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HYPOGLYCEAMIC ACTIVITY OF TWO MEDICINAL CLAY; *NZU AND ULO*

Diabetology

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ABSTRACT

Diabetes mellitus (DM) is a global health problem affecting million people in Africa. The consumption of medicinal clay (geophagy) by DM patients in North Eastern Nigeria, as a traditional remedy for the ailment has become very popular. The hypoglycaemic activity of two medicinal clay: *Nzu* and *Ulo* were evaluated. The elemental constituents of the medicinal clay were investigated using Atomic Absorption Spectrophotometric (AAS) analysis. Hyperglycaemia was induced in 20 of 28 normoglycaemic male rats (170 – 260 g) using the Oral Glucose Tolerance Test (OGTT) protocol, and the rats were shared into seven (7) groups of four (4) rats each and treated with 100 or 200 mg kg^{-1} of Nzu or Ulo, respectively. Elemental analysis of both clay samples, showed the presence of Zn, Pb, Mg and Ni above WHO recommended levels per day. The mean blood glucose level of the hyperglycaemic treated rats in group III, IV, V, VI and VII after administration of the medicinal clay (*Nzu* and *Ulo*) was compared with values in the control groups (I and II) and the Glibenclamide treated group. Significant ($p < 0.05$) reduction in the blood glucose concentrations after 1st, 2nd, 3rd hours were observed between the hyperglycaemic treated and untreated rats. The control drug Glibenclamide (600 µg/kg) showed much lover blood glucose concentration at the 3rd hour above the medicinal clay. It was concluded therefore, that the possible reason for the observed hypoglycaemia, could be due to delayed digestion and assimilation of food, within the gut of the rat or slowed to enable a gradual absorption and catalysis of the glucose within the cells. The consumption of 100 and 200 mg/kg b.wt. *Nzu* and *Ulo* reduced blood glucose levels in hyperglycaemic rats and it justified in the management of diabetes mellitus.

KEYWORDS

Diabetes mellitus, hyperglycaemic, geophagy, medicinal clay, blood glucose level.

INTRODUCTION

Diabetes mellitus (DM) is a global problem affecting about 10% of the world's population and projection suggested an 8.3% increase in 2011 and 9.9% by 2030 (IDF Atlas, 2011). Being one of the largest health emergencies of the 21st century, it is presumed to become the 7th leading cause of death by 2030 (Mukundi *et al*., 2015 and Arumugan *et al*., 2013).

Africa is presently experiencing an epidemiological transition with the affliction of non-communicable diseases particularly DM, which is in the process of overwhelming the poor healthcare system already overstretched by HIV/Aids, tuberculosis, malaria and hepatitis (Agyei- Mensah and Aikins, 2010).

The world health organization (WHO) report (2015), stated that about 14.2 million adults in Africa suffered from or were leaving with DM, while the International diabetic federation (IDF, 2010) reported that more than two thirds of people leaving with DM in Africa are undiagnosed, and more than 321, 100 deaths were attributed to DM (IDF Atlas, 2010). The African continent is expected to have the highest increase in the number of diabetics by 2030 compared to other continents (Whiting *et al*., 2011).

In sub-saharan Africa, which is still plagued by civil and political unrests, massive Government corruption and the prioritizing of non-essentials over healthcare, education and so on, over 66.7% of diabetic patients are said to be undiagnosed (Mbaya *et al*., 2010).

The diabetic association of Nigeria (2011) reported that over six million people suffer from DM. A survey from hospital records indicated the alarming increase in both prevalence and incidence of DM among all ethnic groups and social classes in Nigeria (Nwamarah *et al*., 2015). The prevalence of DM in Nigeria varies from rural areas to urban settlements, for example, a study showed that Mangu village in the middle belt of Plateau state had about 0.65% of its population with full blown DM, while Lagos in the South West had about 11% (Akinkugbe, 1997). The world health organization (WHO) opined that, Nigeria has the highest number of diabetic patients in Africa with an estimated 1.7 million and projected to go even higher to about 4.8 million by 2030 (Chinenye *et al.,* 2012).

There is paucity of statistical data of diabetics in the North Eastern part of Nigeria as a result of the instability. However, unpublished reports showed that there is high incidence of DM. The region has been known for high use of herbs as medicines especially for DM (Zaruwa[a,and,b] *et al*., 2015). Recent observations on the traditional management of DM in both males and females ingest what looks like solid ash, later identified to be a type of medicinal clay. Eating earthy substances such as clay or mud by humans is called Geophagy. It involves the consumption of clay, mud, ash or stones for various reasons (Landa, 2009). The practice of earth eating observed in recent times is new to the North East except among a few women, it is known to have originated from the South East of Nigeria.

Nzu and *Ulo* are earthy substances from South Eastern, Nigeria, that is commonly eaten among women, for the treatment of morning sickness, stomach cramps, and other ailments relating to early pregnancy, including gestational diabetes (Ekong *et al*., 2015; Agene *et al*., 2014).

The main objective of this work is to study the hypoglycaemic properties of medicinal clay (*Nzu* and *Ulo*) on glucose challenged rats, to ascertain its hypoglycaemic effect by glucose tolerance test (GTT) as claimed by local users in North Eastern Communities of Nigeria. The experiment was restricted to the use of oral glucose administration to induce hyperglycaemia as a preliminary test case, towards understanding if the medicinal clay possesses any hypoglycaemic activity and its possible mechanism of action.

MATERIALS

The equipment and apparatus that was used for the experiments included the following: Sharp razor blade, weighing balance (Jenway Scientific, USA), Mortar and pestle, Measuring cylinder, Syringe and needle, AAS- 220 VAC (Buck Scientific, USA), Wister rats, Fine test Glucometer (Infopia Co., Ltd, Korea), D-glucose and Distilled water. All chemicals and reagents were analytical grade.

SAMPLE IDENTIFICATION

Medicinal clay (*Nzu* and *Ulo*) was purchased from Mubi local market in Adamawa State. The clay was identified by Mr. Hananiah Markus of Faculty of Agric Soil Science Lab, Adamawa State University Mubi, Nigeria.

Figure 1: ***NZU*** **Medicinal Clay**

Figure 2: ***Ulo*** **Medicinal Clay**

Sample preparation and filtration

Medicinal clay (*Nzu* and *Ulo*) was grinded in a mortar and pestle, and

sieved with a mesh (115 µm). Samples were retained for subsequent analysis.

Digestion of Clay Samples

Clay samples were digested according to Vento *et al*., (2008). Accurately 5g of clay samples was weighed into 250 cm^3 conical flask and moistened with few drops of water to prevent sputtering. 3 cm^3 of 30% H_2O_2 was then added and left to stand for 60 min until the vigorous reaction ceased. About 75 cm^3 of 0.5mol/dm^{-3} solution of H*Cl* was added and the content heated gently at low heat on the hot plate for 2 hr. The digest was allowed to cool, and then filtered into 50 cm^3 standard flask. The content was then diluted to 50 cm^3 mark with the same acid solution. Triplicate digestions of each sample together with a blank were carried out.

Atomic Absorption Spectrophotometric (AAS) Analysis

This was achieved by means of absorbance measurements of the dilute solutions using the advantage of the sensitivity of the AAS spectrophotometer. The concentrations in mg/l of six elements was determined in all the samples namely Zn, Pb, Mg, Ni, Mn and Cr with the Atomic AAS. The instrument was calibrated by allowing some warm up time for the AAS machine and the control knob adjusted until the meter reads 0% transmittance. Three glass cuvettes were obtained and blotted with tissue paper. One of them was used for the solvent blank, distilled water, while the others were used for the analyte samples. The absorbance reading was measured for each element serial dilution of the various samples. Calibration curve for each element was prepared from which unknown concentration in the samples was extrapolated. A plot of the absorbance (A) versus concentration gave a straight line graph.

Induction of Hyperglycaemia by Oral Glucose Administration

Normal healthy male rats (170 – 260 g), housed under standard environmental conditions with temperature (27 ± 2 ^{0}C) and equal light and dark (12/12 hr). The rats were fed with standard animal Chow (Moraal *et al*., 2012) and water *ad libitum*.

Hyperglycaemia was induced in rat *via* oral glucose solution ingestion with a feeding tube using standard procedures (Steffes, 2008). The effectiveness of the procedure was confirmed by the presence of high fasting blood glucose (hyperglycaemia) level determined an hour after induction. Graded dose of the medicinal clay solution were administered *via* feeding tube to the rats. Physical wellness, water and food consumption pattern was monitored during the experimental period.

EXPERIMENT DESIGN:

The method of Irshaid *et al*., (2010) was modified and adopted. A total of 28 rats were divided into 7 experimental groups of 4 rats each. **Group I** served as control group negative. **Group II** were hyperglycaemic control group, positive. **Group III** were hyperglycaemic rats, treated with 100 mg kg^{-1} body weight of *Nzu*. **Group IV** were hyperglycaemic rats that were treated with 200 mg kg^{-1} body weight of *Nzu.* **Group V** were hyperglycaemic rats that were treated with 100 mg kg^{-1} body weight *Ulo*. **Group VI** were hyperglycaemic rats that were treated with 200 mg kg^{-1} body weight *Ulo*. **Group VII** were hyperglycaemic rats treated Glibenclamide (600 µg/kg p.o.).

Blood collection and glucose determination:

Blood samples were collected from the fasted rats of the seven groups prior to the treatment with above schedule, and three times within a week after oral glucose challenge and treatments. Blood sample was collected by snipping tail with sharp razor blade and blood glucose level was measured immediately with a glucometer.

Statistical analysis

The results were expressed as mean ± standard deviation. Difference between groups was analysed with student's t-test. Difference between groups was considered significant at the conventional level of significance (95% confidence limit and probability level of 0.05). The results were significant if $p<0.05$.

RESULTS AND DISCUSSION:

The quantitative analysis of some elemental constituents of the medicinal clay are shown in Table 1. It indicated the presence of Zn, Pb, Mg, Ni, except Cr in both samples at varying concentrations. A perusal of elemental data in Table 1 show that Zn (1.15 to 1.20 mg/kg) Pb (0.50 to 1.00 mg/kg) Mg (2.00 to 2.40 mg/kg) Ni (2.20 to 2.50mg/kg) Cr (BDL) *Nzu* and *Ulo*.

Table 1.0: Elemental Concentrations in Clay Samples in (mg/kg)

Sample (Clay)	Zn	Pb	Mg	Ni	Cr
Ulo	1.20 ± 0.01	0.50 ± 0.00	2.00 ± 0.00	2.50 ± 0.00	BDL
Nzu	1.15 ± 0.01	1.00 ± 0.00	2.40 ± 0.00	2.20 ± 0.00	BDL
Range	1.15 - 1.20	0.50 - 1.00	2.00 - 2.40	2.20 - 2.50	BDL
FAO/WHO	0 - 15	0 - 0.15	-	0 - 1.5	0 - 0.40

Values are in mean ± standard deviations of 3 replications.
BDL = below detection limit,

Zinc and lead were present at the normal range. Nickel was present at levels higher than WHO recommendations. Chromate was not detected.

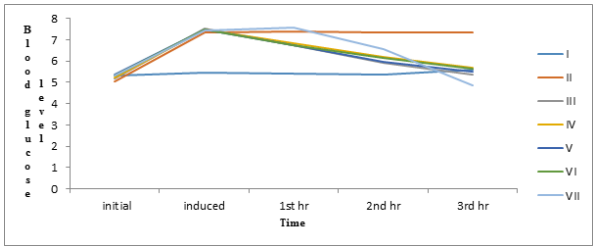

Fig. 3: Effect of oral administration of *Nzu* and *Ulo* on blood glucose level on the first day of treatment.

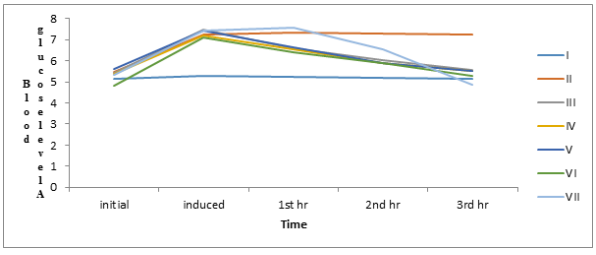

Fig. 4. Effect of oral administration of *Nzu* and *Ulo* on blood glucose level on the second day of treatment

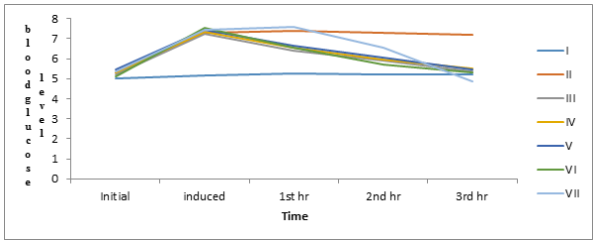

Fig. 5: Effect of oral administration of *Nzu* and *Ulo* medicinal clay on fasting blood sugar on the blood glucose level

DISCUSSION

The analysis of various elements in the clay samples in Table 1 showed the presence of Zn, Pb, Mg, and Ni as constituents of both clay samples. Clinical research suggests that diabetes causes the disruption of mineral trace elements in the body (Soetan *et al*., 2010; Khan and Awan, 2014). The presence of these trace elements in the medicinal clay (*Nzu* and *Ulo*) therefore plays important role in exerting some pharmacological (hypoglycaemic) effects, though the exact mechanism of these active metabolites is not fully understood. The elements Mn, Cu, Fe, and Zn were previously reported to be responsible for the secretion of insulin from the beta cells of pancreas (Ngugi *et al*., 2015; Ekosse and Jumbam, 2010; Chausmer, 1998). Therefore, the presence of same elements in the medicinal clay gave some credence to the hypoglycaemic effect of the medicinal clay as observed. For Oral Glucose Tolerance Test, the blood samples were analyzed for glucose concentration at 0, 1st, 2nd and 3rd hour, respectively. Induction of hyperglycaemia in the experimental rats was confirmed by the presence of high blood glucose levels above (5.5 mmol/l). No mortality was recorded during the experimental period. Figure 3, 4 and 5 showed the effect of medicinal clay (*Nzu* and *Ulo*) on blood glucose levels of the fasted hyperglycaemic rats, as there was gradual decrease in blood sugar levels after 1, 2 and 3 hours of administration of the medicinal clay solution (*Nzu* and *Ulo*) in the

doses of 100 and 200 mg kg^{-1} body weight of both samples, respectively. The mean blood glucose level of the hyperglycaemic treated rats in group 3, 4, 5, 6 and 7 after administration of the medicinal clay (*Nzu* and *Ulo*) was compared with the values in control groups (I and II) and the Glibenclamide treated group. Significant reductions in the blood glucose concentrations after 1st, 2nd, 3rd hours were observed between the hyperglycaemic treated and untreated rats. The blood glucose concentration decreased, when compared with the Glibenclamide treated group. The difference between the two was significant, however the glucose level remained unchanged in the hyperglycaemic untreated group. The control drug Glibenclamide (600 µg/kg) showed much lower blood glucose concentration at 3rd hour above the medicinal clay. Though normoglycaemic models for diabetes was used, previous reports by other researchers has shown that, reduction in blood glucose concentration in the normoglycaemic models translates to the induced diabetic models as well (Abdourahman and Edwards, 2008; Vessal *et al*., 2003; Anitha *et al*., 2012; Sornalakshmi *et al*., 2016).

From the results, it can be deduced that the likely reason for the medicinal clay's hypoglycaemic effect could be due to delayed digestion and assimilation of food within the gut of the rat or slowed to enable a gradual absorption and catalysis of the glucose within the cells, hence the lowering of the blood glucose observed over the hours. A similar pattern of hypoglycaemic effect was observed with coconut oil (Norman, 2017; Schumacher, *et al*., 2016). This deduction agrees with previous opinion among other possible effect of medicinal clay in the animal diet, that "high cation exchange capacity of many clay soils (good at holding to positively charged ions and even microbes), which makes them efficient at binding with toxins, thus reducing the effects of noxious plant chemicals such as tannins and glycol-alkaloids; the detoxification mechanism which is the way that clays affect digestion by reinforcing the intestinal mucosa and blocking the gut, so digestion is slowed to allow better absorption; the quelling gastrointestinal upset since kaolin and smectite present in clay (Christophe and Vernisse, 2009), are known to reduce nausea and diarrhoea, and increasing the pH level of the gut, thus making any ingested content less acidic than the stomach's hydrochloric acid and in doing so, potentially allaying heartburn". These are the likely effects being exploited by the South Eastern Nigerian women when the medicinal clay is used during pregnancy or other conditions.

The Government of North Eastern Nigeria should prioritize provision of healthcare facilities such hospitals, clinics, maternity and counselling centres, and scholarship grants to deserving young men and women from the affected areas to study health related courses, so as to serve their people. There should also be schemes and program to assist the poor in alleviating poverty and the provision of jobs for the women and youths especially.

CONCLUSION

Medicinal clay (*Nzu* and *Ulo*) administered to hyperglycaemic rats significantly reduced their blood glucose concentration and therefore justified its usefulness as a remedy for hyperglycaemia/diabetes mellitus among local users in North Eastern Nigeria. The medicinal clay studied has ample concentration of Zn, Pb, Mg, and Ni above FAO/WHO permissible limits, but no Cr in it. The use of medicinal clay could be harmful to the users leading to incidences of deformation in babies and different types of cancers among women. Therefore, its use should be with caution since geophagic practice cannot be eliminated among local users. Finally, local Authorities, such as Local and State Government should prioritize the provision of health facilities and set up schemes to alleviate poverty among the teaming population of North Eastern Nigeria.

REFERENCE

1. Abdurrahman, A and Edwards, J. G. (2008). Chromium Supplementation Improves Glucose Tolerance in Diabetic Goto-Kakizaki Rats. IUBMB Life, 60(8): 541–548.
2. Agene I. J., Lar U. A., Mohammed S.O., Gajere E. N., Dang B; Jeb D. N; Ogunmola J. K. (2014). The Effects of Geophagy on Pregnant Women in Nigeria. American Journal of Human Ecology Vol. 3, No.1, 1-9.
3. Agyei-Mensah, S. and Aikins de-Graft, A. (2010). Epidemiological transition and the double burden of disease in Accra, Ghana. J Urban Health. 87(5):879-97.
4. Akinkugbe, O. O. (1997). Non-communicable disease in Nigeria. Final report of national survey. Federal Ministry Health and Social Services, Lagos, 64-90.
5. Anitha, M., Sakthidevi, G., Muthukumarasamy, S. and Mohan, V. R. (2012). Effect of Cynoglossum zeylanicum (Vehl ex Hornem) Thunb. Ex Lehm on Oral Glucose Tolerance in rats. Journal of Applied Pharmaceutical Science Vol. 2 (11), pp. 075-078.
6. Arumugam, G., Manjula, P., Paari, N. (2013). Anti-diabetic medicinal plants used for diabetes mellitus. Journal of Acute Disease. 196-200.
7. Chausmer, A. B. (1998). Zinc, Insulin and Diabetes. Journal of the American College of Nutrition 17(2):109-15.
8. Christophe, D. and Bernard, V. (2009). Anti-Diarrheal Effects of Diosmectite in the Treatment of Acute Diarrhea in Children. Pediatr Drugs 11 (2): 89-99.
9. Chinenye, S., Uloko, A. E., Ogbera, A. O., Ofoegbu, E. N., Fasanmade, O. A., Fasanmade, A. A. and Ogbu, O. O. (2012). Profile of Nigerians with diabetes mellitus – Diabcare Nigeria study group (2008): Results of a multicenter study. Indian J Endocrinol Metab.16(4): 558–564.
10. Ekong, M., Ekanem, T. B., Osim, E. E., and Eluwa, M. A. (2015). Maternal Geophagy of Calabar Chalk and the developing cerebral cortex. Nigerian Journal of Neuroscience 7(1): 16 – 23.
11. Ekosse, E. G. and Jumbam, N. D. (2010). Geophagic clays: Their mineralogy, chemistry and possible human health effects. African Journal of Biotechnology Vol. 9(40), pp. 6755-6767
12. International Diabetes Federation (IDF) Atlas, Fifth Edition (2011). Online version of IDF Diabetes Atlas: www.idf.org/diabetesatlas
13. Khan, A. R. and Awan, F. R. (2014). Metals in the pathogenesis of type 2 diabetes. Journal of Diabetes & Metabolic Disorders 13:16 – 31.
14. Landa, E. R, Feller, C. (2009) 'Soil and Culture' (Springer: Dordrecht, The Netherlands). Pp. 524.
15. Mbaya. J. C., Motala, A. A., Sobngwi, E., Assah, F. K., Enoru, S. T. (2010). Diabetes in sub-Saharan Africa. Lancet. 26; 375(9733):2254-66.
16. Moraal, M., Leenaars, P. P., Arnts, H., Smeets, K., Savenije, B. S., Curfs, J. H., Ritskes-Hoitinga, M. (2012). The influence of food restriction versus ad libitum feeding of chow purified diets on variation in the body weight, growth and physiology of female Wistar rats. Lab Anim 46(2) 101 – 107.
17. Mukundi, J. M., Ngugi, M. P., Njagi, E. N. M., Njagi, J. M., Agyirifo, S. D., Gathumbi, K. P. and Muchugi. N. A. (2015). Antidiabetic Effects of Aqueous Leaf Extracts of Acacia nilotica in Alloxan Induced Diabetic Mice. Diabetes & Metabolism. Volume 6 • Issue 7 • 1000-9.
18. Ngole-Jeme, V. M. and Ekosse, G. I. (2015). A Comparative Analyses of Granulometry, Mineral Composition and Major and Trace Element Concentrations in Soils Commonly Ingested by Humans. Int. J. Environ. Res. Public Health. 12, 8933-8955
19. Ngugi, M. P., Njagi, J., Cromwell, J. N., Cromwell, K., Maina, D., Ngeranwa, J.N., Gathumbi, P. K. (2015). Trace elements content of selected Kenyan antidiabetic medicinal plants. International Journal of Current Pharmaceutical Research. Vol 4, Issue 3, 39 – 42.
20. Norman, L. (2017). Effects of Coconut Oil Supplementation on Biomarkers of Inflammation and Lipid Peroxidation. A Thesis Presented in Partial Fulfilment of the Requirements for the Degree Master of Science. Arizona State University, USA. Pg 13 - 15.
21. Nwamarah, J. U., Otitoju, O., Otitoju, G. T. O. (2015). Chemical composition and anti-diabetic properties of Jatropha curcas leaves extract on alloxan induced diabetic Wistar rats. African Journal of Biotechnology. Vol. 14(12): pp. 1056 – 1066.
22. Schumacher Bianca de Oliveira, Edcarlos Maurino Preuss, Carolina Galarza VargasII Elizabete HelbigI (2016). Coconut oil on biochemical and morphological parameters in rats submitted to normolipidic and hyperlipidic diets. Clinic and Surgery. Ciência Rural, Santa Maria, v.46, n.10, p.1818-1823,
23. Steffes, M. (2008). Laboratory Procedure Manual: Oral Glucose Tolerance Test. University of Minnesota Medical Center, Fairview Collaborative Studies Clinical Laboratory Minneapolis, Minnesota. USA.
24. Sharidah, M.M.A. (1999). Heavy metals in mangrove sediments of the United Arab Emirates shoreline
25. (Arabian Gulf). Water Air Soil Pollut, 116: 523- 534.
26. Sornalakshmi, V., Tresina, S. P., Paulpriya, K., Packia, L. M. and Mohan, V. R. (2016). Oral Glucose Tolerance Test (OGTT) in Normal Control and Glucose Induced Hyperglycemic Rats with Hedyotis leschenaultiana DC. International Journal of Toxicological and Pharmacological Research 2016; 8(1); 59-62.
27. Soetan, K. O., Olaiya, C. O. and Oyewole, O. E. (2010). The importance of mineral elements for humans,domestic animals and plants: A review: African Journal of Food Science Vol. 4(5) pp. 200-222.
28. Vento, J. P., Swartz, M. E., Martin, L. B. E. and Derek, D. (2008). Food Intake in Laboratory Rats Provided Standard and Fenbendazole-supplemented Diets. J Am Assoc Lab Anim Sci. 47(6): 46–50.
29. Vessal, M., Zal, F., Vasei, M. (2003). Effects of Teucrium Polium on Oral Glucose Tolerance Test, regeneration of pancreas islets and activity of hepatic glucosokinase in diabetic rats. Arch Iranian Med 2003; 6 (1): 35 – 39.
30. Whiting, D. R., Guariguata, L, Weil, C., Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 94(3):311-21. doi: 10.1016/j.diabres.2011.10.029.
31. WHO (2015) Guidelines for Drinking-water Quality. retrieved from http://www.who.int/water_sanitation_health/ dwq/fulltext.pdf
32. Zaruwaa, M. Z., I V. Clifford, N.I. Ibok, U. Ibok, S. Dlama, L. Ezra, C. Danchal and J.O. Madu, D.A.
33. Dangiwa. (2015). Therapeutic Potentials of Toka a Traditional Condiment from Northeastern Nigeria: Drug Development from the Farm. Asian Journal of Biochemical and Pharmaceutical Research Issue 3 (Vol. 5) ISSN: 2231-2560.
34. Zaruwab, M. Z., Manosroi, J., Akihisa, T. and Manosroi, A. (2015). Castalagin from Anogeissus acuminata (Roxb.ex.DC) Guill. Ex. Perr, a potent Hypoglycaemic Agent. Int.J.Curr.Res.Aca.Rev. 3(7): 147-152.