008).

Hibiscus sabdariffa l. is used ethnomedicinally for many varied purposes such as delicacy and medicinal properties**.** Tender young leaves and stems-raw or cooked are used in salads and as a seasoning in curries. Fresh calyx (the outer whorl of the flower) is eaten raw in salads, is cooked and used as a flavoring in cakes, jellies, soups, sauces, pickles and puddings. The calyx is rich in citric acid and pectin and so is useful for making jams. It is also used to add a red colour and flavour to herbal teas. Also, *Hibiscus sabdarriffa* is a good source of food and essential nutritional values, medicinal properties and notable physiological effect to life (Okasha *et al*., 2008, Arvind and Alka, 2011). It was reported to have antiseptic digestive, diuretic, purgative and sedative effect (*Sini et al.,* 2011). It is a medicinal herb, used in folk medicine in treatment of hypertension (Wang *et al.,* 2000; Odigie *et al.,* 2003). *Hibiscus* anthocyanin, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis*, have been found to have cardioprotective (Jonadet,1990; Olaleye, 2007), hypocholesterolemic (Chen *et al.,*2003), antioxidative and hepatoprotective (Amin and Hamza, 2005) effects in animals. The plant has also been used in traditional medicine for treating cough cancer, fever and above all diabetic diseases. Recently, it was discovered by Saleh *et al.* (2010) that the plant confers protective activity against gastric ulcer. In the Ayurvedic literature in India, different parts of this plant have been recommended as a remedy for various ailments such as hypertension, Pyrexia, liver disorders and as an antidote to poisoning chemicals (Andreas et al., 2000). Anthocyanins, flavonols and protocatechoic acid (PCA) along with other phytochemicals which have been identified as contributors to the observed medicinal effect of *H. sabdariffa*. This study is to Scientifically evaluate the traditional claim of the aqueous extract of various parts of, *H. sabdariffa* against diabetic disease.

MATERIALS AND METHODS

Plant Materials

*Allium sativum (*AS), *Zingiber officinale* (ZO) and fresh leaves, stem- bark and roots of *Hibiscus sabdariffa* (HS), were collected on a regular basis from various markets of Abeokuta and identified by comparison with voucher specimens at the herbaria in Forest Research Institute of Nigeria, Ibadan, Nigeria.

Preparation of Plant extracts

The leaves, stems and roots of *Hibiscus sabdariffa (*HS) 75g, 100g and 125g each and combination of 300g *Hibiscus sabdariffa* (HS) + 100g *Allium sativum* (AS) +100g *Zingiber officinale* (ZO) were prepared separately according to the method of Akhani *et al*. (2004). The plant samples were boiled in 1L of tap water for one hour, filtered using a piece of sterile white cotton cloth and stored in the refrigerator at 2-8°C in a glass container.

Quantitative secondary plant products Analysis of Extracts.

The plant extracts were screened for phyto-

chemical, alkaloids, tannins, flavonoids, and saponins.

Determination of Alkaloids content

This was carried out by the alkaline precipitation gravimetric method described by Kadiri and Fasidi (1992).

Five grams of the sample were dispersed in 10% acetic acid solution in ethanol in ratio 1:10 (10%). The mixture was allowed to stand for 4h at 28^0C and filtered with Whatman's No. 42 grade filter paper. The filtrate was evaporated and treated with drops of conc. aqueous NH_4OH until the alkaloid was precipitated. The precipitated alkaloid was collected on the filter paper; washed with1% ammonia solution and dried in the oven at 80° C. The alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of saponins content

The qualitative determination of saponins was done according to the method of Kadiri and Fasidi (1992).

Five gram of each powdered sample were added to 100ml of 20% aqueous ethanol and kept on a shaker for 30 min. The sample was heated over water bath for 4h at 55° C. The mixture was filtered and residues were re- extracted with another 200ml of 20% aqueous ethanol. The combined extract was reduced approximately to 40 ml over water bath at 90° C. The concentrate was transferred into a 25ml separatory funnel, extracted twice with 20ml diethyl ether. Ether layer was discarded while aqueous layer was retained and 60 ml n- butanol was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation; the sample was dried in oven (40° C) to constant weight. The saponins content was calculated as percentage of initial weight of sample taken.

Determination of flavonoid content

This was determined according to the method of Kadiri and Fasidi (1992). 5g of the sample were boiled in 50ml of 2MHCL solution for 30min under reflux, allowed to cool and filtered through whatman's paper No. 42. A measured volume of the extract was treated with equal volume of ethyl acetate in drops and filter and using weighed filter paper. The resultant weight difference was that of flavonoids in the sample.

Determination of tannins content

Tannin content was determined by the Folin -Denis colorimetric method described by Kadiri and Fasidi (1992) five grams were dissolved in 50ml of distilled water and shaken. The mixture was allowed to stand for 30min at 28° C after which it was filtered through whatman's filter paper No 42. Two milliliters of the extract was dispersed into a 50ml volumetric flask. Similarly 2ml each of standard tannin solution (tannic acid) and distilled water served as standard was added to each of the flask followed by the 2.5ml of saturated Na_2CO_3 solution. The content of each flask was made up to 50mls with distilled water and allowed to incubate at 28° C for 90 min. Their respective absorbance was measured in spectrophotometer at 260nm using the reagent blank to calibrate the instrument at zero.

Methodology

Experimental Animals

Adult (aged 3-4 months) Wister albino rats weighing 90-175g of either sex were purchased from the disease-free stock of the animal house of the College of Veterinary

Medicine, Federal University of Agriculture, Abeokuta. They were maintained in normal and standard laboratory conditions of temperature 28^{0}C) and relative humidity 66%) with 12-hour light dark cycle and adequate ventilation. The animals were fed with commercial diet (Vital Feed Nig. Ltd.) and water, *ad libitum*. Food was withheld 12 hrs. before the experiments (Adikwu, *et al.,* 2010)

Animal categorization

The animals were allowed 7-day acclimatization period, after which the blood glucose level of all the rats were confirmed using glucometer (One-Touch) to determine the normal sugar level of all the rats (48-54 mg/dL) by withdrawing blood from the tail end and testing. After this the rats were randomly divided into two broad categories: non diabetic (normoglycaemic) and diabetic (hyperglycemic) rats.

Induction of Diabetes.

Diabetes was induced on the latter category by intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate freshly prepared with distilled water. Diabetes was confirmed 24 hours later in alloxan- induced animals showing Random Blood Glucose (RBG) level ≥ 200 mg/dL by using glucometer to monitor the blood sample from the tail vein.

Animal grouping and experimental design

Ninety two (92) Male albino rats, weighing 90-175g (n= 4) were used. They were allowed 7–day acclimatization period under standard rat house conditions before the trial was initiated. In a completely randomized design the rats were divided into 8 groups of which group 1-3 were later subdivided into 3 groups comprising 4 alloxan-induced rats as designated by figure (i), (ii) and (iii). The Alloxan-induced rats in sub group figure (i-iii) were treated with (1ml/kg) of 75g, 100g and 125g of ML leaf, stem and root aqueous extracts respectively. Group 4, 5 and 6 composed of another 4 alloxan-induced diabetic rats each treated with1ml/kg of combination 300gML+100g ZO + 100g AS aqueous extracts and glibenclimade orally on daily basis for seventeen days consecutively. Group 7 composed of alloxan-induced rats as diabetic control (D.C) while group 8 composed of another set of 4 non diabetic rats (normoglycaemic) as normal control (N.C).

The extracts and drug were administered by oral route. Blood sample was withdrawn from the tail vein with the aid of a capillary tube and tested using the glucometer. It was withdrawn just before oral administration of substances 0, 1, 3, 5,7,1,13,15 and 17th day in each case. The percentage of glycaemia reduction was calculated at the 17th day during fasting blood sugar (FBS) monitoring using the formula:

$$\text{Percentage change of glycaemia} = \frac{Gx - Go}{Go} \times 100$$

Where Go and Gx were the values of glucose level on post induction and 17th day of experimental set up respectively.

RESULTS

Table 1 shows the hypoglycemic effect of 1ml/kg/day of 75g leaf, stem and root aqueous extract of *H. sabdariffa*. Results showed that oral treatment with 1ml/kg of 75g *H. sabdariffa* extracts did not cause significant ($p>0.05$) alterations in the blood sugar level most especially from day 3 to 7, but from days 9 through 17 moderate sugar reduction

was recorded. This reduction was not enough to cause hypoglycemic effect in rats because the sugar level of the rats was still very high even till the last day of the experimental set up except alloxan-induced rats treated with *H. sabdariffa* root extract (196.00±00mg/dl) (Table1).

When the weight of the experimental samples were increased from 75g to 100g, the sugar level of rats treated with various parts (leaf, stem and root) of *H. sabdariffa* extracts reduced after 17days of treatment by 37.89%, 37.63% and 54.61% respectively (Table 2).

Similar effect was observed when 1ml/kg of 125g of *H. sabdariffa* extracts were administered. At 125g, the sugar level of rats reduced by 54.08%, 58.95% and 62.44%. However, combination 300g *Hibiscus sabdariffa* + 100g *Allium sativum*+ 100g *Zingiber officinale* produced highest sugar reduction (hypoglycemia) of 71.05% than even the glibenclamide (Table 3).

It was observed that in all the parts of *H. sabdariffa*, the percentage weight gain increased when the experimental samples were increased from 75g to 100g. This effect reduced with further increase in the sample quantities from 100g to 125g. It was also observed that the highest percentage weight gain (14.75) was recorded at 100g of *H. sabdariffa* leaf extract. The hypoglycemic effect of the extract of various parts of H. *sabdariffa* increased from root to leaf while the weight gain increased from leaf to root (Table 4).

Table 5 shows the mean values of phytochemical contents of various parts of H. *sabdariffa*. According to the result, there was significant difference in the amount of flavonoids, saponins, alkaloids and tannins present in the leaf, stem and root extracts of *Hibiscus sabdariffa*. Flavonoids (0.79mg/g) and alkaloids (0.86mg/g) contents were significantly ($P<0.05$) higher in root of H. *sabdariffa* than stem and leaf**.** Tannins contents (0**.**89mg/g) were significantly ($P<0.05$) higher in the stem of *Hibiscus sabdariffa* than in root and leaf**.** Flavonoids (0.51mg/g) and alkaloids (0.42mg/g) contents were significantly ($P<0.05$) higher in *Z. officinale* rhizome than in A. *sativum* bulb (Table 5). Tannins (0.89mg/g) was observed to be the highest phytochemical content recorded in the stem of *Hibiscus sabdariffa*. Also, quantitative analysis of combined experimental samples (300g *Hibiscus sabdariffa* +100g *Allium sativum*+100g *Zingiber officinale))* revealed significantly ($p<0.05$) higher flavonoids (0.85mg/g), saponins (0.95mg/g) alkaloids (1.81mg/g) and tannins (0.56mg/g) concentration.

Table 1: Mean Sugar level (mg/dl) of normal rats, Glibenclamide treated diabetic rats, diabetic rats treated with 75g (1ml/kg) of *Hibiscus sabdariffa* leaf, stem and root and diabetic rats treated with combined graded weights of combination 300g HS+100g AS + 100g ZO.

EXTRACTS/ GROUPS	PRE-INDUCTION	POST-INDUCTION	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15	DAY 17	%sugar decrease
Hibiscus sabdariffa leaf extract	50.50+5.39a	299.50+29.58 bc	295.00+28.72bc	291.25+27.98bc	244.25+16.18bc	269.00+2.00d	268.50+0.50d	260.50+1.50c	254.00+5.00d	246.00+6.00d	17.86
Hibiscus sabdariffa steme xtract	51.75+3.19a	289.25+51.40 bc	285.25+51.03 bc	264.75+34.28bc	228.67+4.70bc	219.00+3.21c	220.00+0.00b	215.50+0.50b	213.50+2.50c	207.50+3.50c	28.30
Hibiscus sabdariffa root extract	52.75+4.76a	280.25+18.55bc	275.50+17.71 bc	268.75+17.56bc	259.00±16.21cd	234.69+8.51c	218.00+5.00b	209.50+4.50b	202.50+7.50b	196.00+1.20bc	30.06
300g HS+100g AS + 100g ZO	50.23+2.56a	268.25+11.25 bc	261.75+10.98bc	221.50+11.99b	183.50+9.35b	130.33+10.4b	78.00+9.64a	76.67+9.95a	78.00+9.45a	77.67+8.81a	71.05
Reference drug (Glibenclamide)	54.50+2.63a	238.25+3.44b	234.50+3.28bc	230.50+3.47b	224.75+3.66bc	218.50+3.30c	210.75+2.83b	202.75+2.87b	194.00+2.91b	84.00+1.73b	22.77
Diabetic control	51.75+2.17a	345.75+ 52.05d	347.25+51.84c	353.00+71.76c	315.5+101.50d	422.00+0.00	422.00+0.00	424.00+0.00	425.00+0.00	426.00+0.00	-23.21
Normal Control	48.00+2.48a	104.75+2.25a	104.75+2.25a	98.75+2.50a	95.00+1.58a	93.75+1.11a	91.75±0.63a	90.25+0.25a	91.25+0.25a	90.25+0.48a	13.84

Means followed by the same letters in the same column are not significantly different according to Duncan's Multiple Range Test at ($P<0.05$)

Table 2: Mean Sugar level (mg/dl) of normal rats, Glibenclamide treated diabetic rats, diabetic rats treated with 100g (1ml/kg) of *Hibiscus sabdariffa* leaf, stem and root and diabetic rats treated with combined graded weights of combination 300g HS+100g AS +100gZO.

EXTRACTS/ GROUPS	PRE-INDUCTION	POST INDUC-TION	DAY 3	DAY 5	DAY 7	DAY 9	DAY 11	DAY 13	DAY 15	DAY 17	% sugar decrease
Hibiscus sabdariffa leaf extract	53.00+4.26a	272.50+17.08b	268.75+1688b	261.75+16.82b	254.00+16.61bc	245.25+15.29c	229.75+13.29c	211.25+12.28c	192.25+7.77c	169.25+4.29c	37.89
Hibiscus sabdariffa stem extract	50.73+2.86a	269.00+20.42b	264.75+19.74b	255.75+19.48b	244.50+19.47b	233.25+1894c	217.00+16.47bc	198.75+12.39bc	180.00+7.91c	167.75+3.42c	37.63
Hibiscus sabdariffa root extract	50.50+2.02a	265.50+12.07b	261.25+12.20b	248.50+11.34b	228.75+11.00b	216.75+2.88c	198.25+2.39b	177.25+1.49b	148.50+3.06b	120.50+3.88b	54.61
300g HS+100g AS +100Gzo	50.23+2.56a	268.25+11.25b	261.75+10.98b	221.50+11.99b	183.50+9.35b	130.33+10.40b	78.00+9.64a	76.67+9.95a	78.00+9.451a	77.67+8.81a	71.05
300g ML + 100g AS+ 100Gzo	50.25+2.13a	274.25+26.67b	266.50+25.59b	230.50+24.56b	192.50+23.48b	130.67+14.17b	84.00+8.00a	82.67+5.69a	83.33+5.36a	82.65+4.17a	69.85
Reference drug (Glibenclamide)	54.50+2.63a	238.25+3.44b	234.50+3.28b	230.50+3.47b	224.75+3.66b	218.50+3.30c	210.75+2.83bc	202.75+2.87c	194.00+2.91c	84.00+1.73d	22.77
Diabetic control	51.75+2.17a	345.75+52.05c	347.25+51.84c	353.00+71.76c	315.50+101.50e	422.00+0.00	422.00+0.00	424.00+0.00	425.00+0.00	426.00+0.00	-23.21
Normal Control	48.00+2.48a	104.75+2.25a	104.75+2.25a	98.75+2.50a	95.00+1.58a	93.75+1.11a	91.75± 0.63a	90.25+0.25a	91.25+0.25a	90.25+0.458a	13.84

Means followed by the same letters in the same column are not significantly different according to Duncan's Multiple Range Test at 5% P <0.05

Table 3:Mean Sugar level (mg/dl) of normal rats, Glibenclamide treated diabetic rats, diabetic rats treated with 125g (1ml/kg) of *Hibiscus sabdariffa* leaf, stem and root and diabetic rats treated with combined graded weights of combination 300g HS+100g AS+100gZO.

Extracts/ Groups	Pre Introduction	Post Introduction	Day 3	Day 5	Day 7	Day 9	Day 11	Day13	Day15	Day 17	%sugar decrease
Hibiscus sabdariffa leaf extract	52.75+4.75a	278.75+30.63bc	263.25+28.98b	275.25+17.99b	254.00+17.67cd	234.00+12.17d	210.00+8.00c	185.50+11.50d	155.50+77.50c	128.00+10.00b	54.08
Hibiscus sabdariffa stem extract	53.00+3.41a	286.25+15.33bc	273.25+11.43b	250.75+8.58b	226.00+5.11bc	200.25+3.63c	195.75+21.12b	176.00+15.63c	138.00+4.00c	117.50+4.50b	58.95
Hibiscus sabdariffa root extract	50.50+1.89a	272.50+16.67bc	261.75+14.92b	243.00+13.73b	219.50+12.09bc	193.50+10.22c	166.50+8.60b	147.75±11.91b	107.00+9.16b	102.33+13.77a	62.44
300g HS+100g AS+100gZO	50.23+2.56a	268.25+11.25bc	261.75±10.98bc	221.50+11.99b	183.50+9.35b	130.33+10.40b	78.00+9.64a	76.67+9.95a	78.00+9.451a	77.67+8.81a	71.05
300gML+100 gAS+100gZO	50.25+2.14a	274.25+26.67bc	266.50+25.59b	230.50+24.56b	192.50+23.48bc	130.67+14.17b	84.00+8.00a	82.67+5.69a	83.33+5.36a	82.65+4.17a	69.85
Reference drug (Glibenclamide)	54.50+2.63a	238.25+3.44b	234.50+3.29b	230.50+3.47b	224.75+3.66bc	218.50+3.30c	210.75+2.83c	202.75+2.87d	194.00+2.91d	84.00+1.73c	22.77
Diabetic control	51.75+5.20a	345.75+ 52.05c	347.25+51.84c	353.00+71.76c	315.50+101.50d	422+00+0.00	422.00+0.00	424.00+0.00	425.00+0.00	426.00+0.00	-23.21
Normal Control	48.00+2.48a	104.75+2.25a	104.75+2.25a	98.75+2.50a	95.00+1.58a	93.75+1.11a	91.75.63a	90.25+0.25a	91.25+0.25ab	90.25+0.458a	13.84

Table 4: Effect of various parts of *Hibiscus sabdariffa* aqueous extracts on body weight of rats.

Plants	Values	Percentage weight gain of rats. weight grades of plant samples (g)								
		Leaf			Stem			Root		
		75g	100g	125g	75g	100g	125g	75g	100g	125g
H. sabdariffa	Initial weigh of rats	120.45+2.0	83.65+2.16	147.05+0.74	130.00+0.5	101.30+3.4	150.85+0.95	139.33+1.74	110.50+1.20	152.95+0.34
	Final weight of rats	124.05+4.5	98.13+2.17	165.95+0.55	132.85+0.5	106.55+3.7	159.95+1.35	142.20+2.20	114.55+1.25	159.00+0.20
	Change (%)	3.00	14.75	11.38	2.15	5.00	5.68	2.02	3.53	3.80

Table 5: Effect of combined experimental sample on body weight of rats

Experimental samples	Percentage change (%)	Bodyweights (kg)
300gHS+100g AS+100g -+ 100gZO,	Initial weight of rats Final weight of rats Change (%)	121.23+5.46 157.60+6.89 23.08
Reference drugs (Glibenclamide)	Initial weight of rats Final weight of rats Change (%)	114.93+1.26 123.93+1.62 7.26
Diabetic control	Initial weight of rats Final weight of rats Change (%)	123.05+.84 91.10+0.00 -35.07
Normal control	Initial weight of rats Final weight of rats Change (%)	104.35+3.41 132.93+3.23 21.50

Table 6: Mean value of phytochemical contents of various parts of *H. sabdariffa*

Plants parts	Phytochemical contents (mg/g)			
	Flavonoids	Saponins	Alkaloids	Tannins
Hibiscus sabdariffa				
Leaf	0.23+0.060a	0.130+0.006a	0.12+0.006a	0.17+0.006a
Stem	0.13+0.006b	0.17+0.006b	0.75+0.006b	0.89+0.006b
Root	0.79+0.103c	0.11+0.006c	0.86+0.006c	0.19+0.006c

Mean followed by the same letters on the same columns are not significantly different according to Duncan's Multiple Range Test at ($P<0.05$)

Table 7: Mean value of phytochemical contents of *A. sativum*, *Z. officinale* and *H. sabdariffa*'s dried calyx.

Plants species	Phytochemicals (mg/g)			
	Flavonoids	Saponins	Alkaloids	Tannin
Allium sativum	0.42+0.007a	0.62+0.006a	0.38+0.006a	0.25+0.006a
Zingiber officinale	0.51+0.006b	0.56+0.006b	0.42+0.006b	0.29+0.006b
Hibiscus sabdariffa (dried calyx)	0.24+0.006c	0.14+0.006c	0.25+0.006c	0.29+0.006b

Means followed by the same letters in the same column are not significantly different according to Duncan's Multiple Range Test at ($P<0.05$)

DISCUSSION

The effect of various parts of *H. sabdariffa* on rats has implications on its use as hypoglycemic agent for diabetes therapeutic purposes**.** The observation of high sugar level in alloxan-induced rats treated with leaf and stem extracts till the last day of the experimental set up is an indication that 75g of the experimental samples were too small, hence the extracts were not effective enough on the sugar level. This hypoglycemic effect increased significantly ($p<0.05$) with increase in experimental samples from 75g to 100g. Also, similar effect was observed as the samples were increased from 100g to 125g. This implies that the concentration of the extracts increased with increase in experimental samples. The efficacy of the extract at 125g on hyperglycemic rats may be more effective on the sugar level but it may be too concentrated for consumption as it reflected in the reduction of the body weight of experimental rats. This result agrees with observation of other researchers, who had systematically demonstrated that extract from the plant possesses anti-diabetic properties (Odutuga *et al.,* 2010, Sini *et al,* 2011). Although many researches on the effect of plant extract on sugar level had indicated that some plant can work singly (Soon and Tan, 2002) but this study has clearly revealed that some of these plants can work better if they are combine with other plants material or any other hypoglycaemic agent as reflected in the combination 300g *Hibiscus sabdariffa*+ 100g *Allium sativum* + 100g *Zingiber officinale* which produced highest sugar reduction (hypoglycemia) than even the glibenclamide in this study. This observation is in agreement with the finding of Adikwu (2010) who reported that combinations of the extract and metformin caused more reduction in glycaemia compared to any of the agents acting alone in either of the two categories of animals.

The hypoglycemic effect of the combined agents suggests that their anti-diabetic activities are addictives and this could mean that the extracts from *H. sabdariffa, Allium sativum and Z. officinale* had similar mode of action (Adikwu *et al.,* 2010; Sini *et al*., 2011).
The efficacy of the extract on hyperglycemic rats correlates the result of other findings, which had methodically demonstrated that the extract from the plant characterizes anti-diabetic properties.

Observation of this study is in agreement with the reports from various findings, in that, the diabetic untreated rats demonstrated steady reduction in the body weight and significant blood glucose level increase (Adewole and Ojewole, 2006) through out the period of the experiment while normal control (normoglycaemic) and the extract control rats exhibited consistent increase in body weight.

The decrease in body weight of the diabetic control group may be due to wasting associated with diabetic patients as a result of increase utilization of fats from the adipose tissue for generation of energy in the body as also observed by (Sini *et al.,* 2010).

Percentage weight gain observed in all the parts of *H. sabdariffa* extracts may indicate the nutritional content of the parts most especially leaf but this effect reduced with further increase in the samples from 100g to 125g. This may be an indication that though the plant extracts produced better sugar level reduction at 125g but the resultant effect of that quantity may equally result into weight loss of consumers. Also highest percentage weight gain recorded at 100g of *Hibiscus sab-*

dariffa leaf extracts implies that the extract can maximally be taken at 100g and that further increase of the samples beyond these quantities may be too concentrated for consumption.

The observation of high hypoglycaemic action in the root extract of *H. sabdariffa* may be as a result of high bioactive and non-nutritional secondary plant products such as flavonoids, saponins, alkaloids and tannins recoded in all the parts most especially root. Similar observation was reported by (Adeneye and Adeyemi, 2009, Mbaka *et al*., 2009; Odutuga *et al*., 2010; Oluwatosin, and Justine, 2010; Arvind and Alka, 2011).

CONCLUSION

In conclusion, combination of 300g *Hibiscus sabdariffa* + 100g *Allium sativum*+100g *Zingiber officinale* produced the best hypoglycaemic effect (71.05%) in alloxan-induced diabetic rats in this study hence the plant should be employed as diabetes therapeutic agent.. However, study on the proximate analysis of the investigated parts that will validate factors that are responsible for the increase in the weight of rats is in progress.

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