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PRELIMINARY STUDY OF THE HYPOGLYCEMIC ACTION OF THE EXTRACT OF LEAF OF *TELFAIREA OCCIDENTALIS* IN NORMOGLYCEMIC GUINEA PIGS

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ABSTRACT

Preliminary study of the hypoglycemic activity of cold ethanoic extract of *T. occidentalis* in normoglycemic guinea pigs was carried out.

Intraperitoneal dose of 250mg/Kg and 500mg/Kg body weight of extract were given to overnight fasted animals. The animals were sacrifised one hour after administration of extract. Serum glucose levels, determined by glucose oxidase method, were significantly different from the control at $P<0.1$ and 0.5 for animals dosed with 250mg/Kg and 500mg/Kg body weight, respectively. Phytochemical screening of the extract was conducted using standard procedures. The presence of alkaloids, saponin, glycosides, tannins and flavonoids were confirmed. Any or a combination of more than one of these constituents could be responsible for the hypoglycemic activity of the extract.

Keywords: *Telfairea occidentalis*, hypoglycemia, phytochemical screening.

INTRODUCTION

Telfairea occidentalis (Hooker) popularly known as fluted pumpkin (English), Ugu (Igbo), Ubong (Efik/Ibibio), (Gbile, Z. O., 1984) is widely cultivated for its palatable and nutritious leaves which are used mainly as vegetables (Sani, S. B., 1982). The leaves are rich in vitamins and flavours (Jeffrey, C., 1980 and Whitaker *et al*, 1979). It seeds which are also nutritious are eaten roasted or boiled like the seeds of breadfruits (Johnson and Johnson, 1976). The oil obtained from the seed is considered useful for candle and soap manufacture (Burkhill, H. M., 1979). The young leaves when stored in a bottle containing sodium chloride and coconut water is useful for the treatment of sudden attack of convulsion (Iwu, M. M.,1983). a review of some of the medicinal values of the leaves is given by Elizabeth Kafaru, 1998. It is generally believed that the leaves of *T. occidentalis* have antidiabetic activity. This belief has however not been investigated scientifically, thereby making this study relevant and necessary

MATERIALS AND METHOD

a. **Plant Material:** The leaves were collected from a garden in the University of Uyo main campus. Voucher specimen was deposited in the Faculty of Pharmacy Herbarium.

b. **Animals:** Guinea pig were purchased from the animal house, University of Port Harcourt, Nigeria. they were allowed free access to food and water.

c. **Extraction:** 1Kg of fresh leaves were cut into small pieces, ground with mortar and pestle and transferred into an amber-coloured bottle containing 1.5 litres of 96% ethanol. This was stored in a dark compartment for 3 days. The extract was filtered, concentrated in vacuo using a rotary flash evaporator and dried in a dessicator.

d. **Phytochemical Screening:** Chemical tests were carried out on the extract using standard procedures to identify the constituents (Trease and Evans, 1987)

(i) Test for Tannins – About 0.5g of fresh leaves were boiled with 20ml water and filtered. 4 drops of 0.1% Ferric chloride was added.

(ii) Test for Flavonoids – 1ml 10% lead acetate solution was added into 1ml of aqueous extract obtained as in (i) above.

(iii) Tests for Alkaloids – 5ml of 2% HCl was added to about 2ml of ethanolic extract. The solution was heated and thereafter filtered. 3 drops of the following reagents were added to 1ml of the filtrate:
Dragendorff, Mayer and Wagner reagents.
Thin layer chromatography was also conducted by spotting 2 plates (20 x 20cm, coated with Silica, 0.25mm) with the ethanolic extract. The plates were developed in Ethanol: ammonia (9:1) simultaneously. One of the plates was viewed under UV lamp and subsequently developed in iodine chamber. The other plate was sprayed with Dragendorff reagent.

(iv) Test for Saponins: 5ml of ethanolic extract was shaken vigorously. For confirmatory test, 2 test-tubes containing 1ml each of 20% blood in normal saline were prepared. To one was added 1ml of ethanolic extract and to the other (control) was added 1ml

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96% ethanol. The tubes were left to stand for 8 hours.

(v) Test for Steriods:
Lieberman – Burchard Test: 2ml acetic an hydride was added to 0.5g of ethanolic extract. The solution was cooled in ice. Conc. Sulphuric acid was carefully added by the side of the tube.

(vi) Test for Glycosides: About 0.1g of fresh plant material was soaked in water for 6 hours after which it was filtered. 5ml of 5% sulphuric acid was added to the residue and boiled for 5 mins. before filtering. 5% aqueous solution of sodium hydroxide was added to neutralise the mixture. Few drops of Fehling's solution was added.

(vii) Test for Cyanogenic Glycosides: 1g of powdered leaves was covered with water in a stoppered flask into which sodium picrate paper was suspended by trapping it with the cork. The flask was placed on a water bath for 1 hour.

(viii) Test for Cardiac Glyosides:
Keller – Killiani Test – 1ml of lead sub-acetate was added to 2ml of ethanolic plant extract. The solution was shaken and filtered. The filtrate was extracted with an equal volume of chloroform. The chloroform layer was evaporated to dryness in a dish over a water bath. Residue was dissolved in 3ml of 3.5% ferric chloride in glacial acetic acid. After about a minute, 1ml of conc. sulphuric acid was run down the side of the tube.

e. **Preparation and Administration of Extract**

(i) 2g of dried, resinous extract was diluted to 20ml with distilled water to form stock solution from which different volumes were taken and diluted to 1ml in order to obtain the required dose.

(ii) Grouping of Animals – Guinea pigs of both sexes (294 ± 95g) were fasted overnight for 12 hours prior to testing, but water was allowed ad libitum. The animals were divided into 3 groups: A, B and C comprising 5,5 and 6 guinea pigs, respectively. Group A served as control and received 1ml distilled water only. Group B and C received 1ml extract of leaves, administered intraperitoneally at arbitrary doses of 250mg/Kg and 500mg/Kg body weight, respectively.

(iii) Collection of Blood – Animals were sacrificed 60 minutes (arbitrarily determined) after administration of extract. 2ml of blood was collected from the heart into clean, dry test tubes. Blood samples were allowed to clot overnight in a refrigerator and centrifuged at 3000 x g for 10mins. to obtain the serum.

(iv) Determination of serum glucose level – this was done using glucose oxidase method (Trinder, P. 1969).

(v) Statistical Analysis – Mean values of serum glucose level was expressed with SEM and student's t-test to check their significance.

RESULTS AND DISCUSSION

(i) Phytochemical Screening:
Results of the Phytochemical screening is as shown in Table 1.

(ii) Thin Layer Chromatography:
Five spots (A, B,C,D and E) fluoresced under UV lamps with RF values of 0.08, 0.15, 0.29, 0.49 and 0.66, respectively. When sample plate was developed in iodine tank an additional spot (F) with RF of 0.9 was observed. Spots A and C with RF value of 0.08 and 0.29, respectively gave positive colour reaction (i.e. orange spots) when sprayed with Dragendorff reagents, indicating that constituents at spot A and C are alkaloids.

(iii) Screening for Hypoglycemic Activity:
Table II, shows the results of hypoglycemic screening. Group A (control) gave serum glucose level of 0.76 ± 0.06 mmol L^{-1}. Serum glucose levels for

TABLE I:

Phytochemistry of Leaf extract of *T. occidentalis*

	CONSTITUENTS	TEST	INFERENCE
A	Tannins	Ferric chloride test	Present
B	Flavonoids	Lead acetate test	Present
C	Alkaloid	(i) Dragendorff test (ii) Mayer test (iii) Wagner test (iv) Thin Layer Chromatography of extract sprayed with Dragendorff reagent.	Present Present Present Present
D	Saponin	(i) Frothing test (ii) Hemolysis test	Present Present
E	Steroids	(i) Salkowski test (ii) Lieberman-Burchardt test	Present
F	Glycosides	Fehlings solution test	Present
G	Cyanogenic Glycosides	Picric acid test	Absent
H	Cardiac Glycosides	(i) Keller Killiani test (ii) Legal test	Absent

TABLE II:

Serum Glucose Levels (mmol/L) of animals.

S/N	Control	Animals Treated With 250mg/Kg Extract	Animals Treated With 500mg/Kg Extract
1	0.822	0.520	0.355
2	0.723	0.546	0.644
3	0.677	0.822	0.434
4	0.743	0.644	0.401
5	0.822	0.598	0.440
6	-	-	0.473
Mean Glucose Level	0.76 ± 0.06	0.63 ± 0.12	0.46 ± 0.10

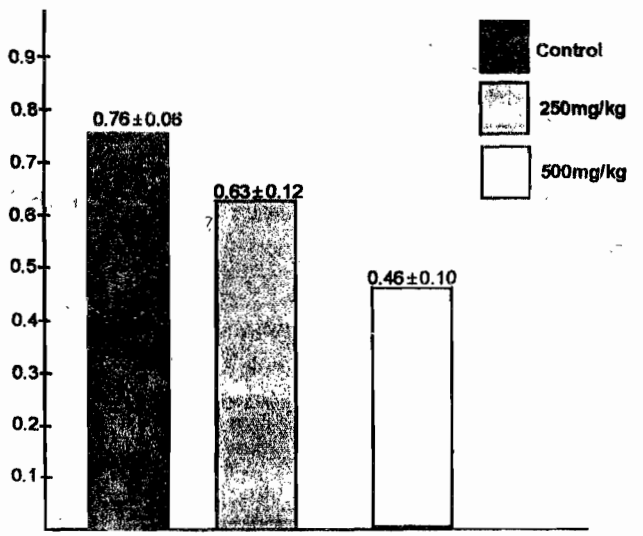

Figure 1: Serum glucose levels in the control and test animals

groups B and C are 0.63 ± 0.12 mmol L^{-1} and 0.46 ± 0.10 mmol L^{-1} respectively.

(iv) Statistical Analysis:
Statistical Analysis revealed that group B (250mg/kg) and group C (dose of 500mg/kg) were significantly different from group A (control) at $P<0.1$ and 0.05.

The present study shows that ethanolic leaf extract of *T. occidentalis* possesses hypoglycemic effect in normoglycemic guinea pigs, the effect being greater at a higher dose (500mg/Kg) than at 250mg/Kg. Figure I depicts this result. Active substance in various plants with hypoglycemic effects have been found to contain polysaccharides, flavonoids, glycoprotein, polypeptide, terpenoids, steriods and alkaloids (Refiye *et al*, 1998). The hypoglycemic activity of *T. occidentalis* leaves may therefore be related to any one of the following constituents: Flavonoids, saponin, steroids or alkaloids. Further investigation is in progress by our team to investigate the antidiabetic activity of leaves of this plant in alloxan-induced diabetic animals and to also identify the constituent(s) responsible for this activity.

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