PHYTOCHEMICALS AND HYPOGLYCEMIC PROPERTIES OF METHANOL LEAF EXTRACT OF *PHYLLANTHUS AMARUS*

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Abstract: *Plants and plant products are continuously being explored in medicine against diabetics. In the present study phytochemical screening and hypoglycemic properties of methanol leaf extract of phyllanthus amarus against alloxan induced diabetic rats were conducted. A total of fifteen (15) albino rats were randomly selected into 5 groups of 3 rats per group. Groups 1-3 rats were treated with 250 and 500 mg/kg methanol extract of phyllanthus amarus and 5 mg/kg of glibenclamide respectively, while groups 4 and 5 served as diabetic and normal controls respectively. All treatments were administered orally, once daily for fifteen days. Results revealed presence of alkaloids, terpenoids, saponins, glycosides, flavonoids, total phenols and tannins. A significant ($P<0.05$) and progressive increase in blood glucose level was observed in diabetic untreated rats throughout the experimental periods. At the end of treatment period rat treated with 250 & 500 mg/kg P. amarus had final glucose level of 117.00±5.43 mg/dl & 106.00±4.56 mg/kg. Rats treated with 2.5mg/kg glibenclimide had final glucose level of 117.33±5.43 while the untreated rat had final glucose level of 562.50±15.45 mg/dl. The extract of phyllanthus amarus at 250 and 500 mg/kg also cause significant improvement in body weight gain of the rat. It is concluded that phyllanthus amarus contains some useful phytochemicals with potential hypoglycemic reputations. Thus, it may be considered as a natural source of drug for therapeutic purposes.*

Keywords*: albino rats, diabetic, glibenclamide and phyllanthus amarus*

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by absolute or total deficiency of insulin as a result of pancreatic β-cell impairment leading to reduction in insulin secretion. It could also result when the insulin receptor are resistance to the function of circulating insulin. Diabetes is a leading cause of adult blindness, amputation, stroked, renal failure and neuropathy (Raija, 2015). There are 2 types of diabetes the insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus. the pharmacological treatment of diabetes mellitus is based on oral hypoglycemic agent and insulin which have many side effects (White, 2014) evaluation of medicinal plant used in traditional treatment of diabetes is of growing interest (Smolek *et al*., 2013) WHO also recommend and encourage this practice especially in country where access to conventional treatment of diabetes is inadequate, it however emphasize the fact that safety should be overriding criteria in the selection of herbal medicine for use m heath care (WHO, 2010) .

The plant *Phyllanthus amarus* Webster (Family: *Euphorbiaceae)* commonly called; gulf leaf-flower, Chanca piedra, quebra pedra, stone braker, arranca-pedras, carry-me-seed, hurricane weed, para-parai mi, quinine weed Mache da goyo (Hausa), Gbogbon owun lese (Yoruba) is a

small, erect, annual herb (Dicotyledonous) that grows 30–40 cm in height (Matur *et al.*, 2009). Closely related species are *P. fraternus, P. sellowianus* and *P. niruri.* The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Oseni *et al.*, 2013). The aim of this study is to determine phytochemicals and hypoglycemic properties of methanol leaf extract of *Phyllanthus amarus* obtained from Niger state, Nigeria.

2. MATERIAL AND METHODS

The freshly harvested leaves of *phyllanthus amarus* was obtained in January, 2017 from Minna, area of Niger state Nigeria. Taxonomic authentication of the plant was conducted at the Department of Biology, Federal University of Technology, Minna Niger State.

2.1 Experimental Animals

Healthy albino rats of average weight 120-150g were purchased from Animal House, Department of Biochemistry, Federal University of Technology Minna, Nigeria. The rats were kept in clean plastic cages and maintained under standard laboratory conditions in the biochemistry laboratory, Federal University of Technology Minna. They were allowed unrestricted access to rat pellets and water *ad-libitum*.

2.2 Sample preparation and Extraction procedure

The fresh leaves of *phyllanthus amarus* were destalked, rinsed in clean water cut into pieces, and freeze dried. The dried leaf of *phyllanthus amarus* were grounded using morta and pestle and then blended to powder using electronic blending machine. Extraction of plant materials was performed by weighing 200 g of the powdered plant and extracted using 600 ml of Methanol. The resulting methanol extract was concentrated in water bath. The concentrated extract were stored in airtight container prior to use

2.3 Qualitative Phytochemical Analysis

Glycoside

A 0.5g portion of aqueous leaf extract of *phyllanthus amarus* was mixed with 2 ml of glacial acetate and 1 drop of ferric chloride solution, after which 1ml of concentrated sulphuric acid were added. The reaction was observed for a brown ring formation (Sofowora, 1993).

Steroids

A 0.5 g portion of aqueous leaf extract of *phyllanthus amarus* was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids (Sofowora, 1993).

Flavonoids

A portion of aqueous leaf extract of *phyllanthus amarus* was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the

filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids (Harborne, 1973; Sofowora, 1993).

Tannins

A 0.5 g portion of aqueous leaf extract of *phyllanthus amarus* was boiled in 20 ml of distilled water in a test tube and filtered. 0.1% ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test samples (Harborne, 1973).

Saponins

A 2.0 g portion of aqueous leaf extract of *phyllanthus amarus* was boiled in 20 ml of distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion characteristic of saponins (Mathew *et al.*, 2014).

Alkaloids

A 0.5g portion of the extract was stirred with 5cm3 of 1% aqueous HCl on a steam bath. Few drops of picric acid solution were added to 2cm3 of the extract. The formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Harborne, 1976: Trease and Evans 1989).

Phlobatannins

A 2.0 g portion of the powdered sample was boiled with 1% aqueous hydrochloric acid; the formation of red precipitate thus indicated the presence of phlobatanins (Harborne, 1973; Sofowara, 1993)

2.4 Antidiabetic Study of the Extract

Induction of diabetes

A freshly prepared solution of alloxan monohydrate (120mg/kg) was injected intra-peritoneally in overnight fasted rats. After 3 days, blood was collected in vials from the tail vein of overnight fasting rats as selected under guidance of a vet using the aseptic conditions and disposable kits. FBS level of blood was checked regularly up to the stable hyperglycemia stage, usually one week after alloxan monohydrate injection. Animals with marked hyperglycemia were selected for the study (Etuk *et al*., 2010).

Experimental design:

Twenty five rats were divided into five groups of five rats each and were given the following treatments:

Group 1: normal control rats,

Group 2: diabetic control rats, given 2ml/kg of normal saline after diabetic induction

Group 3: diabetic rats given *phyllanthus amarus* extract (250mg/kgb.w.) once daily

Group 4: diabetic rats given *phyllanthus amarus*extract (500mg/kgb.w.) once daily

Group 5: diabetic rats given Glibenclamide (2.5mg/kgb.w.) once daily

All the treatment were administered orally once daily for 14 days. After the 14thdose of treatment with the extract, the rats were sacrificed. Blood sample were collected in a centrifugetubes for the estimation lipid profile

Determination of blood glucose level

The blood glucose level in mg/dl was determined during the periods of treatment within three days intervals using Finetest autocoding glucometer which was done by collecting the blood through orbital puncture of the tail vain of rats.

Statistical Analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$ (Adamu and Johnson, 1997)

3. RESULTS AND DISCUSSION

Table 1 shows the phytochemical composition of aqueous leaf extract of *phyllanthus amarus.* The table revealed presence of Alkaloids, terpenoids, saponins, glycosides, flavonoids, Total phenols and tannins, while, steroids, and phlobatannins were not detected.

Table: 1 Qualitative phytochemical composition of aqueous leaves extract of *phyllanthus amarus*

Phytochemicals	**Inferences**
Alkaloids	+
Flavoinoids	+
Saponins	+
Steroids	-
Total phenols	+
Terpenoids	+
Tannins	+
Glycosides	+
Phlobatannins	-

+=positive, -=Negative

3.1 Antidiabetic Study

Fasting blood sugar (FBS)

Effect of methanol leaf extracts of *phyllanthus amarus* on fasting blood glucose level of alloxan induced diabetic rats is presented in Figure 1. A significant (P<0.05) and progressive

increase in blood glucose level was observed in diabetic untreated rats throughout the experimental periods. The group of rats treated with methanol leaf extracts of *phyllanthus amarus* at dose of 250 and 300 mg/kg and rats treated with 2.5mg/kg glibenclimide produce a significant ($p<0.05$) dose dependent and progressive decrease in blood glucose level compare to the untreated rats. At the end of treatment period rat treated with 250 & 500 mg/kg *P. amarus* had final glucose level of 117.00±5.43 mg/dl & 106.00±4.56 mg/kg. Rats treated with 2.5mg/kg glibenclimide had final glucose level of 117.33±5.43 while the untreated rat had final glucose level of 562.50±15.45 mg/dl

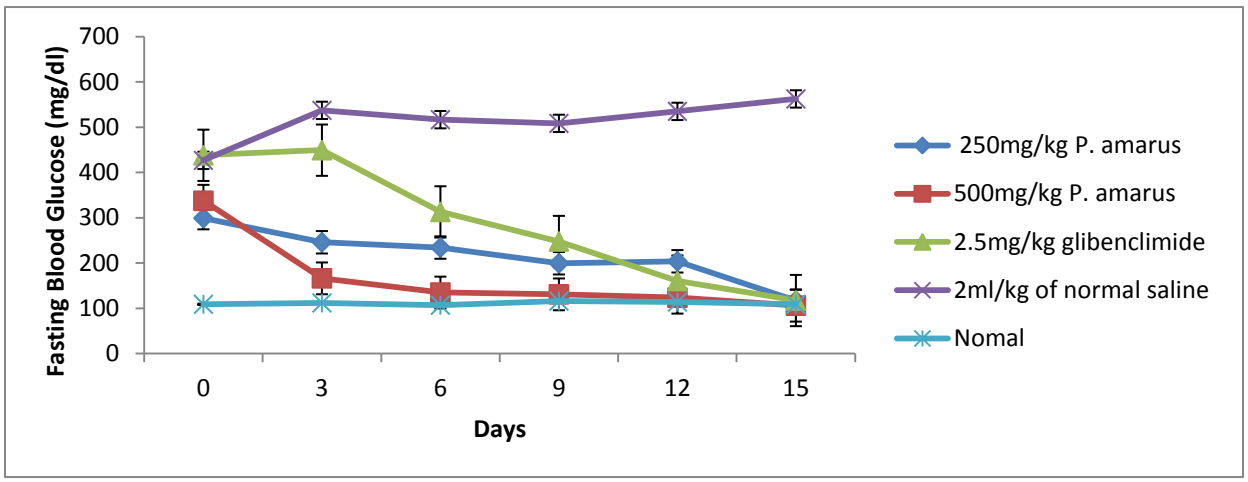

Figure 1: Effect of methanol leaf extract of *phyllanthus amarus* on fasting blood glucose level of alloxan induced diabetic rat

Body Weight

Administration of alloxan cause significant ($p<0.05$) and progressive decrease in body weight of the rats compare to the normal glycemic rat which shows a progressive increase in body weight with time. The methanol leaf extract of *phyllanthus amarus* at 250 and 500mg/kg cause significant improvement in body weight gain of the rat.

Table 2: Effect of methanol leaf extract of *phyllanthus amarus* on body weight change in alloxan induced diabetic rat

Groups	Day 0	Day 3	Day 6	Day 12	Day 15
250mg/kg *p. amarus*	156.88±3.59	148.07±2.37	141.08±2.34	145.97±1.46	152.07±2.35
500mg/kg *p. amarus*	164.37±4.69	150.76±4.12	148.21±0.12	154.46 ±2.14	161.67±0.72
Normal glyceamic	157.32±5.78	166.73±3.61	171.90±3.45	177.69±5.89	185.78±0.99
Positive control	173.58±4.40	169.42±3.31	172.36±4.67	179.42±3.56	182.97±3.03
Negative control	158.80±4.58	148.95±4.33	134.89±4.75	130.92±4.83	123.50±3.96

3.2 Discussion

Plant use in treatment of disease are said to contain active compounds called phytochemicals some of which are responsible for the characteristic adours, purgensies and colour of plant while

others give a particular plant its culinary medicinal or poisonous virtues (Evans, 2002). There is considerable interest by phytochemist to identify the therapeutic agent contained in this plant in order to establish the basis for their uses in traditional medical practice. This study revealed the presence of various important medicinal phytochemicals in methanol leaves extract of *phyllanthus amarus* The presence of these phytochemicals has been previously reported for this plant (Obianime and Uche, 2008),

Saponin has been reported to have anti-inflamatory, cardiac depressant and hyper-cholesterolemic (Trease and Evans 1985) Saponin also appear to kill or inhibit cancer cells without killing the normal cells in the process thus making them suitable as tumor inhibiting agent (Lewis and Elvin-Lewis, 1995). Flavonoids are the most diversified groups of phenolic compound found in plant. It biological activity include, antibacterial, anti-inflammatory, anti-allergic, protect against ulcers, vineses and antitumor effect (Okwu and Okwu, 2004). Flavonoids are free radical scavengers, super antioxidant and potential water soluble which prevent oxidative cell, damage and have strong anti-cancer activity (Birben *et al.*, 2012). Alkaloid are the most efficient therapeutically significant plant substance pure isolated alkaloids and their synthetic derivatives are use as basic medicinal agent for their analgesis, antispasmodic and antibacterial effect (Stray, 1988), Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive antifungal, antiinflammatory, antifibrogenic effect (Ghosal *et al*., 1996). The amounts of flavonoids and Alkaloids obtained in this study could translate strong antioxidative potentials of this plant. Furthermore, flavonoids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models (Kim *et al*., 2004). Saponins are glycosides of triterpenes, steroids or alkaloids. Previous researchers have demonstrated the hypoglycemic activity of triterpenoid glycosides (Kako *et al*., 1997). Thus the phytochemical constituents indicate that the methanol root extract of *phyllanthus amarus* could have potentials to be an antidiabetic agent.

The presence of important phytochemical in this plant is an indication that this plant if properly screened could yield a drug of pharmaceutical significance. However the absence of Phlobatannins and steroids agree with early studies which also found that not all phytochemicals are present in all plant and those that present differs according to the solvent use in the extraction process (Tsado *et al*., 2015)

Blood glucose is a key marker for diagnosis and prognosis of diabetes mellitus. Since alloxan selectively destroy the pancreatic insulin secreting B-cell leaving the less active cell and resulting in diabetic state, the evaluation of hypoglycemic activity of antidiabetic agent using alloxan-induce hyperglycemia model has been widely accepted (Szkudelski 2001).
Results from the present study revealed that administration of alloxan results in hyperglycaemic state as evident in by marked increase in blood glucose level. However, dose dependent reduction in blood glucose level was observed in alloxan-induced diabetic rats treated with methanol leaf extract of *phyllanthus amarus* . In this study, the extract produced a sustained significant ($p<0.05$) reduction in blood glucose level of the diabetic rats compared to non-diabetic rats. The hypoglycaemic effect of *phyllanthus amarus* in this study may be linked to the presenceof flavonoids and terpenes in the extracts. These compounds have been implicated in the antidiabetic activities of many plants (Okokon *et al*., 2006).

The blood glucose reducing activity was accompanied by increase in the body weights of rats the probable mechanism of the reduction in blood glucose level-body weight loss; could be through increased insulin secretion by pancreatic stimulation and the prevention of absorption of glucose in the gut (Zarei *et al*., 2015). The insulin deficiency cause drastic elevation in glucose level as a result of excessive production of endogenous glucose and cause changes in body weight (Ramachandran *et al*., 2011) which may be due to excessive breaking all down of tissue protein and lipid cause by insulin insufficiency. The result of the present study is in line with the finding of Aladodo *et al.*, (2013),who reported decrease in blood glucose level and increase in body weight gain of alloxan induced diabetic rat following treatment with aqueous root extract of *Jatropha curcas*

4. CONCLUSIONS

From the result obtained from this study it can stated that the methanol leaf extract of *phyllanthus amarus* contains important phytochemicals that has beneficial effect in reducing the elevated blood glucose level.

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