

# Anti-diabetic Properties and Phytochemical Studies of Ethanolic Leaf Extracts of *Murraya Koenigii* and *Telfairia Occidentalis* on Alloxan-Induced Diabetic Albino Rats

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## Abstract

The antidiabetic properties and phytochemical studies of fractions of ethanolic leaf extract (400mg/Kgb.w) of *Murraya koenigii* (*M. koenigii*) and *Telfairia occidentalis* (*T. occidentalis*) was carried out on alloxan induced diabetic albino rats. Four and three fractions were obtained from *M. koenigii* and *T. occidentalis* extract respectively using column chromatography. Phytochemical screening of each fraction, indicate the presence of saponins, alkaloids, flavonoids, tannins and cardiac glycosides. The alloxan induced diabetic rats were treated with fractions of the extract, and fraction 1 and 2 of *M. koenigii* and *T. occidentalis* respectively which decreased blood glucose level significantly ( $p < 0.05$ ) by 72% and 78% respectively when compared within the group and showed no significant different when compared to normal control group. All treated groups showed no significant changes ( $p < 0.05$ ) in their body weight with the exception of groups treated with 3<sup>rd</sup> fractions of *M. koenigii* and *T. occidentalis*. Spectroscopic studies indicated the presence of biological active compound in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fractions of *M. koenigii* that absorbed maximally at 200-350nm and the 4<sup>th</sup> fraction showed absorption maximally at 270nm and 290nm; while the 1<sup>st</sup> fraction of *T. occidentalis* absorbed maximally at 320nm, 2<sup>nd</sup> fraction at 290nm and 340nm and 3<sup>rd</sup> fraction at 320nm and 350nm. The 1<sup>st</sup> fraction of *M. koenigii* and 2<sup>nd</sup> fraction of *T. occidentalis* showed high antidiabetic properties at  $\lambda$  max 320nm and 340nm respectively. Our findings certainly suggest among others the use of plants as a source of potentially useful antidiabetic therapy for diabetics.

**Keywords:** Diabetes mellitus, Antidiabetic components, *Telfairia occidentalis* and *Murraya koenigii*

## 1. Introduction

One of the biggest challenges to public health globally is diabetes mellitus. Diabetes is a chronic condition occasioned by abnormally high level of glucose in the blood. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Ahmed et al.,2010). If it is left untreated, it can elicit a number of debilitating consequences such as blindness, kidney disease, nerve disease, cardiovascular disease among others. It is a common chronic disease that affects over 245 million people worldwide and the number simply keep on growing with each passing day( Prohp and Onaogbe,2009; Rahimi,2015). This pattern of prevalence is also experience in Nigeria, were an exponential socioeconomic growth over the past few decades has led to a sedentary and affluent lifestyle of the people in the urban region (Saxena. et al. 2010). A recent follow up on epidemiological study shows an alarming increase in the prevalence of diabetes mellitus during the past few years resulting to public awareness (Al-Daghri et al., 2011). It has been estimated that by 2030 the figure is expected to double (Wild et al., 2004).

For many people, diabetes mellitus is a reality that they have to leave with every single day. Diabetes kills one person every 10 seconds and infects two persons in the same short time. This is the scary reality behind this silent killer which is taking its toll on lives worldwide and even more than HIV/AIDS and Cancer put together (Sy et al.,2005)

Currently available antidiabetic therapies include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides (Patel et al., 2012). As a result of the number of serious adverse effects reported for these therapies, there is therefore the need for hypoglycemic agents that will not only be more effective but should be relatively safe. Aldol reductases, a key enzyme in the polyol pathway catalyzes the reduction of glucose to sorbitol. The accumulation of sorbitol has been shown to cause various complications in the body including cataract, neuropathy and nephropathy (Patel et al.,2012).

One of the therapeutic options mostly explored is the use natural plant derivatives. Herbal medicines from traditional plant have been recommended and are used throughout the world for a range of diabetic presentation.

*Murraya koenigii* (L.) or its common name curry leaf tree is a small strong smelling perennial shrub commonly found in forests as undergrowth. It was originally cultivated in India for its aromatic leaves and for

ornament is normally used for natural flavoring in curries and sauces. Originated in Tarai regions of Uttar Pradesh, India. It is now widely found in all parts of India and it adorns every house yard of southern India and also now cultivated throughout the world including Nigeria. The plant is used in Indian system of medicine to treat various ailments (Kumar et al., 1999). Parts of the plant have been used as raw material for the traditional medicine formulation in India. This plant is known to be the richest source of carbazole alkaloids (Tachibana et al, 2003). It has been reported by authors that carbazole alkaloids present in *M.koenigii* (L.) and display various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities (Mandal et al.,2010; Muthumani et al., 2010). *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching (Kumar et al.,1999).

The aromatic leaves, which retains their flavor and other qualities even after drying, are slightly bitter, acrid, cooling, weakly acidic in tastes and are considered as a tonic, anthelmintic, analgesic, digestive, appetizing and are widely used in Indian cookery for flavoring food stuffs (Adebajo et al., 2004; Mandal et al., 2010). The phytoconstituents isolated so far from the leaves are alkaloids viz., mahanine, koenine, koenigine, koenidine, girinimbiol, girinimibine, koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline and other phytoconstituents such as coumarin glycoside viz., scopotin, murrayanine, calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid (Adebajo et al., 2006; Tachibana et al., 2003 Adebajo and Reisch, 2000). It also reported for anti-microbial, antioxidant (Adebajo et al, 2005; Priya et al, 2014). *M. koenigii* leaves is reported to possess antioxidant, antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and antihypertensive activity (Ranav et al ,2004; Iyer and Uma, 2008; Rao et al,2011).

This tropical vine known as *Telfairia occidentalis* is well grown in West Africa as a leaf vegetable and also for its edible seed. It belongs to a member of the Curcubitaceae family and is indigenous to southern Nigeria. Ugu is mostly cultivated in Nigeria by the south eastern Nigeria, and widely distributed among the Igbo speaking people, particularly Imo state. It is mainly used in soups and herbal medicines (Oyekunle and Oyerele,2012) . The seed produced by the gourd are high in protein and fat, and can therefore contribute to a well-balanced diet. It is widely cultivated in the West and Central Africa (Benin Republic, Cameroon, Nigeria, Sierra Leone to Angola, and up to Uganda in east Africa). It is called ‘ugu’ by the Igbos, ‘ugwu’ by the Yorubas and ‘ekobon’ by the Cameroonians (Francis,2015). The various extracts of *Telfairia occidentalis* leaves reported to be anticancer, antioxidant (okokon et al., 2012), anti-HIV reverse transcriptase (Dowluru et al,2015), hepatoprotective (Obboh,2005;Dowluru et al,2015) , anti in-flammatory (Okokon et al., 2012) , anti-hepatitis (Rupam et al.,2012) and aphrodisiac. It has also been reportedly used in the management of hyperchlesterolaemia, liver problems, and impaired immune system (Usman et al.,2015), remedy reproductive and fertility issues (Obboh,2005),treatment of malaria as well replenish lost blood due to its high iron content among others

To curtail this metabolic disorder and its implication, many synthetic therapeutic agents has been introduce with minimal significant success, giving raise for a search of a better drug. In recent years, ethnobotanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. This quest has led to the employment of plants for the management of diabetes mellitus. A review of the medicinal plants used in Nigeria for the treatment of diabetes mellitus indicates that a rich flora diversity exist in Nigeria. They obviously deserve scrutiny on modern scientific lines such as phytochemical investigation, biological evaluation on experimental animal models, toxicity studies, isolation of active components, investigation of molecular mechanism of action s of isolated phyto-principles and their clinical trials (Audu, 1989 and Harish *et al.*, 2012).

With the prevalence increase in diabetes mellitus in Nigeria and it subsequent management, its effect on human socio-economic and its challenges worldwide in term of therapeutic management, therefore the need to search for drug that is cheaper, safer and available from plants products. With respect to search for drug that is cheaper, safer and available from plants products; Iliyasu (2013) and Endas (2014) investigated the antidiabetic properties of *Murraya koenigii* and *Telfairia occidentalis*, and reported that these plants posses’ antidiabetic properties but their work did not specify the antidiabetic components nor its mechanism of action.

It is in the light of this background that the present was initiated to investigate the antidiabetic properties and phytochemical studies of fractions of ethanolic leaf extract of *Murraya koenigii* (*M. koenigii*) and *Telfairia occidentalis* (*T. occidentalis*) on alloxan induced diabetic albino rats.

## 2 Materials and Methods

### 2.1 Materials

#### 2.1.1 Sample Collection and Processing

The fresh leaves of the plants *Telfairia occidentalis* and *Murraya koenigii* were purchased from traditional herb dealers in Kaduna central Market, Kaduna State of Nigeria. The plants were further authenticated in the Botany unit, Department of biological science, Kaduna State University.

## 2.2 Chemicals

Alloxan monohydrate, silica gel and meyer's reagent were supplied by Sigma –Aldrich Co. USA. Ethanol, dimethyl sulphoxide, chloroform, concentrated sulphuric acid and ammonia solution were supplied by British Drug House (BDH) chemicals, Poole, England . Sodium dihydrogen phosphate, disodium hydrogen phosphate, ferric chloride, glacial acetic acid were products of Pharmacia Fine Chemicals, Upsala, Sweden. All other chemicals and reagents used for the study were of analytical grade and supplied by reputable chemical manufacturers.

## 2.3 Sample preparation

The samples were prepared according to the method described by Gohil *et al.*, (2010). In this method, fresh leaves of the plants *Telfairia occidentalis* and *Murraya koenigii* were washed and dried at room temperature and were pounded into smooth powder. 100gm of each the pounded leave powder was soaked in 400ml of 70% ethanol and were allowed to stand for 24hrs. After 24hrs, the mixtures were filtered through several folded clean muslin cloth to remove debris, the filtrates collected through muslin cloth were re-filter through cotton wool and through 2-folded Whatman filter paper. The volumes of the filtrates were taken and were evaporated to dryness using rotary evaporator (NCY model R-205D). The weight of the extracts was taken and the extracts were transferred to plastic containers and store in the refrigerator for the next step.

## 2.4 Preparation of chromatography column (Silica gel).

The column was washed and rinsed with deionized water, a plastic funnel was placed on top of the column which serves as the packing and buffer reservoir. The chromatography column was filled to about one third with phosphate buffer (pH 6.30).

In a beaker 160ml (103g) of silica gel was measured out, in a separate beaker 350ml of phosphate buffer was measured and used to dissolve the silica gel to form slurry. The prepared slurry was poured into the column while allowing the tap open to prevent overflow. The column was shake for 2mins to ensure proper packing. The excess buffer in the column was removed and the gel ready for use according to standard procedure.

## 2.5 Fractionation of plant's crude extracts.

The ethanolic plant extracts of *Telfairia occidentalis* and *Murraya koenigii* were reconstituted with 30ml of phosphate buffer (pH 6.30) followed by addition of 5ml of dimethyl sulphoxide to aid in the solubility of the plant extracts. The reconstituted solution of the plant extract was loaded into the prepared column above and was allowed to move through the inlet to outlet of the column and collection of different fractions base on the number of layers formed on the column, the retention time, volume and concentration in mg/ml of each fraction was noted. Each of the fraction collected was used to administered orally to a particular group of induce diabetes rats for the period of the experiment.

## 2.6 Experimental animals

Albino rats of both sexes weighing between 70-230g were obtained from National Veterinary Research Institute (NVRI) Vom, plateau state was used. The animals were allowed to have free access to food and water under well ventilated conditions daily. Grower's mash and tap water were used to feed the animals throughout the experimental period.

## 2.7 Weighing of animals/grouping

The rats were weighed using top loading weighing balance (Jenway, Model KL 191-200g/0.01). A suitable container was placed on the weighing balance and the machine was zero. The rats were placed one after the other from each group and their weighs were noted.

The animals were grouped into nine groups and label accordingly. Each group contains four rats. The groups were as follows: TL1, TL2 and TL3 were treated with 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fractions of *Telfairia occidentalis* respectively, ML1, ML2 ML3 and ML4 were treated with 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> fractions of *Murraya koenigii* collected from the earlier fractionation.

## 2.8 Induction of diabetes

The rats in ML1, ML2, ML3, ML4, TL1, TL2, TL3 and DC groups were made to fast for 12 hrs before the induction of diabetes. Thereafter they rats were injected intraperitoneally with freshly prepared alloxan monohydrate (150mg/kg body weight). Two days after injection, the blood glucose of the rats was determined using ON-CALL glucometer (AVC model), the rats with fasting blood glucose higher than 6.2mmol/L were considered diabetic and used for the experiment. Feeding was stopped 12 hrs before blood sampling was taken.

## 2.9 Blood glucose level estimation

Fasting blood glucose was determined using the strip of digital ON-CALL glucose meter (AVC model). A drop of blood was obtained from the tip of conscious rat's tail and placed on the strip. The reading on the meter was noted and recorded as the blood glucose concentration. The ON-CALL glucometer strip uses glucose the chemistry of dehydrogenase. The glucose dehydrogenase in the strip converts the glucose in the blood sample to gluconolacton and through series of reaction a small current is generated that is read by the meter as glucose level.

## 2.10 Phytochemical screening

Phytochemical screening test was carried out on each fractions of the plants extract *Telfairia occidentalis* and *Murraya koenigii*.

### 2.10.1 Test for flavonoids

This was determined according to the method described by Ayoola *et al.*, (2008). In this method, 2ml of aqueous plants extract was taken together with 5ml of dilute ammonia followed by addition of 1ml concentrated sulphuric acid. A yellow colouration that disappears on standing indicates the presence of flavonoids.

### 2.10.2 Test for saponins

This was determined according to the method described by Sofowora (1993). In this method, 5ml of the plants extract fraction was dispensed into a test tube and agitated vigorously till a persistent froth is observed followed by further addition of 3 drops of olive oil. Formation of emulsion on agitation indicate the presence of saponins.

### 2.10.3 Test for tannins

This was determined according to the method described by Ayoola *et al.*, (2008). In this method, about 5ml aliquot of the plant extract was boiled in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed. Brownish green or a blue-black colouration indicate the presence of tannins.

### 2.10.4 Test for alkaloids

This was determined according to the method described by Trease *and* Evans (1989), in this method, 5ml aliquot of plant extract was diluted to 10 ml with acidified alcohol which was boiled and filtered. To 5 ml portion of the filtrate, 2 ml of dilute ammonia was added, together with 5ml of chloroform and the reaction mixture shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. Mayer's reagent was added to the extracted portion. The formation of a cream colour indicates positive result for the presence of alkaloids.

### 2.10.5 Test for glycosides (Keller-Killiani test)

This was determined using the Keller-Killiani's test as described by Trease and Evans (1989), in this method, 5ml of plant's extract fraction was taken into a test tube with 2 ml of glacial acetic acid in which one drop of ferric chloride solution was added and the reaction mixture under laid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

## 2.11 Spectroscopy studies of the plant's extracts fraction

Each fraction of the plant's extracts was subjected to spectroscopy studies at different wavelength according to standard procedure. The absorbance of each of the fraction was measured at wavelength range of 200-700nm at the interval of 5nm. The absorbance obtained was used to plot the spectrum of each fraction so as to determine the  $\lambda_{max}$  of each fraction from the spectra.

## 2.12 Statistical analysis

Results were expressed as mean  $\pm$  S.D. Data were analyzed with t - test for comparison between the groups. The significance level was set at  $P < 0.05$ .

## 3.0 Results

In our study we report the result of the antidiabetic properties and phytochemical studies of fractions of ethanolic leaf extract (400mg/Kgb.w) of *Murraya koenigii* (*M. koenigii*) and *Telfairia occidentalis* (*T. occidentalis*) that was carried out on alloxan induced diabetic albino rats for a period of fourteen days.

The yield of the ethanolic leaf extracts obtained from 100g of *Telfairia occidentalis* and *Murraya koenigii* was 5.1g and 5.5g respectively.

Four and three fractions were obtained from *M.koenigii* and *T. occidentalis* extract respectively (see table 1) Phytochemical screening of each fraction, indicate the presence of saponins, alkaloids, flavonoids, tannins and cardiac glycosides (see table 2.)

Throughout the experiment period there was follow up sleep in all the rats in the treated groups immediately after oral administration of the fractions of the plant extracts which suggested that the plants under study possess sedative properties. Moreso, two rats in both ML3 and ML4 treated groups developed ulcer after five days of treatment which disappeared after twelve days of treatment.

**Table 1:** Retention time, volume and concentration of various fractions obtained from column chromatography process of ethanolic leaf extracts of *Telfairia occidentalis* and *Murraya koenigii*

Fractions	Retention time (min)	Volume (ml)	Concentration of bioactive component (mg/ml)
ML1	9.5	40.0	42.0
ML2	12.5	43.0	39.0
ML3	14.0	96.5	26.0
ML4	16.5	120.5	24.0
TL1	8.5	42.5	34.0
TL2	12.5	47.5	28.0
TL3	13.9	47.0	24.0

The ML1, ML2, ML3 and ML4 represent 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> fraction of *M. koenigii* respectively while TL1, TL2 and TL3 represent 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fraction of *T. occidentalis* respectively. The fraction was obtained from column chromatography packed with silica gel size (8cmx500mm). Retention time is the time taken for the plants extract solution to move from the inlet of the column, separate and come out of the column as eluent.

The alloxan induced diabetic rats were treated with fractions of the extract, and ML1 and TL2 fraction decreased blood glucose level significantly ( $p < 0.05$ ) by 72% and 78% respectively when compared within the group but showed no significant difference when compared to normal control group at the end of the experiment. However rats in groups treated with ML2, ML3, ML4, TL1 and TL3 fraction showed no significant decrease in blood glucose level. (see table 3). Diabetic control group rats showed no significant increase ( $p < 0.05$ ) in blood glucose level at the end of the experiment while normal control group also showed no significant difference ( $p < 0.05$ ) in blood glucose level. Moreso, all rats in both treated groups and control groups showed no significant changes ( $p < 0.05$ ) in their body weight with the exception of groups treated with ML3 and TL3 (see table 3).

**Table 2:** Phytochemical screening on fractions of ethanolic extracts of *Telfairia occidentalis* and *Murraya koenigii*.

Fractions	Saponins	Flavonoid	Tannins	Alkaloids	Glycosides
ML1	-	-	+	+++	-
ML2	+++	+++	++	+	-
ML3	++	++	-	-	+
ML4	-	+	-	-	-
TL1	-	+	-	+	-
TL2	+++	++	-	+++	-
TL3	+++	+	+	-	++

The + sign indicate the presence of the molecule while (-) sign indicate absence of the molecules in the fraction, triple, double and single plus sign (+) indicate presence of molecules in high, moderate and low concentration respectively.

**Table 3:** The effect of fractions of ethanolic leaf extracts of *Telfairia occidentalis* and *Murraya koenigii* on body weight on alloxan induced diabetic albino Wister rats

Group	Initial weight $\pm$ SD (g)	Body weight after induction and commencement of treatment of diabetes (g)	
		1 <sup>st</sup> Day	14 <sup>th</sup> Day
ML1	133.5 $\pm$ 17.20	132.11 $\pm$ 12.55	139.73 $\pm$ 11.5
ML2	123.66 $\pm$ 15.22	119.01 $\pm$ 0.88	131.94 $\pm$ 3.66
ML3	69.27 $\pm$ 14.90 <sup>A</sup>	71.97 $\pm$ 14.04*	100.99 $\pm$ 17.0*
ML4	139.14 $\pm$ 16.51	140.04 $\pm$ 13.77	131.62 $\pm$ 21.42
TL1	169.68 $\pm$ 11.65	166.92 $\pm$ 11.62	152.67 $\pm$ 29.56
TL2	142.36 $\pm$ 2.59	143.89 $\pm$ 3.96	149.42 $\pm$ 19.48
TL3	68.92 $\pm$ 13.35 <sup>A</sup>	70.95 $\pm$ 10.86	83.58 $\pm$ 13.19*
DC	137.66 $\pm$ 12.66	141.74 $\pm$ 12.97	134.72 $\pm$ 14.85
NC	135.24 $\pm$ 41.06	135.33 $\pm$ 41.17	139.85 $\pm$ 35.78



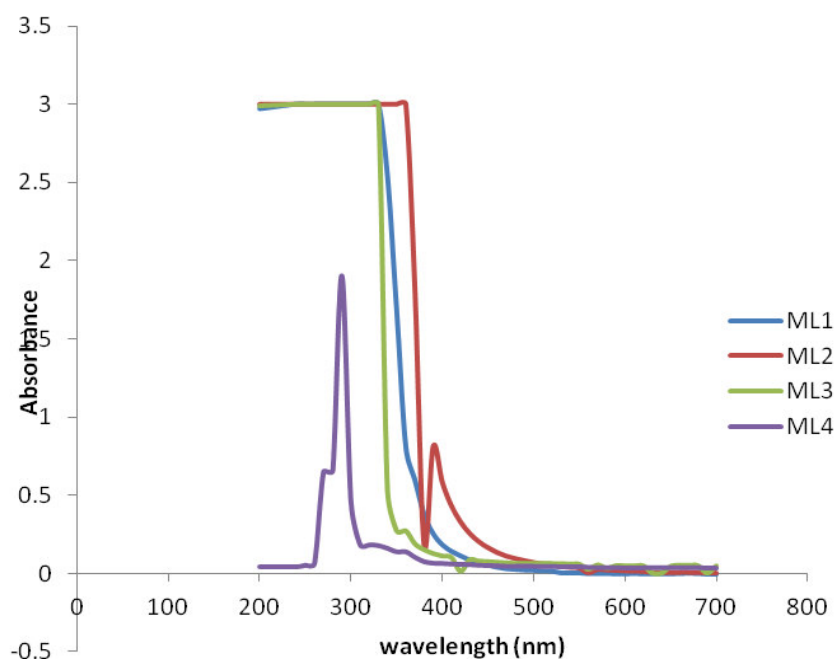
Values are given as mean  $\pm$  standard deviation (SD), (n=4); Values followed by different letters are significantly different at  $p < 0.05$  between the groups and those followed by different number of asterisk are significantly different within the groups at  $p < 0.05$ . Unpaired sample student t-test was used for comparing between the groups, while paired sample t-test was used to compare within the groups.

The fractions of both extracts were scanned using UV-spectrophotometer (Model: UV 752) at wavelength between 200-700nm wavelengths at interval of 5nm. The fractions TL1, TL2, TL3 and ML4 gave spectra with heterogeneous peaks which absorbed maximally at wavelength between 200-350nm (see figure 1 and 2). The following absorptions were observed: ML1 fraction, 320nm (being the  $\lambda_{max}$  of the fraction); ML2 fraction, 390nm (minor Peak) and 350nm (major peak and being the  $\lambda_{max}$  of the fraction); ML3 fraction, 350nm (minor peak) and 320nm (major peak and being the  $\lambda_{max}$  of the fraction); ML4 fraction, 270nm (minor peak) and 290nm (major peak and being the  $\lambda_{max}$  of the fraction); TL1 fraction 340nm (being the  $\lambda_{max}$  of the fraction); TL2 fraction, 310nm (minor peak) and 340nm ((major peak and being the  $\lambda_{max}$  of the fraction) and TL3 fraction, 300nm (minor peak) and 320nm (major peak and being the  $\lambda_{max}$  of the fraction see figure 1 and 2).

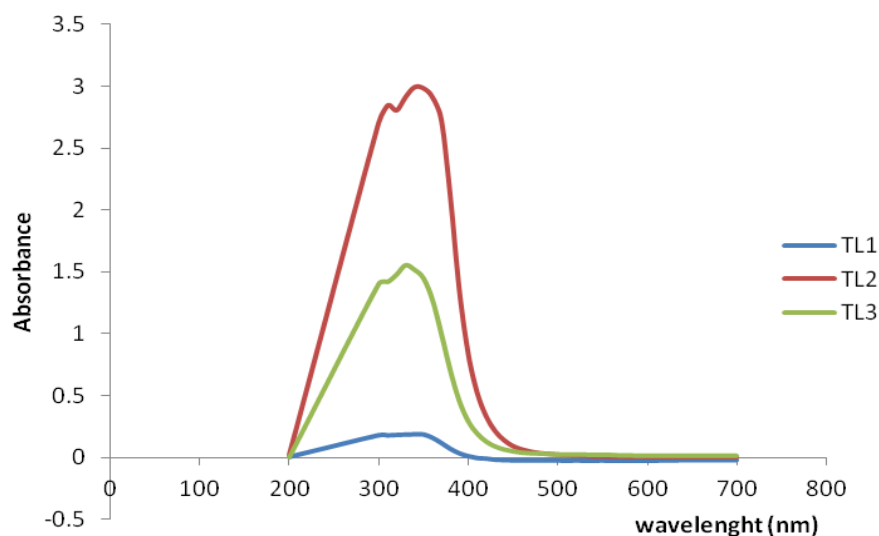
**Table 4:** The effect of fractions of ethanolic leaf extracts of *Telfairia occidentalis* and *Murraya koenigii*, on blood glucose levels on alloxan induced diabetic albino Wister rats

Group	Initial blood glucose (mmol/L)	Blood glucose level after induction and commencement of treatment of diabetes (mmol/L)		
		1 <sup>st</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
ML1	4.39 $\pm$ 1.12	20.93 $\pm$ 10.12 <sup>B*</sup>	16.47 $\pm$ 10.22 <sup>B*</sup>	5.73 $\pm$ 1.49 <sup>A**</sup>
ML2	4.02 $\pm$ 0.71	7.70 $\pm$ 1.47 <sup>*</sup>	7.20 $\pm$ 1.57 <sup>B*</sup>	6.75 $\pm$ 1.48 <sup>B*</sup>
ML3	4.45 $\pm$ 0.64	10.93 $\pm$ 4.60 <sup>B*</sup>	8.67 $\pm$ 5.81 <sup>B*</sup>	7.85 $\pm$ 4.29 <sup>*</sup>
ML4	3.48 $\pm$ 0.62 <sup>B</sup>	9.87 $\pm$ 5.47 <sup>*</sup>	6.75 $\pm$ 1.61 <sup>*</sup>	6.50 $\pm$ 1.15 <sup>B*</sup>
TL1	4.38 $\pm$ 0.92	16.82 $\pm$ 13.37 <sup>B*</sup>	19.17 $\pm$ 10.88 <sup>B*</sup>	13.63 $\pm$ 6.89 <sup>B*</sup>
TL2	4.30 $\pm$ 10.09	20.88 $\pm$ 10.32 <sup>B*</sup>	5.03 $\pm$ 0.81 <sup>A**</sup>	4.55 $\pm$ 0.90 <sup>A**</sup>
TL3	3.70 $\pm$ 0.78	9.05 $\pm$ 1.26 <sup>*</sup>	7.00 $\pm$ 1.26 <sup>*</sup>	7.05 $\pm$ 1.58 <sup>B*</sup>
DC	3.48 $\pm$ 1.01	11.02 $\pm$ 4.66 <sup>B*</sup>	12.18 $\pm$ 5.82 <sup>B*</sup>	12.15 $\pm$ 5.57 <sup>A B*</sup>
NC	4.98 $\pm$ 0.98 <sup>A</sup>	4.90 $\pm$ 1.19 <sup>A</sup>	5.95 $\pm$ 0.93 <sup>A</sup>	5.13 $\pm$ 0.39 <sup>A</sup>

Values are given as mean  $\pm$  standard deviation (SD), (n=4); Values followed by different capital letters are no significantly different at  $p < 0.05$  between the groups and those followed by different number of asterisk are significantly different within the groups at  $p < 0.05$ . Unpaired sample student t-test was used for comparing between the groups, while paired sample t-test was used to compare within the groups.



**Figure 1:** The spectrum of four fractions of ethanolic leaf extract of *M. koenigii*. [The blue, red, green and purple line represent the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> fraction]



**Figure 2:** The spectrum of four fractions of ethanolic leaf extract of *T. occidentalis*. [The blue, red, green and purple line represent the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> fraction].

#### 4 Discussion

One of the ultimate goal of diabetes research and management is the prevention of long-term complication; where agents under study for either remedy or abating is aimed at improving and maintaining the glycemc control over time. Peyrot *et al* (2005) noted that these goals cannot come so easy as the progressive nature of the disease affects optimization time of treatment leading in a majority of cases being observed with the available drugs.

In the present study the antidiabetic properties of the fraction of ethanolic extracts of *Telfairia occidentalis* and *Murraya koenigii* was investigated in alloxan induced diabetes rats. The phytochemical screening of the fractions revealed some differences in the constituents of the phytochemical components in each of the fraction which indicated that plants under study contain phytochemical components that are of different molecular weight since fractionation of the extract was obtained via size exclusion chromatography, suggesting that each fraction may have different therapeutic effects.

The significant decrease in blood glucose observed in the groups treated with these fractions ML1 and TL2 was an indication of presence of antidiabetic components. The hypoglycemic effect observed in ML1 and TL2 treated groups may be due to action of the flavonoid, saponins, alkaloid and tannins present in the fractions since diabetic control group rats showed no significant decrease in blood glucose level. Although other fractions (ML2, ML3, ML4, TL1 and TL3) phytochemical screening results indicated the presence of flavonoids, alkaloids, tannins and saponins as ML1 & TL2 indicated, but no significant decrease ( $p < 0.05$ ) in blood glucose was observed in groups treated with ML2, ML3, ML4, TL1 and TL3 fraction, this indicated that; the alkaloids, saponins, and tannins present in ML1 & TL2 fraction are structurally and functionally different from the one contained in ML2, ML3, ML4, TL1 and TL3 fractions. The flavonoids, alkaloids, saponins and tannins may have scavenged all the free radicals that were introduced into the beta-cells by the alloxan which led to the quick regeneration of beta-cells and ultimately improved insulin secretion or they may have inhibit the activity of the alpha amylases making carbohydrate food biologically unavailable ( Harish *et al.*, 2012 and Dineshkumar *et al.*, 2010).

Lass'a (1991) noted that every organism has a growing stage, and when the organism is out of growing stage, the increase in weight within a short period of time is not always significant. Findings from present study ( shown in table 3) suggest that the significant increase in weight observed in ML3 and TL3 was not a function of components present in the fraction and does not depend on the blood glucose level. Hence, the gain in weight by the rats is as a result of the growing stage they were able to utilize the available nutrients in building up their bodies; while others treated group showed no significant increase which may be possibly attributed to the fact that they were out of their growing stage. Moreover, weight loss is known to be associated with diabetes mellitus due to inability of the body's cells to utilized glucose for energy production resulting to constant hunger ( Cortran *et al.*, 1999). These combine effect has the potential of influencing a rapid weight loss as a result of constant hydrolysis of protein and store fats for energy production leading to the body weight loss being observed (Verdich *et al.*, 2001).

Spectra of the fractions showed peaks at different wavelength which suggest that the fractions contain difference components. The ML1 and TL2 fraction which showed high antidiabetic properties have  $\lambda_{max}$  320nm and 340nm respectively, suggesting that ML1 fraction may contain alkaloid such as Mahanimbine ( $\lambda_{max}$ = 326nm),

murrayanol ( $\lambda_{\max}$ = 330nm), Hydrolysable tannins such as chibulagic acid ( $\lambda_{\max}$ = 276nm), Altagic acid ( $\lambda_{\max}$ = 253nm), ethyl gallate ( $\lambda_{\max}$ = 271nm) and cardiac glycoside ( $\lambda_{\max}$ = 253nm); while TL2 fraction may contain Mahanine alkaloids ( $\lambda_{\max}$ = 340nm), berberine alkaloid ( $\lambda_{\max}$  = 342nm), Saponins ( $\lambda_{\max}$ = 350nm) (), and Hispidulin flavonoids ( $\lambda_{\max}$ = 338nm) ((Pfoze *et al.*, 2014; Anil *et al.*, 2013; Hediat *et al.*, 2012; Mahajan and Pai, 2010 and Knolker and Peddy, 2002). The  $\lambda_{\max}$  of ML1 TL2 fraction are great agreement with  $\lambda_{\max}$  of Murrayanol alkaloids and Mahanine alkaloids respectively.

## 5 Conclusion

The present study has demonstrated that ethanolic extracts of *Telfairia occidentalis* and *Murraya koenigii* possess antidiabetic activity. Further spectroscopic studies have shown that the observed components responsible for the antidiabetic properties of the plants *Telfairia occidentalis* and *Murraya koenigii* absorbed UV-light maximally at 340nm and 320nm respectively. This strongly suggest that the antidiabetic components of these plants (*Telfairia occidentalis* and *Murraya koenigii*) may have structural similarity with Mahanine alkaloids and Murrayanol alkaloids respectively. At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of orthodox therapy and  $\alpha$ -glucosidase inhibitors in addition to insulin. However, due to unwanted side effects, the efficacies of these compounds are contestible thus fuelling the potential for alternative therapy in the treatment of diabetes (Oubre *et al.*, 1997; Fabricant and Farnsworth, 2001;). It is for this reason that plants have been suggested as a rich, but largely unexplored source of potentially useful antidiabetic therapy. Furthermore, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available in vitro assays procedures (Habeck, 2003 and Rahimi, 2015). Nonetheless our modest effort is another addition to the library of data and further justification for the novel role of *traditional plants* in the treatment of diabetes mellitus.

## 6. Conflict of Interest Statement

The authors declared no conflict of interest whatsoever.

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