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Research Article

In vitro antidiabetic effect of *Leptadenia hastata* leaves fractions

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ABSTRACT

Leptadenia hastata (Pers.) Decne is often used traditionally for hypertension, catarrh, skin diseases, wound-healing and in the management of diabetes mellitus. Hydromethanolic leaves extracts of *Leptadenia hastata* were analysed for their phytochemical contents while its fractions were subjected to *in vitro* antidiabetic activity using non-enzymatic glycosylation of hemoglobin, increase in glucose uptake by yeast cell and α-amylase inhibition assays respectively. Phytochemical screening of *L. hastata* leaf indicated the presence of tannins, flavonoids, alkaloids and saponins. The results indicated significant inhibition of glycosylation of haemoglobin, glucose uptake by yeast cells and α- amylase inhibition was in a dose-dependent manner respectively. The present findings suggest that *Leptadenia hastata* fractions exhibited antidiabetic effects thus validating the traditional claim of this plant.

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Keywords: Leptadenia hastata; in vitro antidiabetic; fractions; glycosylation; glucose uptake.

1. INTRODUCTION

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin [1]. It is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally [2]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects and as such, there is an increased interest in the search for more effective and safer hypoglycemic agents.

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes [3]. *Leptadenia hastata* is a voluble herb with creeping latex stems, glabescent leaves, glomerulus and racemes flowers as well as follicle fruits. It is an edible non-domesticated vegetable found in wild throughout Africa. In Nigeria, this plant is commonly used in Hausa-speaking communities as a spice and used in sauces [4,5]. Traditionally, it is used for the treatment of hypertension, catarrh, skin diseases and diabetes [4]. Thus, objective of our study is to investigate guided fractions of *Leptadenia hastata* leaves for its in vitro antidiabetic activity.

2. MATERIAL AND METHODS

2.1 Plant collection/identification

The plant part (leaves) was collected in May, 2014 from the wilds of Aliero Local Government

Area, Kebbi state, Nigeria. Herbarium specimens were prepared and photographs taken to aid in the confirmation of the identity of the plants. Voucher specimens (number 503) were deposited at the Herbarium, Botany unit; Biological Sciences Department, Kebbi State University of Science and Technology, Aliero.

2.2 Preparation of extracts

Leaves of this plant were open air dried under the shade and pulverized into moderately coarse powder using motar and pestle. Powdered leaves (200g) were successively extracted with 70% ethanol for 72hours. The hydroethanolic extracts were concentrated using a water bath at 40^{o}C till dry powder wasformed which was kept in refrigerator for further use. In another experiment, crude extract was prepared by macerating 60g of the leaves with distilled H_2O for 72hours. This was then filtered and concentrated to dryness and used for phytochemical analysis.

2.3 Activity guided fractionation of *L. hastata* leaves

Hydroethanolic leave extract (15.88g) was dissolved in distilled H_2O (100mls) in a separating funnel and partitioned with n- hexane (100mls). The resulting n-hexane phase was separated and concentrated to dryness in vacuo and the resulting powder (5g) was kept in freezer in air–tight container. The resulting aqueous phase was further partitioned with petroleum- ether. The petroleum- ether fraction obtained was also concentrated in vacuo to dryness and the recovered powder (2.7g) was kept in freezer for further use. The resulting aqueous phase was further partitioned with butanol, the butanol fraction obtained was also concentrated in vacuo to dryness and the recovered powder (1.28g) was kept in freezer for further use . The last remaining aqueous fraction was also concentrated in vacuo to dryness and the recovered powder (6.9g) was kept in freezer for further use.

2.4 Preliminary phytochemical screening

Phytochemical screening of *L. Hastata* was carried out qualitatively for the presence of sterols, tannins, flavonoids, saponins, alkaloids, carbohydrates, and steroids using standard procedures [6,7].

2.5 *In vitro* anti-diabetic activity of *L. hastata* leaves

The fractions of *L. hastata* leaves were subjected to non-enzymatic glycosylation of haemoglobin assay, Glucose uptake in Yeast cells, and Alpha Amylase inhibition assay.

2.5.1 Preparation of *Leptadenia Hastata* stock solution

Hydroethanolic leaves extract of *leptadenia hastata* (10 mg) of was dissolved in 1 ml of Dimethylsulphoxide (DMSO), which is used as stock solution with the concentration of 10,000µg/ml. From this stock solution, different concentration viz, 10, 20, 30 mg/ml were prepared using DMSO solution [8].

2.5.2 Non-enzymatic glycosylation of haemoglobin assay

Procedure: Antidiabetic activity of *Leptadenia Hastata* was investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay [9]. All tests were performed in triplicate.

2.5.3 Glucose uptake in yeast cells

Commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1mL of glucose solution (5, 10 and 25mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug [9]. All tests were performed in triplicate.

2.5.4 Alpha amylase inhibition assay

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 dinitro salicylic acid solution 96mM. In alpha amylase inhibition method 1ml substrate- potato starch (1%w/v), 1 ml of plant extracts of four different concentration (20, 40, 60, 80 and 100µg/ml), 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) were added. NOTE- Potato starch solution, alpha amylase solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water). Both control and plant extracts were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. This reaction is detectable at 540 nm [10].

Table 1. percentage yield of *L. Hastata* leaves

Extract/Fraction	*Weight (g)*	*Percentage yield (%)*
Crude extract	15.9	7.9
Hexane fraction	8.0	4.0
Petroleum Ether fraction	2.7	1.4
Buthanol fraction	1.2	0.6
Last remaining aqueous fraction	6.8	3.4

Table 2. Phytochemical analysis of *L. Hastata* leaves

Phytochemical	*Result*
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Starch	ND
Reducing sugar	ND

(+)present, (ND)Not Detected

3. RESULTS AND DISCUSSION

3.1 Percentage yield and phytochemistry

A low percentage yield of *L. hastata* leaves was observed (Table 1). This suggests that *L. Hastata* leaves contain more fibers which turn out to be residue after extraction. Sequential extraction involving solvent of varying polarity (n-hexane, petroleum ether, butanol and water) was used to extract polar compound from the leaf of *L. Hastata*. A sequential extraction procedure was chosen mainly because the nature and polarity and hence the solubility of the bioactive compound in the leaves of the *L.Hastata* were unknown [11]. In general n-hexane was used to extract compounds of low polarity such as fatty acids, waxes fatty acids some alkaloid and terpenoid [12, 13]. Petroleum ether is known to extract both medium polarities and some polar compound such as flavonoid, tannin and some terpenoid [14, 15]. On the other hand butanol and water are known to extract hydro polar compound such as carbohydrate, amino acids and their derivatives [15].

The preliminary phytochemical screening tests for of *L. hastata* leaves (Table 2) revealed the presence of alkaloids, flavonoids, tannins and saponins. Phytochemical such as flavonoids are present in the extract, constitute a major group of compounds that act as primary antioxidants with high redox potentials and singlet oxygen quenchers [16]. Patients with diabetes mellitus are characterized with elevated level of oxidative damage, decreased level of antioxidant defenses and are prone to lipid abnormalities due to lipid peroxidation [17]. The potential beneficial effects of flavonoids in the prevention of diabetes mellitus and its associated complications have been investigated both *in vitro* and *in vivo* studies [18].

Alkaloids have been shown to exhibit hypoglycemic activity in animal studies [18,21]. Three major alkaloids viz., palmatine, jatrorrhizine and magnoflorine of *T. cordifolia* stimulated insulin secretion from the RINm5F cell line [19], also *L. sativum* alkaloid have potential antidiabetic effect against alloxan-induced diabetes [20]. Saponin extract from the root of Garcinia kola demonstrated remarkable antidiabetic activity even more than a standard antidiabetic drug in alloxan-induced diabetic rats [21]. The findings of Phytochemical screening of L.hastata leaves confirmed the presence of several bioactive compounds like alkaloids, flavoniods, tannins and saponins which could be responsible for its medicinal properties.

3.2 *In vitro* antidiabetic activity

3.2.1 *In vitro* non-enzymatic glycosylation of haemoglobin

Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species [22]. The inhibitory effect of *L. hastata* leaves fractions on non-enzymatic glycosylation of haemoglobin is shown in Table 3. *L. hastata* leaves fractions exhibited a concentration dependent increase in %inhibition of glycosylation, suggesting that the plant fractions decrease the formation of the glucose-haemoglobin complex and thus amount of free haemoglobin increases. The hexane fraction and the last remaining aqueous fraction displayed the highest inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 h which was comparable with the standard drug.

Table 3. Effect of *L. hastata* leaves fraction on heamoglobin glycosyalation

Concentration (µg/ml)	Standard		Hexane fraction		Petroleum ether fraction		Butanol fraction		Last remaining aqueous fraction	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	absorbance	% Inhibition	Absorbance	% Inhibition
20	0.118±0.01	15.3	0.363±0.02	72.5	0.244±0.00	59.0	0.167±0.00	40.1	0.321±0.00	68.9
40	0.212±0.01	52.8	0.436±0.01	77.1	0.278±0.00	64.0	0.173±0.00	42.1	0.336±0.00	70.2
60	0.459±0.00	78.2	0.469±0.02	78.7	0.364±0.00	71.1	0.191±0.00	47.6	0.344±0.00	71.0
80	0.504±0.00	80.2	0.552±0.00	81.9	0.429±0.00	76.7	0.351±0.01	72.0	0.347±0.00	71.2
100	0.559±0.00	82.1	0.652±0.00	84.5	0.491±0.00	79.6	0.376±0.01	73.4	0.364±0.00	72.5

Results are presented as mean± SEM (n=3). Absorbance of blank = 0.100 ± 0.00.

Table 4. Effect of *L. hastata leaves fractions* on the uptake of glucose (in 25mM glucose concentration) by yeast cells.

Concentration (µg/ml)	Standard		Hexane fraction		Petroleum ether fraction		Butanol fraction		Last remaining aqueous fraction	
	Absorbance	% Glucose uptake	Absorbance	% Glucose uptake	Absorbance	% Glucose uptake	absorbance	% Glucose uptake	Absorbance	% Glucose uptake
20	0.033±0.00	63.6	0.031±0.00	61.3	0.056±0.00	57.1	0.032±0.00	63.0	0.021±0.00	43.1
40	0.037±0.00	67.6	0.043±0.00	72.1	0.071±0.00	83.1	0.048±0.00	75.0	0.027±0.00	56.1
60	0.044±0.00	73.0	0.052±0.00	77.0	0.084±0.00	85.7	0.063±0.00	81.1	0.031±0.00	61.3
80	0.047±0.00	74.1	0.068±0.00	82.4	0.080±0.00	85.0	0.063±0.01	81.1	0.054±0.00	78.1
100	0.055±0.00	78.2	0.070±0.00	83.1	0.111±0.00	89.2	0.101±0.01	88.1	0.070±0.00	83.1

Results are presented as mean± SEM (n=3). Absorbance of blank = 0.012 ± 0.00.

Table 5. Effect of *L. hastata leaves fractions* on the uptake of glucose (in 10mM glucose concentration) by yeast cells.

Concentration (µg/ml)	Standard		Hexane fraction		Petroleum ether fraction		Butanol fraction		Last remaining aqueous fraction	
	Absorbance	% % Glucose uptake	Absorbance	% % Glucose uptake	Absorbance	% % Glucose uptake	absorbance	%% Glucose uptake	Absorbance	%% Glucose uptake
20	0.017±0.00	29.4	0.037±0.00	67.6	0.030±0.00	60.0	0.022±0.00	45.5	0.043±0.00	72.1
40	0.040±0.00	70.0	0.044±0.00	72.7	0.037±0.00	67.6	0.023±0.00	47.8	0.053±0.00	77.4
60	0.071±0.00	83.1	0.062±0.00	80.6	0.047±0.00	74.5	0.053±0.00	77.4	0.040±0.00	70.0
80	0.114±0.01	89.5	0.075±0.00	84.0	0.058±0.00	79.3	0.054±0.01	78.1	0.051±0.00	76.5
100	0.156±0.01	92.3	0.099±0.00	87.9	0.063±0.00	80.9	0.091±0.01	86.8	0.113±0.01	89.4

Results are presented as mean± SEM (n=3). Absorbance of blank = 0.012 ± 0.00.

Table 6. Effect of *L. hastata leaves fractions* on the uptake of glucose (in 5mM glucose concentration) by yeast cells.

Concentration (µg/ml)	Standard		Hexane fraction		Petroleum ether fraction		Butanol fraction		Last remaining aqueous fraction	
	Absorbance	%% Glucose uptake	Absorbance	% Glucose uptake	Absorbance	% Glucose uptake	absorbance	% Glucose uptake	Absorbance	% Glucose uptake
20	0.029±0.00	58.6	0.025±0.00	52.0	0.033±0.00	63.6	0.016±0.00	25.0	0.028±0.00	57.1
40	0.034±0.00	64.7	0.032±0.00	62.5	0.050±0.00	76.0	0.045±0.00	73.3	0.034±0.00	64.7
60	0.042±0.00	71.4	0.045±0.00	73.3	0.052±0.00	76.9	0.046±0.00	73.9	0.045±0.00	73.3
80	0.052±0.01	76.9	0.052±0.00	76.9	0.050±0.00	76.0	0.048±0.01	75.0	0.057±0.00	78.9
100	0.058±0.01	79.3	0.067±0.00	82.1	0.058±0.00	79.3	0.081±0.01	85.2	0.085±0.01	85.9

Results are presented as mean± SEM (n=3). Absorbance of blank = 0.012 ± 0.00.

Table 7. Effect of L. hastata leaves fractions on alpha amylase inhibition

Concentration (µg/ml)	Hexane fraction		Petroleum ether fraction		Butanol fraction		Last remaining aqueous fraction	
	Absorbance	% Inhibition	Absorbance	% Inhibition	absorbance	% Inhibition	Absorbance	% Inhibition
20	0.243±0.01	75.3	0.337±0.00	82.2	0.186±0.00	67.7	0.203±0.01	70.4
40	0.273±0.00	78.0	0.334±0.00	82.0	0.232±0.00	74.8	0.233±0.00	74.2
60	0.290±0.01	79.3	0.227±0.00	73.6	0.273±0.00	78.0	0.326±0.00	81.6
80	0.311±0.01	80.7	0.209±0.01	71.3	0.298±0.01	79.9	0.407±0.00	85.3
100	0.355±0.01	83.1	0.189±0.00	68.3	0.361±0.00	83.4	0.445±0.01	87.0

Results are presented as mean± SEM (n=3). Absorbance of blank = 0.060 ± 0.00.

The glycosylated haemoglobin is an important clinical marker in diabetes which helps to determine the degree of protein glycation during diabetes [23]. In persistent hyperglycemic state, formation of glycated heamoglobin (HbA1c) occurred by nonenzymatic reaction between glucose and free amino groups of haemoglobin. HbA1c level in diabetes helps to evaluate long-term glycemic control, and it helps to assess the risk of the development or progression of diabetic complications. Published studies supported that reduction in HbA1c levels during the diabetes treatment considerably reduced microvascular complications [24].

3.2.2 Glucose uptake action of *L. hastata* leaves fraction

The rate of glucose transport across cell membrane in yeast cells system at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively is presented in Tables 4 – 6. In this study, glucose uptake rate increased with increasing concentration of the plant fraction which was comparable to the standard. Glucose transport across yeast cell membrane occurs via facilitated diffusion down the concentration gradient. Hence, glucose transport occurs only if the intracellular glucose is efficiently reduced or utilized [25]. The results obtain suggests that fractions of *L. hastata* leaves were capable of enhancing glucose uptake (utilization) thereby controlling the blood glucose level.

3.2.3 Alpha amylase inhibitory effect of l. hastata leaves fraction

Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide [25]. There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme (Table 7). At a concentration 100µg/ml, *L. hastata* last remaining aqueous fraction showed the highest percentage inhibition of 87. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. The α- amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates [1]. This study reveals that *L.hastata* leaves efficiently inhibits α-amylase enzyme *in vitro*. The reaction mechanisms involved in inhibition of α-amylase enzymes by plant protein inhibitors are not clearly understood. But there are some suggestions that the plant protein (flavanols) might cause conformational changes in structure [26].

4. CONCLUSION

Fraction of *L.hastata* leaves exhibited potential antidiabetic activity via inhibition of hemoglobin glycosylation, increased glucose uptake and inhibition of α-amylase activity. Further studies on the isolation and characterization of the active compound responsible for this inhibitory effect are recommended.

AUTHOR CONTRIBUTIONS

ANU designed the study, fractionated the extract and proofread the final draft of the manuscript. MOI carried out Phytochemistry, in vitro antidiabetic activity and prepared the first draft. All authors read and approved the final manuscript.

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