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In vitro antidiabetic potentials of crude saponins extract from *Leptodenia hastata* and *Adansonia digitata* leaves

Asmau Niwoye Abubakar *, Zainab Bolajoko Lawal, Elisha Japeth, Ibrahim Olatunji Yunus and Rahinat Garba

Department of Biochemistry Federal University of Technology, Minna, Niger State, Nigeria.

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Abstract.

Diabetes mellitus is a metabolic disorder of public health concern since it is associated with complications, poor life quality and mortality. Synthetic drugs used in the treatment/management of diabetes are associated with one or more adverse side effects which could harm the patient even though they cure the patient of the condition. Hence, this study was undertaken to investigate the *in vitro* anti-diabetic properties of crude saponins extracts from *Leptodenia hastata* and *Adansonia digitata L*. leaves. The inhibition of glucose uptake by yeast cells and haemoglobin anti-glycation potential of the saponin extracts were evaluated at concentrations ranging between 4~20 mg/ml and 10~25 mg/mL respectively. Results revealed that the highest percentage inhibition of glucose uptake by the yeast cells was observed at 4 mg/ml, however, the percentage increase in glucose uptake by yeast cells for crude saponins from *L. hastata* (90.17±2.88 %) was significantly higher ($P < 0.05$) than crude saponins from *A. digitata* (88.57±0.23 %) but significantly lower ($P < 0.05$) than the metronidazole percentage increase in glucose uptake by yeast cells (97.22±0.32%). In the haemoglobin anti-glycation potential testing, the crude saponins extracts, as well as the metformin, showed a concentration-dependent activity with the highest percentage of haemoglobin anti-glycation being obtained at the highest tested concentration of 25 mg/mL. No significant difference was observed in the activities of crude saponins of *L. hastata* (57.50±0.39 %), *A. digitata* (58.73±1.04 %) and standard drug metformin (59.22±2.95 %). From the results obtained, it is rational to infer that both extracts showed anti-diabetic potentials and may be used in the management/treatment of diabetes or serve as good future promising candidates for the development of antidiabetic agents.

Keywords: *Leptodenia hastata; Adansonia digitata L*; Saponins; Glucose; Yeast cells; Haemoglobin

1. Introduction

Diabetes mellitus (DM) is considered one of the major public health problems. It is a chronic metabolic disorder characterized by an elevated level of blood glucose owing to the inability of the body system to regulate blood glucose concentration due to either absence or insufficient insulin secretion or loss of sensitivity to insulin by insulin-sensitive receptors in many cells alongside disturbances of carbohydrate, fat and protein metabolism (1). DM can result in the development of cardiovascular diseases, neuropathy, nephropathy, and retinopathy. Rubin *et* al., (2) reported that this chronic condition may also lead to the development of atherosclerosis (hardening of blood arteries) that could advance to stroke. Coronary heart diseases and other blood vessel diseases, nerve damage kidney failure, and blindness may also result.

In 2015, according to Ogurtsova *et al.*, (1), there were 415 million (which is approximately 8.8%) adults (aged 20-79) worldwide were estimated to be diabetic. However, this number is expected to rise up to 642 million (10.4%) by 2040, or one adult in ten persons. An estimated 14.2 million adults aged 20-79 had diabetes in the African region that stands

* Corresponding author: Abubakar AN
Department of Biochemistry Federal University of Technology, Minna, Niger State, Nigeria.

for a regional prevalence of about 3.2% in 2015, which can be projected to 7.7% (34.2 million) by 2040. South Africa (2.3 million), the Democratic Republic of Congo (1.8 million), Nigeria (1.6 million), and Ethiopia (1.3 million) are among the highest number of African people living with diabetes (1).

Diabetes mellitus can be managed by diet, physical exercise, and medicinal drugs (like insulin and or oral hypoglycemic drugs such as sulfonylureas and biguanides (3). Extracts from different medicinal plants have been used traditionally in the management of this chronic health condition globally. The use of these medicinal plants in the management of this condition is relatively low cost, health-friendly, easily available, less toxic with relatively little or no side effects in contrary to the synthetic drugs (4). The side effects associated with synthetic drugs may include hypoglycemia (Sulfonylureas), lactic acidosis and folate and vitamin B12 malabsorption (Metformin), gastrointestinal disturbances (Acarbose), weight gain (sulfonylureas and thiazolidinediones). Hence, the search for safer, more effective hypoglycemic agents is of major concern in the treatment/management of this disorder. Globally, natural products particularly medicinal plants have been used as a source of medicine and 80-85% of the world population rely on these medicinal plant extracts or their active constituents to meet their primary health care needs (5).

Leptadenia hastata (Pers.) Decne is medicinal plant used traditionally for the management of diabetes, hypertension, catarrh, skin diseases, prostate complaints and as an aphrodisiac. In traditional systems of medicine, different parts are used including the leaves, latex, roots and even whole plant (6). *Leptadenia hastata* is reported to contain alkaloids, saponins, phenolic glycosides, tannins, flavonoids, proanthocyanidins and triterpenes (6). Adansonia digitata L. (Malvaceae) is a majestic tree revered in Africa for its medicinal and nutritional value. The plant parts are used to treat various ailments such as diabetes, diarrhoea, malaria and microbial infections. It is reported that it is an excellent anti-oxidant due to the vitamin C content which is seven to ten times higher than the vitamin C content of oranges (7). Baobab has numerous biological properties including antidiabetes, antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities amongst others. Phytochemical investigation revealed the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals (7).

Saponins are amphipathic glycosides synthesized by many plant species, have a higher molecular weight consisting of a sugar moiety united to a triterpenoid or steroid sapogenins. Saponins have received numerous attention due to their biological activities that include hepatoprotective, antitumor, antimicrobial, and anti-inflammatory activities (8, 9). Saponins have been known to possess anti-diabetic properties and are promising compounds with the potential to be developed into new drugs for anti-diabetes (10, 11). The present study therefore, investigated the anti-diabetic properties of crude saponins extracts from *Leptodenia hastata* and *Adansonia digitata L*. leaves using the *in vitro* antidiabetic protocols

2. Material and methods

2.1. Materials

2.1.1. Plant sample collection and identification

The leaves of *Leptadenia hastata* and *Adansonia digitata* L. were obtained from El Waziri Bosso Local Government, Minna, Niger State. The plants were identified at the department of Plant biology, Federal University of Technology, Minna.

2.1.2. Reagents and Chemicals

All the solvents and reagents used were of analytical grade. Solvents used include ethanol (JHD), diethyl ether (JHD), n-butanol (JHD), Carbon tetrachloride (CCl_4) and distilled water. Reagents used include 5% starch solution, 6.7mM, and 0.14M Sodium chloride, 20mM and 0.01M phosphate buffer, 3,5-Dinitrosalicyclic acid solution, glucose solution, commercial baker's yeast, Acarbose (Glucobay), Metronidazole, Metformin,

2.1.3. Apparatus and Equipment

The apparatus and equipment used for the analysis include: weighing balance (model GM602), separating funnel (Pyrex), water bath (Griffin 322), hand gloves, sample bottles, EDTA bottles, filter paper (Whatman), pH meter, syringes, pipette and pipette dropper, centrifuge (model LRIO 2.4A) and UV-spectrophotometer (Model 752).

2.2. Methods

2.2.1. Extraction of Crude Saponins

Crude saponins from *Leptadenia hastata* and *Adansonia digitata L*. leaves was extracted by heating 100g of the powdered sample for 4 hours with 600 ml of 70% ethanol in a water bath. The extract was filtered using Whatman filter paper and residue were re-extracted with another 600ml of 70% ethanol. The extract was concentrated on a water bath to a volume of about 200 ml, which was then mixed with 100ml diethyl ether in a separating funnel. The mixture was shaken vigorously and then the separating funnel was fixed on a retort stand till the development of aqueous and diethyl layer. The aqueous portion was collected in a beaker while the diethyl ether portion was discarded. To the aqueous layer n- butanol (300ml) was added and properly mixed by vigorous shaking. The n- butanol extract was treated with 20ml of 5% NaCl solution. The resultant solution was concentrated on a water bath and the crude saponins were kept in a sample bottle (12).

2.2.2. Glucose Uptake Capacity by Yeast Cells.

This assay was performed according to the method of Cirillo, (13) with some modifications. Commercial baker's yeast was dissolved in distilled water to prepare 1% suspension. The suspension was kept overnight at room temperature (25^{o}C). The next morning, yeast cells suspension was centrifuged at 4000 rpm for 5 minutes. This process was repeated by the addition of distilled water to the pellet until a clear supernatant was obtained. Exactly 10 parts of the clear supernatant fluids were mixed with 90 parts of distilled water to get a 10% v/v suspension of the yeast cells. Five concentrations (4, 8, 16 and 20 mg w/v) of crude saponins extract was prepared. The mixture was then supplemented with 5mM of 1ml of glucose solution and incubated for 10 min at 37^{o}C. To start the reaction, 100 µl of yeast suspension was poured in the mixture of glucose and extract, then incubated for 60 minutes at 37^{o}C. After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. Control prepared is the solution having all reagents except the test sample. Metronidazole was used as standard drug (Crag et al., 2009).

The percentage increase in glucose uptake was calculated by the formula:

% = (Ac- As)/Ac x 100

Where AC is absorbance of control and AS is absorbance of the sample.

2.2.3. Haemoglobin anti-glycation potential

Evaluation of hemoglobin glycation was estimated by the method of Adisa et al. (14). Blood was collected from healthy rats and transferred into EDTA bottles containing. Hemolysate was prepared based on the principle of hypotonic lysis. The red blood collected was washed thrice with 0.14 M NaCl solution and one volume of red blood cells suspension was lysed with 2 ml of 0.01 M phosphate buffer pH 7.4 and 0.5 ml of CCl_4. The hemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The hemoglobin rich fraction in the upper layer was separated and dispensed into a sample bottle for further analysis. To 1 ml of hemoglobin solution, 5µl of gentamycin and 25µl of crude saponins extract of *Adansonia digitata* L. at different concentrations (10, 15, 20, and 25 mg/ml) were added. The reaction was started by the addition of 1mL of 2 % glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature for 24 hours. The concentrations of glycated hemoglobin at the incubation period of 24 hours was estimated using UV-spectrophotometer at a wavelength of 443 nm. Metformin was used as a standard drug for the assay and the percentage inhibition of non-enzymatic glycation of hemoglobin was calculated using the formula:

I% = (Ac- As)/Ac x 100

Where ACs absorbance of control and ASs absorbance of the sample.

3. Results

3.1. Effect of Crude Saponins Extract from Leptadenia hastata Leaf and Adasonia digitata on Glucose Uptake by Yeast Cells.

Table 1 below depicts the effect of crude saponins extracts of Leptadenia hastata and Adasonia digitate leaves. The highest percentage increase in glucose uptake by yeast cells for the standard drug (metronidazole) and the two saponins

crude extracts was found to be at the lowest tested concentration (4 mg/mL), wherein the percentage increase in glucose uptake by yeast cells for both crude saponins extracts from L. hastata and A. digitate was significantly lower (*P < 0.05*) than that of metronidazole. However, the percentage increase in glucose uptake by yeast cells for crude saponins extract of L. hastata was significantly higher (*P < 0.05*) than that exerted by crude saponins of A. digitata when compared to the standard (metronidazole). The percentage increase in glucose uptake decreases with increase in concentration of metronidazole and the crude saponins extracts.

Table 1 Effect of crude saponins extracts from Leptadenia hastata and Adasonia digitata leaves on glucose uptake by yeast cells.

Concentration (mg/mL)	Increase in glucose uptake by yeast cells (%)		
	Metronidazole	L. *Hastata*	A. *digitate*
4	97.22±0.32^{c}	90.17±2.88^{b}	88.57±0.23^{a}
8	93.64±0.11^{c}	72.59±0.10^{a}	82.58±0.20^{b}
16	87.22±0.43^{c}	37.59±0.16^{a}	65.86±0.08^{b}
20	88.49±0.13^{c}	26.52±0.09^{a}	57.46±0.09^{b}

Values are presented as mean ± standard error of mean of four replicates
Values with the same alphabet as superscript in a row have no significant difference (*P > 0.05), value with superscript a, b, c indicate the increasing order of significant (p< 0.05); a<b<c*

3.2. Haemoglobin anti-glycation potential of crude saponins extracts from *Leptadenia hastata* and *Adasonia digitata* leaves

Haemoglobin anti-glycation potential of crude saponins extracts of *L. hastata* and *A. digitata* is shown in Table 2 below. The haemoglobin anti-glycation potential of the standard drug (metformin) and crude saponins extracts was found to be concentration-dependent (increase in concentration results in increased activities). The optimum haemoglobin anti-glycation was observed at the highest tested concentration (25 mg/mL) for both metformin and the crude saponins extracts. No significant difference (*P > 0.05*) was found in the activities of metformin and crude saponins extracts at this concentration.

Table 2 Haemoglobin anti-glycation potential of crude saponins extracts from *Leptadenia hastata* and *Adasonia digitata* leaves.

Concentration (mg/mL)	Hemoglobin anti-glycation potential (%)		
	Metformin	*L. hastata*	*A. digitata*
10	2.05±1.20^{b}	0.00±1.42^{a}	27.39±0.14^{c}
15	17.75±2.92^{b}	13.19±0.23^{a}	47.24±2.59^{c}
20	42.76±0.75^{a}	51.04±1.75^{b}	55.92±0.07^{c}
25	59.22±2.95^{a}	57.50±0.39^{a}	58.73±1.04^{a}

Values are presented as mean ± standard error of mean of four replicates
Values with the same alphabet as superscript in a row have no significant difference (*P > 0.05), value with superscript a, b, c indicate the increasing order of significant (p< 0.05); a<b<c*

4. Discussion

Antidiabetic properties of a drug can be exploited through several *in vitro* assays, which in turn provide the researchers with clues for its in vivo anti-diabetic potential. Such assays include glucose uptake across cell membranes such as yeast cells, adipose cells, or muscle cells; hemoglobin anti-glycation potential, and inhibition of alpha-amylase to mention just a few. The *in vitro* assays of the present study indicated that the crude saponins tested possess anti-diabetic properties.

Blood glucose regulation in diabetic individuals can avert numerous health complications associated with the disease (diabetes). In the mammalian system, blood glucose concentration is regulated at intervals in order to maintain stable glucose concentration in the blood under a variety of dietary conditions for a long period of time (15).

Ahmed *et al*., (16) stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport (i.e uptake by the cells) occurs only if the intracellular glucose is effectively reduced (utilized). A significant increase ($P < 0.05$) in the percentage of glucose uptake by yeast cells at a glucose concentration of 5mM in crude saponins extract from *L. hastata* (90.17±2.88%) than *A. digitata* (88.57±0.23%) all at 4 mg/mL (see Table 3.1) infers that crude saponins extract from *L. hastata* facilitate the use of glucose by yeast cells and thus may reduce blood glucose concentration (stimulate glucose uptake by mammalian cells) than the crude saponins from *A. digitata.* By effect, the percentage increase in glucose uptake by yeasts for the crude saponins extract was observed to be lower than the standard drug (metronidazole) with (97.22±0.32%).

Garber, (17) reported that despite insulin therapy, diabetic patients suffer from some chronic clinical complications owing to high blood glucose levels which induce non-enzymatic glycosylation of natural proteins such as hemoglobin, lens proteins, bio-membrane proteins, albumin, collagen, and myelin. Glycated hemoglobin has received significant attention as the scale used in controlling long-term diabetes mellitus in modern medicinal biology (18). Increased level of blood glucose instigates its non-enzymatic binding to the hemoglobin which may result in the formation of reactive oxygen species. Therefore, the non-significant difference ($P > 0.05$) observed in the hemoglobin anti-glycation potential at 25 mg/mL of crude saponins extracts from *L. hastata* (57.50±0.39%) and *A. digitata* (58.73±1.04%), and metformin (59.22±2.95%) (as shown in Table 3.2) shows that both the crude saponins extracts and metformin can prevent hemoglobin glycation to the same extent and thus prevent soar blood glucose concentration, though in vivo assays have not been carried out to confirm this but the possibility is higher with the obtained results.

5. Conclusion

From the results obtained from glucose uptake by yeast cells and hemoglobin anti-glycation, it is rational to infer that both crude saponins extracts possess anti-diabetic properties and may be used in the treatment/management of diabetes or may serve as future promising candidates for the development of antidiabetic agents.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists

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