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# Hypoglycemic effect of *Hydrocotyle bonariensis* Comm. Ex Lam. leaf extract in an experimental type 2 diabetes rat model

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## Abstract

**Background** *Hydrocotyle bonariensis* Comm. Ex Lam. (Araliaceae) leaf water infusion is traditionally used in the treatment of diabetes mellitus (DM) in Côte d'Ivoire and other parts of Africa. However, scientific studies validating these claims are limited.

**Objective** The study was designed to investigate the hypoglycemic activity of water extract from *H. bonariensis* leaf with the hope to validate the traditional usage of *H. bonariensis* leaf in the treatment of DM.

**Methods** The *H. bonariensis* leaf infusion was prepared and administered at 250 and 500 mg/kg body weight (bw) once daily to high-fat fed streptozotocin-induced diabetes rats for three (3) weeks.  $\alpha$ -Amylase and  $\alpha$ -glucosidase inhibitory activities were employed to reveal the mechanism of hypoglycemic potential of the extract. Gas chromatography-mass spectrometry (GC-MS) was used to detect the possible phytochemicals of the extract. In addition, in silico study based on molecular docking was conducted for possible interaction of the compounds with  $\alpha$ -amylase (PDB ID: 1B2Y) and  $\alpha$ -glucosidase (PDB ID: 3W37).

**Results** The results revealed that *H. bonariensis* leaf water extract showed significant ( $p < 0.05$ ) reduction in fasting blood glucose (FBG) levels, ameliorated insulin resistance and improved pancreatic  $\beta$ -cell function compared to diabetic untreated animals and comparable to metformin treated group. However, the extract demonstrated weak modulatory effects on lipid profiles. Notably binding energies were observed between the compounds and the target proteins involving both hydrophilic and hydrophobic interaction. With regards to the compounds 1,2,3-trimethoxy-5-(1E)-1-propen-1-ylbenzene and  $\alpha$ -amylase, a docking score of -5.8 kcal/mol was uncovered, while thymol and hexadecanoic acid, methyl ester showed a docking score of -5.1 and -5.0 kcal/mol with  $\alpha$ -glucosidase, respectively.

**Conclusion** The data confirms the traditional usage of *H. bonariensis* leaf water extract in improving glycemia and recommend further detailed molecular and toxicological studies to support the present data.

**Clinical trial number** Not applicable.

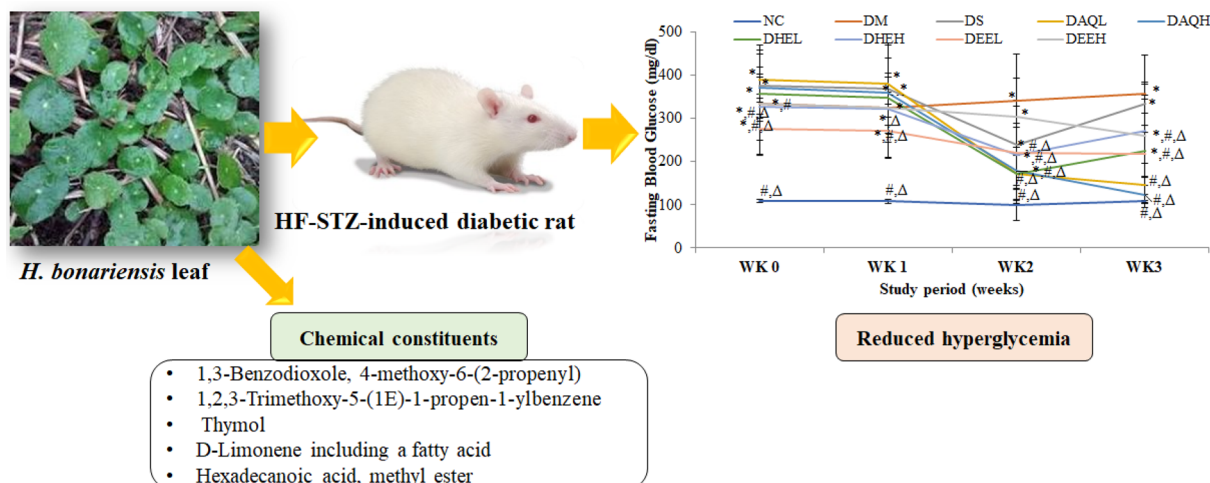
**Keywords** Diabetes mellitus, *Hydrocotyle bonariensis*, Hypoglycemic, Leaf, Water extract

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## Graphical Abstract



## Background

Diabetes mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia resulting from absolute or relative deficiencies in insulin secretion and/or action [1]. Presently, the disease affects an estimated 537 million adults worldwide and is expected to increase to 783 million adults by 2045 [2]. The DM and its complications cause substantial morbidity, disability, and premature death and impose serious economic problems to the patients, attributed majorly to the persistent hyperglycemia [3]. Consequently, hyperglycemia and insulin resistance (IR) have been reported to be the key players in the development of diabetes-induced oxidative stress, leading to macro- and micro-vascular complications [4, 5]. Oxidative stress arises due to imbalance between the generations of reactive oxygen species (ROS) and the reducing potential of the ROS by in vivo antioxidant system (AS), altering the ROS/AS ratio in favor of stress [6]. Furthermore, studies have reported the strong correlation of ROS-induced oxidative stress in the development of IR and vice versa [7, 8]. Therefore, for effective treatment of diabetes, addressing hyperglycemia and insulin resistance are crucial to curtail the upsurge of the complications associated with diabetes.

The major treatment option has been the use of conventional hypoglycemic drugs, that pose serious adverse effects such as excessive hypoglycemia, body weight increase, flatulence and other gastric disturbances [9]. For this reason, search for alternative therapies for DM has been shifted to natural products, particularly plants because plants and their metabolites are made in a biological system, and may interact more effectively with biological targets and are more likely to succeed as drugs.

*Hydrocotyle bonariensis* Comm. Ex Lam. (Araliaceae) commonly known as large leaf pennywort is herbaceous plant with prostrate, creeping or floating stems and roots forming at nodes. It is indigenous to South and West African countries such as South Africa, Côte d'Ivoire, Nigeria and Togo. The *H. bonariensis* leaf is traditionally used to treat various ailments such as hypertension, rheumatism and DM [10]. The *H. bonariensis* leaf is taken either in form of infusion or consume as raw for the treatment of DM and related disorders [10]. Available information revealed that *H. bonariensis* leaf is non-cytotoxic and safe for oral usage with no acute or chronic toxicities [11]. Previous pharmacological studies have reported that *H. bonariensis* leaf possessed antioxidant [12, 13], anti-inflammatory [14], hypotensive [15] properties and attenuated cataract [13, 16]. Two compounds, quercetin and 3-O- $\beta$ -D-glucopyranosyl-sitosterol with strong antioxidant activity were isolated from *H. bonariensis* leaf [12], supporting the potential of the plant to attenuate diseases associated oxidative stress such as DM. However, to the best of our knowledge, studies evaluating the hypoglycemic activity of *H. bonariensis* leaf using either in vitro or in vivo models as well as human subjects remain limited. Therefore, the aim of our present study is to investigate the hypoglycemic effect of water extract from *H. bonariensis* leaf as way of validating the traditional claim in DM remedy. In addition, gas chromatography–mass spectrometry (GC-MS) was used to detect the phytochemicals present and also conducted molecular docking assessment of the bioactive compounds to predict probable outcomes between the compounds and some key enzymes relevant to diabetes.

## Materials and methods

### Chemical compounds/reagents used in the study

Absolute ethanol (CID 702, 99%), Citric acid (CID 311, 99%), Ethyl acetate (CID 8857, 99.5%), Fructose (CID 5984, 99%), Glucose (CID 5793, 98.5%), Hexane (CID 8058, 98.84%), Metformin (CID 4091, 99%), Sodium citrate (CID 6224), Streptozotocin (CID 29327,  $\geq 97\%$ ). The chemicals were procured from Sigma-Aldrich.

### Plant material and preparation of extracts

Fresh leaf of *H. bonariensis* was collected from a garden in Ibadan, Oyo State between July–August, 2019, and authenticated at the herbarium (19783), Department of Botany, Ahmadu Bello University, Zaria, Kaduna State of Nigeria by Mr Namadi Sunusi. The leaf was rinsed with tap water to remove all debris, air-dried at room temperature (25 °C) for about three (3) weeks and pulverized mechanically using a blender. One hundred grams (100 g) of the dry powdered plant material was infused in 1000 ml of water with intermittent shaking for 48 h and filtered through Whatman No. 1 filter paper. The filtrate (15.5%) was then concentrated at 45 °C using water bath.

### Experimental animals

Six-week-old male Wistar rats weighing  $188.20 \pm 3.01$  g were procured from the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and were kept five (5) per cage in a well-ventilated, temperature (25 to 28 °C) and humidity-controlled room with a 12-hour light-dark cycle. The animals were allowed to acclimatized for two (2) weeks before the commencement of the study. A standard rat pellet diet was supplied *ad libitum* to all animals during the study period. They were maintained strictly in accordance with the rules and regulations of the institutional ethical committee on scientific research, Ahmadu Bello University, Zaria (ABUCAUC/2020/41).

### Animal grouping

Twenty-five (25) rats were randomly divided into five (5) groups of five (5) rats namely; NC: Normal Control, DM: Diabetic Model, DMF: Diabetic + metformin (300 mg/kg bw), DWEL, Diabetic + low dose (250 mg/kg bw) of water extract, DWEH, Diabetic + high dose (500 mg/kg bw) of water extract.

### Induction and confirmation of DM

The DM was induced by feeding the animals in DM groups (DC, DMF, DWEL, DWEH) with high fat diet regimen which comprises of 55% fat (lard), 35% carbohydrate (corn starch and sucrose) and 10% protein (casein) for fifteen (15) days. Rats in the NC group were fed with normal diet (carbohydrate 66.65%, proteins 15%, lipid 7%). We adopted the method of Magalhães et al. [17]. The

most important thing is that the high fat diet contained more calories from lipids (55%) and less carbohydrate (35%) and protein (10%) compared to the control diet. On the 15th day, streptozotocin at 40 mg/kg dissolved in phosphate buffer (pH 7.4) was injected intraperitoneally to all rats in DM groups, whereas animals in NC were injected with relative amounts of phosphate buffer. Hyperglycemia was confirmed by raised fasting blood glucose (FBG) over 200 mg/dl measured by a glucometer (Accu-Chek Active Glucometer, Roche, Germany), Rats with a FBG level less than 200 mg/dl were excluded from the study [18].

### Intervention

The extract, standard drug (metformin) and the vehicle were administered via oral gavage once daily in the morning (9 am) for the three weeks. The dosages used (250 and 500 mg/kg bw) were selected based on a pilot study. During the experimental period, weekly FBG and body weight (Data not shown) were measured.

### Blood and organ collection

Animals were euthanized by chloroform (0.94%v/v) anaesthesia at the end of the experimental period and blood samples were collected. Whole blood was collected via cardiac puncture into tubes; the blood was centrifuged at 3000 rpm for 15 min and the serum was separated and preserved at -30 °C until further analysis.

A portion of pancreatic tissue from each animal was harvested and placed in a 10% neutral buffered formalin solution and preserved at room temperature for histopathological study. The neutral buffered formalin of each pancreatic tissue sample was replaced weekly during the entire preservation period.

### Analytical methods

Plasma insulin concentration was measured by ELISA method using ultra-sensitive rat insulin ELISA kit ((Merckodia, Uppsala, Sweden ((10-1220-01)) in a 96 wells multi plate reader at an optical density of 450 nm. The serum lipid profiles and the liver function enzymes; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), and some markers of kidney damage (urea, and creatinine) were assayed with commercial kit (Randox laboratory UK). LDL-cholesterol was calculated according to Friedewald et al. [19].

$$\begin{aligned}\text{LDL} - \text{cholesterol (mmol/l)} \\ = \text{TC} - \text{HDL} - (\text{TG}/5)\end{aligned}$$

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Homeostatic Model Assessment for assessment for  $\beta$ -cell function (HOMA- $\beta$ ) were calculated according to the following formula as reported

by Gayoso-Diz et al. [20] and Albareda et al. [21], respectively:

$$\text{HOMA} - \text{IR} = \left( \frac{\text{Fasting Plasma Insulin in U/l} \times \text{Fasting Plasma Glucose in mmol/l}}{22.5} \right)$$

$$\text{HOMA} - \beta = \left( \frac{\text{Fasting plasma insulin in U/l} \times 20 / \text{fasting blood glucose in mmol/l} - 3.5}{1} \right)$$

Conversion factor : insulin 1 U/l = 7.174 pmol/l

### Histopathological examination of pancreatic tissue

A standard procedure of paraffin embedding was used to treat the formalin preserved pancreatic tissues. Tissue sections were cut in to a size of 4 mm before fixing on the slides. Then, the slides were deparaffinized in p-xylene and rehydrated in ethanol gradient (100%, 80%, 70% and 50%) and rinsed with water. Slides were stained in hematoxylin for 5 min and rinsed with water, and were counter stained in eosin, mounted in DPX, cover-slipped and viewed with scanner.

### Investigation of $\alpha$ -amylase and $\alpha$ -glucosidase activities

The activities of  $\alpha$ -amylase and  $\alpha$ -glucosidase of the aqueous extract were investigated using the methods of McCue and Shetty [22] and Kim et al. [23] for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitions at various concentrations (30–240  $\mu\text{g/ml}$ ) respectively.

### Gas chromatography-mass spectrometry (GC-MS) of the *H. bonariensis* leaf extract

The GC-MS analysis was conducted with an Agilent Technology 6890 gas chromatograph. The aqueous extract that shows promising blood glucose lowering activity was subjected to Gas Chromatography-Mass spectrometry (GCMS) analysis (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 mm film thickness as reported by Ibrahim et al. [24]. The carrier gas was ultra-pure helium at a flow rate of 0.7 ml/min and a linear velocity of 37 cm/s. The injector temperature was set at 250 °C. The initial oven temperature was 60 °C which was programmed to 280 °C at the rate of 10 °C/min with a hold time of 3 min. Injections of 2  $\mu\text{L}$  were made in the splitless mode with a manual split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230 °C, quadrupole temperature 150 °C, solvent delay 4 min and

scan range 50–700 amu. The spectrum of the compounds was compared with the spectrum of National Institute of Standard and Technology (NIST) library database.

### Molecular docking analysis

Molecular docking examination was carried out between the identified compounds in *H. bonariensis* leaf and two diabetic protein targets (PDB ID: 1B2Y and 3W37) using PyRx-Python Prescription 0.8. The chemical structures of the compounds were retrieved from the PubMed (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format, while the crystal structure of the proteins were downloaded in PDB format from <https://www.rcsb.org/>. Thereafter, each of the proteins were loaded into PyRx and prepared for the docking. Similarly, the compounds were imported into PyRx, prepared for docking and completed in pdbqt format. Subsequently, AutoDock Vina wizard was used to execute the docking process within the grid box (1B2Y: center x: 17.9827 y: 21.3078 z: 49.3509, size x: 55.0309 y: 73.2159 z: 54.2445), (3W37: center x: 14.192 y: -16.9877 z: -28.6316, size x: 82.7341 y: 73.4676 z: 79.6546) and an exhaustiveness of 8. Upon completion, binding energies were collected in excel sheet and saved for further use.

Discovery Studio Visualizer v21.1.0.20298 (BIOVIA, San Diego, CA, USA) was used to assess the 2-dimensional and 3-dimensional binding interactions. The ligand-protein interactions involving hydrophobic and hydrophilic bonds will demonstrate the possible antidiabetic properties of the identified compounds in *H. bonariensis* leaf.

### Data analysis

Statistical analysis was conducted using statistical software package (SPSS for Windows, version 20, IBM Corporation, NY, USA). The data was presented as mean  $\pm$  SD ( $n = 5$ ). Differences between mean values were assessed using one-way analysis of variance after confirming normal distribution of data followed by a post hoc test (Tukey's-HSD multiple range post hoc test). For all analysis  $P < 0.05$  was considered significant.

## Results

### Effect of *H. bonariensis* leaf water extract on fasting blood glucose levels

The results of weekly fasting blood glucose (FBG) are depicted in Fig. 1. From the result it is deduced that induction DM significantly ( $p < 0.05$ ) elevated the FBG of the diabetic animals compared to the NC (Fig. 1). After 3 weeks of intervention with the *H. bonariensis* leaf water extract, the FBG significantly ( $p < 0.05$ ) decreased compared to DC group. The extract at both doses reduced FBG by 61.4% and 66.4% at the low and high dosages respectively. The reduction of FBG exhibited by the water

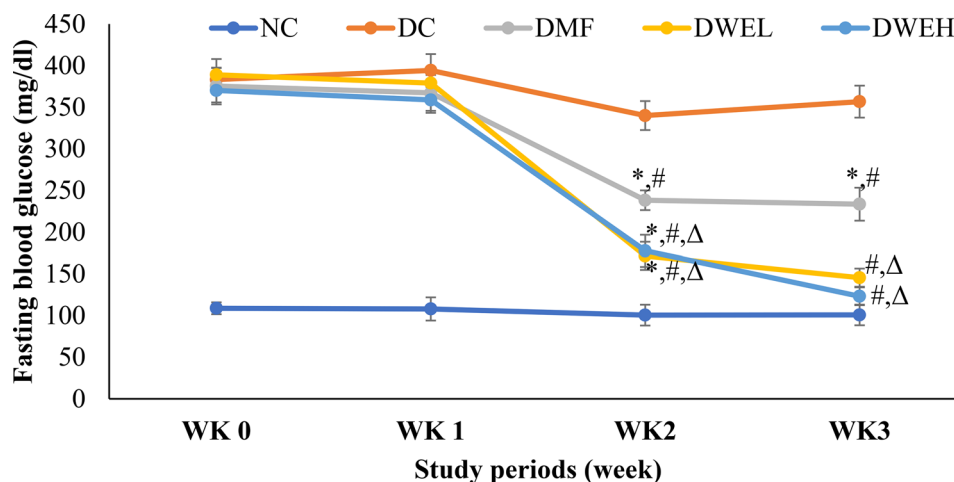


**Fig. 1** Effect of *H. bonariensis* leaf water extract on the fasting blood glucose levels in HFD-STZ-induced diabetic rats. Data are presented as the mean  $\pm$  SD of 5 animals. \* $P < 0.05$ , when comparing with the normal control, # $P < 0.05$  when comparing with diabetic model,  $\Delta P < 0.05$  when comparing with metformin (Tukey's-HSD multiple range post hoc test,  $p < 0.05$ ). NC, Normal control; DC, Diabetic control; DMF, Diabetic metformin; DWEL, Diabetic water extract low (250 mg/kg bw); DWEH, Diabetic water extract high (500 mg/kg bw)

**Table 1** Effect of *H. bonariensis* leaf extracts on serum insulin levels, calculated HOMA-IR and Homa- $\beta$  scores in HFD-STZ induced diabetic rats

| Groups | Insulin (pM)                    | HOMA- IR                       | HOMA- $\beta$                   |
|--------|---------------------------------|--------------------------------|---------------------------------|
| NC     | 349.22 $\pm$ 27.72 <sup>b</sup> | 12.95 $\pm$ 0.87 <sup>a</sup>  | 159.22 $\pm$ 15.43 <sup>e</sup> |
| DC     | 370.12 $\pm$ 21.97 <sup>c</sup> | 44.99 $\pm$ 9.19 <sup>d</sup>  | 51.75 $\pm$ 17.83 <sup>a</sup>  |
| DS     | 386.07 $\pm$ 17.19 <sup>c</sup> | 42.46 $\pm$ 3.64 <sup>d</sup>  | 54.73 $\pm$ 7.06 <sup>a</sup>   |
| DHEL   | 379.49 $\pm$ 23.29 <sup>c</sup> | 29.35 $\pm$ 16.81 <sup>c</sup> | 101.92 $\pm$ 53.38 <sup>c</sup> |
| DHEH   | 384.91 $\pm$ 41.64 <sup>d</sup> | 34.87 $\pm$ 13.15 <sup>c</sup> | 64.45 $\pm$ 19.74 <sup>b</sup>  |
| DEEL   | 391.42 $\pm$ 19.72 <sup>d</sup> | 29.73 $\pm$ 14.14 <sup>c</sup> | 99.61 $\pm$ 39.94 <sup>c</sup>  |
| DEEH   | 353.23 $\pm$ 30.08 <sup>b</sup> | 31.93 $\pm$ 9.76 <sup>c</sup>  | 62.58 $\pm$ 17.05 <sup>b</sup>  |
| DAQL   | 306.48 $\pm$ 37.62 <sup>a</sup> | 16.22 $\pm$ 4.44 <sup>b</sup>  | 106.99 $\pm$ 35.96 <sup>c</sup> |
| DAQH   | 354.88 $\pm$ 31.14 <sup>b</sup> | 13.97 $\pm$ 2.32 <sup>a</sup>  | 144.88 $\pm$ 31.69 <sup>d</sup> |

Data are presented as the mean  $\pm$  SD of 5 animals. a-d values with different superscript letters for the parameter are significantly different. (Tukey's-HSD multiple range post hoc test,  $p < 0.05$ ). NC, Normal control; DC, Diabetic control; DS, Diabetic standard (metformin); DHEL, Diabetic hexane extract low (250 mg/kg bw); DHEH, Diabetic hexane extract high (500 mg/kg bw); DEEL, Diabetic ethyl acetate extract low (250 mg/kg bw); DEEH, Diabetic ethyl acetate extract high (500 mg/kg); DAQL, Diabetic aqueous extract low (250 mg/kg bw); DAQH, Diabetic aqueous extract high (500 mg/kg bw)

extract was significantly ( $p < 0.05$ ) higher compared to the metformin drug treated group (Fig. 1).

#### Effect of *H. bonariensis* leaf water extract on insulin level and insulin resistance

The results of serum insulin level including the calculated HOMA-IR and HOMA- $\beta$  scores are presented in Table 1. According to the results, induction of DM significantly ( $p < 0.05$ ) elevated serum insulin level and insulin resistance and reduced pancreatic  $\beta$ -cell function compared to the NC group (Table 1). Administration of *H. bonariensis* leaf water extract significantly ( $p < 0.05$ ) improved the pancreatic  $\beta$ -cell function via reduction on circulating serum insulin level and HOMA-IR index as well as

increasing HOMA- $\beta$  score higher than that of metformin treated group (Table 1).

#### Effect of *H. bonariensis* leaf water extract on pancreatic histology

The effect of the extract on pancreatic structural integrity is presented in Fig. 2. There was a damage of pancreatic islets with subsequent reduction on the number of  $\beta$ -cells in the DC group compared to the NC group. However, treatment of *H. bonariensis* leaf water extract restored the diabetes induced pancreatic damage with improved number of  $\beta$ -cells and pancreatic morphology especially at the high dose comparable with that of the NC group (Fig. 2).

#### Effect of *H. bonariensis* leaf water extract on serum lipid profiles

The data for serum lipid profiles is presented in Table 2. The serum TC, TG and LDL-cholesterol were significantly ( $p < 0.05$ ) elevated whereas HDL was significantly ( $p < 0.05$ ) decreased in DC group compared to NC group. Oral administration of the extract demonstrated weak lipid lowering activity with significant ( $p < 0.05$ ) increase only on HDL-cholesterol (Table 2). In fact, the levels of TC, TG and LDL-cholesterol significantly ( $p < 0.05$ ) increased after administration of the extract when compared with the diabetic control group (Table 2).

#### Effect of *H. bonariensis* leaf water extract on some serum biochemical parameters

The results of other serum biochemical parameters (ALT, AST, ALP, urea, and creatinine) are presented in Table 3. The serum levels of ALT, AST, ALP, urea, and creatinine were elevated in DC group compared to NC group. Oral

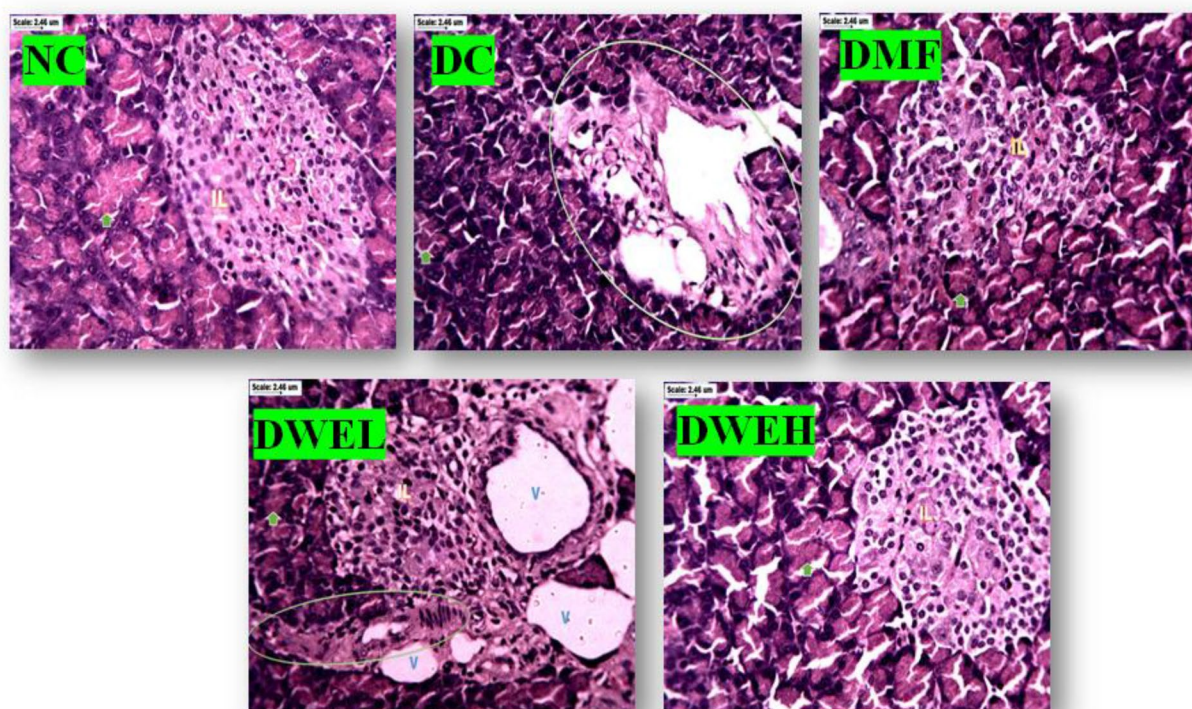


**Fig. 2** Histopathological examination of the pancreatic islets of different animal groups at the end of the intervention period. The NC had a larger islet with high number of  $\beta$ -cells while the DC had a smaller islet and morphologically deformed  $\beta$ -cells. The DWEL and DMF groups had relatively larger islets with higher number of  $\beta$ -cells compared to the DC group. NC, Normal control; DC, Diabetic control; DMF, Diabetic metformin; DWEL, Diabetic water extract low (250 mg/kg bw); DWEH, Diabetic water extract high (500 mg/kg bw)

**Table 2** Effect of *H. bonariensis* leaf extracts on serum lipid profiles in HFD-STZ induced diabetic rats

| Groups | TC (mg/dl)                       | TG (mg/dl)                     | HDL-cholesterol (mg/dl)        | LDL-cholesterol (mg/dl)          |
|--------|----------------------------------|--------------------------------|--------------------------------|----------------------------------|
| NC     | 85.66 $\pm$ 12.79 <sup>d</sup>   | 20.20 $\pm$ 13.32 <sup>b</sup> | 57.72 $\pm$ 5.31 <sup>f</sup>  | 23.74 $\pm$ 12.03 <sup>b</sup>   |
| DC     | 129.40 $\pm$ 9.63 <sup>e</sup>   | 27.20 $\pm$ 14.16 <sup>c</sup> | 21.60 $\pm$ 7.53 <sup>a</sup>  | 102.37 $\pm$ 15.43 <sup>f</sup>  |
| DS     | 69.68 $\pm$ 13.06 <sup>b</sup>   | 11.80 $\pm$ 1.19 <sup>a</sup>  | 39.55 $\pm$ 11.80 <sup>c</sup> | 31.09 $\pm$ 18.58 <sup>d</sup>   |
| DHEL   | 55.45 $\pm$ 17.65 <sup>a</sup>   | 10.90 $\pm$ 1.16 <sup>a</sup>  | 38.33 $\pm$ 12.00 <sup>b</sup> | 14.98 $\pm$ 11.48 <sup>a</sup>   |
| DHEH   | 75.48 $\pm$ 11.32 <sup>c</sup>   | 12.68 $\pm$ 1.64 <sup>a</sup>  | 46.46 $\pm$ 7.78 <sup>e</sup>  | 22.98 $\pm$ 4.59 <sup>c</sup>    |
| DEEL   | 63.26 $\pm$ 12.74 <sup>b</sup>   | 7.96 $\pm$ 3.84 <sup>a</sup>   | 42.30 $\pm$ 4.48 <sup>d</sup>  | 20.30 $\pm$ 15.02 <sup>b</sup>   |
| DEEH   | 62.40 $\pm$ 7.03 <sup>b</sup>    | 12.52 $\pm$ 2.44 <sup>a</sup>  | 39.16 $\pm$ 4.76 <sup>c</sup>  | 24.58 $\pm$ 9.55 <sup>c</sup>    |
| DAQL   | 199.88 $\pm$ 100.15 <sup>f</sup> | 51.40 $\pm$ 11.72 <sup>d</sup> | 41.98 $\pm$ 14.38 <sup>c</sup> | 124.33 $\pm$ 104.29 <sup>f</sup> |
| DAQH   | 122.42 $\pm$ 25.25 <sup>e</sup>  | 49.40 $\pm$ 6.49 <sup>d</sup>  | 37.83 $\pm$ 4.89 <sup>b</sup>  | 74.38 $\pm$ 25.69 <sup>e</sup>   |

Data are presented as the mean  $\pm$  SD of 5 animals. a-d values with different superscript letters for the parameter are significantly different. (Tukey's-HSD multiple range post hoc test,  $p < 0.05$ ). NC, Normal control; DC, Diabetic control; DS, Diabetic standard (metformin); DHEL, Diabetic hexane extract low (250 mg/kg bw); DHEH, Diabetic hexane extract high (500 mg/kg bw); DEEL, Diabetic ethyl acetate extract low (250 mg/kg bw); DEEH, Diabetic ethyl acetate extract high (500 mg/kg bw); DAQL, Diabetic aqueous extract low (250 mg/kg bw); DAQH, Diabetic aqueous extract high (500 mg/kg bw)

intervention of *H. bonariensis* leaf water extract reduced the activities of the serum ALT, AST and ALP as well as the levels of urea and creatinine in the diabetic treated rats when compared with the DC group (Table 3).

#### Effect of *H. bonariensis* leaf water extract on the activities of $\alpha$ -amylase and $\alpha$ -glucosidase

The effect of *H. bonariensis* leaf water extract on the activities of  $\alpha$ -amylase and  $\alpha$ -glucosidase is presented in Table 4. The data revealed that *H. bonariensis* leaf

water extract significantly ( $P < 0.05$ ) inhibited the activities of the enzymes particularly the  $\alpha$ -glucosidase, although lower than the acarbose used as a standard drug (Table 4). It was further observed that the inhibition depicted by *H. bonariensis* leaf water extract for  $\alpha$ -amylase was significantly ( $P < 0.05$ ) higher than that of acarbose (Table 4). Furthermore, for both the extract and acarbose, the inhibitory activity of  $\alpha$ -glucosidase by the extract was greater than that of  $\alpha$ -amylase.

**Table 3** Effect of *Hydrocotyle bonariensis* leaf extracts on liver and kidney function markers

| Groups | ALT(UI/L)                  | AST(UI/L)                 | ALP(UI/L)                    | Urea (mg/dl)               | Creatinine (mg/dl)       |
|--------|----------------------------|---------------------------|------------------------------|----------------------------|--------------------------|
| NC     | 26.20 ± 6.46 <sup>b</sup>  | 23.50 ± 5.20 <sup>d</sup> | 30.72 ± 7.21 <sup>c</sup>    | 28.00 ± 1.30 <sup>b</sup>  | 0.80 ± 0.10 <sup>b</sup> |
| DC     | 35.19 ± 6.54 <sup>d</sup>  | 34.56 ± 8.30 <sup>e</sup> | 40.80 ± 6.56 <sup>f</sup>    | 34.20 ± 11.19 <sup>c</sup> | 1.07 ± 0.21 <sup>d</sup> |
| DS     | 31.75 ± 9.67 <sup>c</sup>  | 17.50 ± 6.40 <sup>b</sup> | 24.08 ± 7.78 <sup>a</sup>    | 25.33 ± 2.65 <sup>a</sup>  | 0.67 ± 0.12 <sup>a</sup> |
| DHEL   | 39.56 ± 10.57 <sup>d</sup> | 19.60 ± 9.81 <sup>c</sup> | 56.16 ± 9.69 <sup>d</sup>    | 27.10 ± 9.63 <sup>b</sup>  | 0.93 ± 0.15 <sup>c</sup> |
| DHEH   | 35.8 ± 3.27 <sup>d</sup>   | 21.80 ± 3.35 <sup>d</sup> | 26.56 ± 4.15 <sup>a, b</sup> | 28.27 ± 2.27 <sup>b</sup>  | 0.80 ± 0.36 <sup>b</sup> |
| DEEL   | 20.80 ± 2.28 <sup>a</sup>  | 6.16 ± 2.46 <sup>a</sup>  | 28.36 ± 2.27 <sup>b</sup>    | 29.80 ± 11.66 <sup>b</sup> | 0.67 ± 0.21 <sup>a</sup> |
| DEEH   | 21.00 ± 2.74 <sup>a</sup>  | 16.20 ± 3.35 <sup>b</sup> | 30.10 ± 0.60 <sup>c</sup>    | 28.70 ± 7.39 <sup>b</sup>  | 0.77 ± 0.12 <sup>b</sup> |
| DAQL   | 26.80 ± 4.44 <sup>b</sup>  | 19.26 ± 9.34 <sup>c</sup> | 37.02 ± 8.45 <sup>e</sup>    | 29.77 ± 7.37 <sup>b</sup>  | 0.83 ± 0.15 <sup>b</sup> |
| DAQH   | 30.60 ± 7.89 <sup>c</sup>  | 21.20 ± 2.59 <sup>d</sup> | 33.88 ± 4.20 <sup>d</sup>    | 32.70 ± 6.50 <sup>c</sup>  | 0.77 ± 0.12 <sup>a</sup> |

Data are presented as the mean ± SD of 5 animals. a-g values with different superscript letters for the parameter are significantly different. (Tukey's-HSD multiple range post hoc test,  $p < 0.05$ ). NC, Normal control; DC, Diabetic control; DS, Diabetic standard (metformin); DHEL, Diabetic hexane extract low (250 mg/kg bw); DHEH, Diabetic hexane extract high (500 mg/kg bw); DEEL, Diabetic ethyl acetate extract low (250 mg/kg bw); DEEH, Diabetic ethyl acetate extract high (500 mg/kg); DAQL, Diabetic aqueous extract low (250 mg/kg bw); DAQH, Diabetic aqueous extract high (500 mg/kg bw)

**Table 4** IC<sub>50</sub> value for the inhibition of α-glucosidase and α-amylase by aqueous extract of *H. bonariensis* leaf

| Extract  | α-Glucosidase             | α-Amylase                  |
|----------|---------------------------|----------------------------|
| Aqueous  | 86.36 ± 1.21 <sup>*</sup> | 122.60 ± 1.58 <sup>*</sup> |
| Acarbose | 12.92 ± 1.08              | 168.97 ± 23.10             |

Data are presented as the mean ± SD of triplicate determinations. \*Indicate significant different at  $p < 0.05$  compared to the standard acarbose (Tukey's-HSD multiple range post hoc test,  $p < 0.05$ )

**Table 5** Possible compounds identified from *H. bonariensis* leaf extract by GC-MS

| Compounds                                    | Retention time (min) | Mass/amu             |
|----------------------------------------------|----------------------|----------------------|
| D-Limonene                                   | 9.80                 | 136 [M] <sup>+</sup> |
| 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)   | 13.20                | 192 [M] <sup>+</sup> |
| Thymol                                       | 14.40                | 150 [M] <sup>+</sup> |
| 1,2,3-Trimethoxy-5-(1E)-1-propen-1-ylbenzene | 15.16                | 208 [M] <sup>+</sup> |
| Selinane                                     | 25.01                | 209 [M] <sup>+</sup> |
| Hexadecanoic acid, methyl ester              | 34.11                | 270 [M] <sup>+</sup> |

#### Possible compounds present in *H. bonariensis* leaf water extract

The GC-MS analysis was conducted to detect some phytochemicals in *H. bonariensis* leaf water extract. Major compounds detected were aromatic in nature such as 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), 1,2,3-trimethoxy-5-(1E)-1-propen-1-ylbenzene, thymol and D-limonene including a fatty acid, hexadecanoic acid, methyl ester (Table 5).

#### Molecular docking assessment

The binding energies, interacting amino acid residues, bond type are presented in Table 6. Notably, 1,2,3-trimethoxy-5-(1E)-1-propen-1-ylbenzene with a docking score of -5.8 kcal/mol had a strong binding interaction with α-amylase involving hydrogen (His305), carbon-hydrogen (Tyr62) bonds, as well as other pi-hydrophobic bonds (Fig. 3a). In a similar fashion, thymol interacts with

α-glucosidase (-5.1 kcal/mol) forming hydrogen bonds with Ile106, Arg113 while having pi-anion (Glu105) and Alkyl (Arg102) bonds (Fig. 3b). In the interaction between hexadecanoic acid, methyl ester and α-glucosidase, two amino acid residues (His626, Asp357) were involved in the hydrogen bonds while Ala234, Phe236, Trp329, Trp432, Phe601, Ala602, Ala628 forms the alkyl/pi-alkyl interaction (Fig. 3c). All the best docking outcomes showed that the compounds were well fitted into the binding pocket of the proteins (1B2Y, 3W37) with varying orientation around the protein to exert the predictable inhibitory activity of the plant.

#### Discussion

The target approach to the treatment of DM has been the control of hyperglycemia, a key factor that contributes to DM-associated complications [25]. In our present study, there was a sustained hyperglycemia in the DC group throughout the study period, indicating successful induction of diabetes. The promising blood glucose lowering activity demonstrated by the groups treated with *H. bonariensis* leaf water extract particularly the high dose indicated that the extract contained compound(s) with greater potential to reduce hyperglycemia. This great potential in reducing hyperglycemia supports the ethnobotanical usage of *H. bonariensis* leaf infusion in the treatment of DM. To the best of our knowledge, this study is among the first to validate the traditional claims of using *H. bonariensis* leaf infusion in the treatment of DM. The potent reduction on hyperglycemia is further supported by the amelioration of insulin resistance and improvement of pancreatic β-cell function.

It has been reported that the hyperglycemia in type 2 diabetes cause an impairment in insulin secretions [26], with subsequent distortion of pancreatic β-cell structure and functions [27] reflected by damages at the pancreatic tissues, increased HOMA-IR and decrease HOMA-β scores which are clearly manifested in DC group. The data aligned well with the results of FBG as the group

**Table 6** Molecular docking scores on the interaction between the identified compounds from *H. bonariensis* leaf water extract by GC-MS and  $\alpha$ -Amylase (1B2Y) and  $\alpha$ -glucosidase (3W37)

| S/No | Compounds                                    | PubChem CID | Binding energy (kcal/mol) |      | Interaction                                                                                                                                                            |                                                                                                                                 |
|------|----------------------------------------------|-------------|---------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|
|      |                                              |             | 1B2Y                      | 3W37 | 1B2Y                                                                                                                                                                   | 3W37                                                                                                                            |
| 1    | D-Limonene                                   | 440,917     | -6.0                      | -5.6 | Pi-Sigma: Trp62<br>Alkyl/Pi-Alkyl: Leu165, His299                                                                                                                      | Pi-Sigma: Trp329<br>Alkyl/Pi-Alkyl: Trp329, Ile358, Trp432                                                                      |
| 2    | 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)   | 4276        | -5.8                      | -5.1 | Carbon-Hydrogen Bond: Asp300<br>Alkyl/Pi-Alkyl: Tyr62, Leu162, Leu165, His299                                                                                          | Carbon-Hydrogen Bond: Pro112<br>Alkyl/Pi-Alkyl: Pro114, Lys309                                                                  |
| 3    | Thymol                                       | 6989        | -6.2                      | -5.1 | Pi-Sigma: Tyr62<br>Pi-Pi Stacked: Tyr62<br>Pi-Alkyl: His299                                                                                                            | Hydrogen Bond: Ile106, Arg113<br>Pi-Anion: Glu105<br>Alkyl: Arg102                                                              |
| 4    | 1,2,3-Trimethoxy-5-(1E)-1-propen-1-ylbenzene | 5,318,557   | -5.8                      | -5.1 | Hydrogen Bond: His305<br>Carbon-Hydrogen Bond: Tyr62<br>Pi-Sigma: Trp59, Tyr62<br>Pi-Pi Stacked/Pi-Pi T-shaped: Trp58, Trp59<br>Alkyl/Pi-Alkyl: Leu165, His299, His305 | Pi-Anion: Asp568<br>Pi-Sigma: Trp329<br>Pi-Sulfur: Met470<br>Alkyl/Pi-Alkyl: Trp329, Ile358, Trp432, Phe476, Phe601, Ala628     |
| 5    | $\beta$ -Selinene                            | 442,393     | -7.8                      | -5.9 | Pi-Alkyl: Trp58, Trp59, Tyr62, His299                                                                                                                                  | Pi-Sigma: Phe476<br>Alkyl/Pi-Alkyl: Trp432, Met470, Lys506                                                                      |
| 6    | Hexadecanoic acid, methyl ester              | 8181        | -5.3                      | -5.0 | Carbon-Hydrogen Bond: Glu233<br>Alkyl/Pi-Alkyl: Trp58, Trp59, Tyr62, Leu162, Leu165                                                                                    | Hydrogen Bond: His626<br>Carbon-Hydrogen Bond: Asp357<br>Alkyl/Pi-Alkyl: Ala234, Phe236, Trp329, Trp432, Phe601, Ala602, Ala628 |

treated with high dose of the extract showed greatest amelioration of insulin resistance and improved pancreatic  $\beta$ -cell structure and function. This further reveal that the *H. bonariensis* leaf water extract contains relevant phytochemicals, that could mitigate insulin resistance and regenerate pancreatic  $\beta$ -cell structure and function.

Substantial evidence has accumulated linking insulin resistance in the etiology of dyslipidemia in DM. Insulin resistance triggers lipolysis from the fats depots, which promote the activities of HMG-CoA reductase and decreases lipoprotein lipase, which lead to the elevation of TG and TC levels [28]. In our present study, the water extract that depicted highest glucose-lowering ability showed weak control of diabetes-induced lipid alterations of which the reason is not clear. One possible explanation could be that the 3 weeks administration period was short to revealed significant improvement on lipid metabolism. The most interesting fact is that the extract was able to improves the HDL-cholesterol level which could go a long way in ameliorating the dyslipidemia induced by diabetes.

Hepato- and reno-protective effects of any potential drug are crucial for successful attenuation of diabetes-induced complications. It is known that DM induces

aggressive elevations in the activities of ALT, AST, ALP, and the serum levels of urea and creatinine that was observed in DC group. The promising attenuation of the alterations of the liver and kidney markers stated above is an indication that the extract could ameliorate the deleterious effects associated with the organs in DM condition.

Due to the fact that *H. bonariensis* leaf water extract showed promising potential in mitigating hyperglycemia and insulin resistance, the extract was subjected to  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory potential to understand the possible antihyperglycemic activity of the aqueous extract. Inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase is one of the approaches developed for the treatment of DM. These enzymes are crucial to the breakdown of dietary carbohydrates into absorbable monosaccharides. Inhibitors of  $\alpha$ -glucosidase and  $\alpha$ -amylase activities delay the overall digestion of carbohydrates by increasing the digestion period, thereby reducing the rate of intestinal glucose absorption, which in turn diminishes postprandial hyperglycemia [29]. The extract showed promising inhibition against the  $\alpha$ -amylase and  $\alpha$ -glucosidase activities and could be the possible mechanism behind the antihyperglycemic activity of the extract.

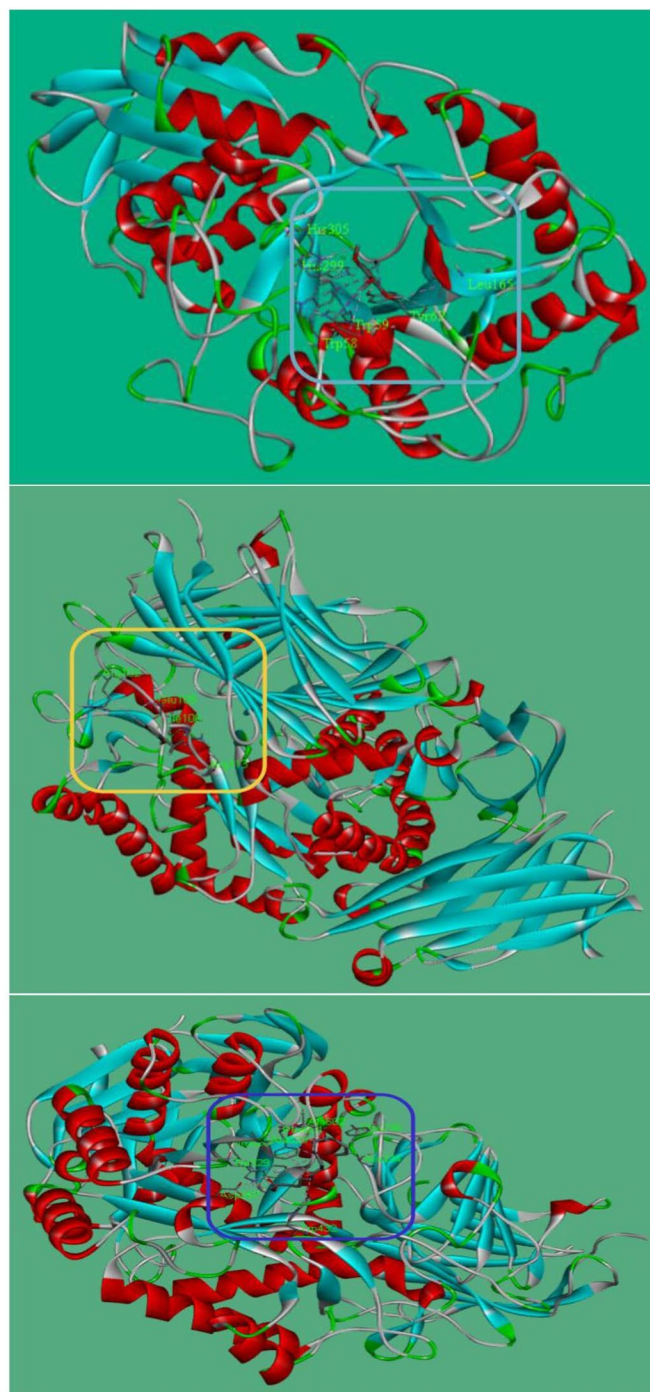
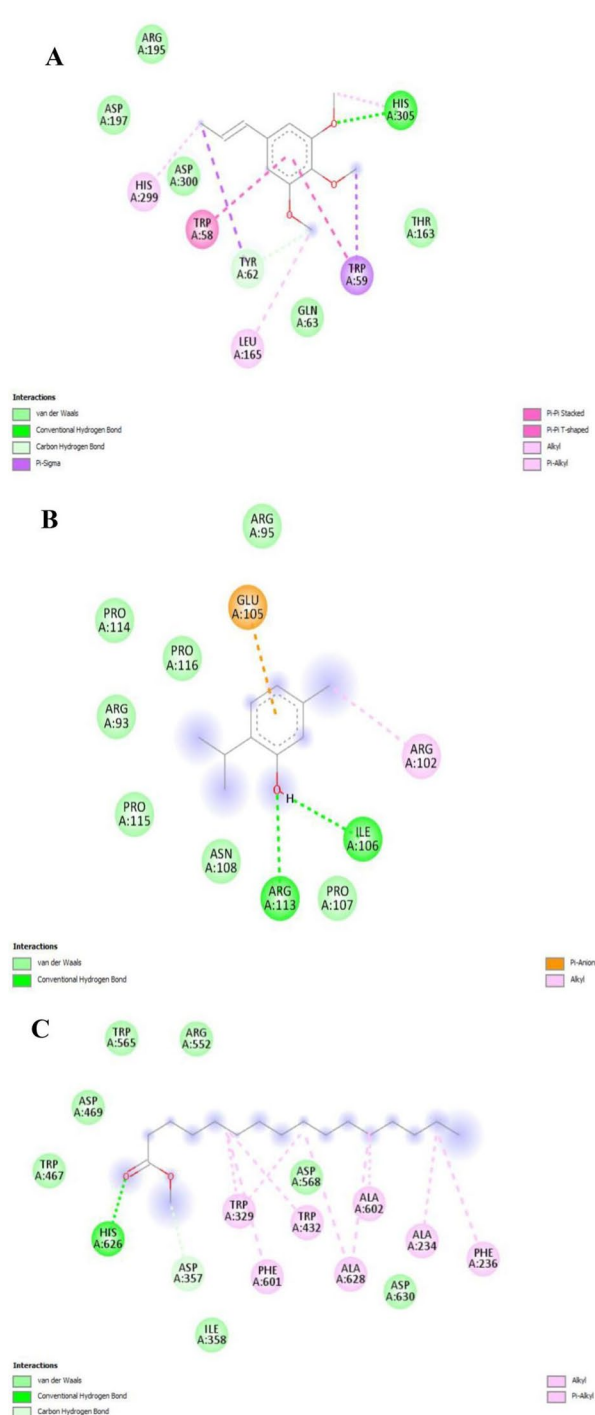


**Fig. 3** Two-dimensional (2D) and three-dimensional (3D) binding interaction of (A) 1,2,3-Trimethoxy-5-(1E)-1-propen-1-ylbenzene with  $\alpha$ -amylase (1B2Y), (B) Thymol with  $\alpha$ -glucosidase (3W37) and (C) Hexadecanoic acid, methyl ester with  $\alpha$ -glucosidase (3W37)

It is a known fact that the pharmacological activity of plants depends entirely on the nature of chemical compounds present. Despite the fact that Ajani et al. [12] have reported the isolation of quercetin and 3-O- $\beta$ -D-glucopyranosyl-sitosterol from *H. bonariensis* leaf methanol extract, the GC-MS analysis was conducted to detect

some possible compounds present in water extract. The GC-MS analysis revealed some major aromatic compounds that some were reported to ameliorate hyperglycemia and oxidative damages due to diabetes. Agarwal et al. [30] reported that thymol attenuated hyperglycemia and other parameters associated with diabetes in

animals. Similarly, D-limonene was reported to reduced hyperglycemia and associated oxidative damages in diabetes animal models [31]. Therefore, the promising anti-diabetic activity of *H. bonariensis* leaf water extract could be due to the individual or combine effects of the compounds detected.

To further support our data, in-silico evaluation via molecular docking assessed the antidiabetic potential of *H. bonariensis* leaf with  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes due to their crucial role in carbohydrate metabolism. Accordingly, the type of binding interaction resulting from this study focused on the possible inhibitory effect of the compounds, wherein the network parameters of a good inhibitors are fairly documented. This is contained in the good formation of binding energy, binding complex and the amino acid residues accommodating the catalytic pocket of the protein targets. Evidently, the strength and nature of the interaction between the molecules, typically expressed by the binding energy of 1,2,3-trimethoxy-5-(1E)-1-propen-1-ylbenzene (-5.8 kcal/mol) with His305, Tyr62, Trp59, Trp58, Leu165, His299 of  $\alpha$ -amylase suggest a high affinity around the binding pocket of the enzyme, thus indicating a stronger interaction and potentially greater biological activity. A similar mode of binding interaction exists between thymol (-5.1 kcal/mol), hexadecanoic acid, methyl ester (-5.0 kcal/mol) with  $\alpha$ -glucosidase as supported by strong hydrogen, carbon-hydrogen, alkyl, pi-alkyl typed bonds (Fig. 3b and c). This observation is in agreement with Khan et al. [32] and Ahmed et al. [33].

Major limitations to the present study include the lack of any molecular or expressions of some genes and/or proteins to clarify some findings. In addition, GC-MS analysis was used to detect the phytochemicals present that could be more appropriate for less polar extract. We considered these limitations as critical and hoped to present the data in our subsequent publications.

## Conclusion

Oral administration of *H. bonariensis* leaf water extract demonstrated antidiabetic properties via reduction of FBG and improving glucose utilization and insulin sensitivity in DM model of rats. The data support and confirm the traditional use of the plant against DM and its complications. For this reason, detailed toxicity study and molecular studies are recommended to further validate the efficacy of *H. bonariensis* leaf in DM treatment.

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## Author contributions

Maryam Zakari: Formal analysis, Methodology, Writing-original draft, Writing-review & editing. Ismaila Alhaji Umar: editing & Funding acquisition. Aminu Mohammed: Methodology, Validation, Formal analysis. Auwal Garba:

Validation, Investigation, Writing-review & editing, Mukhtar Adeiza Suleiman, Methodology.

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## Data availability

No datasets were generated or analysed during the current study.

## Declarations

### Ethical approval

Ethical approval for animal experimentation was obtained from the Ahmadu Bello University Animal Care and Use Committee (ABUCAUC/2020/41).

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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