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(RESEARCH ARTICLE)



# Phytochemicals, hypoglycemic and hypolipidemic effects of methanol leaf extract of *Hibiscus sabdariffa* in alloxan induced diabetic rats

Tsado Amos Ndarubu <sup>1</sup>, Onukogu Stella Chiamaka <sup>2</sup>, Suleiman Alfa <sup>3</sup>, Mustapha Aishatu <sup>4, 5</sup>, Osuigwe Ekeoma Chinedu <sup>2</sup>, Dannana Luke Wenawo <sup>2</sup>, Alawode Rhamatalah Adenike <sup>6</sup>, Lawal Bashir <sup>2,\*</sup> and Berinyuy Bonghan Eustace <sup>7</sup>

- <sup>1</sup> Department of Biological Sciences, Niger State Polytechnic Zungeru, P.M.B 001, Zungeru, Niger State.
- <sup>2</sup> Department of Biochemistry, Federal University of Technology Minna, P.M.B. 65, Nigeria.
- <sup>3</sup> Department of Biological Sciences, Federal Polytechnic Bida, Nigeria.
- <sup>4</sup> Department of Microbiology, Federal University of Technology Minna, P.M.B. 65, Nigeria.
- <sup>5</sup> Niger State Primary Health Care Development Agency. Station: PHC Tunga, Niger State Nigeria.
- <sup>6</sup> Forestry research institute of Nigeria-Southern Guinea Research Station, Mokwa, Niger State, Nigeria.
- <sup>7</sup> Faculty of medicine and biomedical sciences, University of Yaounde 1, Yaounde, Cameroon.

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#### **Abstract**

This study investigates the phytochemicals, antidiabetic and hypolipidemic activities of *Hibiscus sabdariffa* methanol leaves extract in alloxan induced diabetic wistar rat by administering graded oral doses (100, 200 and 400 mg/kg body weight) for 21 days. Results revealed that the methanol leaf extract of *Hibiscus sabdariffa* contains 51.90±3.89 mg/100g of tannins, 102.56±6.89 mg/100g of saponins, 54.78±3.89 mg/100g of alkaloids, 67.45±3.87 mg/100g of flavonoids and 121.54±5.67 mg/100g of phenols. The extract showed dosed dependent significant (p<0.05) antidiabetic activity with significant improvement in body weight. The extract also resulted in significant (p<0.05) reduction serum cholesterol, triglycerides. LDL-cholesterol level and increase HDL-cholesterol when compared with untreated control. In conclusion, this study demonstrates, for the first time, that *Hibiscus sabdariffa* is effective in inhibiting hyperglycemia and dyslipidemia in diabetes condition.

**Keywords:** Hibiscus sabdariffa; Phytochemicals; Hyperglycemia; Hyperlipidemia; Diabetes

## 1. Introduction

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action [1]. The rapid global increase in the number of people with diabetes is quite alarming. More than 177 million of global population live with diabetes and this figure is likely to increase by 2030 [2]. In sub-Saharan Africa, it is estimated that 10.8 million and that this would rise to 18.7 million by 2025 [3]. In Nigeria, about 3% of adult were reported to have DM [4]. Diabetes is a leading cause of adult blindness, amputation, stroked, renal failure and neuropathy [5]. Dislipidemia are commonly observed in hyperglycemic diabetic patient. Diabetic (NIDDM) patient with mild fasting hyperglycemia commonly present hypertriglyceridemia due to overproduction of hepatic triglyceride-rich lipoproteins, associated with decreased high-density lipoprotein (HDL) cholesterol levels [6].

Plants had been used for medicinal purposes long before recorded history. Researchers found that people in different parts of the world tended to use the same or similar plants for the same purpose [7]. Evaluation of medicinal plant

\* Corresponding author

E-mail address: bashirlawal12@gmail.com

used in traditional treatment of diabetes is of growing interest [8]. WHO also recommend and encourage this practice especially in country where access to conventional treatment of diabetes is inadequate [2].

Fresh or dried calyces of *H. sabdariffa* are used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes in Sudan and Nigeria, the calyces are boiled with sugar to produce a drink knownas "Karkade" or "Zoborodo" [9]. *H. sabdariffa* exhibited significant hypolipidemic and has antidiabetic effect [10]. The plant also showed antinociceptive and anti-inflammatory effects 11. Traditionally, the plant has been used for diabetes [12]. Therefore, the present work was undertaken to explore the phytochemicals, antidiabetic and hypolipidemic potentials of *H. sabdariffa* methanol leaves extract in alloxan induced diabetic wistar rat.

#### 2. Material and methods

## 2.1. Sample collection

The freshly harvested leaves of *Hibiscus sabdariffa* was obtained in August, 2016 from Bosso Mina, Niger state, Nigeria. Taxonomic authentication of the plant was conducted at the Department of Biology, Federal university of technology, Minna.

## 2.2. Experimental Animals

Healthy albino rats of average weight 120-150g were purchased from Animal House, School of life sciences, Federal University of Technology Minna, Nigeria. The rats were kept in clean plastic cages and maintained under standard laboratory conditions. They were allowed unrestricted access to rat pellets and water *ad-libitum*.

#### 2.3. Sample preparation and phytochemical analysis

The leaves of *Hibiscus sabdariffa* was washed and air dried for 2 weeks (37°C) and finally grounded using a grinder mill. Extraction of plant materials was performed by weighing 200 g of the powdered plant and extracted using 600 ml of methanol. The resulting extract was concentrated in water bath. The concentrated extract was stored in airtight container prior to use. Quantitative **p**hytochemical analysis of the plant was carried out using standard procedures as described previously [13-16].

## 2.4. In vivo antidiabetic study

Twenty-five (25) albino rats were intra-peritoneally administered a freshly prepared solution of alloxan monohydrate (120 mg/kg) to overnight fasted rats. Diabetic state was confirmed by glucose level above 200 mg/kg bw [17]. The animals were divided into 4 groups and were treated with 2 ml/kg of normal saline, 100 mg/kg, 200 mg/kg, 400 mg/kg bw extract and 5 mg/kg b. wt glibenclamide. All treatments were administered daily through oral route for 21days. Five (5) rats were also set up as normal control. The blood glucose level was checked and the weight taken on a weekly basis.

## 2.5. Blood collection

Collection of samples for lipid profile analyses was as described previously [18-19]. The animals were anasthesized with chloroform and blood was collected through cardiac puncture into a clean, dry centrifuge tubes. The blood sample was allowed to stand for 10minutes at room temperature and then centrifuged at 1000rpm for 15minutes to get the serum.

## 2.6. Analysis of lipid profile

Serum concentrations of lipid profile including total cholesterol, triglycerides and high density lipoprotein (HDL)-cholesterol were assay by enzymatic colorimetric methods using commercially kits according to the manufacturer's instructions [20-21]. VLDL-cholesterol was estimated as TG/5, and LDL-cholesterol was calculated using Friedewald formula [22] as follows:

LDL (mg/dl) = TC - (HDL + VLDL)

## 2.7. Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means  $\pm$  SE of the mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at P < 0.05 [23].

#### 3. Results and discussion

Plant use in treatment of disease are said to contain active compounds called phytochemicals some of which are responsible for the characteristic adours, purgensies and colour of plant while others give a particular plant its culinary medicinal or poisonous virtues [24]. Qualitative phytochemical composition of methanol leaf extract of Hibiscus sabdariffa revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, terpenes, cardiac glycoside, steroids and anthraquinone while phlobatannins was absent (Table 1). Saponin has been reported to have anti-inflamatory, cardiac depressant and hypo-cholesterol emic effect [25]. Flavonoids are the most diversified groups of phenolic compound found in plant. It biological activity include, antibacterial, anti-inflammatory, anti-allergic, protect against ulcers, vineses and antitumor effect [26]. Flavonoids are free radical scavengers, super antioxidant and potential water soluble which prevent oxidative cell, damage and have strong anti-cancer activity [27]. Alkaloid have been reported for antiplasmodial analgesic, antispasmodic, antidiabetic, anti-inflammatory properties [28]. Ouantitatively, methanol leaf extract of *Hibiscus sabdariffa* contains 51.90±3.89 mg/100g of tannins, 102.56±6.89 mg/100g of saponins, 54.78±3.89 mg/100g of alkaloids, 67.45±3.87 mg/100g of flavonoids and 121.54±5.67 mg/100g of phenols (Table 2). The high flavonoids and alkaloids obtained in this study could be an indication of strong antioxidative and hypoglycemic potentials of this plant. Furthermore, flavonoids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models [29]. Previous researchers have demonstrated the hypoglycemic activity of triterpenoid glycosides [30]. Thus, the phytochemical constituents indicate that the methanol leaf extract of Hibiscus sabdariffa could have potentials to be an hypoglycemic agent. The presence of important phytochemical in this plant is an indication that this plant if properly screened could yield a drug of pharmaceutical significance. However, the absence of phlobatannins agree with early studies which also found that not all phytochemicals are present in all plant and those that present differs according to the solvent use in the extraction process [31-32].

**Table 1** Qualitative phytochemical composition of methanol leaf extract of *Hibiscus sabdariffa* 

| Phytochemicals    | Inference |
|-------------------|-----------|
| Phenol            | +         |
| Flavonoids        | +         |
| Tannins           | +         |
| Saponins          | +         |
| Alkaloids         | +         |
| Terpens           | +         |
| Phlobatannins     | -         |
| Cardiac glycoside | +         |
| Anthraquinone     | +         |
| steroids          | +         |

Table 2 Quantitative Phytochemical composition of methanol leaf extract of Hibiscus sabdariffa

| Phytochemicals | Quantity                 |  |
|----------------|--------------------------|--|
| Phenol         | 121.54±5.67 <sup>d</sup> |  |
| Flavonoids     | 67.45±3.87 <sup>b</sup>  |  |
| Tannins        | 51.90±3.89 a             |  |
| Saponins       | 102.56±6.89 c            |  |
| Alkaloids      | 54.78±3.89a              |  |

Data are Mean ± SEM of triplicate determination. Values followed by different superscript alphabet were significantly different (p<0.05)

Alloxan induced diabetic untreated rats shows progressive increase in blood glucose level throughout the study period. Methanol leaf extract of *Hibiscus sabdariffa* exhibited dose dependent decrease blood glucose level throughout the 21 days' study period (figure 1). Methanol leaf extract of *Hibiscus sabdariffa* produce 68.49 %, 73.59 % and 80.47 % hypoglycemic effect at 100, 200 and 400 mg/kgb.wt respectively while the standard drug (gilbenclamide) at 5 mg/kg bodyweight caused 81.10 % hypoglycemic effect (table 3).

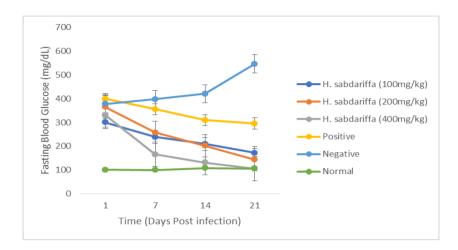


Figure 1 Effect of methanol leaf extracts of Hibiscus sabdariffa on blood glucose levels in alloxan induced diabetic rat

**Table 3** Effect of methanol leaf extracts of *Hibiscus sabdariffa* on glucose reduction and weight gain in alloxan induced diabetic rat

|                          | Fasting Blood Glucose (mg/dL) | Glucose Reduction (%) | Weight gain  |
|--------------------------|-------------------------------|-----------------------|--------------|
| H. sabdariffa (100mg/kg) | 172.34±8.45 <sup>c</sup>      | 68.49                 | -5.78±0.67*  |
| H. sabdariffa (200mg/kg) | 144.46±7.03 <sup>b</sup>      | 73.59                 | 6.09±0.97    |
| H. sabdariffa (400mg/kg) | 106.82±7.65 a                 | 80.47                 | 7.70±0.87    |
| Positive                 | 103.35±11.93 <sup>a</sup>     | 81.10                 | 9.55±0.02    |
| Negative                 | 547.09±23.45 <sup>d</sup>     |                       | -32.00±0.55* |
| Normal                   | 106.56±6.56a                  |                       | 9.42±0.87    |

Data are Mean  $\pm$  SEM of triplicate determination. Values followed by different superscript were significantly different (p<0.05). "\*" indicates weight loss

The hypoglycemic effect of methanol leaf extract of *Hibiscus sabdariffa* reported in this study may be attributed to the presence of phenols, flavonoids, alkaloids, tannins and saponins that have been associated with hypoglycemic activity [33]. Leaves of *Hibiscus sabdariffa* have also been reported by Sachdewa & Khemani, [12], to be hypoglycemic.

Alloxan induced diabetic untreated rats showed a progressive decrease body weight (figure 2) with final body weight loss of 32.00±0.55. Rats treated with 100 mg/kg bw of methanol leaf extract of *Hibiscus sabdariffa also exhibited* progressive decrease body weight with loss of 5.78±0.67 g. however rats treated with 200 and 400 mg/kg bw of the extract as well as those treated with the standard drug shows improvement in body weight with a gain of 6.09±0.97 g, 7.70±0.87 g and 9.55±0.02 g respectively (Table 3). During diabetic conditions, insulin deficiency prevents the body from the utilization of glucose for energy source. Thus, the body switched to the stored fats and muscle proteins, leading to the reduction in overall body weight as observed in untreated groups. The anti-diabetic activity of *Hibiscus sabdariffa* methanol leaf extract is also supported by the significant weight gain of the treated animals in comparison with the untreated animals. This shows the improvement in metabolic activity of the treated animals.

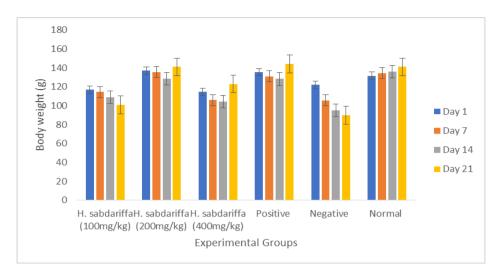
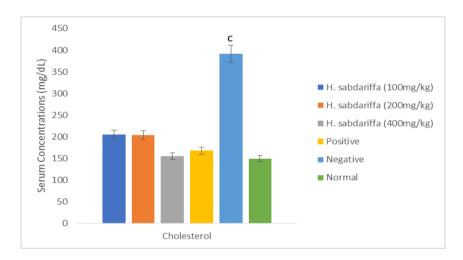
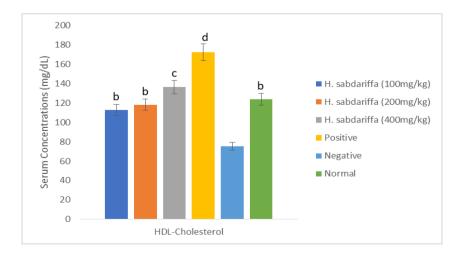


Figure 2 Effect of methanol leaf extracts of Hibiscus sabdariffa on body weight in alloxan induced diabetic rat



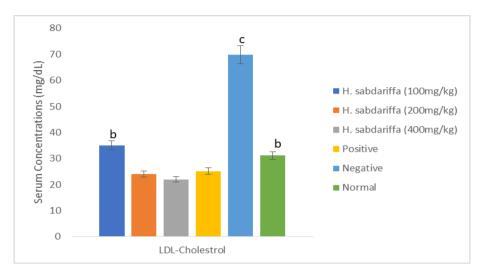
**Figure 3** Effect of methanol leaf extracts of *Hibiscus sabdariffa* on serum cholesterol in alloxan induced diabetic rat. Bars with different superscript alphabet were significantly different (p<0.05)



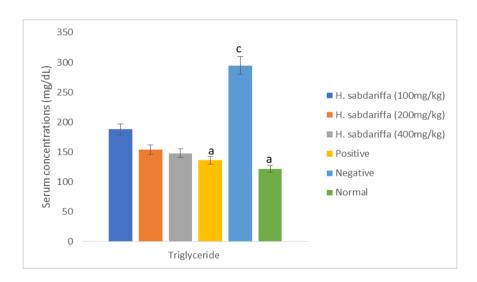
**Figure 4** Effect of methanol leaf extracts of *Hibiscus sabdariffa* on serum triglyceride in alloxan induced diabetic rat.

Bars with different superscript alphabet were significantly different (p<0.05)

Since, lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore, ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. In this study, there was a significantly (p<0.05) increase in serum levels of total cholesterol, triglycerides, low density lipoprotein (LDL) cholesterol and decreases in high density lipoprotein (LDL) cholesterol in diabetic untreated rats when compared to the control group. Oral administration of methanol extract of leaf extract of *Hibiscus sabdariffa* at 100, 200 and 400 mg/kg bw produced significant (p<0.05) reduction in total cholesterol, triacylglycerol, low density lipoprotein and also increase the high-density lipoprotein when compared with untreated control (Figure 3, 4, 5 and 6), thus indicating the hypolipidemic property of this plant.



**Figure 5** Effect of methanol leaf extracts of *Hibiscus sabdariffa* on serum LDL-cholesterol in alloxan induced diabetic rat. Bars with different superscript alphabet were significantly different (p<0.05)



**Figure 6** Effect of methanol leaf extracts of *Hibiscus sabdariffa* on serum triglyceride concentration in alloxan induced diabetic rat. Bars with different superscript alphabet were significantly different (p<0.05).

The reductions in total cholesterol concentration in serum of rats following the administration of the extract might have resulted from the presence of flavonoids, tannins and saponins that were reported to inhibit cholesterol biosynthesis in the liver [34], and the inhibition of the absorption of cholesterol via the small intestine [35]. The dose dependent reduction in triacylglycerol recorded in this study could also be attributed to the presence of alkaloids and saponins, which could result in the reduction of the absorption of dietary glucose in the gastrointestinal tract due to 'autointoxication' or "leaky gut' [35]. The dose dependent increase in serum HDL levels of rats following the administration of methanol leaf extract of Hibiscus sabdariffa suggested a possible boost of HDL-C biosynthesis in the liver promoted by the presence of flavonoids [36]. Therefore, more cholesterol would be transported from peripheral

tissues to the liver for excretion and could be the reason for the reported trend in the serum cholesterol concentration. The observed increase and decrease in the serum HDL C and LDL-C levels respectively as compared to diabetic untreated rat, suggests a reduced risk of developing atherosclerosis following administrations of the methanol leaf extract of Hibiscus sabdariffa to diabetic rats.

## 4. Conclusion

From the result obtained from this study it can stated that the methanol leaf extract of *Hibiscus sabdariffa* contains important phytochemicals that has significant ameliorative effects on alloxan induced hyperglycemia and dyslipidemia.

# Compliance with ethical standards

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## Disclosure of conflict of interest

This work is a collaboration of all the authors. All authors read and approved the final manuscript. The authors declared no conflict of interest exist.

## Statement of ethical approval

The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

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