In *vivo* antidiabetic efficacy, mineral element composition, and qualitative phytochemistry of the aqueous leaf extracts of *Pentas zanzibarica* (Klotzsch.) Vatke and *Olea europaea* subspecies *africana* (Mill.)

Gervason Apiri Moriasi[1*], Cromwell Mwiti Kibiti[3], Mathew Piero Ngugi[2]

[1]Mount Kenya University, Department of Medical Biochemistry, PO Box 342-01000, Thika-Kenya.
[2]Kenyatta University, Department of Biochemistry, Microbiology, and Biotechnology, PO Box 43844-00100, Nairobi-Kenya.
[3]Technical University of Mombasa, Department of Pure and Applied Sciences, PO Box 90420-80100, Mombasa-Kenya.

*Corresponding author
Gervason Apiri Moriasi
Mount Kenya University,
Department of Medical
Biochemistry, PO Box 342-01000,
Thika-Kenya.
e-mail: gmoriasi@outlook.com

ABSTRACT

Persistent hyperglycemia is the hallmark of diabetes and is accountable for the devastating complications, which cause high morbidity and mortality. Conventional anti-diabetic agents are only palliative and characterized with limited efficacy, adverse effects, high costs, inaccessibility, prompting the need for better alternatives. Therefore, we investigated the *in vivo* hypoglycemic activities, elemental composition, and qualitative phytochemistry of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* as potential sources of affordable, safer, accessible, and potent anti-diabetic therapies. *In vivo* hypoglycemic activities of the studied plant extracts were evaluated at three dose levels of 50, 100, and 150 mg/kg body weight (bw) in Alloxan-induced hyperglycemic Swiss albino mice. The elemental composition of the plant extracts was analyzed using the energy dispersive x-ray fluorescence spectroscopy (EDXRF System) and the atomic absorption spectrometry (AAS). Qualitative phytochemical screening was done following standard procedures. In this study, the aqueous leaf extract of *P. zanzibarica*, significantly (p<0.05) reduced alloxan-induced hyperglycemia in mice from 163.26±2.24 mg/dL (at 0 h) to 52.12±1.16 mg/dL (at the 4th h) at a dose of 50 mg/kg body weight, 166.98±1.56 mg/dL (at 0 h) to 48.90±1.40 mg/dL (at 4th h) at a dose of 100 mg/kg body weight, and 168.64±2.96 mg/dL (at 0 h) to 42.46±1.80 mg/dL (at 4th h) at a dose of 150 mg/kg body weight, respectively. Similarly, the aqueous leaf extract of *O. europaea* subspecies *africana* significantly (p<0.05) reduced hyperglycemia from 165.74±3.66 mg/dL (at 0 h) to 65.26±1.46 mg/dL (at the 4th h) at a dose of 50 mg/kg body weight, 158.14±3.49 mg/dL (at 0 h) to 53.68±1.48 mg/dL (at the 4th h) at a dose of 100 mg/kg body weight, and from 161.66±2.19 mg/dL (at 0 h) to 44.48±1.35 mg/dL (at the 4th h) at a dose of 150 mg/kg body weight, respectively. Furthermore, the extracts contained chromium (Cr), zinc (Zn), magnesium (Mg), among other elements, and phytochemicals like phenols, flavonoids, and alkaloids, among others. Generally, the studied plant extracts exhibited significant hypoglycemic efficacy in alloxan-induced hyperglycemic mice, indicating their antidiabetic potential, and possess pharmacologically active phytochemicals and valuable minerals.

INTRODUCTION

Diabetes mellitus (DM) is a broad term referring to a group of metabolic defects that are caused by abnormalities in insulin secretion, insensitivity of target tissues to insulin, or both [1,2]. Inherent genetic defects and acquired factors initiate and promote the advancement of DM in affected subjects [3]. The hallmark feature of DM is uncontrolled hyperglycemia, which causes severe complications, including ketosis, neuropathy, nephropathy, retinopathy, polydipsia, polyphagia, polyuria, cardiovascular impairment, among others [2,4]. Death of the affected patients ensues when diabetes is not adequately controlled due to metabolic shock and multiple organ failure [2].

The number of people suffering from DM is rapidly increasing worldwide. It is estimated that 578 million people will be affected by the year 2030, over 240 million by the year 2040 and over 700 million will be diabetic by the year 2050 [5,6]. The low- and middle-income countries, especially in the African continent, bear over 80 % of the diabetes burden [6–8]. Worryingly, the International Diabetes Federation (IDF) projects over a 143 % increase in DM incidences in Africa, from the current 19 million to over 47 million by the year 2045 [5,6,9].

Unfortunately, the management of DM is a global conundrum due to the lack of curative therapies [10]. Several drugs such as biguanides, inhibitors of α-glucosidase, thiazolidinediones, sulfonylureas, non-sulfonylureas secretagogues, and insulin that are currently prescribed for DM are palliative in nature with a limited usage timeframe. Furthermore, the costs of anti-diabetic drugs are beyond the reach of many diabetic persons living in rural regions of low-income countries [11]. Worse still, the synthetic oral hypoglycemic agents cause adverse side effects like brain atrophy, anorexia nervosa, fatty liver, among others, thus further exacerbating diabetes complications in affected patients [12,13]. Therefore, the search for novel, accessible, affordable, and safe remedies for DM is warranted as an urgency [8].

Recently, the search for novel anti-diabetic drugs has focused on medicinal plants due to their easy availability, accessibility, affordability, cultural acceptability, efficacy, and fewer side effects associated with their use [14–20]. Despite the longstanding utilization of medicinal plants or their products to manage DM, only a few of them have been scientifically scrutinized for their anti-diabetic activities. As a result, the World Health Organization recommends the scientific investigation of ethnomedically utilized plants for the management of DM [21–23].

Traditional medicine practitioners of Kenya use various plants such as *Pentas zanzibarica* (Klotzsch) Vatke and *Olea europaea* subspecies *africana* (Mill.) to treat DM [24–28]. *Pentas zanzibarica* is a herb of the Rubiaceae family that grows up to 2.6 m tall along forest edges, grassland, especially in sub-Saharan Africa [29]. Locally, *P. zanzibarica* is known as 'Mdimi' among the Shambaa and 'Mdobe' in the Digo community [30]. Traditionally, the juice of the pound leaves of *P. zanzibarica* is mixed with little water and as a drastic purgative. Additionally, a leaf decoction of *P. zanzibarica* is taken to manage the blood sugar level [30]. On the other hand, a root decoction is taken as a remedy for gonorrhoea and syphilis. Besides, a tea preparation of roots and leaves is given to children as a tonic (the decoction of the roots may be mixed with skimmed milk) [30]. Besides, the root is used with other plants in the treatment of cerebral malaria, headaches, rheumatic pain, gonorrhoea, and [31]. Previously, the ethanolic and leaf extracts of *Pentas spp.* have been demonstrated to possess inhibitory properties against hyaluronidase, phospholipase A_2, and protease, antiplasmodial, and antifungal activities [32,33].

Olea europaea subspecies *africana* (Mill.) is a shrub or tree in the Oleaceae family, which grows 3-24 m tall [34]. It is found in dry upland evergreen forests, in woodlands, and on lava flows. In Kenya, it is known as 'Mutamaiyu' or 'Mutero' in the Agikugu, 'Ol-Orien' among the Maasai, 'Muthata' in Ameru, 'Molialundi' in the Akamba, and 'Lorien' among the Dorobo communities [30,35]. Traditionally, the bark infusion or decoction of *O. europaea* subspecies *africana* is drunk as an antihelminth against tapeworm infestation. The bark decoction is used in a steam bath while some are drunk for the treatment of itchy rash. On the other hand, the leaf decoction is taken to treat hepatic diseases and control blood sugars [30]. Previous studies show that *O. europaea* subspecies *africana* has antihypertensive, anticancer, anti-inflammatory, antibacterial, and antioxidant activities [36,37]. Despite the ethnomedical usage of *P. zanzibarica* and *O. europaea* subspecies *africana* in the management of blood sugar levels among other complications associated with diabetes, these plants have not been empirically evaluated, hence the present study.

MATERIALS AND METHODS

Collection and processing of plant materials

The fresh leaves of *P. zanzibarica* and *O. europaea* subspecies *africana* were harvested from Gachoka Division, Embu County, Kenya, in their natural habitat, based on their ethnomedical usage. A taxonomist at the Department of Plant Sciences, Kenyatta University, authenticated the collected plant samples and assigned them voucher specimen numbers (*Pentas zanzibarica* - PZC-002.03/2004; Olea europoea subspecies *africana* - OEC-003.01/2004). Voucher specimens were prepared, and duplicates were deposited for future reference. The collected

leaves were separately spread on a bench at the Biochemistry Laboratory and regularly grabbled to dry uniformly for two weeks. The dried plant materials were separately ground using an electric plant mill (Christy and Norris Ltd., England) into powders, and stored in well-labelled khaki envelopes awaiting aqueous extraction.

Aqueous extraction procedure

The method described by Harborne [38] was followed. Briefly, 100 g of each powdered plant material was indirectly heated for 60 minutes, in 1 liter of distilled water in a water bath set at 60°C for 1 hour. Then, the mixtures were cooled to room temperature, decanted carefully, and filtered through folded cotton gauzes three times into dry conical flasks. The filtrates were transferred into clean flasks and freeze-dried using a Modulyo freeze dryer (Edwards, England) for 48 hours. The dry-lyophilized extracts were weighed and stored in clean vials at -20°C awaiting biological and chemical investigations.

Preparation of appropriate doses the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana*

Three dose levels (50 mg/kg body weight,100 mg/kg body weight, and 150 mg/kg body weight) of the studied plant extracts were selected for the determination of anti-diabetic activity after a pilot study. The experimental dose preparation guidelines of Erhierhie *et al.* [39]. Briefly, 125 mg, 250 mg, and 375 mg of respective extracts were dissolved in 10 mL of physiological saline to obtain 50 mg/kg body weight,100 mg/kg body weight, and 150 mg/kg body weight, respectively, for administration into mice. Besides, 25 insulin units of insulin were diluted in 100 mL of normal saline to give 1IU/kg body weight for administration. The studied plant extracts were administered orally (*p.o*) while insulin was administered intraperitoneally (*i.p*) at a volume of 0.1mL into mice.

Experimental animals

In this study, the experimental animals were 3-4 weeks old male Swiss albino mice with an average body weight of 24±2 g. The mice were bred and kept at 25 ºC, with a 12-hour/12-hour day/night cycle in the animal house of Biochemistry, Microbiology, and Biotechnology Department of Kenyatta University. The mice were offered standard rodent pellets and water *ad libitum.* The experimental mice were acclimatized for 72 hours before the assay.

The experimental animals were fasted for 12 hours but allowed offered drinking water *ad libitum* before inducing hyperglycemia. Alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil; 10 %) (Sigma, Steinheim, Switzerland), at a dose of 150 mg/kg body weight, was intraperitoneally administered into all mice except to those of the normal control group to induce diabetes (hyperglycemia). The normal control mice were administered with physiologic saline at a dose level of 10 mL/kg body weight orally. Hyperglycemia was confirmed in experimental mice on the fourth day after alloxan administration by the fasting blood glucose levels of ≥150 mg/dL, measured using a glucometer (Hypoguard, Woodbridge, England) [40].

This study was approved by the Department of Biochemistry, Microbiology and Biotechnology ethical review committee of Kenyatta University and research permit was granted by the Kenya National Commission for Science, Technology and Innovation (NACOSTI/P/16/7212/132765), and did not involve human subjects. Appropriate protocols for experimental animal handling, use, and disposal [46] were followed. Furthermore, all the chemicals and reagents used in this study were handled and disposed of according to the set procedures and manufacturers' material safety data sheets (MSDS) and instructions.

Experimental design

In this study, a completely controlled randomized study design was adopted from which an experimental design was derived. Mice were randomly assigned into six treatment groups, each consisting of 5 mice. The normal control group mice were administered with physiologic saline only. The negative (diabetic) control group mice were administered with alloxan and physiologic saline. The positive control group mice received alloxan and insulin. Experimental groups 1, 2 and 3 were administered with 50 mg/kg body weight, 100 mg/kg body weight, and 150 mg/kg body weight, respectively, of the respective studied plant extracts. This experimental design is summarized in Table 1.

Table 1. Experimental design for the determination of the hypoglycemic activity of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* Subspecies *africana* in Alloxan-induced diabetic mice.

Treatment Groups	Treatment
Normal control	Physiologic saline only (10 mL/kg body weight; *i.p*)+ Physiologic saline (10 mL/kg body weight; *p.o*)
Negative control	Alloxan (150 mg/kg body weight; *i.p*) + Physiologic saline (10 mL/kg body weight; *p.o*)
Positive control	Alloxan (150 mg/kg body weight; *i.p*) + Insulin (1 IU/kg body weight; *i.p*)
Experimental group 1	Alloxan (150 mg/kg body weight ; *i.p*) + Extract (50 mg/kg body weight ; *p.o*)
Experimental group 2	Alloxan (150 mg/kg body weight ; *i.p*) + Extract (100 mg/kg body weight ; *p.o*)
Experimental group 3	Alloxan (150 mg/kg body weight ; *i.p*) + Extract (150 mg/kg body weight ; *p.o*)

Extract: Aqueous leaf extract of *P. zanzibarica* or Aqueous leaf extract of *O. europaea* subspecies *africana*; *i.p*: Intraperitoneal injection; *p.o*: *Per os* (oral route of administration). The tails of experimental mice were swapped with 70 % ethanol, and blood samples were collected at the start of the experiment and repeated after 1 hour, 2 hours, 3 hours, and 4 hours. A glucose analyser model (Hypoguard, Woodbridge, England) was used to measure blood glucose levels.

Energy dispersive x-ray fluorescence spectroscopy (EDXRF System) analysis

The previously described method of Hdyhska [41] using the EDXRF system comparing an X-ray spectrometer and a radioisotope excitation source, was adopted to determine the concentration of various elements (manganese (Mn), copper (Cu), iron (Fe), strontium (Sr), nickel (Ni), molybdenum (Mo), zinc (Zn), and Lead (Pb)) in the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* Subspecies *africana*. Briefly, one gram of each studied plant extract was accurately weighed and pressed using a press pellet machine to obtain 2-3 pellets of 300-1000 mg/cm^2 for analysis The radiation from the radioactive source, [Cd^{109} (T$_{1/2}$= 453 days, and activity =10 mCi)] was incident on the sample emitting distinct X-rays, which were were distinguished by a Si (Li) detector (EG&G Ortec, 30mm^2×10mm sensitive volume, 25 μm Be window) with an energy resolution of 200 eV at 5.9keV Mn K$_\alpha$–line. The spectral data for analysis were collected using a personal computer-based Canberra S-100 multichannel analyser (MCA). The acquisition time for EDXRF quantification was set at 1000 seconds. The X-ray spectral analysis and quantification were done using IAEA QXAS software (QXAS 1992), based on the Fundamental Parameters Method (FPM) [42,43].

The atomic absorption spectrophotometric (AAS) technique was used to quantify the concentration of magnesium (Mg), chromium (Cr), and Vanadium (V) in the studied plant extracts using the AAS equipment (Model: 210VGP; Scientific Equipment) [44,45].

The glassware (beakers, funnels, volumetric flasks, measuring cylinders, pipettes, and burettes) used in this study, were meticulously cleaned in a mixture of concentrated nitric acid, concentrated hydrochloric acid, and detergent in deionized triple-distilled water. They were then rinsed and dried in a hot-air oven maintained at 105 ^{0}C for 2 hours, then were removed and kept in a clean and dry place awaiting use. Cleaning and drying of glassware were done routinely before elemental analysis.

Standard stock solutions of Cr and Mg (1000 ppm) were used as provided by the manufacturer (Aldrich Chemical Co., Inc). Besides, 1.7852 g of Vanadium pentoxide was accurately weighed and dissolved in a small amount of concentrated sulphuric acid, and then gently heated for complete dissolution. The solution was cooled and transferred into a clean volumetric flask and made up to 1 liter with triple-distilled deionized water. This gave a Vanadium stock solution of 1000 ppm for use.

New standard stock solutions were prepared freshly before each analysis. Briefly, appropriate volumes of the standard stock solutions of the analyzed elements were transferred into clean volumetric flasks (100 mL capacity). The flasks' contents were made up to the mark with triple triple-distilled deionized water and mixed well. Furthermore, blank solutions comprising all the reagents except the target elements were prepared and used to control for background noise.

Lanthanum chloride (12.6263 g) was dissolved in triple-distilled water to obtain a working solution of 50 mg/mL. After that, triple-distilled water was added into the prepared solution in a 250 mL volumetric flask, thoroughly mixed and used for quantification of magnesium in the studied plant extracts.

Preparation of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* for V, Mg, and Cr determination

The wet oxidation method was used to solubilize the studied plant extracts. Briefly, 1 g of each extract were separately transferred into Pyrex beakers (100 mL capacity), after which concentrated nitric acid (10 mL) was added. The contents were mixed well, and three milliliters of 60 % perchloric acid was added. The mixtures were initially warmed up to rid frothing and later evaporated to remove the nitric acid. Upon charring, the mixtures were brought to room temperature, and after the addition of concentrated nitric acid (10 mL), the solutions were heated further till the appearance of white fumes of perchloric acid. The resultant solutions were cooled, and then 25 % hydrochloric acid (25 mL) was added and filtered through Whatman filter papers into 100mL-volumetric flasks. Triple-distilled water was then added into each volumetric flask to the mark and then mixed well. The procedure was repeated thrice for each studied plant extract, and analysis was done immediately.

Determination of Cr, V, and Mg in the studied plant extracts by atomic absorption spectroscopy (AAS)

The solutions of the digested extracts were aspirated at a volumes 1mL of into volumetric flasks (100 mL capacity) and topped up to the mark using triple-distilled water. Similarly, 5 mL of lanthanum solution was transferred into a 100 mL flask and made up to the mark with triple-distilled deionized water. Besides, the digested sample solutions were analyzed for Cr and V without further dilution. The appropriate analysis conditions for each studied element were set in the AAS instrument, and the standard stock solutions and the samples were aspirated in turns into the flame whereby respective absorbances were measured. A minimum of four standard solutions were aspirated after 6-10 sample analyses to monitor and maintain the stability of the assay conditions. The flame was always flushed using triple-distilled deionized to re-establish the baseline (zero absorbance). The obtained absorbances were converted to concentrations, which were then corrected by multiplying with respective dilution factors. The amounts of the analyzed elements were expressed as µg/g dry matter of sample.

Qualitative phytochemical screening

The qualitative phytochemical composition of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* was determined as per the standard phytochemical screening methods described by Harborne [38].

Test for Alkaloids

About 2 g of the studied plant extracts were transferred into boiling tubes into which 2 mL of 1 % aqueous concentrated hydrochloric acid (HCL). The solutions were heated in a boiling water bath for 10 minutes and then filtered while hot. The filtrates were treated with Dragendorff's reagent. The appearance of turbidity or precipitation is an indication of the presence of alkaloids.

Test for Sterols and Terpenoids

Approximately 2 g of the studied extracts were washed with *n*-hexane, after which the resultant residues were extracted with 2mL of dichloromethane and dehydrated over anhydrous sodium sulphate. Afterward, 0.5mL acetic acid anhydride was added, followed by the addition of two drops of concentrated sulphuric acid. The appearance of green to blue colour is indicative of the presence of sterols, whereas a colour change from pink to purple indicates the presence of terpenoids.

Test for Saponins

About 1g of the studied plant extracts were transferred into clean test tubes. Then, 2 mL of distilled water were added, and the mixture was shaken vigorously for 30 seconds. The existence of persistent frothing (≥30 minutes) is an indication of the presence of saponins in the test sample.

Test for Flavonoids

About 1g of the studied plant extracts were washed in 5 mL thrice of *n*-hexane to remove fat and then in 4 mL 80% methanol before filtering. The resultant filtrates were divided into two portions and treated were treated as follows: To the first portion of the filtrate (2 mL), 1 mL of 1% aluminum chloride (prepared in methanol) was added and gently shaken. The occurrence of yellow colour indicates the presence

of flavanols, flavones, and chalcones in the test sample. Into the second portion (2 mL), 1mL of 1% potassium hydroxide was added, and the contents were shaken gently. The appearance of a dark yellow colour indicates the presence of flavonoids in the test sample.

Test for Tannins

The studied plant extracts (1g each) were transferred into clean test tubes, and 2 mL of distilled water was added. The mixtures were shaken and filtered through Whatman filter paper No 1. Afterwards, three drops of 5 % ferric chloride were added into the filtrates and swirled. The occurrence of a blue-black to green precipitate indicates the presence of tannins in the sample.

Test for Anthraquinones

About 2 g of the studied plant extracts were extracted with 10mL of benzene and filtered. Into the respective filtrates, 5 mL of 10 % ammonium hydroxide was added and mixed well. The appearance of violet colour in the ammoniacal phase indicates the presence of free anthraquinones in the sample. Similarly, 2 g of benzene-extracted samples were boiled with 10 mL of 1 % HCl and filtered while hot. Then, 10 mL of benzene were added, and the contents were mixed before separating the benzene layer. Afterwards, 5 mL of 10 % ammonium hydroxide was added and gently mixed. The occurrence of a violet colour indicates the presence of bound anthraquinones in the test sample.

Data management and statistical analysis

Anti-diabetic activity and elemental analysis data were first tabulated on an Excel spreadsheet (Microsoft 365) and then exported to Minitab v19.2 statistical software for analysis. The data were subjected to descriptive statistics, after which the results were presented as $\bar{x} \pm SEM$. One-Way ANOVA was performed to determine significant differences among means, followed by Tukey's test for pairwise comparison and separation of means. The comparisons between the two extracts were done using the unpaired student t-test statistic. In each case, *p<0.05* was considered statistically significant. Qualitative phytochemical screening data were only tabulated.

RESULTS

Effects of the aqueous leaf extract of *P. zanzibarica* and *O. europaea* subspecies *africana* on blood glucose levels in alloxan-induced diabetic mice

The blood glucose levels in the normal control mice significantly reduced between zero hour (0 h) and the first hour (1st h) (p<0.05); however, no significant differences in blood glucose levels were observed after the second hour (2nd h) through to the fourth hour (4th h) (p>0.05; Tables 2 and 3). Besides, the negative control mice recorded significantly higher blood glucose levels than those recorded in all the other experimental mice (p<0.05), and significantly increased from 0 h through to the 4th h (p<0.05; Tables 2 and 3). Additionally, the blood glucose levels in the positive control group mice significantly decreased in a time-dependent manner from 0 h through to the third hour (3rd h) (p<0.05; Tables 2 and 3); however, the blood glucose levels in the positive control group mice recorded at the 2nd h and those recorded in the 4th h were not significantly different (p>0.05; Tables 2 and 3).

Notably, the fasting glucose levels of mice that were administered with the aqueous leaf extract of *P. zanzibarica* significantly decreased in a dose-dependent manner at each hour across the four-hour experimental period (p<0.05; Table 2), except at 0 h where the differences in blood glucose levels were not significantly different (p>0.05; Table 2). Moreover, the recorded blood glucose levels in mice treated with the aqueous leaf extract of *P. zanzibarica* significantly decreased in a time-dependent fashion from 0 h through to the 4th h (p<0.05; Table 2).

Besides, the positive control group mice recorded significantly lower blood glucose levels than the experimental mice that were treated with the aqueous leaf extract of *O. europaea* Subspecies *africana* and those in the negative and normal control groups at the 1st h, 2nd h, and 3rd h, respectively (p<0.05; Table 3). No significant differences in blood glucose levels of experimental mice that were administered with the aqueous leaf extract of *O. europaea* Subspecies *africana*, all three dose levels, and those in the positive and negative control group were observed at 0 h (p>0.05; Table 3).

Notably, dose-dependent reductions in blood glucose levels were observed in experimental mice that were administered with the aqueous leaf extract at the 1st h, 2nd h, 3rd h, and 4th h, respectively (p<0.05; Table 3).

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Additionally, the experimental mice that received the aqueous leaf extract of *O. europaea* Subspecies *africana* had significant reductions in blood glucose levels in a time dependent manner from the 1st h through to the 4th h (p<0.05; Table 3).

Table 2. Effects of the aqueous leaf extract of *P. zanzibarica* on blood glucose levels in alloxan-induced diabetic mice.

Treatment group	Blood glucose levels (mg/dL)				
	0 h	1st h	2nd h	3rd h	4th h
Normal control	$60.82\pm1.78^{b}_{a}$	$55.38\pm1.13^{e}_{b}$	$52.48\pm1.23^{e}_{c}$	$52.66\pm1.60^{b}_{c}$	$51.80\pm2.48^{b}_{c}$
Negative control	$170.86\pm4.39^{a}_{b}$	$199.38\pm1.64^{a}_{b}$	$210.16\pm1.19^{a}_{ab}$	$201.10\pm20.30^{a}_{b}$	$232.89\pm2.53^{a}_{a}$
Positive control	$165.31\pm1.77^{a}_{a}$	$151.32\pm1.21^{f}_{b}$	$148.10\pm1.31^{f}_{c}$	$46.92\pm1.26^{b}_{c}$	$151.25\pm1.36^{bc}_{b}$
Experimental group 1	$163.26\pm2.24^{a}_{a}$	$115.36\pm1.50^{b}_{b}$	$85.46\pm1.97^{b}_{c}$	$66.96\pm2.30^{b}_{d}$	$52.12\pm1.16^{b}_{e}$
Experimental group 2	$166.98\pm1.56^{a}_{a}$	$97.80\pm1.24^{c}_{b}$	$75.30\pm1.35^{c}_{c}$	$57.32\pm1.25^{c}_{d}$	$48.90\pm1.40^{c}_{e}$
Experimental group 3	$168.64\pm2.96^{a}_{a}$	$84.94\pm1.42^{d}_{b}$	$65.74\pm1.44^{d}_{c}$	$51.08\pm1.07^{d}_{d}$	$42.46\pm1.80^{d}_{e}$

Values are presented as $\bar{x} \pm SEM$; Values with different alphabet superscripts within the same columns, and those with different alphabet subscripts across the same rows, are significantly different (One-Way ANOVA with Fisher's LSD *post hoc* test). Normal control: Physiologic saline (10 mL/kg body weight); Negative control: Alloxan (150 mg/kg body weight; *i.p*) + Physiologic saline (10 mL/kg body weight; *i.p*); Positive control: Alloxan (150 mg/kg body weight; *i.p*) + Insulin (1 IU/kg body weight; *i.p*); Experimental group 1: Alloxan (150 mg/kg body weight ; *i.p*) + *P. zanzibarica* (50 mg/kg body weight ; *i.p*); Experimental group 2: Alloxan (150 mg/kg body weight; *i.p*) + *P. zanzibarica* (100 mg/kg body weight ; *i.p*); Experimental group 3: Alloxan (150 mg/kg body weight; *i.p*) + *P. zanzibarica* (150 mg/kg body weight; *i.p*).

Table 3. Effects of the aqueous root extract of *O. europaea* Subspecies *africana* on blood glucose levels in alloxan-induced diabetic mice.

Treatment group	Blood glucose levels (mg/dL)				
	0 h	1st h	2nd h	3rd h	4th h
Normal control	$60.82\pm1.78^{e}_{a}$	$55.38\pm1.13^{e}_{b}$	$52.48\pm1.23^{e}_{c}$	$52.66\pm1.60^{d}_{c}$	$51.80\pm2.48^{d}_{c}$
Negative control	$170.86\pm4.39^{a}_{b}$	$199.38\pm1.64^{a}_{b}$	$210.16\pm1.19^{a}_{ab}$	$201.10\pm20.30^{a}_{b}$	$232.89\pm5.53^{a}_{a}$
Positive control	$165.31\pm1.77^{a}_{a}$	$51.32\pm1.21^{f}_{b}$	$48.10\pm1.31^{f}_{c}$	$46.92\pm1.26^{e}_{c}$	$51.25\pm1.36^{d}_{b}$
Experimental group 1	$165.74\pm3.66^{a}_{a}$	$94.08\pm1.35^{b}_{b}$	$84.34\pm1.41^{b}_{c}$	$71.56\pm1.48^{b}_{d}$	$65.26\pm1.46^{b}_{e}$
Experimental group 2	$158.14\pm3.49^{a}_{a}$	$79.66\pm1.38^{c}_{b}$	$69.78\pm1.33^{c}_{c}$	$61.70\pm1.54^{c}_{d}$	$53.68\pm1.48^{c}_{e}$
Experimental group 3	$161.66\pm2.19^{a}_{a}$	$73.42\pm1.24^{d}_{b}$	$66.00\pm1.32^{d}_{c}$	$54.96\pm1.43^{d}_{d}$	$44.48\pm1.35^{e}_{e}$

Values are presented as $\bar{x} \pm SEM$; Values with different alphabet superscripts within the same columns, and those with different alphabet subscripts across the same rows, are significantly different (One-Way ANOVA with Fisher's LSD test). Normal control: Physiologic saline (10 mL/kg body weight); Negative control: Alloxan (150 mg/kg body weight; *i.p*) + Physiologic saline (10 mL/kg body weight; *i.p*); Positive control: Alloxan (150 mg/kg body weight ; *i.p*) + Insulin (1 IU/kg body weight; *i.p*); Experimental group 1: Alloxan (150 mg/kg body weight; *i.p*) + *O. europaea* subspecies *africana* (50 mg/kg body weight; *i.p*); Experimental group 2: Alloxan (150 mg/kg body weight; *i.p*) + *O. europaea* subspecies *africana* (100 mg/kg body weight; *i.p*) ; Experimental group 3: Alloxan (150 mg/kg body weight; *i.p*) + *O. europaea* subspecies *africana* (150 mg/kg body weight; *i.p*).

Comparisons between the effects of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* on blood glucose levels in Alloxan-induced diabetic mice

Upon comparison, the results revealed no significant difference in blood glucose levels in mice that were treated with 50 mg/kg body weight of the aqueous leaf extracts of the the two studied plants at 0 h (p>0.05; Figure 1). However, at the same time (0 h), the mice that received the aqueous leaf extract of *O. europaea* subspecies *africana* at doses of 100 mg/kg body weight and 150 mg/kg body weight, respectively, recorded significantly lower blood glucose levels, than those that were administered with similar doses of the aqueous leaf extract of *P. zanzibarica* (p<0.05; Figure 1). Besides, at the 1st h, the mice that were treated with the aqueous leaf extract of *O. europaea* subspecies *africana*, had significantly lower blood glucose levels than those treated with the aqueous leaf extract of *P. zanzibarica* at all the three studied dose levels (p<0.05; Figure 1).

At the 3rd h, differences in blood glucose levels recorded in mice that administered with the two studied plants at a dose of 50 mg/kg body weight were not significant (p>0.05; Figure 1). However, at dose levels of 100 mg/kg body weight and 150 mg/kg body weight, the mice that received the aqueous leaf extract of *P. zanzibarica*, had significantly lower blood glucose

levels than those treated with similar doses of the aqueous leaf extract of *O. europaea* subspecies *africana*, in the 3rd h (p<0.05; Figure 1). On the other hand, the mice that received the aqueous leaf extract of *P. zanzibarica* at dose levels of 100 mg/kg body weight and 150 mg/kg body weight recorded significantly lower blood glucose levels than their counterparts that were administered with similar doses of the aqueous leaf extract of *O. europaea* subspecies *africana* in the 4th h (p<0.05; Figure 1). However, no significant differences in blood glucose levels were observed in nice that received 150 mg/kg body weight of the two studied plant extracts at the 4th h (p>0.05; Figure 1).
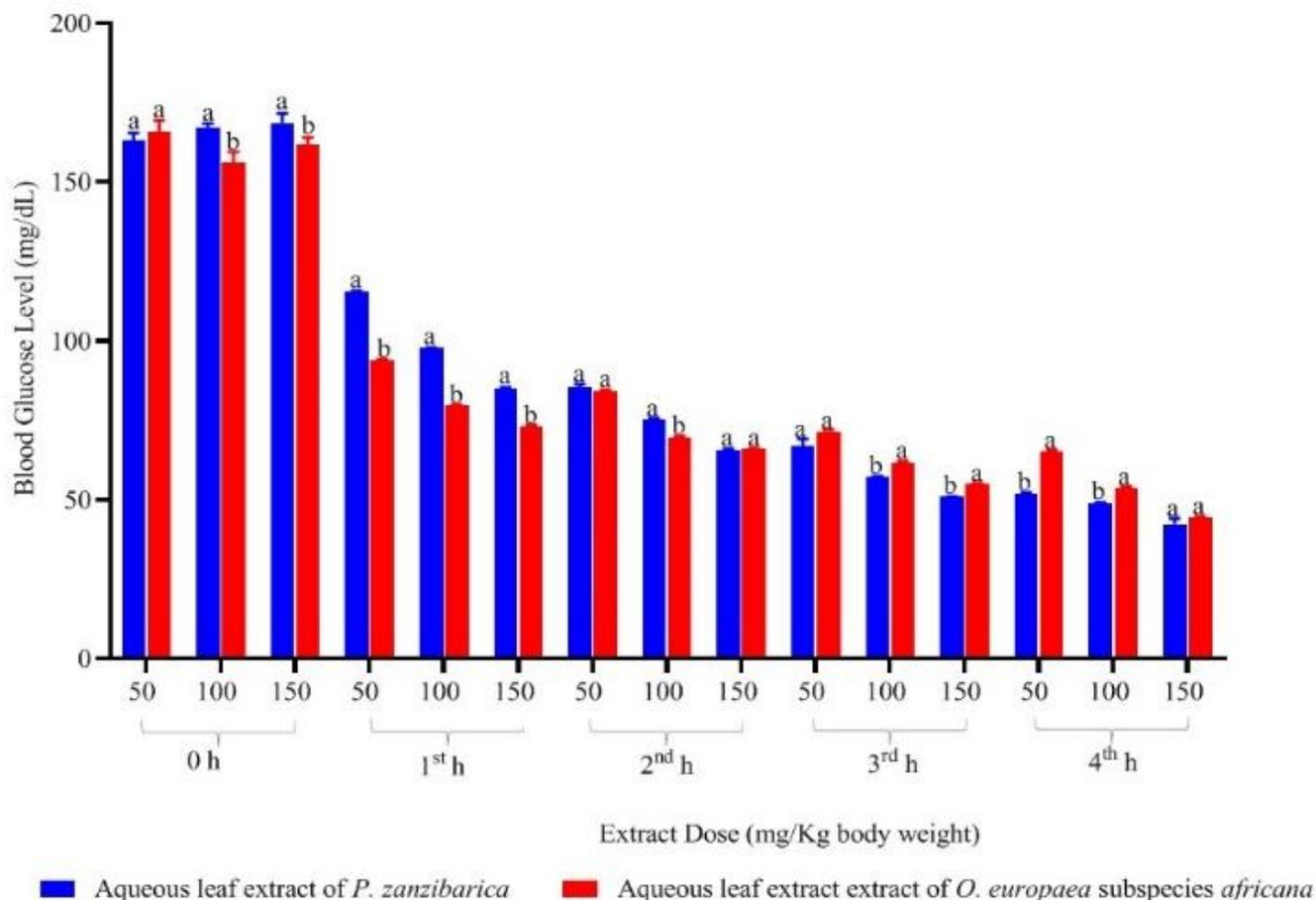
Figure 1. Comparison between the blood glucose levels in experimental mice treated with the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* measured at different periods. Bars with the same letter within the same dose level at each hour are not significantly different (p>0.05; Unpaired student t-test).

Elemental composition of the aqueous leaf extracts *P. zanzibarica* and *O. europaea* subspecies *africana*

Elemental analysis revealed significantly higher concentrations of Cr, Cu, and Fe in the aqueous leaf extract of *P. zanzibarica* than in the aqueous leaf extract of *O. europaea* subspecies *africana* (Table 4). Conversely, the concentrations of Mg and Mn were significantly higher in the aqueous leaf extract of *O. europaea* subspecies *africana* than in the aqueous leaf extract of *P. zanzibarica* (Table 4). However, Ni, Sr, and V were undetectable in both extracts of the studied plant extracts, while Mo was below the detection limit in the aqueous leaf extract of *P. zanzibarica* (Table 4).

Table 4: Elemental composition of the aqueous leaf extract of *P. zanzibarica* and *O. europaea* subspecies *Africana*.

Element	Concentrations in ppm (µg/g)	
	P. zanzibarica	*O. europaea* subspecies *africana*
*Cr	114.15±0.28[a]	90.30±0.72[b]
Cu	30.70±0.59[a]	8.10±0.06[b]
Fe	674.33±7.62[a]	131.00±1.15[b]
Ni	<5	<5
*Mg	3570.20±1.30[b]	3692.30±7.88[a]
Mn	18.0±0.58[b]	148.33±1.67[a]
Mo	<1	3.4±0.1
Sr	<1	<1
*V	<1	<1
Zn	91.13±2.08[b]	102.67±1.45[a]

Elements with an asterisk (*) were analyzed using AAS; <5: below the limit of detection (LOD) for EDXRF; <1: below the limit of detection for AAS; Values are presented as $\overline{x}$±SEM; means with different superscript alphabets across the rows are significantly different (Un-Paired student t-test; $p<0.05$).

Qualitative phytochemical composition of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana*

Qualitative phytochemical screening revealed the presence of saponins, flavonoids, sterols, terpenoids, tannins, and phenols, and the absence of alkaloids and anthraquinones in the aqueous leaf extract of *P. zanzibarica* (Table 5). On the other hand, Alkaloids, flavonoids, sterols, terpenoids, phenols, and anthraquinones were detected in the aqueous leaf extract of *O. europaea* subspecies *africana* (Table 5). However, tannins and saponins were absent in the aqueous leaf extract of *O. europaea* subspecies *africana* (Table 5).

Table 5. Qualitative phytochemical composition of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *Africana*.

Phytochemical class	*P. zanzibarica*	*O. europaea* subspecies *africana*
Alkaloids	-	+
Saponins	+	-
Tannins	+	-
Flavonoids	+	+
Phenols	+	+
Anthraquinones	-	+
Sterols	+	+
Terpenoids	+	+

+: Present; -: Absent

DISCUSSION

The complications of diabetes mellitus cause devastating morbidity and mortality, financial strain, and poor quality of life [47–49]. Furthermore, persistent hyperglycemia damages blood vessels, nerves, kidneys, eyes, among other organs and machinery of the body, resulting in devastating sequelae [48]. The currently available anti-diabetic therapies, including insulin and synthetic oral hypoglycemic drugs such as biguanides, sulfonylureas, and glinides [12], are inaccessible, unaffordable, palliative, and cause serious side effects [48,50–52]. Consequently, the search for potent, curative, safer, accessible, and affordable alternative antidiabetics is warranted. In light of this, we investigated the hypoglycemic activities, mineral element composition, and qualitative phytochemical phytochemistry of the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* as potential sources of accessible, affordable, curative, and safe anti-diabetic drugs.

Hyperglycemia (diabetes) was experimentally induced in Swiss albino mice using alloxan [40]. This chemical causes oxidative damage to the β-cells of the pancreas, thereby impairing insulin secretion and action resulting in hyperglycemia, which is characteristic in diabetes [40]. As a result, alloxan is widely used to screen for anti-diabetic efficacies of plant extracts and other drug agents [53–57]. Research has established that when alloxan (10 %) is administered intraperitoneally at a dose of 150 mg/kg body weight , into experimental animals, especially laboratory rodents, sufficiently causes hyperglycemia (≥150 mg/dL) [40,55–57]. Consequently, experimental drugs/ plant extracts that can deter or reverse hyperglycemia to normoglycemia are potential anti-diabetic therapies.

In this study, 10 % alloxan (150 mg/kg body weight) successfully and sufficiently induced hyperglycemia in experimental mice, as witnessed by the persistently high fasting blood glucose levels in the negative control group mice. Conversely, insulin effectively reversed hyperglycemia, as evidenced by significantly reduced blood glucose levels in the positive control group mice. Besides, the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* ameliorated alloxan-induced hyperglycemia in experimental mice in a dose- and time-dependent manner, demonstrating their anti-diabetic efficacy.

Additionally, the aqueous leaf extract of *O. europaea* subspecies exhibited better hypoglycemic efficacy between zero hours and the second hour, while the

aqueous leaf extract of *P. zanzibarica* demonstrated higher hypoglycemic efficacy after the second hour. Perhaps, the mode of action of the aqueous leaf extract of *O. europaea* subspecies *africana* could be like that of thiazolidinediones. In contrast, the mode of action of the aqueous leaf extract of *P. zanzibarica* could be related to that of biguanides and other similar antidiabetics. These findings corroborate those of earlier reports that indicate the appreciable hypoglycemic potential of various medicinal plants at various time points [15,54,57–60].

The anti-diabetic efficacy of medicinal plants is attributable to the presence of various bioactive principles, which protect the pancreatic cells against damage, foster the regeneration of the pancreatic β-cells, or modulate insulin secretion, activity, and sensitivity [61]. These bioactive principles could be specific mineral elements or phytochemicals that modulate various metabolic pathways at the cellular and molecular levels, thereby preventing, controlling, or reversing diabetes in affected patients. The present study's findings revealed the presence of antidiabetic-associated mineral elements in the aqueous leaf extracts of the studied plants, which may have played significant roles in averting alloxan-induced hyperglycemia in mice [62]. Research has indicated that Zn modulates the metabolism of proteins, carbohydrates, and lipids by targeting affecting various molecules and pathways [63]. For instance, Zn modulates the activity of glyceraldehyde-3-phosphate dehydrogenase in the glycolytic pathway to optimize energy metabolism [64,65].

Also, Zn is a critical regulator of insulin synthesis by the pancreatic β-cells and modulates its action on the responsive cells and tissues [65]. Furthermore, Zn quenches toxic free radicals and amends oxidative stress-associated damage in the pancreatic β-cells, thus promoting regeneration and proper functioning [65,66]. Our study's findings suggest that the concentrations of Zn in the studied plant were sufficient to thwart alloxan-induced oxidative damage to the pancreatic β-cells or to modulate insulin activity and metabolism in experimental mice.

Besides, Mg and Mn are cofactors of different oxidation and phosphorylation enzymes that mediate glucose metabolism in the body [67]. These minerals also facilitate insulin synthesis, proper binding on its cell-surface receptor, and its activity [68]. Moreover, studies have demystified the role of Mg and Mn in preventing and averting hyperglycemia by

modulating the synthesis, secretion, and activity of insulin in the body [67,69]. As a result, their deficiency has been implicated in insulin resistance and impaired metabolism, which are consequences of diabetes [67]. Therefore, the Mg and Mn quantities in the aqueous leaf extracts of the studied plants are partly accountable for the hypoglycemic properties reported herein.

Research has indicated that Cr enhances the expression of insulin receptors on the surface of target cells, thereby facilitating the binding of insulin to its receptor. This modulates the uptake of glucose by responsive cells, thereby deterring hyperglycemia [70]. Accordingly, previous studies show that Cr supplementation forestalls various symptoms associated with diabetes, such as polyuria, hyperglycemia, and fatigue [71,72]. Undeniably, the Cr concentrations in the aqueous leaf extracts of the studied plants may have played significant roles in either preventing or reversing alloxan-induced hyperglycemia in mice.

Furthermore, Cu facilitates the regeneration of pancreatic β cells following an assault, promotes lipogenesis, and regulates blood glucose levels [73]. Also, Cu has been shown to exhibit insulin-mimetic properties and promotes the activity of various enzymes involved in the metabolism, transportation, and utilization of glucose [74]. However, elevated amounts of Cu cause oxidative damage to cellular components and have been associated with hypercholesterolemia [75]. This study demonstrates that the concentrations of Cu in the aqueous leaf extracts of the studied plants were optimal and may have significantly contributed to their hypoglycemic efficacy without eliciting toxicity.

On the other hand, Fe overload causes hemochromatosis, thus inhibiting glucose metabolism and proper insulin functioning in the body [73]. Hemochromatosis damages the liver and impedes insulin's ability to regulate gluconeogenesis, glycogenolysis, and glucogenesis resulting in hyperglycemia [76,77]. Therefore, the quantities of Fe in the aqueous leaf extracts of the two studied plants did not limit their hypoglycemic effects.

Besides, medicinal plants synthesize secondary metabolites to counter biotic and abiotic stresses [78,79]. The medicinal value of plants is attributable to these secondary metabolites (phytochemicals) whose pharmacologic effects have been demonstrated [80,81]. Upon consuming medicinal plants or their products,

the phytoactive principles are delivered into the subject, thereby providing dietary and medicinal benefits [78]. In the present study, qualitative phytochemical screening of the aqueous leaf extracts of studied plants revealed the presence of hypoglycemic-associated phytochemicals, which may have partly contributed to the hypoglycemic efficacy. Alkaloids are nitrogen-containing secondary metabolites produced by medicinal plants and are widely known for their analgesic and anticancer properties. Research has indicated that berberine alkaloids drive glucose-stimulated insulin secretion in experimental diabetic rats by modulating the activity of nuclear factor 4α [82,83]. Besides, catharanthine, uropyranose, kinenoside, schulzeines, mahanimbine, among other alkaloidal compounds, have been shown to inhibit the activity of α-glucosidase. Furthermore, these alkaloids exhibit hypolipidemic, antihyperglycemic activities and promote the insulin hormone's proper functioning [84]. Indeed, the presence of alkaloids in the aqueous leaf extract of *O. europaea* subspecies *africana* played a critical role in the amelioration of hyperglycemia in experimental mice. However, the hypoglycemic activity of the aqueous leaf extract of *P. zanzibarica* may not be associated with alkaloids as they were absent.

Phenolic phytocompounds account for the antioxidant capacity of plants and exert broad pharmacologic effects by interacting with cellular machinery [85]. Consumption of polyphenolic-rich plants or their products has been shown to regulate the body's glycemic index by modulating the α-amylase activity [86]. Notably, gallic acid, betulic acid, chlorogenic acid, 4-hydroxy-3-methoxycinnamic acid, among other phenolics, possess antihyperglycemic and anti-diabetic activities when consumed [86–89]. Therefore, the presence of phenols in the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* could be responsible for their hypoglycemic effects.

Like phenols, flavonoids comprise polyphenolic compounds, which are made up of 15 carbons and two benzene rings linked by a 3-carbon linear chain [90]. Flavonoids possess antioxidant, antidementia, anti-inflammatory, anti-cancer, and anti-diabetic properties [90–92]. Previous research shows that quercetin, a flavonoid, modulates insulin secretion, promotes insulin action on target tissues, enhances glucose uptake, and facilitates the regeneration of pancreatic β-cells following alloxan-induced damage in experimental rodents [93,94]. Other flavonoid compounds like silymarin, 6-hydroxyapigennin,

chrysin, myricetin, among others, possess hypoglycemic activity and demonstrable antihyperglycemic activities and boost the synthesis and functioning of insulin [90,92,95]. Thus, the presence of flavonoids in the aqueous leaf extracts of the studied plants may have greatly contributed to their hypoglycemic efficacies in alloxan-induced hyperglycemic mice.

Research has also shown that terpenoids, glycosides, and sterols were detected in the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* and possess anti-diabetic activities by modulating insulin production, binding, and activity. Moreover, these phytoconstituents inhibit the α-glucosidase enzyme's activity, regulate glycemic index, and facilitate pancreatic β-cell recovery following Alloxan-induced oxidative injury [55,56,96,97]. Therefore, a combination of the anti-diabetic associated phytochemicals and mineral elements in the studied plant extracts could be responsible for the hypoglycemic effects we report for the first time in our study.

Owing to the ethnomedical usage of *P. zanzibarica* and *O. europaea* subspecies *africana* in the management of diabetes complications, especially hyperglycemia [30,31], the findings of our study demonstrate that indeed the aqueous leaf extracts of the two studied plants are potential sources of efficacious hypoglycemic agents. However, further empirical investigations should be conducted to fully establish their antidiabetic potential, safety and possibly develop potent, safe, accessible, and affordable anti-diabetic therapies.

CONCLUSIONS

Considering this study's findings, the aqueous leaf extracts of *P. zanzibarica* and *O. europaea* subspecies *africana* possess appreciable hypoglycemic activities in alloxan-induced hyperglycemic swiss albino mice. The aqueous leaf extracts of the studied plants contain phytoactive constituents and mineral elements associated with hypoglycemic activity. Further studies aimed at establishing the specific modes(s) through which the studied plant extracts exert hypoglycemic effects should be done. Moreover, isolation and characterization of the pure hypoglycemic bioactive molecules should be conducted for possible development. Additionally, studies focused on

extensive toxicological studies and safety evaluation of the studied plant extracts should be performed.

AUTHOR CONTRIBUTIONS

Gervason Moriasi conceived the research idea, performed the experiments, and wrote the manuscript under the close guidance and supervision of Mathew Ngugi and Cromwell Kibiti. All the authors read, reviewed, and approved the final manuscript for publication.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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