Original Research Article

Evaluation of Antihperlipidaemic Activities of Hydromethanolic Extracts of Dioscorea bulbifera

ABSTRACT

Introduction
Hyperlipidemia is a leading cause of cardiovascular diseases (CHDs) with treatment ranging from dietary management and the use of antihyperlipidaemic drugs. The desire for anti-hyperlipidaemic drugs with less side effects has led to the screening of medical with anti-hyperlipidaemic properties.

Aim
The present study is aimed at evaluating the effects of hydromethanolic extracts of *Dioscorea bulbifera* on high fat diet, tyloxapol and dexamethasone induced hyperlipidaemia using Wistar rat models.

Methodology
Fifty five (55) adult male Wistar rats weighing 180-250g were used for the study. Natural induction of hyperlipidaemia was done using a formulated High fat diet made from commercial rat chow and rendered cow fat while chemical induction of hyperlipidaemia was done using tyloxapol (200mg/kg) and dexamethasone (20mg/kg). The Wistar rats where divided into eleven (11) groups comprising four (5) control groups and seven (6) experimental groups. The extracts were used to treat the hyperlipidaemic rats at 200mg/kg and 400mg/kg while Simvastatin was used as a standard. Blood samples of the animals were analyzed for Total cholesterol (TC), Triglycerides (TG), High density lipoproteins (HDL), Low density lipoproteins (LDL), Very low density lipoprotein (VLDL) and Serum glucose were determined by standard enzymatic methods

Results
The results indicate that the hyperlipidaemic rats treated with extracts of *Dioscorea bulbifera* had significantly reduced TC, TG, LDL, VLDL and serum glucose compared with the control (untreated hyperlipidaemic rats) ($P<0.05$). In the same way, the HDL was found to be significantly higher among the treated hyperlipidaemic rats compared with the untreated controls.

Conclusion
The present study shows that hydromethanolic extracts of *Dioscorea bulbifera* has a possible antihyperlipidaemic potentials as demonstrated by its ability to significantly improve lipid profile and lower serum glucose levels in hyperlipidaemic rat models

Key words: Dioscorea bulbifera, lipid profile, serum glucose, hyperlipidaemia

INTRODUCTION

Hyperlipidemia refers to abnormally high levels of lipids (fats) in the blood stream. Triglycerides, cholesterol esters, phospholipids and or lipoproteins are the various forms of lipids in the blood [1-3]. Lipids are generally divided into cholesterol and triglycerides. While cholesterol are the forms which circulate in the blood stream carried or attached to lipoproteins, triglycerides are stored in fat cells where they function mostly as energy stores[4-6]. The excessive buildup of these lipids in the blood leads to thickening and narrowing of blood vessels leading high blood pressure, atherosclerosis, ischemic and coronary heart diseases [2, 7, 8]. Studies have shown that reduction a blood cholesterol significantly reduced mortality due to atherosclerosis, coronary heart diseases and other lipid associated disorders [9-11]. Dietary management of hyperlipidaemic patients in addition to the use of antihyperlipidaemic drugs has remained the main stay of the treatment and management of hyperlipidaemia[12, 13]. However, the undesirable side effects associated with many synthetic anti-hyperlipidaemic drugs continues to mask their effectiveness[14, 15]. These side effects include but not limited to various degree of gastrointestinal disorders, choestryamine drug interactions, hepatic injury, gall stone formation as well as arrhythmias [12, 15-17]. These undesirable effects associated with antihyperlipidaemic drugs has continued to fuel research in natural plants and herbs with antihyperlipidaemic properties.

Dioscorea bulbifera commonly known as aerial yam has remained one of the available yam in the West Africa, Asia and the Caribbean where they are grown mostly for food their wild range of application in folk remedy [18-20]. Common names in Nigeria include; adu, aduinu, isu-emina, emina, isu-ahun[21, 22]. It is used in many parts of the world alone or in combination with other herbs in the treatment and management of various ailments. In Nigeria, it is used in the treatment of fever, constipation as well as for memory enhancement[21]. In Zimbabwe, Cameroun and Madagaster it is used in the management of wounds and sores [23, 24]

Over one hundred (100) compounds have been isolated from *Dioscorea bulbifera* conferring on it a wide range of documented pharmacological properties [25] as reports have shown that extracts of *Dioscorea bulbifera* has haematopoietic potential[26] , wound healing potential [27, 28], analgesic and anti-inflammatory properties[18] and antioxidant and gastroprotective effects[29, 30], antitumor[31, 32] and antibacterial properties[33, 34]. Though recent studies have shown its ability to improve lipid profile[35, 36], no study have demonstrated its efficacy in the possible improvement of lipid profile of natural and chemically induced hyperlipidaemia. The aim of this study therefore is to determine the effects of hydromethalonic extracts of *Dioscorea bulbifera* on high-fat-diet, tyloxapol and dexamethasone induced hyperlipidaemia using Wistar rat models.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The aerial tubers of *Dioscorea bulbifera* were freshly harvested from a farm in Amiri, Imo State, Nigeria. They were sliced into smaller pieces and shade dried for a period of 2 weeks. They were grinded into a coarse powder and then extracted to exhaustion with a soxhalet apparatus using hydromethanol (80:20). Extraction and phytochemical screening were carried out according to methods described by Odebiyi and Sofowora [37]. The extracts were then filtered and concentrated using a rotatory evaporator at 40-50°C to dryness. The hydromethanol extracts were stored at 2-5°C until required.

Experimental Animals

Fifty five (55) adult male Wistar rats weighing 180-250g were source from the Animal house of the Department of Human Physiology, University of Port Harcourt and used for the study. The rats were allowed three weeks of acclimatization before the start of the study under standard laboratory conditions: Temperature at 25 - 29°C, 55 - 65% relative humidity under natural light/dark natural cycle. During the period of acclimatization, the animals were fed a balanced commercial rat chow (Top Feed LTD., Sapele, Nigeria) ad libitum.

Induction of hyperlipidaemia

Natural induction of hyperlipidaemia was done using a formulated High fat diet[38] using 80% commercial rat chow (Top Feed LTD., Sapele, Nigeria) and 20% rendered cow fat. Cow fat was sourced from Slaughter market, Port Harcourt, Nigeria. The fat was dried, rendered and thoroughly mixed with the rat chow. The animals were allowed to feed on the formulated diet *ad libitum* for eight (8) weeks. Animals with a weight increase up to 30% were considered obese/hyperlipidaemic and used for the study.

Chemical induction of hyperlipidaemia was done using tyloxapol and dexamethasone. A single intra-peritoneal administration of Tyloxapol at 200mg/kg [39-41] (Carbosynth, Ltd., UK) was used while a continuous oral five (5) days oral administration of dexamethasone [14, 42] (Carbosynth, Ltd., UK) was used at 20mg/kg. A standard ant-hyperlipidaemic drug, Simvastatin (TEVA, UK) at 10mg/kg was used as the positive control. All experiments were examined and approved by the appropriate ethics committee.

Experimental Design

The male Wistar rats where divided into eleven (11) groups comprising four (5) control groups and seven (6) experimental groups as follows:

Group 1 – Control [Distilled water]
Group 2 – Negative control I [High fat diet only]
Group 3 – Negative control II [Tyloxapol only]
Group 4 – Negative control III [Dexamethasone only]
Group 5 – Positive control [Simvastatin]
Group 6 – High fat diet + 200mg/kg of *Dioscorea bulbifera*

Group 7 - High fat diet + 400mg/kg of *Dioscorea bulbifera*
Group 8 – Tyloxapol + 200mg/kg of *Dioscorea bulbifera*
Group 9 – Tyloxapol + 400mg/kg of *Dioscorea bulbifera*
Group 10 – Dexanmethasone + 200mg/kg of *Dioscorea bulbifera*
Group 11 – Dexanmethasone + 400mg/kg of *Dioscorea bulbifera*

The oral treatment of hyperlipidaemic rats with of hydromethanolic extracts of *Dioscorea bulbifera* and standard drug lasted for fourteen (14) days. All the animals fasted overnight before being sacrificed by cardiac puncture under chloroform anaesthesia. The blood samples were collected into dry sample bottles and allowed to cloth for about 20mins. They were centrifuged at 5000rpm and the supernatant serum was collected and stored at 4°C prior to biochemical analysis. Total cholesterol (TC), Triglycerides (TG), High density lipoproteins (HDL) and Serum glucose were determined by enzymatic methods, using standard laboratory test kits (Randox, UK). Low density lipoproteins (LDL) was calculated as TC-HDL/TG/2.2[43], Very low density lipoprotein (VLDL) was calculated as TG/2.2[44] while atherogenic index was calculated as $\log_{10}$TG/HDL.

Statistical Analysis

The mean and standard error of mean were determined using SPSS v.20.The one way ANOVA followed by an LSD post hoc analysis were used to determine the difference in means among the groups. The results were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The result of the preliminary phytochemical screening of hydroethanolic extract of *Dioscorea bulbifera* showed the presence of carbohydrates, cholesterol, alkaloids, steroids/triterpenoids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, phenols, proteins and amino acids as reported elsewhere [18, 23, 45-47].

The lipid profile, serum glucose and atherogenic index of high fat diet, tyloxapol and dexamethasone induced hyperlipidaemic Wistar rats treated with hydromethonolic extract of *Dioscorea bulbifera* are shown in Tables 1, 2 and 3 respectively.

Table 1. Lipid profile, serum glucose and atherogenic index of high fat diet induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera*

Parameters	Group 1 Control	Group 2 High fat diet only	Group 5 Simvastatin	Group 6 200mg/kg *D.bulbifera*	Group 7 400mg/kg *D.bulbifera*
TC (mmol/l)	5.50±0.10	7.20±0.34	5.84±0.14	5.50[a] ±0.07	5.20[a]±0.09
TG (mmol/l)	2.44±0.12	3.12±0.26	2.50±0.08	2.14[ab]±0.12	1.43[ab]±0.26
HDL (mmol/l)	1.46±0.09	1.06±0.09	1.98±0.07	2.05[a]±0.90	2.30[ab]±0.07
LDL (mmol/l)	2.70±0.22	5.58±0.62	3.01±0.24	1.76[ab]±0.33	1.20[ab]±0.05
VLDL (mmol/l)	1.10±0.68	2.41±0.17	1.14±0.04	0.97[ab]±0.19	0.75[ab]±0.17
Serum glucose (mmol/l)	6.40±0.10	8.80±0.11	6.54±0.17	5.90[a]±0.11	5.41[a]±0.11
Atherogenic Index	0.22±0.02	0.47±0.04	0.24±0.03	0.18[ab]±0.3	-0.48[ab]±0.04

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin.

Table 2. Lipid profile, serum glucose and atherogenic index of tyloxapol induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera*

Parameters	Group 1 Control	Group 3 Tyloxapol Only	Group 5 Simvastatin	Group 8 200mg/kg *D.bulbifera*	Group 9 400mg/kg *D.bulbifera*
TC (mmol/l)	5.50±0.10	8.89±0.37	5.84±0.14	6.52[a]±0.53	5.38[a] ±0.08
TG (mmol/l)	2.44±0.12	4.54±0.07	2.50±0.08	2.56[ab] ±0.08	2.14[ab] ±0.07
HDL (mmol/l)	1.46±0.09	1.34±0.07	1.98±0.07	3.14[ab] ±0.04	3.54[ab] ±0.08
LDL (mmol/l)	2.70±0.22	4.59±0.16	3.01±0.24	1.98[ab] ±0.14	1.23[ab] ±0.16
VLDL (mmol/l)	1.10±0.68	2.06±0.04	1.14±0.04	1.16[a] ±0.03	0.99[a] ±0.03
Serum glucose (mmol/l)	6.40±0.10	8.39±0.16	6.54±0.17	5.19[a] ±0.11	4.20[a] ±0.12
Atherogenic Index	0.22±0.02	0.53±0.03	0.24±0.03	-0.09[ab] ±0.01	-0.22[ab] ±0.01

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin.

Table 3. Lipid profile, serum glucose and atherogenic index of dexanmathasone induced hyperlipidaemic Wistar rats treated with hydromethanolic extract of *Dioscorea bulbifera*

Parameters	Group 1 Control	Group 4 Dexanmeth-asone Only	Group 5 Simvastatin	Group 10 200mg/kg *D.bulbifera*	Group 11 400mg/kg *D.bulbifera*
TC (mmol/l)	5.50±0.10	10.7±0.53	5.84±0.14	6.82 [ab]±0.41	5.54[ab] ±0.41
TG (mmol/l)	2.44±0.12	4.41±0.15	2.50±0.08	1.90[ab] ±0.07	1.31[ab] ±0.06
HDL (mmol/l)	1.46±0.09	1.81±0.07	1.98±0.07	2.05[ab] ±0.08	3.10[ab] ±0.07
LDL (mmol/l)	2.70±0.22	6.00±0.30	3.01±0.24	1.45[ab] ±0.12	0.98[ab] ±0.03
VLDL (mmol/l)	1.10±0.68	2.00±0.03	1.14±0.04	0.96[ab] ±0.05	0.81[ab] ±0.04
Serum glucose (mmol/l)	6.40±0.10	12.50±0.87	6.54±0.17	7.50[a] ±0.87	6.70[a] ±0.87
Atherogenic Index	0.22±0.02	0.52±0.06	0.24±0.03	-0.19[ab] ±0.02	-0.44[ab] ±0.03

Results are given as mean±standard error of mean, a=significantly different compared to control, b=significantly different compared to standard drug, simvastatin

The mean values for Total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins, serum glucose and atherogenic index were found to be significantly lower in all experimental groups (groups 6-11) compared to untreated hyperlipidaemic rats (groups 2-4) ($P<0.05$). However the mean values obtained for high density lipoproteins was found to be higher in in all experimental groups (groups 6-11) compared to untreated hyperlipidaemic rats (groups 2-4) ($P<0.05$). For the high fat diet induced hyperlipidaemic rats (Table 1), treatment with the extract caused significant reduction in the mean values for triglycerides, low density lipoproteins, very low density lipoproteins in the experimental groups (groups 6,7) when compared with the antihyperlipidaemic standard drug, simvastatin ($P<0.05$) and a significantly increased high density lipoprotein among the rats treated with 400mg/kg of *Dioscorea bulbifera* (group 7) when compared with the hyperlipidaemic standard drug, simvastatin ($P<0.05$). Similarly, it was observed that mean values for triglycerides and low density lipoproteins among tyloxapol induced hyperlipidaemic rats (Table 2) were found to be significant lower compared with the the antihyperlipidaemic standard drug, simvastatin ($P<0.05$) with a significantly increased high density lipoprotein among the rats treated with hydromethanolic extract of *Dioscorea bulbifera* (groups 8,9) ($P<0.05$). For the dexamethasone induced hyperlipidaemic rats (Table 3), it was observed that the mean values for total cholesterol, triglycerides and low density lipoproteins and very low density lipoproteins were significantly lower in rats treated with hydromethanolic extract of *Dioscorea bulbifera* (groups 10,11) compared with the hyperlipidaemic standard drug, simvastatin ($P<0.05$) with) with a significantly increased high density lipoprotein among the rats treated with hydromethanolic extract of *Dioscorea bulbifera* compared with the hyperlipidaemic standard drug, simvastatin ($P<0.05$)

This observed antihyperlipidaemic activity of *Dioscorea bulbifera* could be attributed the effect of diosgenin which has been noted as the main active saponin in the family Dioscoreaceae [48] with significant active quantities found in *Dioscorea bulbifera* [49-51]. Diosgenin suppresses cholesterol absorption and increases cholesterol secretion. It has also been reported to interfere with both exogenous and endogenous cholesterol accompanied by depressed rates of hepatic and intestinal cholesterol synthesis [52]. Also, saponins are known to exhibit cholesterol-lowering ability by binding dietary cholesterol and bile acids in the intestinal lumen. Binding of cholesterol decreases their absorption and subsequently their fecal excretion while binding of bile acids leads to an increase synthesis of bile acids from cholesterol causing cholesterol already absorbed via the gastrointestinal tract to be inhibited and secreted into feces [7, 52-56]. Saponins have also been reported to exhibit anti-lipase activity, reduced adipocyte differentiation and lipogenesis leading to the low degradation of cholesterol, triglycerides and lipoproten [7, 57, 58]. More so, the anti-oxidant properties of *Dioscorea bulbifera* [29, 30] due to their flavonoid content has been shown to prevent the oxidative modification of LDL by scavenging free radicals [59, 60], they are known to reduce LDL levels while increasing the levels of HDL[61, 62]. Similarly, triterpenoids, has been demonstrated to reduce the levels of TG, TC and phospholips [63]. The result of the present study concurs with previous studies where *Dioscorea bulbifera* was found to reduce serum cholesterol and lipoproteins [35, 36, 64].

The ability of extracts of *Dioscorea bulbifera* to cause a significant reduction in serum glucose could also be attributed to the presence of diosgenin, a steroidal saponin, a potent glucosidase and α-amylase inhibitor [65]. While α-glucosidase inhibitors prevents the action of carbohydrate

digestion enzymes α- glucosidase which typically break down complex carbohydrates such as glycogen and starch to their monomers, the α-amylase inhibitor prevents the action α-amylase on long chain carbohydrates like amylose and amylopectin to yield simpler sugars (Rubilar *et al.*, 2011). Hence, through delayed carbohydrate absorption, the rate of glucose absorption is reduced leading to the observed decrease in serum glucose. Also, flavonoids as contained in the extract has also been also been shown to be a potent glucosidase and α-amylase inhibitors [66-69]. Alpha-glucosidase inhibitors represent the one of the most common oral agents used in ameliorating the effect of hyperglycaemia due to their lack of hypoglycemic threat and also their ability to control blood glucose without causing body weight gain and hyperinsulinemia. They also do not cause any nutritional calorie loss as they only slow down carbohydrate absorption without altering the total amount of carbohydrate absorbed [66, 70]. It is also possible that the extract may have through some extra pancreatic mechanism, inhibited hepatic glucose production [71, 72]. Another possible mechanism of action of the extract could be the stimulation insulin secretion from the β-cells of the islets of Langerhans [72, 73]. The result of the present study concurs with previous studies where *Dioscorea bulbifera* was found to have an antihyperglycaemic activity [47, 51, 65, 73].

The observed antihyperlipidaemic activity of *Dioscorea bulbifera* extract consequently improved the mean values of atherogenic index in all the experimental groups compared to the control groups. The values for atherogenic index has remained an important tool for analyzing the values for lipid profile as the association of TG and HDL reflects the important balance between risk and protective lipoprotein forces[74] and has remained a significant predictor for cardiovascular diseases [75-78]. It is useful in the evaluation of response to treatment. The result from the present study suggests that extracts of *Dioscorea bulbifera* significantly reduced the risk of cardiovascular diseases (CVDs) for the hyperlipidaemic rats.

In conclusion, the present study has shown that hydromethanolic extracts of *Dioscorea bulbifera* has a possible antihyperlipidaemic potentials as demonstrated by its ability to significantly improve lipid profile and lower serum glucose levels in high fat induced, tyloxapol and dexamethasone induced hyperlipidaemic rat models.

REFERENCES

1. Sartaj B, Sheeba D, Navitha H, Ramesh C. Evaluation of Antihyperlipidemic and Antioxidant Activity of Albizia amara. International Journal of Biological and Pharmaceutical Research. 2012;3(7):875-82.
2. Shattat GF. A review article on hyperlipidemia: types, treatments and new drug targets. Biomedical and Pharmacology Journal. 2015;7(1):399-409.
3. Ginghina C, Bejan I, Ceck C. Modern risk stratification in coronary heart disease. Journal of medicine and life. 2011;4(4):377-86.
4. Onwe P, Folawiyo M, Anyigor-Ogah C, Umahi G, Okorocha A, Afoke A. Hyperlipidemia: etiology and possible control. Journal of Dental Medical Sciences. 2015;14(10):93-100.
5. Anderson TJ, Grégoire J, Hegele RA, Couture P, Mancini GJ, McPherson R, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. Canadian Journal of Cardiology. 2013;29(2):151-67.
6. Singh R, Nain S. A Mini-Review on Hyperlipidemia: Common Clinical Problem. Interventional Cardiology Journal. 2018;4(3(10)):1-3.
7. Kumar VS, Inamdar MN, Viswanatha GL. Protective effect of lemongrass oil against dexamethasone induced hyperlipidemia in rats: possible role of decreased lecithin cholesterol acetyl transferase activity. Asian Pacific Journal of Tropical Medicine. 2011;4(8):658-60.
8. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. The lancet. 2005;365(9455):217-23.
9. Zamani M, Rahimi AO, Mahdavi R, Nikbakhsh M, Jabbari MV, Rezazadeh H, et al. Assessment of anti-hyperlipidemic effect of Citrullus colocynthis. Revista Brasileira de Farmacognosia. 2007;17(4):492-6.
10. Muldoon MF, Manuck SB, Matthews KA. Lowering cholesterol concentrations and mortality: a quantitative review of primary prevention trials. Bmj. 1990;301(6747):309-14.
11. Assmann G, Cullen P, Jossa F, Lewis B, Mancini M. Coronary heart disease: Reducing the risk: The scientific background to primary and secondary prevention of coronary heart disease a worldwide view. Arteriosclerosis, thrombosis, and vascular biology. 1999;19(8):1819-24.
12. Farmer JA, Gotto AM. Antihyperlipidaemic agents. Drug safety. 1994;11(5):301-9.
13. Katan M. Dietary management of the hyperlipidaemic patient. Lipid Insight. 1991;1:2-5.
14. Pragda SS, Kuppast IJ, Mankani KL, Ramesh L. Evaluation of antihyperlipidemic activity of leaves of *Portulaca oleracea* linn against dexamethasone induced hyperlipidemia in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(4):279-83.
15. Steiner A, Weisser B, Vetter W. A comparative review of the adverse effects of treatments for hyperlipidaemia. Drug Safety. 1991;6(2):118-30.
16. Knodel LC, Talbert RL. Adverse effects of hypolipidaemic drugs. Medical Toxicology and Adverse Drug Experience. 1987;2(1):10-32.
17. Tonstad S, Pometta D, Erkelens D, Ose L, Moccetti T, Schouten J, et al. The effect of the gastrointestinal lipase inhibitor, orlistat, on serum lipids and lipoproteins in patients with primary hyperlipidaemia. European journal of clinical pharmacology. 1994;46(5):405-10.
18. Mbiantcha M, Kamanyi A, Teponno RB, Tapondjou AL, Watcho P, Nguelefack TB. Analgesic and anti-inflammatory properties of extracts from the bulbils of *Dioscorea*

bulbifera L. var sativa (Dioscoreaceae) in mice and rats. Evidence-Based Complementary and Alternative Medicine. 2010;2011:1-9.
19. Ghosh S, Parihar VS, More P, Dhavale DD, Chopade BA. Phytochemistry and therapeutic potential of medicinal plant: *Dioscorea bulbifera*. Medicinal Chemistry. 2015;5:154-9.
20. Martin FW. Tropical yams and their potential. Part 2. *Dioscorea bulbifera*. Virginia: Agricultural Research Service; 1974.
21. Odugbemi T. A textbook of medicinal plants from Nigeria. Lagos: University of Lagos Press; 2008.
22. Okeke EC, Eneobong HN, Uzuegbunam AO, Ozioko A, Kuhnlein H. Igbo traditional food system: Documentation, uses and research needs. Pakistan Journal of Nutrition. 2008;7(2):365-76.
23. Subhash C, Sarla S, Abhay MP, Anoop B. Nutritional profile and phytochemical screening of Garhwal Himalaya medicinal plant *Dioscorea bulbifera*. International Research Journal of Pharmacy. 2012;3(5):289-94.
24. Cogne AL. Phytochemical investigation of plants used in African medicine: *Dioscorea sylvatica* (Dioscoreaceae), *Urginea altissima* (Liliaceae), *Jamesbrittenia fodina* and *Jamesbrittenia elegantissima* (Scrophulariaceae). Doctorat Theses University of Lausanne, Lausanne. 2002.
25. Guan X-R, Zhu L, Xiao Z-G, Zhang Y-L, Chen H-B, Yi T. Bioactivity, toxicity and detoxification assessment of Dioscorea bulbifera L.: a comprehensive review. Phytochemistry Reviews. 2017;16(3):573-601.
26. Chinko BC, Dapper DV, Adienbo OM, Egwurugwu JN, Joffa PPK. Preliminary Assessment of the Effects of Hydromethanolic extract of Dioscorea bulbifera on Haematological Parameters and Serum Electrolytes of Wistar rats. European Journal of Biomedical and Pharmaceutical Sciences. 2016;3(10):83-7.
27. Panduraju T, Bitra VR, Vemula SK, Reddy PRV. Wound healing activity of Dioscorea bulbifera Linn. Journal of Pharmacy Research. 2010;3(12):3138-9.
28. R Vasanthi H, ShriShriMal N, K Das D. Phytochemicals from plants to combat cardiovascular disease. Current medicinal chemistry. 2012;19(14):2242-51.
29. Suriyavathana M, Indupriya S. Screening of antioxidant potentials in *Dioscorea bulbifera*. International Journal of Pharmacy and Life Sciences. 2011;2(4):661-4.
30. Balasubramanian J, Dhanalakshmi R, Jibnomen P, Manimekalai P. A preclinical evaluation on antioxidant and gastroprotective effect of *Dioscorea bulbifera* in Wistar rats. Indian Journal of Innovations and Developments. 2012;1(3):149-54.
31. Gao H, Hou B, Kuroyanagi M, Wu L. Constituents from anti-tumor-promoting active part of Dioscorea bulbifera L. in JB6 mouse epidermal cells. 亚洲传统医药. 2007;2(3):104-9.
32. Gao H, Kuroyanagi M, Wu L, Kawahara N, Yasuno T, Nakamura Y. Antitumor-promoting constituents from *Dioscorea bulbifera* L. in JB6 mouse epidermal cells. Biological and Pharmaceutical Bulletin. 2002;25(9):1241-3.
33. Kuete V, BetrandTeponno R, Mbaveng AT, Tapondjou LA, Meyer JJM, Barboni L, et al. Antibacterial activities of the extracts, fractions and compounds from *Dioscorea bulbifera*. BioMed Central Complementary and Alternative Medicine. 2012;12(1):1.
34. Adeosun OM, Arotupin DJ, Toba OA, Adewole A. Antibacterial activities and phytochemical properties of extracts of Dioscorea bulbifera Linn (Air Potatoe) tubers and peels against some pathogenic bacteria. 2016.
35. Chinko BC, Dapper DV, Adienbo OM, Egwurugwu JN, Uchefuna RC. Biochemical Evaluation of the Effects of Hydromethanolic Extracts of Dioscorea bulbifera in Wistar Rats. IOSR Journal of Dental and Medical Sciences. 2016;5(9):105-10.

36. Ahmed Z, Chishti MZ, Johri RK, Bhagat A, Gupta KK, Ram G. Antihyperglycemic and antidyslipidemic activity of aqueous extract of *Dioscorea bulbifera* tubers. Diabetologia Croatica. 2009;38(3):63-72.
37. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Journal of Natural Products (Lloydia). 1977;41(3):234-46.
38. Gajda AM. High fat diets for diet-induced obesity models. Open Source Diets. 2009.
39. Ngoc TH, Ngoc QN, Tran A, Phung NV. Hypolipidemic effect of extracts from Abelmoschus esculentus L.(Malvaceae) on Tyloxapol-induced hyperlipidemia in mice. J Pharm Sci. 2008;35:42-6.
40. Bertges LC, Mourão Jr CA, Souza JB, Cardoso VAC. Hyperlipidemia induced by Triton WR1339 (Tyloxapol) in Wistar rats. Braz J Med Sci Health. 2011;1:32-4.
41. Ansarullah A, Jadeja R, Thounaojam M, Patel V, Devkar R, Ramachandran A. Antihyperlipidemic potential of a polyherbal preparation on triton WR 1339 (Tyloxapol) induced hyperlipidemia: A comparison with lovastatin. International Journal of Green Pharmacy (IJGP). 2009;3(2).
42. Nvl SR, Aveti S, Anjum M. Anti-hyperlipidemic activity of methanolic extract of Syzygium alternifolium bark against high-fat diet and dexamethasone-induced hyperlipidemia in rats. Asian J Pharm Clin Res. 2015;8(6):165-8.
43. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
44. Crook M. Clinical Chemistry & Metabolic Medicine. London: Edwarld Arnold publishers Ltd.; 2006.
45. Sheikh N, Kumar Y, Misra A, Pfoze L. Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. Journal of Medicinal Plants. 2013;1(6):62-9.
46. Malode UG, Mohammad N, Quazi SA, Mahajan DT, Masand V. Phytochemical investigations of *Dioscorea bulbifera* linn. Indian Journal of Research in Pharmacy and Biotechnology. 2015;3:20-3.
47. Okon JE, Ofeni AA. Antidiabetic effect of *Dioscorea bulbifera* on alloxan induced diabetic rats. CIBTech Journal of Pharmaceutical Sciences. 2013;2(1):14-5.
48. Cayen MN, Dvornik D. Effect of diosgenin on lipid metabolism in rats. Journal of lipid research. 1979;20(2):162-74.
49. Jyothishwaran G, Seetharam YN. Biotransformation of cholesterol to diosgenin by freely suspended and immobilised cells of Dioscorea bulbifera L. Journal of Asian Natural Products Research. 2008;10(2):139-45.
50. Jayachandran KS, Vasanthi AHR, Gurusamy N. Steroidal saponin diosgenin from *Dioscorea bulbifera* protects cardiac cells from hypoxia-reoxygenation injury through modulation of pro-survival and pro-death molecules. Pharmacognosy Magazine. 2016;12(1):14-20.
51. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, et al. Diosgenin from Dioscorea bulbifera: novel hit for treatment of type II diabetes mellitus with inhibitory activity against α-amylase and α-glucosidase. PloS One. 2014;9(9):e106039.
52. Estiasih T, Ariestiningsih AD, Wardani NAK. The effect of crude diosgenin extract from purple and yellow greater yams (*Dioscorea alata*) on the lipid profile of dyslipidemia rats. Emirates Journal of Food and Agriculture. 2016;28(7):506-12.

53. Elekofehinti OO, Omotuyi IO, Kamdem JP, Ejelonu OC, Alves GV, Adanlawo IG, et al. Saponin as regulator of biofuel: implication for ethnobotanical management of diabetes. Journal of Physiology and Biochemistry. 2014;70(2):555-67.
54. Sidhu GS, Oakenfull DG. A mechanism for the hypocholesterolaemic activity of saponins. British Journal of Nutrition. 1986;55(3):643-9.
55. Oakenfull D, Sidhu GS. Could saponins be a useful treatment for hypercholesterolaemia? European Journal of Clinical Nutrition. 1990;44(1):79-88.
56. Malloy MJ, Kane JP. Basic & Clinical Pharmacology: Agents used in dyslipidemia. New York: McGraw Hill Companies; 2012.
57. Chantre P, Lairon D. Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. Phytomedicine. 2002;9(1):3-8.
58. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C, et al. Epigallocatechin gallate supplementation alleviates diabetes in rodents. The Journal of nutrition. 2006;136(10):2512-8.
59. Sudheesh S, Vijay Kumar S, Sandhya C, Vijayalakshmi NR. Toxic effects of condensed tannins from Solanum melongena on rats. Journal of Ecotoxicology and Environmental Monitoring. 1996;6:221.
60. Bahorun T, Neergheen VS, Aruoma OI. Phytochemical constituents of Cassia fistula. African journal of Biotechnology. 2005;4(13).
61. Patel D, Patel K, Patel U, Thounaojam M, Jadeja R, Padate G, et al. Assessment of lipid lowering effect of Sida rhomboidea. Roxb methanolic extract in experimentally induced hyperlipidemia. Journal of Young Pharmacists. 2009;1(3):233-8.
62. Otunola GA, Oloyede OB, Oladiji AT, Afolayan AA. Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. Afr J Biochem Res. 2010;4(6):149-54.
63. Sudhahar V, Kumar SA, Sudharsan PT, Varalakshmi P. Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vascular Pharmacology. 2007;46(6):412-8.
64. Pessoa L, Rego T, Asht L, Rego I, Fortunato M, Feijo M. Serum and liver lipids distributions in streptozotocin induced diabetic rat treated with diet containing Yam (*Dioscorea bulbifera*) flour. Nutrición Hospitalaria. 2015;31(4):1647-53.
65. Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat Dusane M, Joshi BN, et al. Antidiabetic activity of *Gnidia glauca* and *Dioscorea bulbifera*: potent amylase and glucosidase inhibitors. Evidence-Based Complementary and Alternative Medicine. 2011;2012:1-10.
66. Kim JS, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Bioscience, Biotechnology, and Biochemistry. 2000;64(11):2458-61.
67. Rubilar M, Jara C, Poo Y, Acevedo F, Gutierrez C, Sineiro J, et al. Extracts of maqui (*Aristotelia chilensis*) and murta (*Ugni molinae Turcz*.): sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors. Journal of Agricultural and Food Chemistry. 2011;59(5):1630-7.
68. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-Glucosidase and α-Amylase by Flavonoids. Journal of Nutritional Science and Vitaminology. 2006;52(2):149-53.
69. Franco OL, Rigden DJ, Melo FR, Grossi-de-Sá MF. Plant α-amylase inhibitors and their interaction with insect α-amylases. European Journal of Biochemistry. 2002;269(2):397-412.
70. Mooradian AD, Thurman JE. Drug therapy of postprandial hyperglycaemia. Drugs. 1999;57(1):19-29.

71. Swanston-Flatt SK, Day C, Bailey CJ, Flatt P. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia. 1990;33(8):462-4.
72. Oyedemi SO, Yakubu MT, Afolayan AJ. Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. Journal of Medicinal Plants Research. 2011;5(1):119-25.
73. Sharma SB, Nasir A, Prabhu KM, Murthy PS. Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. Journal of Ethnopharmacology. 2006;104(3):367-73.
74. Nwagha UI, Ikekpeazu EJ, Ejezie FE, Neboh EE, Maduka IC. Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. African health sciences. 2010;10(3):248-52.
75. Dobiášŏvá M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FERHDL). Clinical biochemistry. 2001;34(7):583-8.
76. Onat A, Can G, Kaya H, Hergenç G. "Atherogenic index of plasma"(log 10 triglyceride/high-density lipoprotein− cholesterol) predicts high blood pressure, diabetes, and vascular events. Journal of clinical lipidology. 2010;4(2):89-98.
77. Niroumand S, Khajedaluee M, Khadem-Rezaiyan M, Abrishami M, Juya M, Khodaee G, et al. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Medical journal of the Islamic Republic of Iran. 2015;29:240.
78. Bhardwaj S, Bhattacharjee J, Bhatnagar MK, Tyagi S. Atherogenic index of plasma, castelli risk index and atherogenic coefficient-new parameters in assessing cardiovascular risk. Int J Pharm Biol Sci. 2013;3(3):359-64.