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Phytochemical and antidiabetic evaluation of the solvent fractions of *Coccinia barteri* Hook F (Cucurbitaceae) on Alloxan induced diabetic rats

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Abstract
The use of natural anti-diabetic medications in treating diabetes is receiving more attention daily. This study evaluated the phytochemical constituents and anti-diabetic properties of the leaves of *Coccinia barteri* on alloxan induced diabetic rats. The powdered leaf was macerated in methanol for 72hrs and then fractionated with ethyl acetate, chloroform and aqueous methanol solvents. Phytochemical and analytical evaluations were done using standard methods. The toxicity study was carried out on mice using Lorke's method. The Albino rats were divided into 8 groups of 4 rats each, 6 groups for the fractions and 2 for negative control and positive controls. Alloxan monohydrate (120 mg/kg) was used to induce diabetes. Treatment were with oral doses of the fractions (100 and 300mg/kg body weight) and Glibenclamide 5mg for 7days and their glucose levels were checked daily. The macroscopic and microscopic analysis showed dark green leaf, reticulate veination, smooth texture, fresh leafy odour, glandular trichome, wavy epidermal cell and anisocytic stomata. Analytical evaluation produced total ash (7.00), acid insoluble ash (0.93), water soluble ash (2.96), moisture content (8.40), alcohol extractive value (29.67), water extractive value (40.17) and ethyl acetate extractive value (21.00). The phytochemical analysis revealed flavonoids, alkaloids, steroids, glycosides and saponins are present. The anti-diabetic test revealed a significant decrease in the rat's fasting blood glucose especially in the aqueous methanol fraction (300 mg/kg). The least reduction was seen in chloroform fraction (100 mg/kg).The phytochemical constituents *C.barteri* has good anti-diabetic effects and can be used for further studies.

Keywords: *Coccinia barteri*, glibenclamide, alloxan, diabetes mellitus

Introduction
There is a growing interest in the increasing prevalence of non-communicable diseases (NCD) in Nigeria. Diseases with a lengthy duration and typically slower progression, also known as chronic diseases, NCDs are driven on by a combination of genetic, physiological, environmental, and behavioral factors [4]. Diabetes mellitus (DM), a complex metabolic ailment in which the pancreas generates insufficient amounts of insulin (type 1 DM), or in which a person's system fails to respond effectively to insulin, type 2 DM) is one of these diseases with an increasing prevalence in Nigeria [5] Glucose levels rise in the blood and urine of patients with diabetes mellitus, leading to increased urination, thirst, hunger, and abnormalities with the metabolism of both protein and fat (Ayoade, Oluwole and Barbara, 2009) [5]. A few chemotherapeutic drugs have been in use to manage Diabetes mellitus since the discovery of the hypoglycaemic action of sulfonamides. However, it has been highlighted that traditional medicine must be combined with orthodox medicine in order for effective health to be achieved in Africa [6]. Phytotherapy (herbal medicine) is highly regarded and used frequently in traditional African communities [7].
Currently, It has been reported that a number of plants are effective in the treatment of DM [8]. Many drugs and chemotherapeutic agents have been developed from these plants. Parts of plants such as the leaves, stems, roots, barks are being extracted in different ways to produce certain herbal formulations which are then packaged and used for medical purposes [9]. These plants' extracts have proven to be effective sources of antibiotics for a variety of bacterial and fungal diseases [10]. These plants are known to have various bioactive substances such as phytochemicals which have therapeutic potentials.

These phytochemicals are actively being researched for their potential direct application as therapeutic agents as well as their potential as prototype lead compounds for the creation of novel synthetic or semisynthetic medicines. One of such medicinal plant is *Coccina bartei*- a climbing perennial herb from the family *Cucurbitaceae*. *C. barteri* is a climbing perennial plant from the family *Cucurbitaceae*; also known as gourd, melon, and pumpkin family of flowering plants, belonging to the order *Cucurbitales* [11]. It is widespread in Asia and Africa and is renowned for bearing fruit throughout the year. *Coccinia barteri* occurs in evergreen forest [22]. It grows as a wild plant in several nations in west and east Africa's lowland rainforests and swamp forests. It is a 15-meter-long herbaceous climber with a tuberous rootstock [13]. *Coccinia barteri* (Hook f) is distinguished by unbranched tendrils that grow from the leaf axils and allow it to cling to supports. The leaves have a variety of shapes, are shiny, bright, and dark green in color.

Fig 1: *Coccinia barteri* (Hook f)

Traditional uses for the fruits of *C. barteri* include as food and medicine. The Coccinia plant's therapeutic applications date back to ancient times when the juice of the roots and leaves was used to treat diabetes, gonorrhea, and constipation [14]. In addition, various parts of the herb (including the leaves, stems, and roots) are traditionally used for the treatment of bronchitis, jaundice, burns, skin eruptions, fever, insect bites, allergy, eye infections, gonorrhoea, syphilis, etc. [15]. According to reports, *C.barteri* leaves have antioxidant qualities and a high concentration of phenolic and flavonoid components [16]. Pharmacologically, the anti-glycation and insulinotropic properties of *C. barteri* fruit have been examined [17]. Particularly, the fruits of the *C. barteri* plant, which are employed in cooking, have evolved into a pure source of herbal medicine. *C. barteri* is a very significant herb with known biological properties, as evidenced by the discovery of components having aldose reductase inhibitor and antioxidant capabilities [11]. In some regions of Nigeria and west Cameroon, the plant's cold infusion is used to treat venereal illnesses [3].

The number of Diabetes mellitus (DM) patients is increasing globally with 79% of they were residing in low- and middle-income nations [18]. According to a systematic and meta-analysis study conducted in Nigeria, the prevalence rate of diabetes mellitus among individuals aged 20 to 79 is 5.77%, accounting for more than 11.2 million cases [19]. In Nigeria, the DM-related death rate was reported to be 30.2 per 100,000 people in that year, with a case fatality rate of 22.0% [5].

Despite these alarming statistics, there is presently no specific and definite therapy for Diabetes mellitus management [7]. Although oral hypoglycaemic medications have been helpful in the management of diabetes mellitus, their usage is restricted due to their adverse effects and the heavy financial burden they impose on developing countries.

This study's primary goal was to evaluate the anti-diabetic properties of *Coccina barteri*'s methanol extract and its solvent fractions.

Materials, general reagents and detecting reagents

The general reagents and detecting reagents used are of analytical grade were obtained from scientific laboratory of Pharmacognosy Department, Enugu State University of Science and Technology. These include: Methanol 99.8%, Ethyl acetate, Chloroform, Ferric chloride, Million reagent, Alpha naphthanol, Benedict's solution, Dragendorff reagent, Dilute hydrochloric acid, Sodium hydroxide, Ammonia and Sulphuric acid. Material used Includes; the leaf of the plant, sterile distilled water, standard ant diabetic drug (glibenclamide), alloxan, portable animal cage, feeding syringe. Equpments used include Analytical balance, Conical flask, Accu check glucometer, Beaker, Measuring cylinders, Rotary evaporator, Vacuum pump, Water bath, Glass jar, Autoclave, Refrigerator, Whatman filter paper, Separating funnel, Bucket, Funnel, Grinder, Stirrer Spatula. The plant part used is Leaf of *Coccinia barteri*. Animals used includes; Albino mice and Albino Rats. The animals were acclimatized to the environmental conditions and were housed in aluminum cages. The animals were fed on provision of food and water orally for a week prior to the experiment. The leaves of *Coccinia barteri* were collected from Nru, in Nsukka, LGA of Enugu State, Nigeria in November, 2021 and authenticated by a taxonomist Mr. Felix of the Pharmacognosy Department in the University of Nigeria Nsukka. The collected leaves were ground into a coarse powder using a suitable grinder, dried at room temperature in the shade for approximately 13 days, and then weighed and stored in an airtight container in preparation for extraction.

Methods

1.35kg of the pulverized leaf was macerated in 5litres of methanol for 72 hours and then filtered with Whatman (No1) filter paper. The marc was rewashed until all the extractable constituents were completely washed out and then filtered. The filtrates were concentrated using a rotary evaporator, the weight was recorded and the percentage yield was calculated. The methanol extract (30 g) was washed with Ethylacetate, Chloroform and Aqueous methanol using column chromatography (liquid – liquid fractionation) and the individual fractions were concentrated with rotary evaporator set at 40 °C and 120 rpm and the resultant extracts were

further dried at room temperature. The dried extracts were tested for the presences of secondary metabolites such as alkaloids, steroids, tannins, flavonoids, glycosides, saponins [1] and also for ant diabetic assay [20] Using the Lorke-described standard approach, the acute oral toxicity of the plant extract in mice was assessed [3].

Diabetes induction in rat

9 groups of overnight fasted rats were selected randomly, each group consisting of 4 rats. The bodies of each rat was marked using marker on different parts of their body for easy identification after weighing. A dose of 120 mg/ml kg1 bodyweight of Alloxan monohydrate was dissolved in 2 ml of sterile normal saline and administered intraperitoneally to each of the rats in 8 groups, with 1 group receiving no treatment. Steady diabetes was confirmed the next day by measuring the blood glucose level before starting an experiment with the different solvent extract, which was >200 mg/dl.

Sensitivity study of fractions and standard drug

After the induction of diabetes, the positive control group was treated with 5mg/ml of the standard drug (Glibenclamide) while the negative control group (toxic group) was not treated. Groups D and E were treated with 100mg and 300mg/kg body weight of aqueous methanol fraction respectively while groups F and G were treated with 100mg and 300mg/kg body weight of Ethylacetate fraction respectively. To the remaining groups H and I, 100mg and 300mg/kg body weight of the chloroform extract was administered respectively. A Stock preparation of each extract/fraction dissolved in 1ml of water and 50mg of Glibenclamide in 10mls of normal saline were made separately. The required dose corresponding to each rats in each group was administered orally, daily for 7 days and their blood glucose level was measured after every 2 days of treatment using blood glucose test strips with Accucheck glucometer and was recorded.

Results

Table 1: Showing the phytochemical constituents of *Coccinia barteri*

S/N	Constituent	Methanol Extract	Ethyl Acetate	Chloroform	Acqueous Methanol
1	Flavonoid	+	+	+	+
2	Protein	_	_	_	_
3	Tannins	+	+	+	+
4	Reducing sugar	_	_	_	_
5	Saponins	+	+	+	+
6	Alkaloid	+	+	+	+
7	Glycosides	+	_	+	+
8	Steroids	+	_	+	+

Key: positive = + and negative = -

Table 2: Showing result of second phase of acute toxicity study using mice

Group A (1600mg/kg of mother extract)				
S/N	**Label**	**Weight (g)**	**Dose of extract (ml)**	**Remark**
1	Head	10.79	0.43	No death
Group B (2900mg/kg of mother extract)				
S/N	**Label**	**Weight (g)**	**Dose of extract (ml)**	**Remark**
1	Leg	10.60	0.54	No death
Group C (5000mg/kg of mother extract)				
S/N	**Label**	**Weight (g)**	**Dose of extract (ml)**	**Remark**
1	Tail	11.83	0.70	No death

Table 2: Showing the results for the sensitivity study of the fractions and standard drugs.

Group	Treatment	Dose	Before Induction	After induction	3 day	5 day	7 day
A	Normal Rat	Normal saline	74.00±5.35	75.75±4.03	75.75±3.60	74.50±1.29	74.50±5.07
B	Negative control	Normal saline	72.50±5.20	456.50±48.53	493.00±78.16	493.00±83.13#	496.75±85.34#
C	Positive 5mg/kg	5mg/kg	72.00±2.45	473.25±31.43	384.75±12.69	242.00±18.31*	146.50±7.14*
D	Aqueous methanol	100mg/kg	71.50±5.80	416±17.03	361.50±38.14	290.50±18.44*	190.00±12.73*
E	Aqueous methanol	300mg/kg	75.25±5.00	429.50±43.13	356.25±27.71	255.00±12.08*	187.75±18.87*
F	Ethylacetate	100mg/kg	70.25±1.71	407.25±5.62	352.25±13.20	287.50±23.73*	213.50±10.34*
G	Ethyl acetate	300mg/kg	72.25±1.71	414.25±57.89	328.00±36.94*	284.75±18.96*	208.00±19.65*
H	Chloroform	100mg/kg	70.00±2.45	437.00±97.21	410.25±130.80	386.00±142.87	280.00±82.00*#
I	Chloroform	300mg/kg	73.25±1.71	410.75±67.10	354.50±56.67	326.50±50.84*	245.50±45.68*

Control group; Results are expressed as mean ± SD. N = 36.Significant different is set at *p<0.05 when compared with negative control group at the same day (0, 3, 5 and 7 days); one-way, ANOVA followed by Turkey HSD.

* Represent a group where there is a significant difference in FBG level when compared with the negative control on same day while # represents a group where there is a significant difference in FBG level when compared with the standard drug on same day

Table 3: Showing results of the percentage blood glucose reduction of fractions *Coccinia barteri* in the anti-diabetic study.

Treatment groups	Dose mg/kg	Blood glucose level reduction (%)			Average percentage reduction (%)
		3day	5 day	7 day	
Normal Rat	Normal saline	0.00	1.65	1.65	1.10
Negative control	Normal saline	-8.00	-8.00	-8.82	-8.27
Positive 5mg/kg	5mg/kg	18.70	48.86	69.04	45.53
Aqueous methanol	100mg/kg	13.10	30.17	54.33	32.53
Aqueous methanol	300mg/kg	17.05	40.63	56.29	37.99
Ethylacetate	100mg/kg	13.51	29.40	47.58	30.16
Ethyl acetate	300mg/kg	20.82	31.26	49.79	33.96
Chloroform	100mg/kg	6.12	11.67	35.93	17.91
Chloroform	300mg/kg	13.69	20.51	40.23	24.81

Discussion and Conclusion

Phytochemical screening is usually carried out to show the constituents of the plant extracts and the one that predominates over the others. The qualitative phytochemical screening of the different extracts was performed to identify the main groups of secondary metabolites present using colour reactions or changes. The secondary metabolites present in the leaf of *Coccinia barteri* are flavonoids, tannins, saponins, alkaloids, glycosides and steroids. Those absent are reducing sugar and proteins. These secondary metabolites are responsible for the anti-diabetic properties of this leaf, they act either synergistically or independently to enhance the anti-diabetic activity of the leaf.

The lethal dose of the methanol crude extract of *Coccinia barteri* is greater than the dose of 5000mg/kg body weight in mice. This is due to the fact that no deaths were reported following oral administration of *Coccinia barteri* leaf extract at doses of up to 5000 mg/kg body weight of the animals tested.

After 24 hours of treatment, an increase in the animals' fasting blood glucose levels caused by the administration of alloxan at a dose of 120 mg/kg body weight of the animals was able to induce diabetes. Alloxan monohydrate was chosen as a diabetes-inducing substance because it causes irreversible damage to the insulin-secreting pancreatic beta cells, which reduces endogenous insulin release and causes Diabetes mellitus to develop after a single dose. By having a specific cytotoxic impact on pancreatic beta cells, alloxan causes hyperglycemia. One intracellular phenomenon that contributes to its cytotoxicity has been shown to produce free radicals both *in vivo* and *in vitro* [21].

The *Coccinia barteri* fractions' anti-diabetic assay reveals that, within a week of administration, all doses of the fractions and the usual medication significantly decreased the animals' fasting blood glucose (FBG). The result of the anti-diabetic study shows that of all the different doses of the fractions, high dose (300 mg/kg) of the ethyl acetate fraction has quickest onset of significant anti-diabetic action than the other group in comparison with the negative control by solely producing statistical different in reduction by Day 3. By the 5th and 7th day, all the treated groups produced statistical different reduction in blood glucose with the exception of low dose (100mg/kg) chloroform fraction which produced significant difference only at the 7th day thus having the least reduction in FBG compared to other fractions.

The percentage reduction of the fasting blood glucose in the anti-diabetic assay of different fraction of the plant *Coccinia barteri* reveals that higher dose of the fractions produced higher reduction in FBG. The positive control showed the highest reduction in the FBG on alloxan induced diabetic rats with average reduction of 45.53%. This is followed by 300mg/kg aqueous methanol with 37.99% reduction, 300mg/kg ethyl acetate with 33.96% reduction, then aqueous methanol 32.53% reduction, ethylacetate 100mg/kg with 30.16% reduction, chloroform 300mg/kg produced 24.81% reduction and finally chloroform 100mg/kg with the least reduction in FBG of 17.91%. The significant lowering of the FBG in alloxan - induced diabetic animals after administration of extract and fractions especially aqueous methanol fraction confirms that the plant *Coccinia barteri* has hypoglycemic activity. The studies also shows that all the fractions produced anti-diabetic effect to at least 17.91% as seen in the chloroform fraction (100mg/kg).

In conclusion, the analytical evaluation carried out can be used in the proper identification, collection and authentication of this plant, *Coccinia barteri*. The methanol leaf extracts and solvent fractions of *Coccinia barteri* showed anti-diabetic effects at various doses of the extract by reducing the fasting blood glucose on the alloxan induced anti-diabetic rats.

There is enough evidence to support the claim that the plant has anti-diabetic activity and more studies can be carried out on it to serve as part of a base in establishing a new drug.

Extensive studies should be carried out to further characterize the phytoconstituents, structure elucidation and microscopic analysis, and toxicity studies to ensure its safety and to prevent adulteration.

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