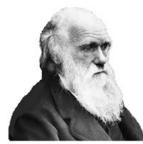
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## Evaluation of *in vitro* antidiabetic and antioxidant activities of different extracts of the root of *Telfairia occidentalis* Hook.fil. (Cucurbitaceae)

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#### ABSTRACT

The leaves of *Telfairia occidentalis* are widely used as vegetable in the preparation of soups and consumed as food although it can also be used as an infusion in medicinal preparations due to its various medicinal properties such as anti-anemic, antidiabetic and antimicrobial activities etc. The present study sought to undertake the screening and quantification of the secondary metabolites and in vitro antioxidant and antidiabetic properties of the different extracts of the root of Telfairia occidentalis. Phytochemical constituents were determined using standard procedures. Antioxidant activity of the extracts was evaluated using DPPH scavenging activity and the antidiabetic activity evaluated using hemoglobin glycosylation and glucose uptake by yeast. The results of the phytochemical screening showed that the root of T. occidentalis contains saponins and alkaloids and quantitatively 4.7% and 1.26% respectively. From the antioxidant evaluation, the different extracts of the root of T. occidentalis were able to scavenge DPPH free radicals in a concentration dependent manner. The antidiabetic evaluation showed that the different extracts could inhibit hemoglobin glycosylation. The different extracts also caused an increase in glucose uptake by yeast than glibenclamide when compared together with Sample C (99.8% ethanol soxhlet extract) having the highest of these properties. In conclusion, the root of *T. occidentalis* can be employed in the treatment of diseases in which participation of reactive oxygen species are implicated such as diabetes.

*Keywords*: Antidiabetic, Antioxidant, *Telfairia occidentalis*, DPPH Activity, Hemoglobin Glycosylation, Yeast Uptake

## **1. INTRODUCTION**

Diabetes mellitus is a group of metabolic disorders that is characterized by elevated levels of glucose in the blood (hyperglycemia) and insufficiency in production or action of insulin produced by the pancreas inside the body. (Maritim *et al.*, 2003) [1]. Insulin is a protein (hormone) synthesized in the beta cells of the pancreas in response to various stimuli such as glucose, sulphonyl ureas and arginine however glucose is the major determinant. (Joshi *et al.*, 2007) [2]. Long term elevation in blood glucose levels is associated with macro- and micro-vascular complications leading to heart diseases, stroke, blindness, kidney disease etc. The American Diabetes Association classified diabetes mellitus into four namely:

Type 1 (insulin dependent), Type II diabetes, Gestational diabetes, and Diabetes from other causes such as Monogenic diabetic syndromes, Drug or chemical-induced diabetes and Diseases of exocrine pancreas. However diabetes mellitus is majorly classified basically into two types namely: Type 1 and Type 2.

The global diabetes prevalence in 2019 was estimated to be 9.3% of which >90% was type 2 diabetes, and projections estimated an increase in the number of diabetes individuals to 578 million by 2030, 642 million by 2040 and 700 million by 2045. (Wang *et al.*, 2022) [3].

Free radicals are reaction chemical entities that are short lived species containing one or more unpaired electrons. Free radicals induce damage to cells by passing the unpaired electrons to the cell resulting in oxidation of cell components and molecules (Bansal and Bilaspuri, 2011) [4].

The body maintains a balance between antioxidant and pro-oxidants. A disturbance of this balance in favor of the pro-oxidants is known as oxidative stress. Hence excess formation or/and insufficient removal of highly reactive molecules such as reactive nitrogen species (RNS) and reactive oxygen species (ROS) is oxidative stress. Oxygen is highly reactive species that has the ability to become part of potentially harmful and damaging molecules (Free radicals) thus implicated in many disease conditions known today.

Since ancient times, humans have depended on herbs and medicinal plants as a source of food and remedy mainly because plants produce a large number of bioactive compounds which can protect against free radical damage and hence prevent diseases. (Swamy and Akhatar, 2019) [5]. The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionalized. (Airaodion *et al.*, 2019) [6]. Nature has been an important source of medicinal agent in the management of diabetes mellitus while also providing antioxidants to manage oxidative stress.

Herbal medicine is based on premise that plants contain natural substances that can promote health and alleviate illness such as flavonoids, alkaloids, tannins, steroids etc. It has been observed that the fruits and seeds of various plants in the cucurbitaceae family (such as the fruit of *Mormodica charanta* and the seed of *Mormodica cochinchinensis*) have antidiabetic effects, hence it is not surprising that the different parts of *Telfairia occidentalis* have been reported to have antioxidant and antidiabetic activities (Eseyin *et al.*, 2006, 2007, Nwanna *et al.*, 2007) [7-9].

The seeds and leaves of the fluted pumpkin (*T. occidentalis*) are healthy sources of lipids, vitamins, fiber and minerals such as iron, potassium, phosphorus and mineral salts. (Akpasi *et al.*, 2023) [10].

This study therefore investigates the phytochemical compositions, the *in-vitro* antioxidant and antidiabetic properties different extracts of *T. occidentalis* root.

## 2. SPECIMEN INFORMATION

The specimen (*Telfaria occidentalis* root) was obtained from the farm of the Pharmacognosy department of the Faculty of Pharmacy, University of Uyo having gotten permission from the Head of Department of the Department of Pharmacognosy. The specimen was then sent to the Herbarium of the Department of Pharmacognosy of the Faculty of Pharmacy, University of Uyo, where it was deposited and given a Taxonomic Serial Number: 505897.

## **3. MATERIALS AND METHODS**

The equipment used for the work includes: Water bath (selecta), Weighing balance (S. Mettler), UV-visible spectrophotometer (Labo Med Inc), Laboratory oven (Uniscope), Extraction tanks, Centrifuge.

#### Solvents, Reagents and Chemicals

Distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Chloroform (SCP), Ethanol (James Burrough Limited), Gallic acid, Folin-ciocalteu reagent, Ammonium hydroxide, n-butanol, Mayer's reagent, Dragendoff's reagent, Tannic acid, Hemoglobin, Glucose, Sodium carbonate, Acetic acid, Magnesium metal (Sigma-Aldrich), Acetic anhydride, Concentrated sulphuric acid, Ferric chloride, Acetone (BDH Chemicals Limited)

#### Collection and Identification of the Root of T. occidentalis

The fresh roots of *T. occidentalis* were collected from Itak in Ikono LGA of Akwaibom State on the 25<sup>th</sup> day of May 2019. Taxonomic identification and authentication were carried out by Professor Margaret Bassey of the Department of Botany, Faculty of Science, University of Uyo and taxonomic serial number given. The roots were washed, pounded in a mortar and dried under shade.

#### **Preparation of Extracts**

#### **Maceration Using 99.8% Ethanol**

The ground root (50 g) was weighed into an extraction tank. 1 litre of 99.8% ethanol was transferred to the container, shaken and covered. The mixture was allowed to stand for 3 days with intermittent shaking. After 3 days, the mixture was filtered using a white cloth and the filtrate dried over a water bath to obtain 99.8% ethanol extract and this was labeled Sample A.

#### **Maceration Using 50% Ethanol**

The ground root (50 g) was weighed into an extraction tank. 500 ml of water and 500 ml of 99.8% ethanol were mixed in a volumetric flask to obtain a 50% ethanol solution which was transferred into the tank, shaken and allowed to stand for 3 days with intermittent shaking. After 3 days, the mixture was filtered and the filtrate dried over a water bath to obtain 50% ethanol extract and this was labeled Sample B.

## Soxhlet Extraction Using 99.8% Ethanol

The ground root (20 g) was wrapped in a white cloth and placed in the tube of the soxhlet apparatus. 250 ml of 99.8% ethanol was transferred to a flat bottom flask and placed over a heating mantle and the soxhlet apparatus setup. The extract obtained was heated to dryness in a water bath to obtain a 99.8% ethanol soxhlet extract and this was labeled Sample C.

## Soxhlet Extraction Using 50% Ethanol

The ground root (20 g) was wrapped in a white cloth and placed in the tube of the soxhlet apparatus. 125 ml of water and 125 ml of 99.8% ethanol were mixed together in a volumetric flask to give a 50% ethanol solution which was transferred to the flat bottom flask of the apparatus and placed over a heating mantle and the apparatus was setup.

The extract obtained was heated to dryness in a water bath to obtain a 50% ethanol soxhlet extract and labeled Sample D

## **Decoction Extraction**

The ground root (50 g) was weighed into a beaker. 1 litre of water was transferred into it and boiled over a heating mantle for one hour. It was then filtered and dried in the oven to obtain an infusion extract and labeled Sample E.

## Phytochemical Screening of the Root of T. occidentalis

Phytochemical analysis of the plant sample was carried out based on the method of (Sofowora, 1993) [11].

## **Test for Saponin (Frothing Test)**

The ground root sample (0.1 g) was boiled with 5 ml distilled water for 5 minutes and decanted while still hot. 1 ml of filtrate was diluted with 4 ml of distilled water and the mixture shaken vigorously persistent frothing indicates the presence of saponin.

## Test for Alkaloids (Dragendorff's test)

The ground root sample (0.1 g) was boiled with 5 ml of 2% hydrochloric acid on a water bath, allowed to cool and then filtered. 1 ml of the filtrate was treated with 2 drops of Dragendorff's reagent. A reddish brown precipitate indicated the presence of alkaloids.

## **Test for Tannins (Ferric Chloride Test)**

About 0.5 g of the decoction extract was stirred with 10 ml of distilled water and filtered. Ferric chloride was added to the filtrate. A blue black, green or blue-green precipitate indicated presence of tannins.

## **Test of Phenols**

About 0.5 g of the decoction extract was treated with 2 ml of 1% aqueous ferric choride solution. A green colour indicates the presence of phenols.

## **Test of Flavonoids**

About 2 g of the ground root sample was heated with 10 ml of 5% ethyl acetate in a boiling water bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of 1% aluminum chloride and 1 ml of 1% dilute ammonia solution. Formation of a yellow coloration of ammonia layer indicates presence of flavonoids.

## Test for Cardiac Glycosides (Salkowski's Test)

Concentrated sulphuric acid (2 ml) was added to an aqueous solution of decoction extract. Formation of a reddish brown colour indicated the presence of steroidal aglycone part of the glycoside.

## **Test for Terpenoids**

Chloroform (2 ml) was added to 5 ml of aqueous solution of decoction extract. 2 ml of concentrated suphuric acid was added. Presence of red colour in the lower chloroform layer indicates presence of steroids.

## Quantitative Analysis of the root of Telfairia occidentalis

## **Determination of Alkaloids**

The ground root sample (10 g) was weighted into a 250 ml beaker. 100 ml of 1% acetic acid in ethanol was added to it. The mixture was shaken and allowed to stand for 4 hours before filtration. The mixture was filtered and the filtrate evaporated to <sup>1</sup>/<sub>4</sub> of the original volume. 1% concentrated ammonium hydroxide was added drop wise to precipitate the alkaloids. The precipitate was filtered, weighed, oven dried at 60 °C for 30 minutes and reweighed (Harbone, 1973) [12].

#### **Determination of Saponins**

The ground root sample (20 g) was weighed into a conical flask and 100 ml of 20% aqueous ethanol was added to it and heated at 55 °C in a water bath for 4 hours. The mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol for 30 minutes and filtered. The filtrates were combined and the volume reduced to 40 ml its original volume over a water bath at 75 °C. 40 ml of the concentrate was transferred into a separating funnel, 20 ml of diethyl ether was added and the aqueous layer obtained, this is repeated. The aqueous layer was transferred to the separating funnel and 60 ml n-butanol added to it. The n-butanol layer is obtained and washed with 10 ml of 5% sodium chloride and heated to dryness over a water bath and to constant weight in the oven. Saponin content was calculated as a percentage. (Obadoni and Ochuko, 2001) [13].

#### Determination of In-vitro Antioxidant Properties

## **DPPH Radical – Scavenging Activity**

From a stock concentration of 1 mg/ml of the standard (Ascorbic acid) and each of the extracts, 5 different concentrations of the standard and extract 20, 40, 60, 80 and 100  $\mu$ g/ml were prepared. 2 ml of each of the concentrations were transferred into 5 separate test tubes. This was followed by 2 ml of DPPH solution (0.04%) to each of the different concentrations in

the test tubes. The test tubes were incubated in a dark cupboard at room temperature for 30 minutes. Absorbance was then measured after 30 minutes using a UV-Visible spectrophotometer at 517 mm. The tests were performed in triplicate. The percentage inhibition of ascorbic acid and each of the extract was then calculated.

Percentage Inhibition (%) =  $Abb - Abs / Abb \times 100$ 

where: Abs = Absorbance of sample and Abb = Absorbance of blank (Liyana-Pathiana *et al* 2005)[14].

#### **Determination of Hemoglobin glycosylation**

## **Preparation of Hemoglobin**

Powdered hemoglobin (20 g) was weighed into a beaker. 100 ml of water was added to it gradually and stirred to form a suspension. It is then filtered.

## **Preparation of Glucose**

A stock concentration of 1% glucose solution was prepared by dissolving 0.1 g of glucose in 100 ml of water.

## **Preparation of Extracts**

Each of the extracts (0.2 g) was dissolved in 20 ml of the solvent used for extraction to give a 10 mg/ml concentration.

## **Estimation of Hemoglobin Glycosylation**

Different concentrations of glucose (10, 20, 30  $\mu$ g/ml) were transferred from the 1% glucose stock concentration. 2 ml of hemoglobin fraction was transferred into these test tubes followed by 2 ml of 99.8% ethanol extract. A control preparation was done for the different glucose concentrations and hemoglobin. The reaction started immediately and the test tubes were incubated inside a dark cupboard. The absorbance of the preparation was taken at 443 mm at 24, 48 and 72 hours respectively using a UV-Visible spectrophotometer. This procedure was carried out for the remaining extracts (samples) in triplicates. The percentage inhibition of hemoglobin glycosylation of the different extracts were calculated using the formula:

Inhibition of hemoglobin glycosylation (%) =  $Abs_{sample} - Abs_{control} / Abs_{sample} \times 100$ 

where: Abs<sub>control</sub> is the absorbance of the control reaction (not containing extract or drug) and Abs<sub>sample</sub> is the absorbance of the test samples. (Adisa *et al* 2004)[15].

## **Determination of Glucose Uptake by Yeast**

## **Preparation of Yeast Solution**

Baker's yeast (20 g) was dissolved in 100 ml of distilled water in a beaker. This was followed by centrifugation at a speed of 3000 rpm for 5 minutes. The supernatant was decanted and centrifuged again at the same speed and time until a clear supernatant was obtained.

## **Extraction of Drug (Glibenclamide)**

The drug Clamide by Hovid (20 tablets) were crushed in a mortar and extracted with two 50 ml acetone, filtered and the filtrate allowed to dry under room temperature and the extract weighed.

## **Preparation of Drug (Glibenclamide)**

The extracted drug after weighing was 0.63 g was then dissolved in 63 ml of water to give a concentration of 10 mg/ml.

## **Estimation of Yeast Uptake**

A stock solution of 1% glucose concentration was prepared and different dilutions of 10, 20, 30, 40, 50  $\mu$ g/ml prepared in different test tubes. 1 ml of yeast solution was transferred into each of the test tubes and vortexed. To another set of 5 test tubes containing different dilutions of glucose concentration (10, 20, 30, 40, 50  $\mu$ g/ml), 1 ml of yeast solution was transferred and vortexed. 1 ml of 99.8% ethanol extract solution was then added to each test tube and incubated in a dark cupboard for 60 minutes at room temperature. The absorbance was taken at 540 nm using a UV-Visible spectrophotometer. This procedure was repeated for the rest of the extracts and the drug. The percentage increase in glucose uptake by yeast was calculated using the formula:

Increase in glucose uptake (%) =  $Abs_{sample} - Abs_{control} / Abs_{sample} \times 100$ 

where,  $Abs_{control}$  is the absorbance of the control reaction (not containing extract or drug) and  $Abs_{sample}$  is the absorbance of the test samples. All the experiments were carried out in triplicates. (Cirillo, 1962) [16].

## **Statistical Analysis**

All results were expressed as mean  $\pm$  SEM and were analyzed by one-way ANOVA using MS excel 2016. P<0.05 was taken as significant.

## 4. RESULTS AND DISCUSSION

## **Phytochemical Screening**

The results of the phytochemical screening of *T. occidentalis* roots are presented in Table 1 below

**Table 1.** Results for the phytochemical screening of *Telfairia occidentalis* root.

| Plant Phytochemicals | Inference |
|----------------------|-----------|
| Alkaloids            | +         |

| Saponins           | + |
|--------------------|---|
| Flavonoids         | - |
| Steroids           | - |
| Tannins            | - |
| Phenols            | - |
| Terpenes           | - |
| Cardiac glycosides | - |
|                    |   |

Key: + = present, - = absent

## **Quantitative Phytochemical Analysis**

Results for quantitative analysis of *T. occidentalis* root extract: Saponins = 4.70%, Alkaloids = 1.26%

## **Antioxidant Evaluation**

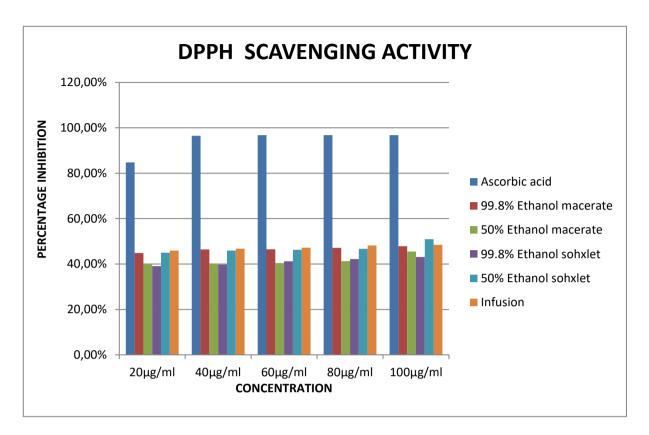
## **DPPH-Scavenging Activity Assay**

The results of the DPPH scavenging activity of Ascorbic acid and the 5 extracts at different concentrations are presented in Table 2 and their percentage inhibition presented in figure 1

| Concent | ration (µg/ml)   | Mean ±SEM<br>BLANK = 1.663 |          |          |          |             |
|---------|------------------|----------------------------|----------|----------|----------|-------------|
|         | Ascorbic acid    | Sample                     | Sample   | Sample   | Sample   | Sample      |
|         | Ascorbic actu    | А                          | В        | С        | D        | Е           |
| 20      | 20 0.252±0.04    | 0.916±0.                   | 1.001±0. | 1.012±0. | 0.914±0. | $0.900\pm$  |
| 20      |                  | 007                        | 02       | 01       | 02       | 0.02        |
| 40      | 0.059±0.04       | 0.892±0.                   | 0.995±0. | 1.002±0. | 0.899±0. | $0.885 \pm$ |
| 40      |                  | 007                        | 02       | 01       | 02       | 0.02        |
| 60      | 0.054+0.04       | 0.889±0.                   | 0.991±0. | 0.978±0. | 0.892±0. | $0.878\pm$  |
| 60      | $0.054{\pm}0.04$ | 007                        | 02       | 01       | 02       | 0.02        |
| 90      | 0.054±0.04       | 0.880±0.                   | 0.976±0. | 0.962±0. | 0.887±0. | $0.862\pm$  |
| 80      |                  | 007                        | 02       | 01       | 02       | 0.02        |
| 100     | 0.054±0.04       | 0.868±0.                   | 0.907±0. | 0.947±0. | 0.816±0. | $0.856\pm$  |
| 100     |                  | 007                        | 02       | 01       | 02       | 0.02        |

**Table 2.** Results of the absorbance at 517nm of different concentrations of DPPHand Ascorbic acid and Samples A, B, C, D, E.

Mean  $\pm$  SEM, n = 5, Values are significantlyws different at p<0.04



**Fig. 1.** A graph comparing the DPPH scavenging activity of ascorbic acid and the 5 extracts at different concentration.

## **Antidiabetic Evaluation**

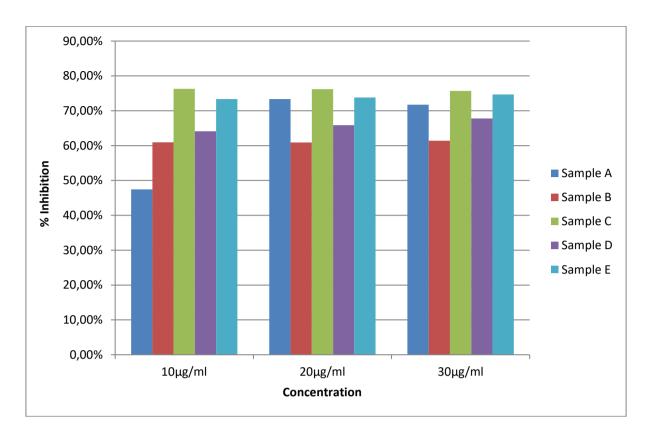
## Hemoglobin Glycosylation Assay

The results of the absorbance at 443nm of the inhibition of hemoglobin glycosylation activities of the 5 extracts at various time intervals are presented in Tables 3-5.

**Table 3.** The results of the absorbance of the inhibition of hemoglobin glycosylation activitiesof the 5 extracts after 24 hours at 443 nm.

| Concentration | Sample A   | Sample B   | Sample C  | Sample D  | Sample E  |
|---------------|--|--|---|---|---|
| 10 µg/ml      | $\begin{array}{c} 0.4743 \pm \\ 0.3 \end{array}$ | $0.6093 \pm 0.01$                                | $\begin{array}{c} 0.7629 \pm \\ 0.02 \end{array}$ | $\begin{array}{c} 0.6412 \pm \\ 0.06 \end{array}$ | $0.7333 \pm 0.006$                                |
| 20 µg/ml      | $\begin{array}{c} 0.7335 \pm \\ 0.3 \end{array}$ | $\begin{array}{c} 0.609 \pm \\ 0.01 \end{array}$ | $0.7621 \pm 0.02$                                 | $\begin{array}{c} 0.6587 \pm \\ 0.06 \end{array}$ | $0.7379 \pm 0.006$                                |
| 30 µg/ml      | $\begin{array}{c} 0.7172 \pm \\ 0.3 \end{array}$ | $\begin{array}{c} 0.614 \pm \\ 0.01 \end{array}$ | $\begin{array}{c} 0.7569 \pm \\ 0.02 \end{array}$ | $\begin{array}{c} 0.6781 \pm \\ 0.06 \end{array}$ | $\begin{array}{c} 0.747 \pm \\ 0.006 \end{array}$ |

Mean  $\pm$  SEM, n = 5



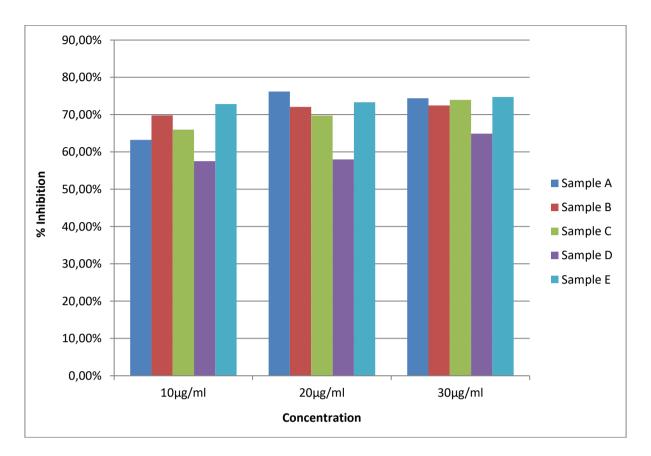
**Fig. 2.** A graph comparing the percentage inhibition of hemoglobin glycosylation of the 5 extracts after 24 hours of incubation.

| <b>Table 4.</b> Results of the absorbance of the inhibition of hemoglobin glycosylation of |
|--|
| the 5 extracts after 48 hours of incubation at 443 nm.                                     |

| Concentration | Sample A     | Sample B     | Sample C     | Sample D        | Sample E       |
|---------------|--------------|--------------|--------------|-----------------|----------------|
| 10 µg/ml      | $0.6323 \pm$ | $0.6979 \pm$ | $0.6598 \pm$ | $0.5754 \pm$    | $0.7285$ $\pm$ |
| 10 µg/III     | 0.2          | 0.08         | 0.04         | 0.03            | 0.02           |
| 20 µg/ml      | $0.7621 \pm$ | $0.7207 \pm$ | $0.6976 \pm$ | $0.58 \pm 0.03$ | $0.7332 \pm$   |
| 20 µg/III     | 0.2          | 0.08         | 0.04         | $0.30 \pm 0.03$ | 0.02           |
| 30 µg/ml      | $0.744 \pm$  | $0.7246 \pm$ | $0.7396 \pm$ | $0.6491 \pm$    | $0.7475 \pm$   |
| 50 μg/III     | 0.2          | 0.08         | 0.04         | 0.03            | 0.02           |

Mean  $\pm$  SEM, n = 5



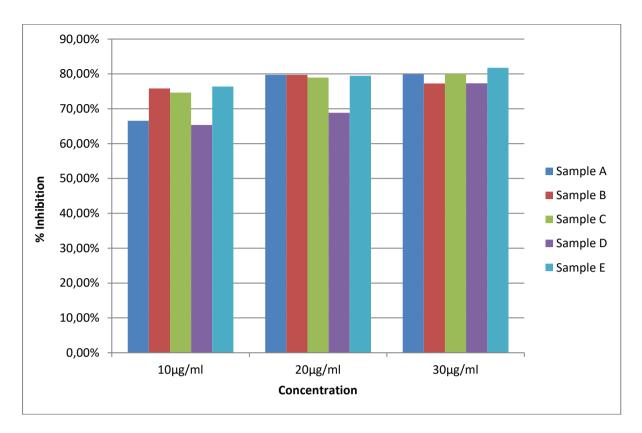


**Fig. 3.** A graph comparing the percentage inhibition of hemoglobin glycosylation of the 5 extracts after 48hours incubation.

| <b>Table 5.</b> Results of the absorbance of the inhibition of hemoglobin glycosylation of |
|--|
| the 5 extracts after 72hours incubation 443 nm.  |

| $2 \pm 0.7462 \pm$ | 0 (520 )         | 0.7(20)   |
|--------------------|------------------|---|
|                    | $0.6538 \pm$     | $\begin{array}{c} 0.7638 \pm \\ 0.01 \end{array}$     |
|                    | 0.04<br>0.6883 ± | 0.01<br>$0.7944 \pm$                                  |
| 2 0.02             | 0.04             | 0.01  |
|                    | 0.7731 ±         | $\begin{array}{c} 0.8175 \pm \\ 0.01 \end{array}$     |
|                    | 2 0.02           | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ |

Mean  $\pm$  SEM, n = 5 The values of the extracts are significantly different from each other at p<0.05



**Fig. 4.** A graph comparing the percentage inhibition of hemoglobin glycosylation after 72 hours incubation.

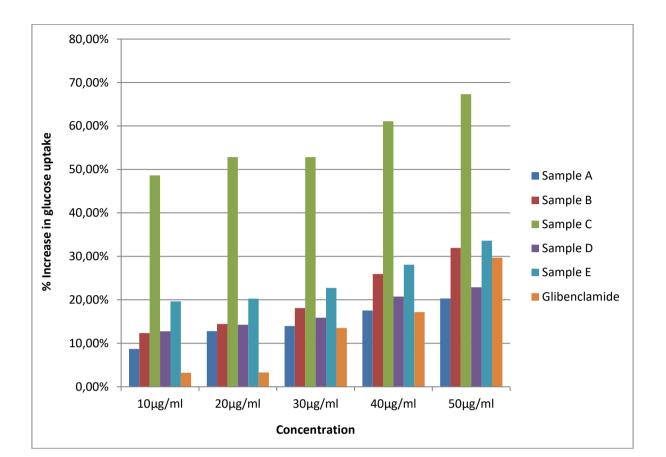
## Glucose Uptake by Yeast Assay

The results of the percentage increase in glucose uptake by yeast for the 5 extracts and drug (glibenclamide) at different glucose concentration is presented in Table 6.

**Table 6.** Results of the increase in glucose uptake by yeast for the 5 extracts and glibenclamide at different glucose concentration.

| Concentration         | Sample<br>A  | Sample<br>B  | Sample<br>C  | Sample<br>D  | Sample<br>E  | Glibenclamide |
|-----------------------|--------------|--------------|--------------|--------------|--------------|---------------|
| 10 µg/ml              | $0.0872\pm$  | 0. 1234±     | $0.4864 \pm$ | 0.1275±      | $0.1964 \pm$ | $0.0323 \pm$  |
| 10 μg/mi              | 0.018        | 0.03         | 0.03         | 0.02         | 0.02         | 0.04          |
| $20 \mu g/m^{1}$      | $0.1279 \pm$ | $0.1444\pm$  | $0.5284\pm$  | $0.1429 \pm$ | $0.2029 \pm$ | $0.0329 \pm$  |
| 20 µg/ml              | 0.018        | 0.03         | 0.03         | 0.02         | 0.02         | 0.04          |
| $20 \mu g/m^{1}$      | $0.1398 \pm$ | 0.1809±      | $0.5285 \pm$ | 0.1591±      | $0.2273 \pm$ | 0.1355 ±      |
| 30 µg/ml              | 0.018        | 0.03         | 0.03         | 0.02         | 0.02         | 0.04          |
| $40 \text{ ug/m}^{1}$ | $0.1754\pm$  | $0.2595 \pm$ | $0.6109 \pm$ | $0.2075\pm$  | $0.2807\pm$  | $0.172 \pm$   |
| 40 µg/ml              | 0.018        | 0.03         | 0.03         | 0.02         | 0.02         | 0.04          |
| $50 \text{ ug/m}^{1}$ | $0.203\pm$   | 0.3197±      | $0.673\pm$   | 0.229±       | $0.3361\pm$  | $0.2971 \pm$  |
| 50 µg/ml              | 0.018        | 0.03         | 0.03         | 0.02         | 0.02         | 0.04          |

Mean  $\pm$  SEM, n = 5



**Fig. 5.** A graph comparing the percentage increase in glucose uptake by yeast of the 5 extracts and glibenclamide at different glucose concentrations.

#### 5. DISCUSSION

The phytochemical screening of the root of *Telfairia occidentalis* revealed the presence of alkaloids and saponins as shown in Table 1 and the quantitative analysis of the metabolites were saponins (4.70%) and alkaloids (1.26%). This is in contrast with the work of Ogbonnaya and Uadia, (2016) [17] where the root of *T. occidentalis* were shown to contain alkaloids, phenols, tannins, saponins, flavonoids, steroids, terpenes.

The absence of these secondary metabolites (steroids, flavonoids, tannins, terpenes, phenols) might be due to variation in age of the plant, geographical location and time of collection of plant sample. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of the 5 extracts and ascorbic acid, a known antioxidant was shown on Tables 2. Their activities were determined as a function of their percentage inhibition and the activities of the extracts compared with that of ascorbic acid at different concentrations of DPPH as shown in Figure 1.

The values of the results were significantly different between extracts and between extracts and ascorbic acid. The results showed that with increase in concentration there was an increase in percentage inhibition this being in agreement with the work of Onocha *et al.*, (2010) [18] although the extracts had a lower DPPH scavenging activity compared to ascorbic acid at different concentrations.

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The decoction extract had the highest scavenging activity of the extracts with the 99.8% ethanol soxhlet extract having the least. This might mean that the secondary metabolites responsible for the antioxidant activity are more soluble in water than organic solvents.

Increased glucose concentration in the blood leads to its binding to hemoglobin which may result in the formation of reactive oxygen species. Plant extracts play an important role in the inhibition of the glycosylation end products (Sindhu *et al.*, 2013) [19].

Tables 3, 4 and 5 shows the percentage inhibition of hemoglobin glycosylation by the different extracts after incubation at room temperature for 24-72 hours respectively and Figures 2 - 4 compares the inhibition of hemoglobin glycosylation of the different extracts at the same time interval.

There was no significant difference between the mean of the extracts at 24 and 48 hours but there was significant difference at 72 hours (p<0.05). It was observed that the extracts inhibited hemoglobin glycosylation after incubation in a concentration dependent manner with Sample C (99.8% soxhlet ethanol extract) having the highest inhibition after 24 hours as shown in Figure 2.

After 48 and 72 hours it was observed that there was progressive increase in the percentage inhibition of hemoglobin glycosylation of other extracts with Sample A (99.8% ethanol macerate) having the highest at 20  $\mu$ g/ml but Sample C (99.8% soxhlet ethanol extract) was the highest at 30  $\mu$ g/ml.

In yeast, glucose transport takes place by facilitated diffusion. Table 6 and Figure 5 showed the percentage increase in glucose uptake by yeast for the extracts and glibenclamide. It was observed from figure 5 that the increase in glucose uptake by yeast was concentration dependent with Sample C (99.8% soxhlet ethanol extract) observed to have the highest percentage glucose uptake across the different glucose concentrations.

This compliments the work done by Eseyin *et al.*, (2006) in which the 96% soxhlet ethanol extract was observed to increase blood glucose level significantly (P<0.05) in normoglycaemic rats thus it may be inferred that the extract sensitizes the cell to the presence of glucose causing increase in uptake thus preventing type 2 diabetes.

The 99.8% ethanol marcerate had the least percentage increase in glucose uptake. This might be due to the presence of some secondary metabolites released by the hot extraction of the soxhlet which are absent in the cold maceration. Glibenclamide a known anti-diabetic agent showed a low percentage increase in glucose uptake by yeast compared to the different extracts which might be due to the absence of insulin as indicated by its mechanism of action which is to stimulate the secretion of insulin from the B-cells of the pancreas.(Jonathan *et al.*, 2021) [20].

#### 6. CONCLUSION

*Telfairia occidentalis* root has been shown from this study to be an important phytomedicine. The results and findings of this study suggest that the different extracts of the root of *T. occidentalis* all have antidiabetic and antioxidant activities but the method of extraction affects the degree of activity with the decoction extract having the highest antioxidant activity while 99.8% sohxlet ethanol extract having highest antidiabetic activity.

Further studies are needed to confirm the *in vivo* potential of *Telfairia occidentalis* root in the management of medical conditions linked to oxidative stress.

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