Introduction
Diabetes mellitus has been described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Walter, 1977, Albert et al., 1998, Kumar and Clark, 2005). It is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries (Mbaka et al., 2012). It is usually irreversible and its late complications result in reduced life expectancy and major health loss (Kumar and Clark, 2005). Currently diabetes is controlled by diet, exercise, oral hypoglycaemic agents and insulin therapy (Mallick et al., 2007). The high level of treatment failures, unpleasant side effects and enormous cost associated with oral antidiabetic drugs have generated an urgent need and desire for alternative treatments (Suneetha et al., 2010). The preferred choice of plant medicine by many might not be unconnected with the historical successes recorded in the use of herbal product in traditional system of medicine in managing diabetes mellitus (Mbaka et al., 2012). Besides, herbal formulations were observed to have fewer side effects and less toxic because of their rich natural source. Based on these and the support provided for its practice by the World Health Organization, several scientific investigations are being conducted with the view of identifying new active ingredient of natural source that would be more effective in the treatment of diabetes mellitus and diabetic complications (WHO, 1980).

Uveria chamae is a medicinal plant that belongs to the family, Annonaceae. It is a climbing plant commonly found in West Africa (Irvin, 1961, Okwu, 2004). In this region of the world, it is identified by numerous names such as: Ogholo by Esan people of Edo state, Ayiloko by the Igala, Kaskaifi by the Hausas, Oko oja by the Yorubas in Nigeria and Akotompo by Fula-fainte people of Ghana (James et al., 2013).
reported to have antivenom activity (James et al., 2013). The antibacterial and antifungal activities have also been reported (Okwu et al., 2004). However, no scientific study has been conducted on the antidiabetic activity of this plant. The aim of this study is to evaluate the hypoglycaemic properties and preliminary phytochemical screening of *U. chamae*.

**Materials and Methods**

*Plant materials:* The roots of *U. chamae* were obtained from a farm in Uromi, Edo State, Nigeria. They were authenticated by a taxonomist, mr T.K Odewo, of Botany department, University of Lagos. The voucher specimen with number LUH 3572 was deposited in the University herbarium.

*Preparation of the plant material for extraction:* The roots were washed with clean water to remove foreign materials, chopped into small pieces and dried in an oven at 45 degrees centigrade for four days. They were powdered to coarse particles with electric grinder. The root powder, weighing 500 g, was extracted with 93.3% aqueous ethanol by maceration with frequent stirring for 5 days. The extract was filtered using Whatman filter paper No. 4 and concentrated with a rotary evaporator. The concentrated extract was dried in an oven at 40 degrees centigrade to obtain 22.4 g dry residue (4.5% yields).

*Animals:* Swiss mice (20-25 g) and Wistar rats (160 ± 20 g) of both sexes were obtained from the Laboratory Animal Center, College of Medicine, University of Lagos, Idi-Ara and were kept under standard environmental condition of 12/12 hours light/dark cycle. They were housed in cages (5 animals per cage), maintained on standard animal pellets (Pfizer Feeds Plc, Nigeria), and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

*Acute toxicity study:* The toxicity study was carried out using 35 (male and female) Swiss albino mice (each weighing 20-25 g). The animals were randomly distributed into a control group and six treated groups, containing five animals per group. After fasting the animals overnight, the control group was given 0.4 mL of acacia (2%) suspension orally. while each treated group received oral solution of the extract prepared with 2% acacia in the doses of 1.0, 2.5, 5.0, 10.0, and 15.0 and 20.0 g/kg body weight respectively. The animals were observed continuously for the first 4 hours and then for each hour for the next 24 hours and at 6 hourly interval for the next 48 hours after administering the extract to observe any death or changes in general behaviour and other physiological activities (Ayub et al., 1997; Bürger et al., 2005). The dose that results in 50% mortality (LD$_{50}$) was then determined (Figure 1).

**Preliminary phytochemical screening:** Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods using the following reagents and chemicals: alkaloids with Mayer’s reagent and Dragendorff’s reagent (Farnsworth, 1966; Harborne, 1998), flavonoids with the use of 10% lead acetate and 20% sodium hydroxide (Sofowora 1993), tannins with 5% ferric chloride solution (Yadav and Agarwala, 2011) and saponins with ability to produce suds (Houghton and Raman, 1998). Terpenes with Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid. Terpenoids with a mixture of extract and chloroform and concentrated H$_2$SO$_4$ (Sofowora, 1993).

**Assessment of hypoglycemic properties of U. Chamae (single dose study):** Fifteen rats were randomly selected into 3 groups, 5 rats per group. The rats were fasted overnight. Fasting blood glucose levels of each group was evaluated. Group I, untreated control was given 0.5 mL of 2% Acacia, while Group II and III were given the extract, orally at doses of 250 mg/kg and 500 mg/kg respectively. Blood samples were collected for estimation of Blood glucose level from the tail vein at 2, 4 and 6 hours after giving the extract (Santosh et al., 2007).

**Effect of the extract on oral glucose tolerance test:** Normal rats male and female were fasted over night and divided into four groups of five rats each. Blood samples were collected from the tail veins of the rats to estimate the fasting blood glucose levels. Group 1, the control, was given 0.5 mL of 2% Acacia and group 2, 3 and 4...
were given 100, 250 and 500 mg/kg of extract respectively. Thirty minutes after administering the extract, the three groups were administered 40% (w/v) glucose at a dose of 1 mL/100 g body weight orally (Ogbonnia et al., 2011). Blood glucose levels monitored at 30 min, 60 min and 120 min intervals and reported as the average glucose level of each group.

**Statistical analysis:** Analysis of data was done using Graph Pad Prism 6. One way analysis of variance and t-test were used to compare means. Means ± SEM are shown in all tables. Level of significance was set at p<0.05 or p<0.01.

**Results**

In the acute toxicity study (Table I), there was no death among the animals that received 1,000-5,000 mg/kg body weight of the extract. The animals that received 10,000-20,000 mg/kg body weight of the extract died within 24 hours. The LD$_{50}$ of the drug was calculated to be 7,080 mg/kg body weight.

Table II shows the hypoglycaemic effects of a single oral administration of two doses 250 and 500 mg/kg body weight of the root extracts of *U. chamae* in normal healthy rats. These doses showed significant reduction (p<0.05) in blood glucose levels at 2 and 6 hours compared to control. Rats treated with 250 mg/kg of the extract showed a maximum reduction of 54.2% in blood glucose level after 6 hours of oral administration. However, the reduction in blood glucose level at the dose of 500 mg/kg body weight at 2 and 4 hours was 17.4% and 3.2% respectively.

**Figure 2** shows the summary of the oral glucose tolerance test. Following oral glucose load in the control group, the rise in blood glucose level reached a peak at 30 min of glucose load. Decrease in blood glucose level occurred after 30 min but the blood glucose level failed to return to baseline after 120 min. There was a significant decrease (p<0.05) in blood glucose levels at 30, 60 and 120 min with a percentage decrease of 57.5%, 28.6% and 41.4% respectively in the group of rats treated with 500 mg/kg dose of extract compared with control.

The preliminary phytochemical screening of the root extract of *U. chamae* revealed the presence of flavonoids, alkaloids, cardiac glycosides, terpenoid and terpenes, saponin, tannin proteins and sugars (Table III).

**Discussion**

The major goals in the treatment of diabetes has been to keep both short-term and long-term glucose levels within acceptable limits, thereby reducing the risk of long term complications (Park et al., 2009). This could be achieved by optimizing both fasting blood glucose and postprandial glucose levels which have been found to be very important in achieving near normal glucose levels. Postprandial glucose levels have been reported to serve as a better maker of glycemic control than fasting blood sugar levels (Park et al., 2009).

Drugs that reduce post-prandial hyperglycemia by suppressing hydrolysis of starch have been found useful in the control of diabetes mellitus (Tundis et al., 2010; Kazeem et al., 2013). Many herbal extracts

**Table I: Oral acute toxicity of root extract of *Uveria chamae* in mice**

| Group | Dose (mg/kg) | Log dose | Mice alive | Dead mice | Cumulative alive | Cumulative dead |
|-------|-------------|----------|------------|-----------|-----------------|----------------|
| I     | 20,000      | 4.30     | 0          | 5         | 15              | 0              |
| II    | 15,000      | 4.18     | 0          | 5         | 10              | 0              |
| III   | 10,000      | 4.0      | 0          | 5         | 5               | 0              |
| IV    | 5,000       | 3.7      | 5          | 0         | 5               | 0              |
| V     | 2,500       | 3.4      | 5          | 0         | 10              | 0              |
| VI    | 1,000       | 3.0      | 5          | 0         | 15              | 0              |

Control received 0.4 mL of 2% acacia; Group I: 20,000 mg/kg; Group II: 15,000 mg/kg; Group III: 10,000 mg/kg; Group IV: 5,000 mg/kg; Group V: 2,500 mg/kg; Group VI: 1,000 mg/kg; n=5

**Table II: Assessment of hypoglycaemic properties of *Uveria chamae***

| Group | Blood glucose levels (mg/dL) | Pre-treatment | Post-treatment |
|-------|-----------------------------|---------------|---------------|
|       |                             | 0 (FBG)       | 2 hours       | 4 hours       | 6 hours       |
| Group I| 62.2 ± 2.5                  | 63.8 ± 2.4    | 53.8 ± 2.5    | 66.3 ± 7.0    |
| Group II| 57.7 ± 1.6                  | 53.3 ± 1.9*   | 50.4 ± 1.5    | 30.4 ± 1.1*   |
| Group III| 60.8 ± 2.4                  | 52.7 ± 1.5*   | 52.1 ± 1.8    | 36.6 ± 3.3*   |

Mean ± SEM; n=5; *p<0.05; **p<0.01 vs. control group; Group I: control received 0.5 mL of 2% acacia; Group II: 250 mg/kg; Group III: 500 mg/kg
have reported for their antidiabetic activities and are currently being used in traditional medicines for the treatment of diabetes. However, such medicinal plants have not yet gained much importance as medicines due to lack of sustained evidence (Sudha et al., 2011).

The results of this study showed that the median lethal dose (LD$_{50}$) of the root extract was determined to be 7.08 g/kg body weight translating to 490 g dose for human adult. According to Loomis and Hayes (1996), the extract can be classified as being practically non-toxic since this value is much higher than Organization for Economic Cooperation and Development (OECD) toxicity index of 2 g/kg (Walum, 1998; OECD, 2001). Therefore, the extract may be considered to be safe for consumption.

This study revealed that the rats treated with 250 mg/kg and 500 mg/kg of the extract had maximum reduction of 54.15% and 44.80% in blood glucose level after 6 hours of oral administration respectively. The extract exerted hypoglycaemic activity by decreasing the blood glucose level significantly. Low blood glucose level reduces the risk of complications associated with diabetes (Attele et al., 2002; Emordi et al., 2014).

The result of oral glucose tolerance test showed that there was a significant decrease (p<0.05) in blood glucose levels at 30, 60 and 120 min with a percentage decrease of 57.5%, 28.6% and 41.4% respectively in the group of rats treated with 500 mg/kg dose of extract compared with control.

Insulin release in response to a glucose load occurs in two phases in humans and in rodents. The early phase peaks within the first 15-30 min and is responsible for limiting the initial rise in glucose upon meal ingestion. The late phase of insulin secretion occurs later than 30 min after a meal, and may persist for several hours. This delayed burst of insulin secretion is responsible for returning glucose to baseline fasting levels. In the face of insulin resistance, the late phase of insulin secretion persists for an extended period and contributes to excessive insulin levels even after a return to the fasted state, resulting in fasting hyperinsulinemia (Oda, 2012). From this study, the marked reduction in plasma glucose concentration may be as a result of increased release of insulin from beta cells. This may also account for the hypoglycaemic activity of the extract.

The health benefits of medicinal plants are attributed in part to their unique phytochemical composition (Okwu, 2005). Phytochemicals are secondary metabolites of plant origin which act as antioxidants and stimulate the protective enzymes in the liver or block damage to genetic materials (Okwu, 2004). They prevent the occurrence of oxidative chemical species, stimulate antioxidant repairing mechanism and scavenging capacity for free radicals in the system.

Flavonoids are phenolic compounds that possess antioxidant and antidiabetic potentials due to the presence...
of hydroxyl groups that confer scavenging ability on them (Mayur et al., 2010). Research has shown that many plants containing flavonoids have been used for the treatment of diabetes (Meiselman et al., 1976; Choi et al., 1991; Hassig et al., 1999).

Tannins induce phosphorylation of the insulin receptors as well as translocation of glucose transporters 4 (GLUT-4), the protein factor involved in the signaling pathway of insulin-mediated glucose transport and the inhibition of the expression of key gene for adipogenesis thereby helping to reduce blood glucose level without increasing adiposity (Liu et al., 2005).

Suba and co-workers reported in 2004 that tannin has antidiabetic activity.

One of the most important carbohydrate-splitting enzymes is the maltase-glucamylase which helps to breakdown dietary disaccharides into monosaccharide in the small intestine. If its activity is inhibited, the digestion and absorption of monosaccharide can be slowed down, decreasing the post-prandial hyperglycaemia. The potential therapeutic use of polyhydroxylated alkaloids in the treatment of type-2 diabetes due to their ability to inhibit maltase-glucamylase has been reported (Shang et al., 2013). It is therefore possible that the phytochemicals present in the root extract of *U. chamae* may be responsible for the observed hypoglycaemic activity.

The LD<sub>50</sub> value 7.1 g/kg obtained was a clear indication that *U. chamae* is safe for use. The study showed that the root extract of *U. chamae* has hypoglycaemic activity which may be as a result of increased release of insulin from beta cells. The hypoglycaemic activity of the extract may also be accounted for by the presence of several bioactive compounds like flavonoids, tannins and alkaloids.

Acknowledgement

The authors wish to thank Mr. M.E Idemudia of Idumu-une Quarters, Uromi, Edo State, Nigeria, for his assistance in plant collection.

References

Albert RGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part I, Provisional report of WHO consultation. Diabetes Med. 1998; 15: 539-53.

Attele AS, Zhou Y, Xie J, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan C. Anti-diabetic effects of *Panax ginseng* berry extract and the identification of an effective component. Diabetes 2002; 51: 1851-58.

Ayub SMA, Garg SK, Garg KM. Subacute toxicity studies on pendimethalin in rats. *Indian J Pharmacol*. 1997; 29: 322-24.

Burger C, Fischer DR, Cordenuzzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosus* in mice. *J Pharmaceut Sci*. 2005; 8: 370-73.

Choi JS, Yokozawa T, Oura H. Improvement of hyperglycaemia and hyperlipidemia in streptozotocin-diabetic rats by a methanolic extract of *Prunus davidiana* stems and its main component, Prunin. Planta Medica. 1991; 57: 208-11.

Colegate SM, Molyneux RJ. Bioactive natural products detection, isolation and structural determination. 1<sup>st</sup> ed. USA, CRC Press, 1993, p 447.

Emordi EJ, Ogbonnia OS, Olayemi OS, Anyika NE, Iribhogbe IO. Hypoglycaemic and hypolipidemic effects of the phytomedicine- bee honey and *Musa paradisica* extract in alloxan-induced diabetic rats. *Int J Herbs Pharmacol Res*. 2014; 3: 16-23.

Farnsworth RN. Review on biological and phytochemical screening of plants. *J Pharm Sci*. 1966; 55: 225-76.

Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3<sup>rd</sup> ed. London, Chapman and Hall, 1998, p 235.

Hassig A, Liang WX, Schwabl H, Stampfli K. Flavonoids and tannins: Plant-based antioxidants with vitamin character. *Med Hypotheses*. 1999; 52: 479-81.

Houghton PJ, Raman A. Laboratory handbook for fractionation of natural extracts. London, Chapman and Hall, 1998, p 199.

Institute of Laboratory Animal Research. Commission on life science. National Research Council, 1996.

Irvin FR. Woody plants of Ghana with special reference to their uses. London, Oxford University Press, 1961, pp 19-20, 695.

James O, Godwin EU, Otini IG. *Uvaria chamae* (Annonaceae) plant extract neutralizes some biological effects of *Naja nigricollis* snake venom in rats. *Bri J Pharmacol Toxicol*. 2013; 4: 41-50.

Kazeem MI, Abimbola SG, Ashafa AOT. Inhibitory potential of *Gossypium arboreum* Linn leaf extracts on diabetes key enzymes (α-amylase and α-glucosidase). Bangladesh *J Pharmacol*. 2013; 8: 149-55.

Kumar P, Clark M. Diabetes mellitus and other disorders of metabolism. Kumar and Clark's Clinical Medicine. 6<sup>th</sup> ed. Philadelphia, Elsevier Saunders, 2005, pp 1101-51.

Liu X, Kim J, Li Y, Li J, Liu F, Chen X. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-Li cells. *J Nutr*. 2005; 135: 165-71.

Loomis TA, Hayes AW. Loomis’s essentials of toxicology. 4<sup>th</sup> ed California, Academic Press, 1996, pp 208-45.

Mallick C, Chatterjee K, Guhabiswas M, Ghosh D. Antihyperglycemic effects of separate and composite extract of root of *Musa paradisica* and leaf of *Coccinia indica* in streptozotocin-induced diabetic male albino rat. *Afr J Trad Cam*. 2007; 4: 362-71.

Mayur B, Sandesh S, Shrutti S, Sung-Yum S. Antioxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides*...
Mbaka GO, Ogbonnia SO, Banjo AE. Activity of Raphia hookeri root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan-induced diabetic rats. J Morphol Sci. 2012; 29: 214-22.

Meiselman HL, Halpern BP, Dateo GP. Reduction of sweetness judgments by extracts from the leaves of Ziziphus jujuba. Physiol Behavior. 1976; 17: 313-17.

Oda E. Metabolic syndrome: Its history, mechanisms, and limitations. Acta Diabetol. 2012; 49: 89-95.

OECD. Acute oral toxicity: Fixed dose procedure. OECD Guide line for testing chemicals. 2001.

Ogbonnia SO, Mbaka GO, Anyika EN, Ladiju O, Igbokwe HN, Emordi JE, Nwakakwa N. Evaluation of anti-diabetics and cardiovascular effects of Parinari curatellifolia seed extract and Anthoclista vogelli root extract individually and combined on postprandial and alloxan-induced diabetic albino rats. Bri J Med Medical Res. 2011; 1: 146-62.

Okwu DE. Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. J Sustainable Agric Environ. 2004; 6: 30-37.

Okwu DE. Phytochemicals, vitamins and mineral content of to Nigerian medicinal plants. Int J Mol Med Adv Sci., 2005; 1: 375-81.

Park J, Bong H, Jeong H, Kim KY, Kim JY, Kwon O. Post-prandial hypoglycemic effect of mulberry leaves in Goto-kakizaki rats and counterpart control Wistar rats. Nutr Res Pract. 2009; 3: 272-78.

Santosh KS, Achyut NK, Prashant KR, Geeta W. Assessment of glycemic potential of Musa paradisiacaal stem juice. Indian J Clin Biochem. 2007; 22: 48-52

Shang Q, Xiang J, Zhang H, Li Q, Tang Y. The effect of polyhydroxylated alkaloids on maltase-glucoamylase. PLoS ONE. 2013; 8: 1-5.

Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria, Spectrum Books limited, 1993, pp 191-289.

Suba V, Murugesan T, Rao BR, Ghosh L, Pal M, Mandal SC, Saha BP. Antidiabetic potential of Barleria lupulina extract in rats. Fitoterapia 2004; 75: 1-4.

Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Compl Alternative Med. 2011; 11: 1-10.

Suneetha B, Sujatha D, Prasad KVSRG. Antidiabetic and antioxidant activities of stem juice of Musa paradisiaca on alloxan induced diabetic rats. Int J Adv Pharmaceut Sci. 2010; 1: 167-76.

Tundis R, Loizzo MR, Menichini F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycemic potential in the treatment of diabetes: An update. Minirev Med Chem. 2010; 10: 315-31.

Walter BJ. An introduction to the principles of disease. Philadelphia, USA, W.B. Saunders Company, 1977, pp 374-75.

Walum E. Acute oral toxicity. Environ Health Perspect. 1998; 106: 498.

World Health Organization. Expert committee on diabetes mellitus. (Technical Report, Series 646). Geneva, WHO, 1980.

Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011; 3: 10-14.