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Antidiabetic, Toxicity Studies and Phytochemical Profile of *Hedranthera barteri* (Hook. f.) Pichon

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Abstract: *Objective:* This work investigated the antihyperglycaemic activities, safety and chemical composition of *Hedranthera barteri* (Hook. f.) Pichon (Apocynaceae) methanol leaf extract aimed at establishing its antidiabetic folkloric usage. *Methods*: Acute and sub acute toxicity tests of the plant extract were assessed with a modified OECD test guidelines. The effects of the extract on glucose, haematological and biochemical parameters were evaluated using standard procedures. Its antidiabetic activities were assayed in α-amylase, α-glucosidase, glucose and streptozotocin-induced diabetic rats' models at various doses with acarbose and glibenclamide (5 mg/kg) as positive controls. Histopathological examination of the pancreas, liver and kidney of rats administered with the extract at 250, 500 and 1000 mg/kg was also carried out. The phytochemical components of the extract were analysed with GC-MS. The results obtained from these studies were subjected to statistical analysis using Analysis of Variance (ANOVA), followed by the Student Newman Keul's test, and $p< 0.05$ was taken as significant. *Results*: The median lethal dose, LD_{50} of the extract was above 5000 mg/kg in rats without adverse effects on blood glucose levels and biochemical components of normal rats especially at 250 and 500 mg/kg while it significantly potentiated PCV, RBC and haemoglobin levels of the rats. The extract caused an inhibition of α-amylase and α-glucosidase enzymes in concentration dependent manner, reduced hyperglycaemia in glucose loaded rats with 200 mg/kg as the most effective dose and elicited hyperglycaemia lowering activity that was comparable to glibenclamide in drug-induced diabetic rats. The extract had no adverse effect on the histology of the pancreas, liver and kidney at 250 mg/kg while 500 and 1000 mg/kg caused moderate and severe effects on these organs, respectively. Tannins, flavonoids, saponins, alkaloids, terpenoids, deoxy-sugars were observed in the extract by preliminary phytochemical screening while some GC-MS identified possible antidiabetic constituents of the plant include 9, 12-Octadecadinoic acid, phytol and catechol. Conclusion: The study validated the folkloric use of the leaves of *Hedranthera barteri* in the management of diabetes mellitus and confirmed its non toxic effect on different blood components of animals and their organs at low doses.

Keywords: *Hedranthera barteri,* Diabetes Mellitus, Toxicity, Phytochemical Profile

1. Introduction

Diabetes mellitus is a long-term metabolic disorder that results from interplay of genetic and environmental factors, chiefly characterized by hyperglycaemia, polyuria, and polyphagia [1]. The soaring number of individuals living with diabetes has rapidly increased (from 108 million in 1980 to 422 million in 2014) within 34 years only, while the worldwide incidence of diabetes among adults over 18 years of age has risen to 8.5% (2014) from 4.7% (1980) [2]. With a current 9.3% prevalence rate worldwide, and by 2030 this prevalence rate is estimated to reach 10.2% and by 2045 to 10.9%, no doubt, this metabolic cankerworm is on the rise in every country, augmented by the global rise in the prevalence of obesity and unhealthy lifestyles [3]. Numerous risk factors are implicated in the disease onset which ranges from genetic factors, environmental factors, loss of very first phase associated with insulin launch, sedentary lifestyle, smoking, alcohol, reduced β-cell sensitivity, improved glucagon activity are the primary risk elements for prediabetes and DM [4-8].

Hedranthera barteri (Hook.f.) Pichon (Apocynaceae), also known as *agbo omode* or *isepe akere* among Yoruba speaking people of Nigeria is a widespread shrub of about 2 m high that is found in the understory in damp situations of the closed-forest in Ghana, Northern and Southern Nigeria, Western Cameroons, and Zaire [9]. Ethnomedically, its leaf decoction is taken by the Igbo speaking people of Southern Nigeria for dizziness while the fruit is used in other parts of Nigeria to treat gonorrhoea and prevent miscarriage in women [9-12]. The leaf decoction is used in the management of diabetes by some herbalists in Osun State, Nigeria (Personal Communication). Isolated compounds from *H. barteri* include: amataine, beninine, goziline, owerreine, subsessiline, isoquinoline and vobstusine which are alkaloids [13, 14]. Other compounds such as, cardenolides, saponins, flavonoids, and polyphenols have also been reported to be present in the leaves and roots of the plant [15]. The leaf of *H. barteri* has been reported for its antidepressant, antimicrobial, anxiolytic, anti-inflammatory, antimalarial, anticonvulsant and antinociceptic activities while the root has been scientifically proved for its antiulcer and antioxidant potentials [12, 15-18]. This study was designed to evaluate the antihyperglycaemic activity, safety of *H. barteri* leaf extract in rats as well as to identify its chemical constituents with a view to establishing its antidiabetic ethnomedicinal usage.

2. Methodology

2.1. Materials and Equipment

Rotary evaporator (RE301/601/801 model, Yamato Scientific America, Inc., U.S.A), chiller (Churchill, Instrument Co. Ltd, U.K), vacuum pump (MB 338618 model, Edwards High Vacuum Int., England), oven (Hearson & Co. Ltd, London), Mettler electronic weighing balance (AB 54 model, Mettler Toledo, U.S.A), Ultra-violet (UV) lamp (254 and 366 nm) (Grant Instrument, U.K), Oral cannula, ACCU-CHEK Glucometer (model GB 11558973, Roche, Germany) with ACCU-CHECK test strips (Roche, Germany), UV spectrophotometer, Dutrao (Model SM 600, Shang Yhai Yong Chuang Medical Instrument Co. Ltd) spetrophotometric microplate reader, Automated haematology analyzer, Centrifuge, Semi-automated biochemistry analyzer. Sodium Citrate, Citric acid, Streptozotocin, Glibenclamide® (Sigma-Aldrich Co. LLC, U.S.A), potassium hydroxide.

2.2. Plant Materials

Hedranthera barteri leaves were collected at Ede Road, Ile-Ife. It was authenticated by Mr. Ogunlowo Ifeoluwa at the Faculty of Pharmacy Herbarium and Herbarium specimen number, FPI 2421. The leaves were air-dried, powdered and macerated in methanol for 72 hours and mechanically agitated at intervals. The extract was filtered and the marc was re-extracted three times and concentrated *in-vacuo* to obtain a yield of 14.52% w/w.

2.3. Animals

Healthy albino rats (120-200 g) of either sex bred under standard conditions (temp. 27±3°C, relative humidity 65%) at the animal house, Department of Pharmacology, Faculty of Pharmacy, Olabisi Onabanjo University, Shagamu, Nigeria were used for the experiment. They were fed on a standard pellet diet (Bendel Feeds, Nigeria) and water was given *ad libitum*.

2.4. Acute and Subacute Toxicity Tests

The acute toxicity test was carried out according to the modified OECD Test Guideline 423 [20] Annex 3 model. Two groups of 8 animals each were administered distilled water and single oral administration of 5000 mg/kg extract, respectively. They were observed for signs of gross toxicity, behavioural changes and mortality, one hour after administration and daily for 14 days [19]. Sub acute toxicity test was carried out following the modified OECD Test Guideline 407 [21]. The extract was solubilised in 1% Tween 80 in distilled water and administered to groups of 8 rats daily for 21 days at graded doses of 250, 500, and 1000 mg/kg. The blood glucose levels in rats were monitored on days 7, 14 and 21. The animals were anaesthetized using chloroform and blood sample (5 mL) collected by cardiac puncture after the 21st day [19].

2.5. Haematological Analysis

Blood sample, 50 µL was used for haematological study using the automated haematology analyzer [22].

2.6. Biochemical Assays

The serum was analyzed for biochemical markers such as total cholesterol (TC), Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatise (ALP) and Creatinine were estimated using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK) and following

the guidelines described by the manufacturer [23].

2.7. Histopathological Examination

Tissue samples of liver, kidney and pancreas were prepared using the method described by Baker and Silverston [24]. The tissue histology slides were viewed under the light microscope at ×400 magnification.

2.8. Antidiabetic Studies

2.8.1. In-Vitro α-amylase Inhibitory Activity of the Extract

The assay was evaluated using modified procedure of Sulaimon [25]. A volume of 100 µL of extract or acarbose (positive control) and 100 µL of 0.02 M phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α- amylase from *Aspergillus oryzae* (0.5mg/mL) were added to each tube and incubated at 25°C for 10 min. After pre-incubation, 100 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube. The reaction was stopped with 200 µL of dinitrosalicylic acid color reagent. The test tubes were incubated in a boiling water bath for 5 min, then cooled to room temperature. The reaction mixture was then diluted by adding 1.5mL distilled water, and absorbance was measured at 540 nm using a microplate reader (SpectraMax, USA) by adding 200 µL in 96-well plates. The α-amylase inhibitory activity was expressed as% inhibition and also the concentrations of extract/ Acarbose resulting in 50% inhibition of enzyme activity (IC_{50}) were determined.

$$\% \text{ Inhibition } = \frac{\text{Absorbance of Control} - \text{Absorbance of Extract/Acarbose}}{\text{Absorbance of Control}} \times 100$$

2.8.2. In-Vitro α-glucosidase Inhibitory Activity of the Extract

The assay was evaluated using modified procedure of Sulaimon [25]. Alpha glucosidase from *Saccharomyces cerevisae* was purchased from Sigma. A volume of 50 µL of extract or standard drug (acarbose) and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) were incubated in 96-well plates at 25°C for 10 min. After pre-incubation, 50 µL of 1 mM p-nitrophenyl-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well. The reaction mixtures were incubated at 25°C for 20 min and stopped by adding 200 µL of 1M Na_2CO_3. The absorbance readings were recorded by micro-plate reader 405 nm and compared to a control which had 50 µL of buffer solution in place of the extract/acarbose. The α-glucosidase inhibitory activity was expressed as% inhibition and also the concentrations of extract/acarbose resulting in 50% inhibition of enzyme activity (IC_{50}) were determined.

$$\% \text{ Inhibition } = \frac{\text{Absorbance of Control} - \text{Absorbance of Extract/Acarbose}}{\text{Absorbance of Control}} \times 100$$

2.8.3. Antihyperglycaemic Effect of Extract on Glucose Induced Hyperglycaemic Rats

Groups of 6 rats each fasted for 18 hours that were given 10 g/kg of glucose (p.o.) were used for the experiment. After 0.5 hour (time point 0), rats having blood glucose levels ≥7.0 mmol/L (126 mg/dL) were considered hyperglycaemic and given (p.o.) vehicle (Tween 80 (1%) in distilled water) (negative control) extract (25, 50, 100 and 200 mg/kg) separately and 5.0 mg/kg glibenclamide (positive control). At 0.00, 0.50, 1.00, 2.00, and 4.00 hours, blood drop from each rat's caudal vein was placed on to a glucometer strip inserted into the glucometer. The percentage decrease in blood glucose level at these time points was calculated and compared to the negative and positive controls [26-29].

2.8.4. Antihyperglycaemic Effect of Extract on Streptozotocin-Induced Diabetic Rats

Overnight fasted rats were intraperitoneally injected with freshly prepared, 65 mg/kg streptozotocin (STZ) solution in 0.1 M sodium citrate buffer (pH 4.5) to induce diabetes. The blood glucose levels of the rats were observed after 72 hours of induction and they were left for 5 days afterwards. Rats with fasting blood sugar (FBS ≥ 11.0 mmol/l) were considered diabetic and separately divided into 4 groups of 8 rats viz; negative control that were orally given 1% Tween 80 in distilled water, test groups that received, 200 and 400 mg/kg (doses with the highest activity from glucose induced hyperglycaemic experiment); the positive control group that were administered with glibenclamide (5 mg/kg). Each group was treated daily accordingly for 14 days while blood glucose levels were monitored on days 1, 4, 7, 10 and 14 and the percentage blood glucose reduction was determined and compared with that of the control [19, 30]. All animal experiments conformed to the guide for the care and use of laboratory animals published by the national academies press [31].

2.9. Gas Chromatographic – Mass Spectroscopy of the Leaf Extract

The GC-MS analysis of the leaf extract *of H. barteri* was carried out at the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos State, Nigeria. The analysis was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl /95% dimethyl polysiloxane) fused a capillary column (30 × 0.25µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1). Five (5) point serial dilution calibration standards (1.25, 2.5, 5.0, 10.0ppm) were prepared from the stock solution of 40ppm and used to calibrate the GC-MS.

The injector temperature was maintained at 300°C, and the ion-source temperature was 250°C, and the oven temperature was programmed from 100°C (isothermal for 0.5 min), with

an increase of 20°C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33min. The data solution software supplied was used to control the system and acquire the data. The separated constituents were passed to the detector which recorded the emergence of the constituents as peaks with a retention time. The percentage compositions of the compound in the entire sample were computed from the peak areas automatically generated by the machine [25]. The results were recorded as retention time against percentage composition in the original sample.

Interpretation of mass spectrum GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns and the National Centre for Biotechnology Information. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The data generated was tabulated to reflect the molecular formula, molecular weight, peak area of each component identified by its retention time [25].

2.10. Statistical Analysis

Results obtained were expressed as mean ± SEM using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). The significance of the difference between the controls and treated groups was determined using one-way and two analyses of variance (ANOVA) $p< 0.05$) followed by the Dunnett multiple comparison test.

3. Results and Discussion

3.1. Acute Effect of Extract in Normal Rats

Hedranthera barteri methanolic leaf extract had no acute toxicity effect on normal rats when its 5000 mg/kg was administered to the animals and observed daily for 14 days. There was no mortality in the rats neither was there any observable changes in behaviour of the rats with respect to breathing, cutaneous effect, sensory and nervous system responses or gastrointestinal effect. This indicated that the median lethal dose, LD_{50} of the extract was greater than 5000 mg/kg, suggesting its safety and low risk of toxicity. The doses of 100 - 1000 mg/kg used in the study were therefore non toxic.

3.2. Sub Acute Effect of H. barteri Extract on Blood Glucose Level in Normal Rats

The negative control normoglycaemic rats that were given distilled water did not show any appreciable decrease or increase in the blood glucose levels of the rats throughout the period of the study confirming that water did not contain any bioactive constituents. The extract at 250 and 500 had comparable ($p>0.05$) effect on the blood glucose level of the rats to the negative control on all the days of the experiment (Table 1). However, its 1000 mg/kg gave a significant reduction in blood glucose levels of the animals on day 21 which indicated possible hypoglycaemic effect of the extract when administered to non diabetic individuals at higher doses especially when taken for a long period.

Table 1. *Effect of H. barteri leaf extract on blood glucose level of normal rats.*

Dose of Extract (mg/kg)	Percentage reduction in blood glucose levels			
	Day 1	Day 7	Day 14	Day 21
DW	100	94.56±6.50[a]	98.54±3.32 [a,b]	96.33±3.68 [b]
HBLE (250)	100	91.05±2.08 [a] (3.71%)	89.86±3.45 [a,b] (8.81%)	85.30±3.25 [a,b] (11.45%)
HBLE (500)	100	93.76±1.60 [a] (0.85%)	88.81±2.61 [a,b] (9.87%)	87.34±2.60 [a,b] (9.33%)
HBLE (1000)	100	89.54±1.88 [a] (5.31%)	85.88±1.87 [a] (12.85%)	81.83±3.18 [a] (15.05%)

Data show the mean ± SEM blood glucose levels at the different time points (Tt) expressed as percentages of level at day 1, percentage reductions in the bgls relative to negative control for each time point, N = 8. Values with similar superscript are comparable ($p>0.05$). One-way analysis of variance followed by the Student Newman-Keuls' post-hoc test). DW: Distilled Water; HBLE (250, 500, 1000): *Hedranthera barteri* methanolic leaf extract.

3.3. Sub Acute Effect of the H. barteri Extract on Haematological Parameters

Table 2. *Effect of extract on haematological parameters of normal rats.*

Blood parameters	DW	HBLE (250 mg/kg)	HBLE (500 mg/kg)	HBLE (1000 mg/kg)
PCV (%)	40.00±0.82[a]	42.00±0.19[b]	44.00±0.22[b]	46.00±0.10[b]
HGB (g/dL)	13.3±0.12[a]	14.0±0.80[a]	14.6±0.15[a]	15.3±0.12[b]
RBC (10^6 /µL)	4.67±0.18[a]	4.99±0.21[a]	5.28±0.13[b]	5.65±0.11[b]
WBC (10^6 /µL	4.40±0.21[a]	4.10±0.62[a]	4.20±0.10[a]	4.25±0.16[a]
MCV (fl)	85.7±0.42[a]	84.2±0.34[b]	81.8±0.31[b]	81.4±0.4[b]
MCH (pg)	28.5±0.33[a]	28.1±0.11[a]	27.1±0.42[a]	27.1±0.02[a]
MCHC (g/dL)	33.25±0.54[a]	33.33±0.10[a]	33.14±0.22[a]	33.26±0.42[a]

Data show the mean ± SEM haematological parameters at the different doses, n=8. Results having separate superscripts within row are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.050$): One-way variance analysis (ANOVA) followed by Student-Newman-Keul's test. DW: Distilled Water; HBLE (250, 500, 1000): *Hedranthera barteri* leaf extract. PCV: Parked cell volume; WBC: White Blood Cells, RBC: Red Blood Cells, HGB: Haemoglobin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration.

Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems [32]. They can also be used to explain blood relating functions of chemical compounds/plant extracts. Such laboratory

investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational research, disease diagnosis, prevention and treatment [32]. The leaf extract of *H. barteri* at 250-1000 mg/kg caused a significant ($p<0.05$) increase in PCV level, 500 and 1000 mg/kg significantly increased RBC level while its 1000 mg/kg significantly potentiated haemoglobin level of the normal rats' blood. This result indicated positive effect of the extract on the haemopoietic system of the experimental animals and showed anti-anaemic effect. It also reflected possible immunomodulatory effects of the extract especially at higher doses [19, 33]. The extract did not show any negative effect on the other components of the blood sample (Table 1). Extracts of *Aspilia Africana* and *Viscum album* (Mistletoe) had been reported to show similar effects on the haematological components in rats [34, 35].

3.4. Effect of the H. barteri Extract on Biochemical Parameters

The extract did not produce a significant increase in all the assayed biochemical parameters compared to the negative control especially at 250 and 500 mg/kg. However, there was a significant increase in cholesterol and aspartate transaminase levels in the animals treated with 1000 mg/kg which indicated caution in the use of the plant at elevated doses and prolonged use (Table 3).

Table 3. *Effect of extract on biochemical parameters of normal rats.*

Biochemical parameters	DW	HBLE (250 mg/kg)	HBLE (500 mg/kg)	HBLE (1000 mg/kg)
AST (µ/L)	20.00 ±0.10^{b}	17±0.11^{a}	19±0.15^{b}	23±0.10^{b}
ALT (µ/L)	21.00±0.30^{a}	20±0.16^{a}	21±0.11^{a}	21±0.21^{a}
CREA (mg/dL)	1.8.00±0.22^{a}	1.5±0.12^{a}	1.7±0.13^{a}	1.5±0.14^{a}
CHOL (mg/dL)	51.00±0.12^{a}	51±0.20^{a}	52±0.10^{a}	58±0.22^{b}
ALP (IU/L)	61±0.15^{a}	60±0.30^{a}	59±0.14^{a}	58±0.30^{a}

Data show the mean ± SEM biochemical parameters at different doses, n=8. Results having separate superscripts within row are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.05$). DW: Distilled Water; HBLE (250, 500, 1000): Extract of *Hedranthera barteri*. AST: Aspartate Transaminase, ALT: Alanine Transaminase CREA: Creatinine, CHOL: Cholesterol, ALP: Alkaline phosphatise.

3.5. Antidiabetic Studies of H. barteri Leaf Extract

Table 4. *In-vitro α-amylase inhibitory activity of H. barteri leaf extract.*

Concentration of the extract (µg/mL)	Percentage α-amylase Inhibition (%)	
	Hedranthera barteri	Acarbose
62.5	84.54±1.26^{b}	67.76±0.43^{a}
125	79.11±1.32^{b}	61.35±2.35^{a}
250	61.35±0.54^{b}	58.39±3.93^{a}
500	58.88±0.54^{b}	50.49±2.35^{a}
1000	55.43±0.54^{b}	46.38±1.76^{a}

Data show the mean ± SEM (n = 6). Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test).

The α-amylase inhibitory activity result of *H. barteri* leaf extract showed a non concentration dependent effect from 62.5-1000 similar to acarbose having highest activity at 62.5 µg/mL. The extract at all the concentrations used gave a significantly more α-amylase inhibitory effect than the positive control indicating that the extract was able to prevent the breakdown of complex sugars to simple sugars which showed the extract as a potential anti hyperglycaemic agent (Table 4). The seed extracts of *Carica papaya* and *Citrillus lanatus* had similarly been reported for α-amylase inhibitory activity [36].

Table 5. *In-vitro α-glucosidase inhibitory effect of H. barteri leaf extract.*

Concentration of extract (µg/mL)	Average percentage inhibition (%)	
	Hedranthera barteri	Acarbose
62.5	74.16±4.90^{a}	84.22±0.20^{b}
125	80.92±4.39^{a}	86.79±1.95^{b}
250	81.10±4.12^{a}	87.51±3.07^{b}
500	82.31±5.05^{a}	96.53±2.45^{b}
1000	83.70±6.15^{a}	98.44±2.73^{b}

Data show the mean ± SEM (n = 6). Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test).

Table 6. *Dose related antihyperglycaemic effect of Hedranthera barteri leaf extract.*

Extract/Drug (mg/kg)	Blood glucose levels as percentages of To (% reduction in blood glucose relative to negative control at To)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	97.16±4.02^{b}	81.92±2.99^{b}	75.67±2.12^{c}	72.87±2.20 ^{c}
HBLE (100)	100.00	92.63±0.84^{b} (4.66%)	83.44±2.21^{b} (-1.86%)	74.41±3.81^{c} (1.67%)	54.43±2.66^{b} (25.31%)
HBLE (200)	100.00	87.01±1.48^{a} (10.45%)	70.75±1.47^{a} (13.64%)	61.23±2.44^{b} (19.08%)	44.82±1.07^{a} (38.49%)
HBLE (400)	100.00	89.80±1.99^{a} (7.58%)	75.79±4.25^{a} (7.48%)	66.66±1.36^{b} (11.91%)	47.39±1.12^{a} (34.97%)
GLI (5)	100.00	79.37±4.0^{a} (18.31%)	66.20±2.86^{a} (19.19%)	51.32±3.87^{a} (32.18%)	44.94±3.80^{a} (38.33%)

Data show the mean±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. GLU (10 g/kg): Glucose 10 g/kg; HBLE: *Hedranthera barteri* leaf extract, GLI: Glibenclamide (Positive Control, 5 mg/kg).

Contrary to the α- amylase inhibitory effect of *H. barteri* leaf extract, its α-glucosidase inhibitory activity increased with increase in concentration of the extract similar to acarbose indicating antihyperglycaemic potential of the

extract. Acarbose however, gave a significantly higher activity than the extract at all the tested concentrations (Table 5). The combination of both α- amylase and α- glucosidase effects of the extract in this study (Tables 5 and 6) will explain the extrapancreatic activity of the extract.

It has been reported that the results of antihyperglycaemic studies using glucose-loaded rat model and insulin-stimulating drugs like glibenclamide as positive controls can be extrapolated to type 2 diabetes state in humans [37]. Glibenclamide with early extra-pancreatic and late insulin stimulating mechanisms of action was used as the standard drug in this study [38] to investigate possible mechanism of action of the extract [39, 40]. There was an observed time dependent (0.5-4 h) reduction in blood glucose levels of normal rats in the negative control group that received 10 g/kg glucose solution of distilled water in 1% Tween 80. This was due to the released insulin by the rats pancreas in response to hyperglycaemia caused by the glucose load [41, 42]. The extract at 100 mg/kg lacked activity at 0.5-2 h but gave 25% effect at 4 h while its 200 and 400 mg/kg elicited a similar profile of activity to glibenclamide (5 mg/kg) which suggested that the extract may have early extrapancreatic and late insulin stimulating mechanism of action of glibenclamide at these doses [38]. The antihyperglycaemic effect of the extract at 200 and 400 mg/kg were comparable ($p > 0.05$) at 0.5-4 h and comparable to glibenclamide at 0.5, 1 and 4 h which showed the effectiveness of the extract at these doses (Table 6).

Table 7. *Dose related antidiabetic activity of Hedranthera barteri leaf extract.*

Extract/Drug (mg/kg)	Blood glucose levels as percentages of To (% reduction in blood glucose relative to negative control at To)				
	Day 1	**Day 4**	**Day 7**	**Day 10**	**Day 14**
DW	100.00	95.36±3.37^{c}	94.26±4.45^{c}	99.34±3.00^{b}	100.26±2.81^{b}
HBLE (200)	100.00	57.29±6.67^{a} (39.92%)	24.17±1.87^{a} (74.35%)	21.69±1.00^{a} (78.17%)	19.51±1.07^{a} (80.54%)
HBLE (400)	100.00	78.84±6.89^{b} (17.32%)	27.70±3.32^{a} (70.61%)	24.31±2.72^{a} (75.53%)	24.17±3.92^{a} (75.89%)
GLI (5)	100.00	80.23±5.94^{b} (15.87%)	51.54±7.42^{b} (45.32%)	27.86±1.60^{a} (71.95%)	22.46±2.38^{a} (77.60%)

Data show the mean±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. Glu (10 g/kg): Glucose 10 g/kg; HBLE: *Hedranthera barteri* leaf extract,, GLI: Glibenclamide (Positive Control, 5 mg/kg).

Two most effective doses, 200 and 400 mg/kg in the glucose-induced antihyperglycaemic study (Table 6) were used in streptozotocin–induced diabetic rats to further validate the antidiabetic ethnomedicinal use of the plant. The result showed that the blood glucose levels of the non treated diabetic rats were consistently high throughout the 14 days of the study which confirmed that the diabetes induced in the rats by the administered streptozotocin was permanent (Table 7). The positive control (glibenclamide 5 mg/kg) caused 16, 45, 72, 78% blood glucose levels reduction on days 4, 7, 10 and 14, respectively, a time dependent antidiabetic activity that was due to insulin stimulating action of the drug on the remaining pancreatic βcells of the diabetic rats (Table 6). The leaf extract of *H. barteri* at 200 mg/kg gave an early onset of antidiabetic action of 34% on day 4 while the activity of its 400 mg/kg only became pronounced on day 7 of the study. Both 200 and 400 mg/kg of the extract were significantly more active than glibenclamide (5 mg/kg) on day 7 but had comparable activity on days 10 and 14. This result indicated the effectiveness of the extract at these doses and further justified its folkloric antidiabetic usage.

3.6. Results of Phytochemical Screening of the Extract

Table 8. *Summary of phytochemical screening of H. barteri leaf extract.*

Test	Inferences
Alkaloids	+++
Saponins	+
Cardiac glycosides	_
Tannins	+++
Flavonoids	+++
Anthraquinones	-
Terpenoids	+++
Steroid	-

Key:
+++ Abundantly present
+ Present
- Absent

3.7. GC-MS Analysis of the Leaf Extract of H. barteri

The GC-MS profile of the methanolic leaf extract of *Hedranthera barteri*, showing the identified compounds, their retention times, molecular weights, molecular formular and peak. The peaks in the chromatogram were automatically integrated for quantitative measurement of the components while their MS data were compared with the database of spectra of known components stored in the GC-MS library.

GC-MS analysis of plant extract has been found to be one of the most powerful tools that are used for identifying the chemical constituents of plants [43]. It was therefore used in this study for the analysis of the methanol leaf extract of *H. barteri* in order to identify its various chemical compounds, especially those that could be responsible for the observed antidiabetic activities of the plant (Tables 4-7). The GC-MS analysis of the extract showed a total of 32 compounds of which 16 were eventually characterised. The first peak had an Rt of 3.24 while the last component was 19.66 spanning from polar to non polar constituents, as is expected from the reversed phase column used for the analysis; this confirms the multicomponent nature of the extract. The most abundant compounds identified are, 2, 3, 5, 6-Tetrafluoroanisole (7), Silane, ethyltrimethyl (6), Hexadecanoic acid, methyl ester (10) and 9, 12,

15-Octadecatrienoic acid, methyl ester, Z, Z, Z (13) in that order based on the peak area. The next in abundance (3-5%) are (Phenol, 2-methoxy-4-(1-propenyl)-(Z) (5), n-Hexadecanoic acid (11), (Methyl stearate 1 (5), Phytol (14), 3-Octadecyne (8) and less than 2% are (2-Methoxy-4-vinylphenol (3), Oxalic acid, monoamide, n-propyl pentadecyl ester (4), (Phytol, acetate (9), (9, 12-Octadecadienoic acid, methyl ester, (E, E (12).

GC-MS analysis of the chloroform leaf extract of *Ximenia* americana had been reported to contain octadecatrienoic acid and n-Hexadecanoic acid its aqueous extract contained 9, 12-Octadecadinoic acid. In vitro antidiabetic studies of the aqueous extract of X. americana which contained 9, 12-Octadecadienoic acid exhibited significant activity [44]. It therefore indicated that 9, 12-Octadecadinoic acid that is similarly present in *H. barteri* may contribute to its observed antidiabetic activities. In addition, phytol that was a major component of the antidiabetic n-butanol fraction of *Piper guinensis* obtained by GC-MS [24] that was also present in *H. barteri* could be said to be one of the antidiabetic constituents of the plant. Phenylpropanoyl esters of catechol glycoside and its dimers from *Dodecadenia grandiflora* with reported antihyperglycemic activity could justify that catechol was one of the antidiabetic compounds in *H. barteri* [45]. The phytochemical screening of the plant extract showed the presence of flavoniods and terpenoids which was confirmed by its GC-MS analysis with the detection of catechol and phytol which are flavonoid and terpene, respectively (Tables 8 and 9).

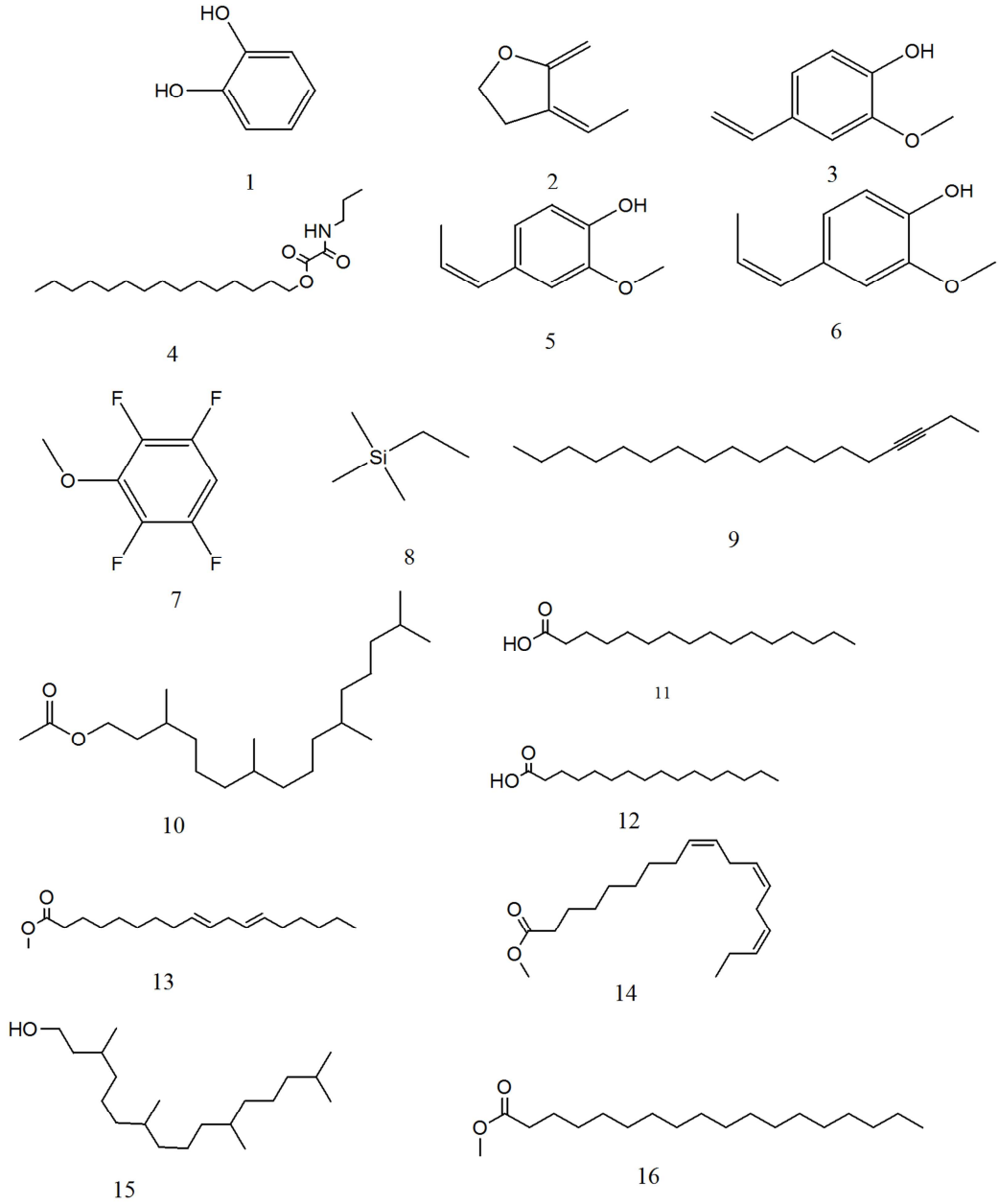

Figure 1. *Chemical structures of the identified and characterised compounds from the methanolic leaf extract of Hedranthera barteri.*

Table 9. *Gas-Chromatographic analysis of the leaf extract of H. barteri.*

CODE	NAMES OF COMPOUNDS	M/W	FORMULAR	CAS NUMBER	RT (mins)	PEAK AREA
1	Catechol	110.1	$C_6H_6O_2$	000120-80-9	6.189	1.67
2	Benzofuran, 2,3-dihydro-	120.1485	C_8H_8O	000496-16-2	6.354	4.59
3	2-Methoxy-4-vinylphenol	150.1745	$C_9H_{10}O_2$	007786-61-0	7.447	2.39
4	Oxalic acid, monoamide, n-propyl pentadecyl ester	173.21	$C_8H_{15}NO_3$	1000309-25-8	8.918	2.82
5	Phenol, 2-methoxy-4-(1-propenyl)-(Z)	164.2011	$C_{10}H_{12}O_2$	005912-86-7	8.952	3.01
6	2,3,5,6-Tetrafluoroanisole	180.10	$C_7H_4F_4O$	002324-98-3	10.131	12.95
7	Silane, ethyltrimethyl	102.2502	$C_5H_{14}Si$	003439-38-1	11.109	16.33
8	3-Octadecyne	250.463	$C_{18}H_{34}$	061886-64-4	12.580	5.2
9	Phytol, acetate	338.5677	$C_{22}H_{42}O$	1000375-01-4	12.946	2.55
10	Hexadecanoic acid, methyl ester	270.4507	$C_{17}H_{34}O$	000112-39-0	13.312	12.8
11	n-Hexadecanoic acid	256.4241	$C_{16}H_{32}O$	000057-10-3	13.644	3.52
12	9,12-Octadecadienoic acid, methyl ester, (E,E)	294.4721	$C_{19}H_{34}O$	002566-97-4	14.680	2.2
13	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	292.4562	$C_{19}H_{32}O$	000301-00-8	14.732	12.80
14	Phytol	296.53	$C_{20}H_{40}O$	000150-86-7	14.823	5.08
15	Methyl stearate	298.5038	$C_{19}H_{38}O$	000112-61-8	14.903	3.53

Key: M/W: Molecular weight; RT: Retention time

3.8. Histopathological Studies of the Effect of Leaf Extract of H. barteri

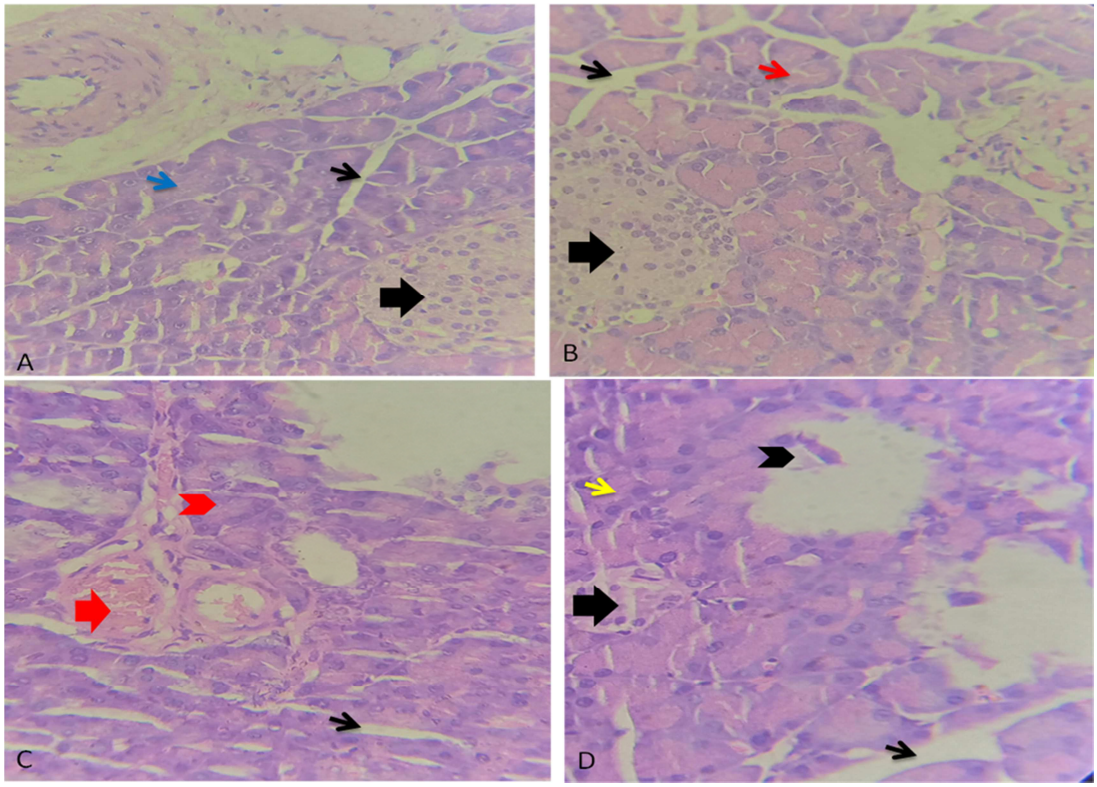

Figure 2. *Photomicrograph of the histology of pancreatic tissue. A: Control group, B-D: Treatment groups with 250, 500 and 1000 mg/kg of HB extract, respectively.*

The histology of the pancreatic tissues of the control group of rats showed well differentiated and normal pancreatic architecture, the pancreatic islets (black thick arrow), pancreatic acini (blue thin arrow), and the interlobular connective tissue (black thin arrow) were all intact. The treated group of rats with 250 mg/kg of HB extract showed no significant histological changes, the pancreatic islets (black thick arrow), pancreatic acini (red thin arrow), and the interlobular connective tissue (black thin arrow) were all intact without any loss of function. However, in the 500 mg/kg of extract treated group, there was constricted interlobular connective tissue (black thin arrow), degenerated pancreatic acini (red arrow head) while 1000 mg/kg caused severe necrotic degeneration and degradation (black arrow head), pancreatic acini (yellow thin arrow), interlobular connective tissue (black thin arrow) and pancreatic islets (black thick arrow) in the rats pancreas. This indicated lethal effect of the extract on the pancreas at high dose.

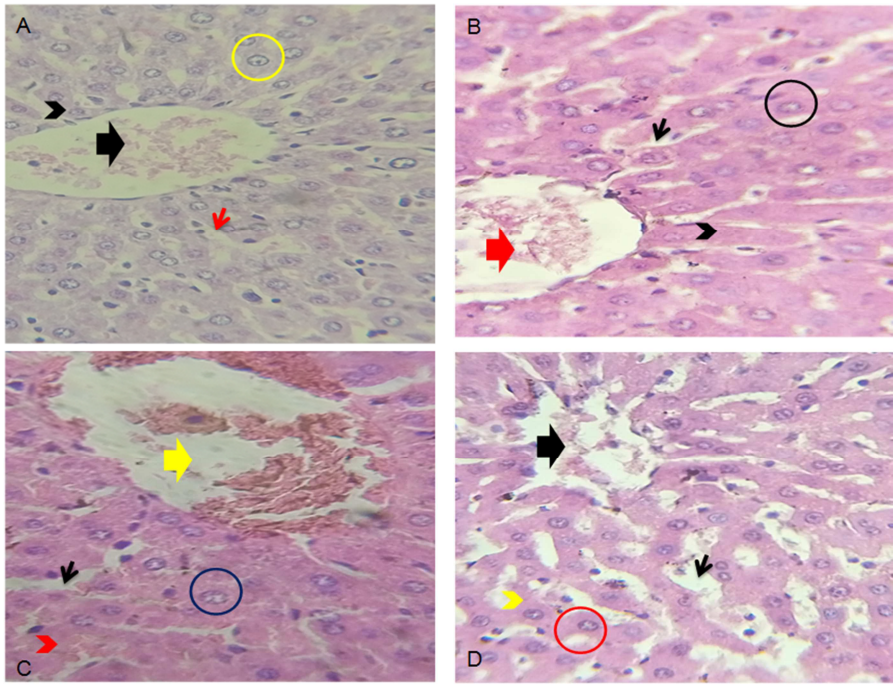

Figure 3. *Photomicrographs of the histology of liver tissue A: Control group, B-D: Treatment groups with 250, 500 and 1000 mg/kg of HB extract, respectively.*

The histology of the liver tissue of the control group of rats showed normal histomorphology of the central vein (black thick arrow), hepatocytes (yellow circle), sinusoids housing the kupfer cells (red thin arrow) and the hepatic plate (black arrow head) were well defined. The liver histology of the rats that received 250 mg/kg of the exract showed slight morphological changes, irregularity of the hepatic plate (black arrow head), slight congestion of the central vein (red thick arrow), sinusoids with reduced kupfer cells (black thin arrow) and hepatocytes (black circle). At 500 mg/kg of the extract there was mild morphological changes, congested central vein (yellow thick arrow), microvesides hepatocytes (blue circle), constricted sinusoids with reduced kupfer cells (black thin arrow) and hepatic plate (red arrow head). The 1000 mg/kg extract showed degradation and hydropic degeneration of the hepatocytes (red circle), congested central vein (black thick arrow), dilated sinusoids (black thin arrow) with loss of function (Figure 3). This indicated the extract could be toxic to the liver at high doses.

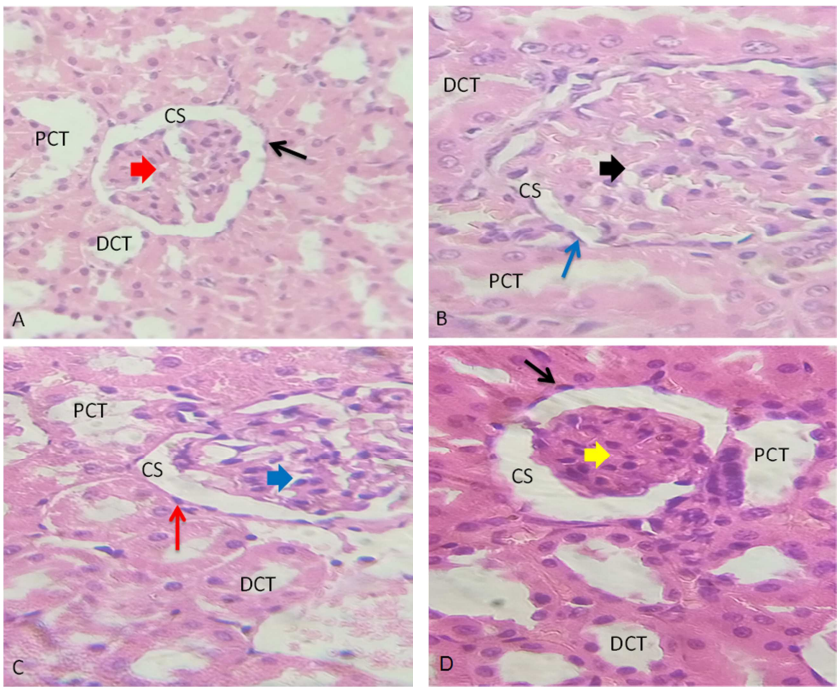

Figure 4. *Photomicrographs of the histology of renal tissue A: Control group, B-D: Treatment groups with 250, 500 and 1000 mg/kg of HB extract, respectively.*

The histology of the control group of animals depicted normal histoarchitecture of renal tissue. The glomerulus (red thick arrow), capsular space (CS), epithelia cells (black thin arrow) and the proximal convulated tubules (PCT) and distal convulated tubules (DCT) appeared normal and intact. The group of rats that were treated with 250 mg/kg extract showed no significant morphological changes, the glomerulus (black thick arrow), epithelial cells (black thin arrow), constricted capsular space (CS), the proximal and distal convulated tubules (PCT and DCT) were well organized without any loss of function. At 500 mg/kg of HB extract, there was distortion of the glomerulus (blue thick arrow), dilated distal and proximal convulated tubules (DCT and PCT) and irregular capsular space (CS) while 1000 mg/kg extract showed collapsed and extrusion of the glomerulus (yellow thick arrow), dilated capsular space (CS) and severe dilated proximal and distal tubules (PCT and DCT) with loss of function (Figure 4). This also showed toxic effect of the extract on the kidney at elevated doses.

4. Conclusion

The results obtained from this study have justified the ethnomedicinal antidiabetic use of *Hedranthera barteri* and confirmed that the leaf can be used as an adjunct in the management of diabetes mellitus. Future work will focus on the isolation and characterization of the antidiabetic constituents of the plant that have been established in this study.

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