International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 8 Issue 5, May 2021

ISSN: 2394-4404

Investigating The Anti-Diabetic Phytoconstituent(S) Of Rauvolfia Vomitoria Leaves By Gas Chromatography-Mass Spectrometry (GC-MS)

Peter Akpojotor

Maureen Isoken Ebomoyi

Department of Physiology, School of Basic Medical Sciences,
University of Benin, Nigeria

Abstract: ***Rauvolfia vomitoria is a medicinal plant that has been implicated for various medicinal actions including anti-diabetic. But among the various literature on its anti-diabetic action, none has provided information on the phytoconstituents responsible for its anti-diabetic action, the mechanisms of action as well as its effect on plasma insulin and glucagon (the primary regulators on blood glucose). Thus the aim of this study was to investigate the active phytoconstituents of Rauvolfia vomitoria leaf extract as well as its possible mechanisms of action with respect to diabetic treatment. GC-MS analysis of the hydromethanolic extract of Rauvolfia vomitoria revealed phytol as its major phytoconstituent with 38.73% percentage abundance. Diabetes was induced via intraperitoneal injection of 55mg/kg dose of streptozotocin and treated with whole leaf extract, phytol and glyburide for 28 days. After treatment, phytol caused significant (P<0.05) decrease in blood glucose level by approximately 95.73%, versus 99.5% for the hydromethanolic extract of Rauvolfia vomitoria leaf and 101.9% for the standard anti-diabetic drug, glyburide. These results therefore, showed that phytol is the major phytoconstituent of Rauvolfia vomitoria leaves responsible for its anti-diabetic action and can be further exploited for anti-diabetic medications.***

Keywords: ***Rauvolfia vomitoria, anti-diabetic, streptozotocin, glyburide, GC-MS, phytoconstituents, phytol***

I. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronically elevated blood glucose level resulting from either a pathophysiology of the Islet cells of the pancreas resulting in deficiency in insulin secretion (a type known as type-1 diabetes mellitus) or the body's insensitivity to insulin (known as type-2 diabetes mellitus) (Ezuruike and Prieto, 2014; N'doua *et al*., 2016). This chronic metabolic disorder is highly dreaded worldwide. This is because, its progression is often associated with various life-threatening complications such as hypertension, myocardial infarction, diabetic retinopathy, diabetic nephropathy (degenerative changes in kidney), diabetic neuropathy (degenerative changes of autonomic and peripheral nerves) etc., (Canadian Diabetes Association, 2003).

The prevalence of diabetes is on a steady exponential increase globally (Ezuruike and Prieto, 2014). In 2016, 422 million people were estimated to have diabetes worldwide (WHO, 2016), up from an estimated 382 million people in 2013 (Shi and Hu, 2014) and from 108 million in 1980 (WHO, 2016). These increases and more have been predicted (WHO 2008; International Diabetes Federation, 2014; Piero *et al.*, 2014). This disorder is posing great threat to humanity due to its exponential increasing rate and the limitations of synthetic drugs in providing complete cure to it (Melander, 1988; Okpuzor *et al.*, 2009; Kumari *et al.*, 2013). Most of the synthetic drugs available for treating diabetes (Sulfonylureas and related compounds) in addition to their various side effects, stimulate insulin release from beta cells of pancreas in the absence of high glucose levels, which is one of their shortcomings (Moller, 2001). According to Moller (2001) an

ISSN: 2394-4404

ideal anti-diabetic drug is that which will cause insulin release in a glucose-dependent fashion.

Thus the need and call by stakeholders including the recommendation by the World Health Organization expert committee on diabetes, for researches on medicinal plants due to their promising results in providing solutions to health issues with little or no side effect (WHO Expert Committee, 1980; Shukla *et al.*, 2000; Ezejindu *et al.*, 2013; Kumari *et al.*, 2013). In response to this call, over the last four decades a handful of literature on the anti-diabetic effect of medicinal plants have been made available through researches. But most of these literature only report the blood glucose lowering action of medicinal plants. They lack vital scientific information needed to justify medicinal plants as a better alternative to synthetic drugs. They lack information on how these medicinal plants effect their blood glucose lowering action (that is, the exact constituent(s)/phytochemical(s) through which they bring about their reported anti-hyperglycemic actions as well as the mechanism(s) of actions), their effect on the organ (pancreas) and its hormones (insulin and glucagon) responsible for blood glucose homeostasis, etc.

Rauvolfia vomitoria is an ever green perennial shrub belonging to the apocynaceae family. It is widely distributed all over the world especially in the tropical forest of Africa, South America and Asia (Amole, 2003, Ogbe *et al*., 2009]. It has been associated with strong anti-diabetic potentials (Campbell-Tofte *et al*., 2011; N'doua *et al*., 2015 & 2016). Available literature on the anti-diabetic action *Rauvolfia vomitoria* mainly report its blood glucose lowering action. There is lack of information on the phytoconstituents responsible for its anti-diabetic action and the mechanisms of action.

Hence, this research study was designed to investigate the active phytochemicals of the plant responsible for its anti-diabetic action using gas chromatography-mass spectrometric (GC-MS) analyzing technique.

II. METHODOLOGY

STUDY DESIGN

This research was conducted in two phases. In the first phase, hydromethanolic extraction and preliminary phytochemical screening of the plant were conducted at the Department of Pharmacognosy, University of Uyo, Akwa Ibom State, Nigeria, using the methods of Akpojotor and Kagbo (2016) and Amita and Shalini (2014) respectively. Thereafter, identification of the phytoconstituents of hydromethanolic extract of *Rauvolfia vomitoria* leaf was done using gas chromatography-mass spectrometry.

In the second phase, the anti-diabetic action of the suspected most potent phytoconstituent of the hydromethanolic extract of *Rauvolfia vomitoria* leaves was investigated using streptozotocin-induced diabetic male wistar rats.

MATERIALS, DRUGS AND CHEMICALS

Streptozotocin (STZ) and phytol were purchased from Sigma Aldrich Co. (St Louis, MO, USA) through BioRapid Diagnostic Nigeria Limited, Abuja; Accu‑Chek Active blood glucose meter and Accu‑Chek Active test strips both manufactured by Roche Diabetes Care GmbH Sandhofer Strasse 116 68305 Mannheim, German and glyburide were purchased from Greenhouse Group Pharmacy, University of Port Harcourt, Nigeria.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the Research Ethics Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria, with approval number: CMS/REC/2021/159. Animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals as contained in Guide for the Care and Use of Laboratory Animals 8th edition (NRC, 2011).

ANIMALS

Twenty-five adult male wistar rats (weighing between 200-220g) used in the second phase the study were obtained from the Department of Pharmacology Experimental-Rat Breeding Facility, University of Port Harcourt.

COLLECTION AND IDENTIFICATION OF PLANT SAMPLES

The leaves of *Rauvolfia vomitoria* (a medicinal plant authenticated by Dr. Edwin Nwosu at the Herbarium Unit of the Department of Plant Science and Biotechnology of the University of Port Harcourt, with Ecoland Herbarium identification number EH – P – 051) were harvested from the farmland in Orhuwhorun community in Udu LGA of Delta State, Nigeria.

PREPARATION AND EXTRACTION OF THE PLANT SAMPLES

The harvested leaves were rid of dirt. Thereafter, air-dried at room temperature for 6 days and then milled to fine powder using manual engine grinder (Model Corene, A.5 lander YCIA S.A). The milled sample of the plant was subjected to hydromethanolic (ratio of water to methanol is 1:4) maceration extraction following the modified extraction method of Akpojotor and Kagbo (2016). 500 grams of the milled sample of the plant was then mixed with 5 litres of the extraction solvent (hydromethanol). The mixture was stirred occasionally for 48 hours at room temperature, thereafter filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated under reduced pressure in a vacuum at 40°C using a rotary evaporator (Searl Instruments Ltd. England) and activated silica gel was used to remove moisture, thereby producing the hydromethanolic extract which was used for the study.

PHYTOCHEMICAL SCREENING OF PLANT SAMPLE (EXTRACT)

SCREENING FOR ALKALOIDS

Extract was dissolved in dilute Hydrochloric acid and filtered. Thereafter, the filtrate was subjected to Mayer's and Dragendorff's tests for confirmation.

- ✓ Mayer's Test: Filtrate treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids (Amita and Shalini, 2014).
- ✓ Dragendorff's Test: Filtrates treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicated the presence of alkaloids (Amita and Shalini, 2014).

SCREENING FOR SAPONINS

- ✓ Froth Test: Extracts was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins (Amita and Shalini, 2014).
- ✓ Foam Test: 0.5 gm of extract was shaken with 2 ml of water. Foam produced persists for ten minutes, which indicated the presence of saponins.

SCREENING FOR CARDIAC GLYCOSIDES

- ✓ Salkowski's Test: Extracts was treated with chloroform and filtered. Thereafter, few drops of concentrated sulphuric acid was added to the filtrate to form a lower layer. Appearance of golden yellow color indicated the presence of triterpenes.
- ✓ Liebermann test: Extracts was treated with chloroform and filtered. Then the filtrate treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicated the presence of cardiac glycosides (Sofowora, 2008; Amita and Shalini, 2014).

SCREENING FOR PHENOLS

- ✓ Ferric Chloride Test: Extracts will be treated with 3-4 drops of ferric chloride solution. Formation of bluish black color will indicate the presence of phenols (Amita and Shalini, 2014).

SCREENING FOR TANNINS

- ✓ Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins (Sofowora, 2008).

SCREENING FOR FLAVONOIDS

- ✓ Shinoda's Test: Extract was dissolved in ethanol and few drops of concentrated HCl added to the filtrate. Thereafter magnesium tunings were added. Formation of pink red coloration was an indication of the presence of flavonoids (Amita and Shalini, 2014).
- ✓ Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid indicated the presence of flavonoids (Amita and Shalini, 2014).

SCREENING FOR TERPENES

- ✓ To the extract, 3ml of chloroform was added and then filtered. To the filtrate, 3ml of conc. Sulphuric acid was added. A pink color at the interphase indicated the presence of terpenes (Amita and Shalini, 2014).

GC–MS ANALYSIS OF RAUVOLFIA VOMITORIA LEAF

The identification of main phytoconstituents of *Rauvolfia vomitoria* leaves was done by subjecting the hydromethanolic extract to gas chromatography – mass spectrometric (GC-MS) technique following the method of Nguyen and Kimaru (2014), using an Agilent 7890A equipped with mass spectrometer MS 5975C (Agilent Technologies, CA, U.S.A). Capillary column used was DB-5MS with column thickness 0.25µm; internal diameter 0.32mm; length 30m (J&W Scientific, CA, U.S.A). Carrier gas was helium with a flow rate of 1ml/min. The temperature programme was set as follows; initial temperature 80°C held for 2 minute at 10°C per minute to 240°C held for 6 min, a total run time of 22 minutes. In brief, 1µL of the sample was injected via the injector port into the GC-MS. Inside the GC-MS, the sample is heated and turned into vapor (gas). The vapor is then blown by an inert gas (helium) from the mobile phase into the column unit. As the sample (now in gaseous form) travels through the column, the different phytoconstituents travelling with different speed due to their different mass and vitality get to the detector unit of the GC at different retention times (RTs) and then pass through the mass spectrometry system operated in electron ionization mode with selected ion monitoring. The RTs, mass and spectra are then compared and matched against those of authentic standard spectra using computer search in NIST LIBRARY 2015.

GROUPING OF EXPERIMENTAL ANIMALS

Twenty-five adult male wistar rats were divided into five groups of five rats each (namely groups 1-5). The groups are as follow;

- ✓ Group 1 (Normal control) - Diabetes was not induced and they were not treated.
- ✓ Group 2 (Diabetic control) - Diabetes was induced but they were not treated
- ✓ Group 3- Diabetes was induced and they received 500mg/kg of Hydromethanolic extract of *Rauvolfia vomitoria* leaves.
- ✓ Group 4- Diabetes was induced and they were treated with 200mg/kg of dose of phytol
- ✓ Group 5- Diabetes was induced and they were treated with 5mk/kg dose of glyburide (standard anti-diabetic

drug). This group served as standard to compare the blood glucose-lowering potency of the extract.

The choice of extract doses for this study are based on the LD_{50} obtained from the first phase of this study and the doses for which medicinal activities have been reported on *Rauvolfia vomitoria* leaves (Olatokunboh *et al.,* 2009; Ezejindu *et al.,* 2014).

INDUCTION OF DIABETES

The method of Rossini *et al*. (1977) was followed. Experimental animals were fasted overnight. Streptozotocin was dissolved in citrate buffer, pH 4.5 and injected intraperitoneally within 20 minutes of dissolution for the induction of diabetes in group 2-5. Dose of 55 mg/kg body weight Streptozotocin (according to the dose used by Rajasekaran *et al*., 2005; Davidson *et al*., 2011) was administered. Thereafter, blood glucose level of animals was measured 72 hours (3 days) after streptozotocin administration for confirmation of diabetes, via collection of blood from the tip of the tail and using a blood glucometer (Accu-Chek active, Germany). Animals with blood glucose level equal to or more than 280 mg/dl were taken as diabetic as used in other studies (Rajasekaran *et al*., 2005; Davidson *et al*., 2011).

ADMINISTRATION OF PLANT EXTRACT, PHYTOL AND STANDARD ANTI-DIABETIC DRUG, GLYBURIDE

Administration (treatment) commenced 3 days after induction of diabetes. Daily administrations were via oral route (between 8am and 10am).

MEASUREMENT OF BLOOD GLUCOSE LEVELS

Blood glucose levels of animals were measured using the method of N'doua *et al*. (2016). In brief, the tip of the tail of the animal was pierced with a lancet and a drop from the blood that oozes out was applied to test strip inserted into an Accu‑Cheek blood glucose meter. The glucose concentration in the blood was displayed on the screen of the Accu‑Cheek blood glucose meter. This procedure was performed on all the animals.

STATISTICAL ANALYSIS

Results were presented as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA). Differences between means were analyzed by applying Scheffe's t- test for comparison with control groups at 95% ($p<0.05$) confidence level. The data were statistically analyzed by SPSS VERSION 23.0.

III. RESULTS

Constituents	Inference
Saponins	+++
Flavonoids	++
Alkaloids	+++
Tannins	+++
Terpenes	++
Cadiac glycosides	+
Cyanogenic glycosides	-
Phlobatannins	-
Anthraquinones	-

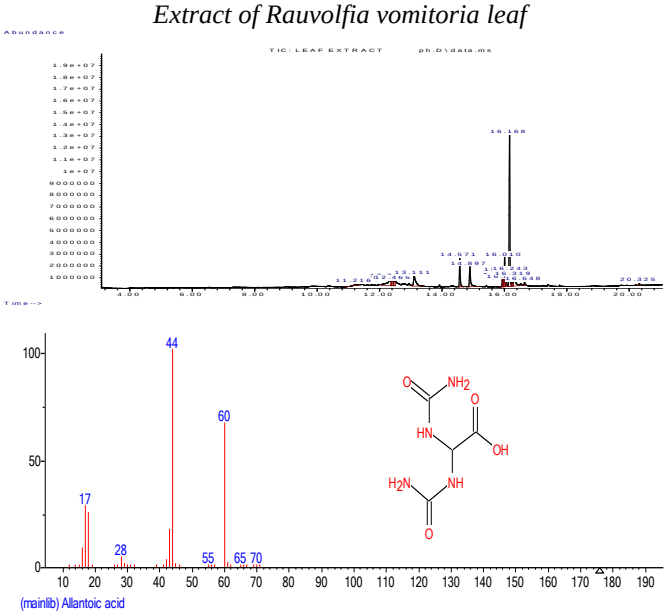
\+ *indicate presence*

\- *Indicate absence*

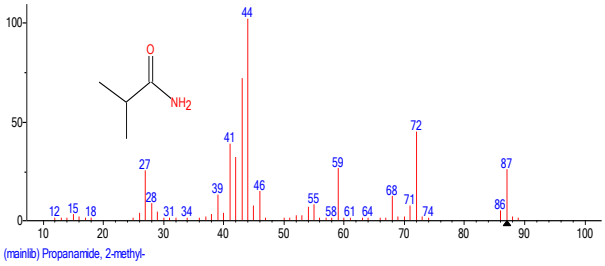
Table 1: Preliminary phytochemical screening of hydromethanolic leaf extracts of Rauvolfia vomitoria

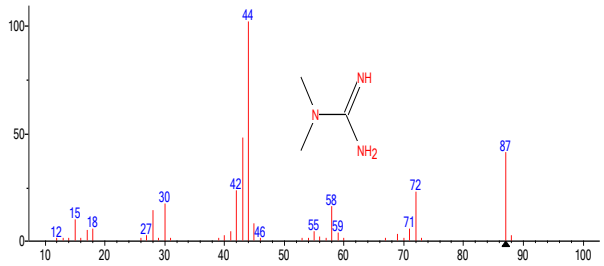
S/N	Name of Compound	Retention Time (Min.)	Percentage abundance	Molecular weight
1	Allantoic acid	11.216	0.841	176
2	Propanamide, 2-methyl-	12.316	7.387	87
3	Guanidine, N,N-dimethyl-	12.397	2.520	87
4	Hydroperoxide, 1,4-dioxan-2-yl	12.465	2.225	120
5	1H-Indole-2-ethanol, β-(3-ethylidene-1-methyl-4-piperidinyl)-3-methyl-	13.111	7.495	298
6	Hexadecanoic acid, methyl ester	14.571	7.663	270
7	n-Hexadecanoic acid	14.897	7.508	256
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.937	2.297	294
9	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)	15.972	3.114	264
10	9-Octadecenoic acid (Z)-, methyl ester	16.010	7.328	296
11	9-Octadecenoic acid (Z)-, methyl ester	16.058	2.166	296
12	Phytol	16.168	38.730	296
13	Methyl stearate	16.243	3.657	298
14	9,12-Octadecadienoic acid (Z,Z)- methyl ester	16.319	5.930	294
15	Methyl 10-trans,12-cis-octadecadienoate	16.648	0.691	294
16	Cyclopentane, bromo-	20.325	0.447	148

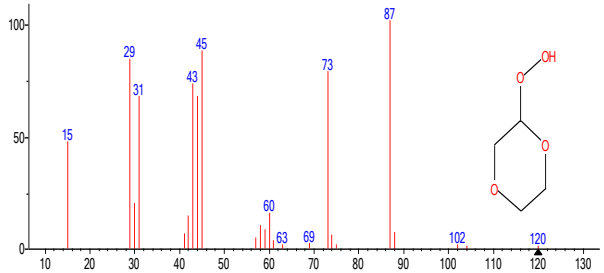
Table 2: GC-MS Phytochemical Profile of Hydromethanolic Extract of Rauvolfia vomitoria leaf

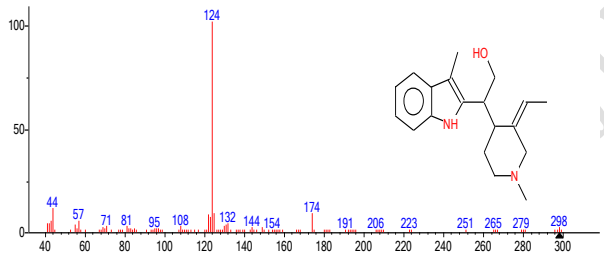


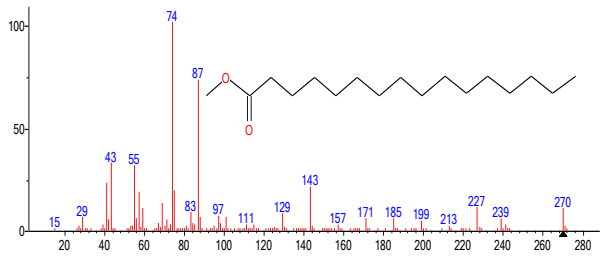
ISSN: 2394-4404

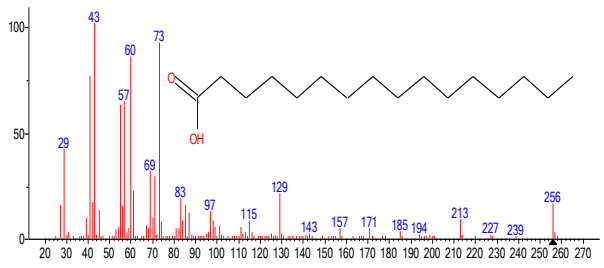

(mainlib) Propanamide, 2-methyl-

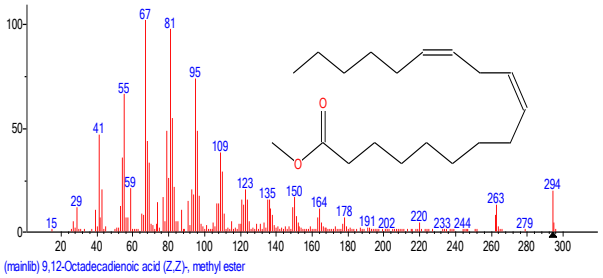

(mainlib) Guanidine, N,N-dimethyl-

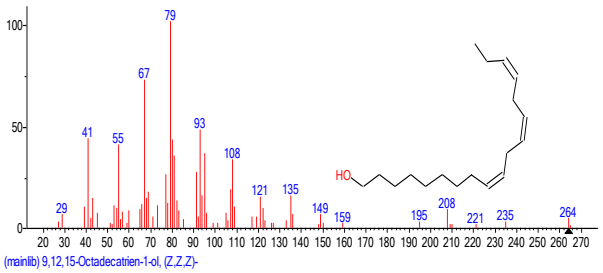

(mainlib) Hydroperoxide, 1,4-dioxan-2-yl

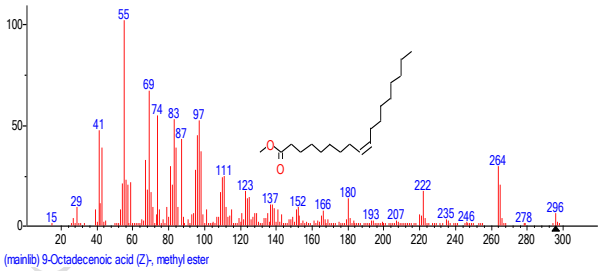

(mainlib) 1H-Indole-2-ethanol, β-(3-ethylidene-1-methyl-4-piperidinyl)-3-methyl-

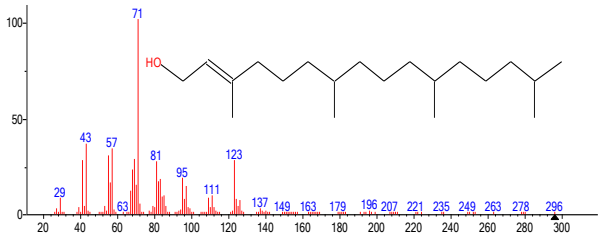

(mainlib) Hexadecanoic acid, methyl ester

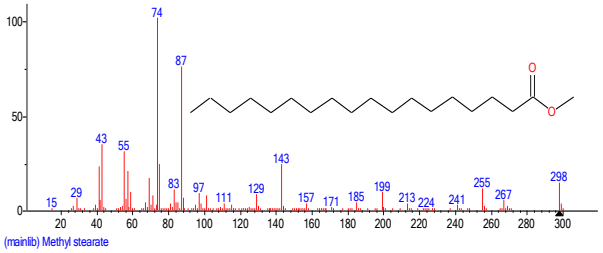

(mainlib) n-Hexadecanoic acid

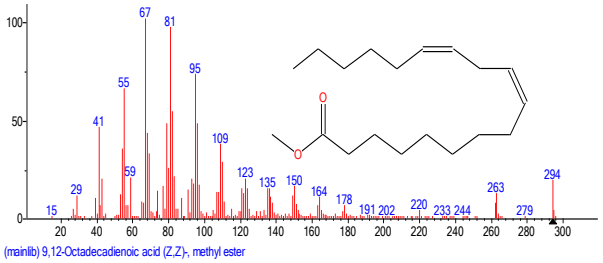

(mainlib) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester


(mainlib) 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-


(mainlib) 9-Octadecenoic acid (Z)-, methyl ester


(mainlib) Phytol


(mainlib) Methyl stearate


(mainlib) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

ISSN: 2394-4404

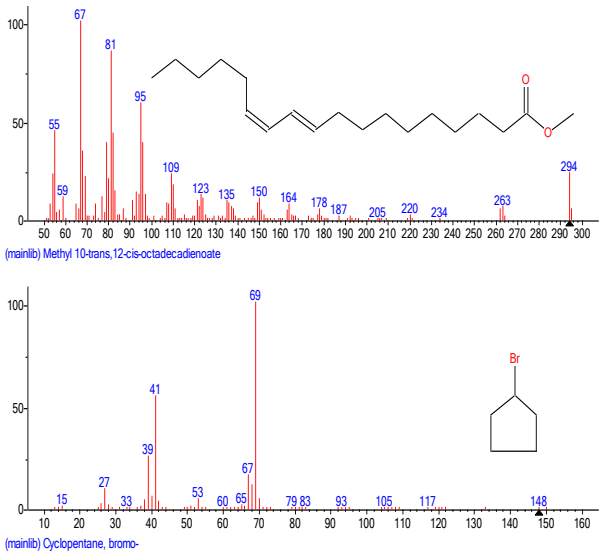


Figure 1: Chromatogram obtained from the GC-MS with the hydromethanolic extract of Rauvolfia vomitoria leaf

S/N	Descript	Just b/4 induct	72 hrs after induc	2 days of treat.	6 days of treat.	10 days of treat.	14 days of treat.	18 days of treat.	22 days of treat.	26 days of treat.	28 days of treat.
1	Normal control	82.80±3.34	81.60±3.61	85.60±2.71	80.40±2.84	82.80 ±3.95	82.00±3.08	85.00 ±3.16	85.00±3.63	89.00±2.10	88.20 ±1.62
2	Diabetic control	89.80±2.92	339.00±6.38	326.60±7.81	315.00±7.56	302.80 ±4.19	299.60 ±7.88	303.40±1.94	299.80±2.48	304.00±2.19	300.00±0.71
3	Diabetes plus HM extract of *Rv* (500mg/kg)	86.60±2.23	324.80±10.72	280.40±11.10	241.60±4.24	185.40 ±6.45*	127.20 ±4.25*	106.20±5.45*	94.20±2.22*	89.40±1.54*	87.80 ±3.31*
4	Diabetes plus phytol (200mg/kg)	87.80±3.12	322.00±9.97	277.40±7.46	237.80±2.48	194.4± 10.36*	151.20 ±7.99*	136.60±10.98*	127.00±2.83*	108.00±4.22*	97.80 ±2.42*
5	Diabetes plus glyburide (5mg/kg)	86.60±2.23	318.00±6.74	279.00±6.72	244.20±3.07	179.40 ±5.97*	130.80 ±3.77*	110.20±4.80*	100.60±2.25*	96.20±1.83*	81.00 ±4.23*

*Values are expressed as Mean±SEM; n=5; *=Significant at p<0.05*

Table 3: Effect of 28 days treatment with hydromethanolic (HM) leaf extract of Rauvolfia vomitoria (Rv) and Phytol on blood glucose level (mg/dl) of Streptozotocin induced-diabetic male wistar rats

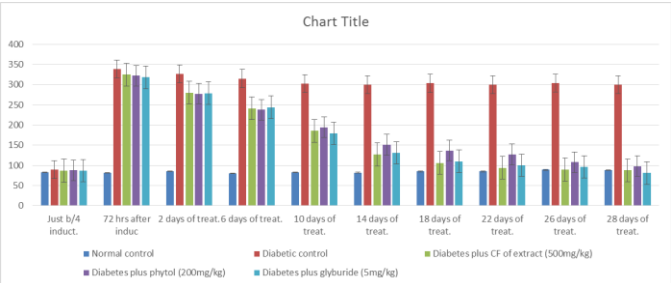


Figure 3: Graphical representation of the effect of 28 days treatment with hydromethanolic leaf extract of Rv and Phytol on blood glucose level (mg/dl) of Streptozotocin induced-diabetic male wistar rats

IV. DISCUSSION

To the best of our knowledge, this is the first study that investigates the phytoconstituent(s) of *Rauvolfia vomitoria* leaf responsible for its anti-diabetic effect.

The result of the preliminary phytochemical screening of *Rauvolfia vomitoria* leaf (Figure 1) shows that it contains saponins, flavonoids, alkaloids, tannins, terpenoids and cardiac glycosides. The result from this study (Table 1) is similar to those of Koffi *et al*. (2009), Ojo *et al*. (2012), Ajayi and Ojalere (2013) and Okereke *et al*. (2015), which indicated flavonoids, Alkaloids, saponins, tannins, terpenoids, cardiac glycosides as the phytochemicals of *Rauvolfia vomitoria* leaf.

Some of these phytochemicals and their metabolites are responsible for the various medicinal actions associated with *Rauvolfia vomitoria*.

GC-MS analysis of the hydromethanolic extract of *Rauvolfia vomitoria* leaf revealed the presence of notable phytochemicals, namely, Propanamide, 2-methyl (7.387%), 1H-Indole-2-ethanol, β-(3-ethylidene-1-methyl-4-piperidinyl)-3-methyl (7.495%), Hexadecanoic acid, methyl ester (7.663%), n-Hexadecanoic acid (7.508%), 9,12,15-Octadecatrien-1-ol, (3.114%), 9-Octadecenoic acid (Z)-, methyl ester (7.328%), Phytol (38.730%), Methyl stearate (3.657%) and 9,12-Octadecadienoic acid (Z,Z)- methyl ester (5.930%), some of which have been identified and characterized (including their mechanisms of action) by previous studies (Rajeswari *et al*., 2012; Sermakkani and Thangapandian, 2012; Elmazar *et al*., 2013; Govindappa *et al*., 2014; Gomathi *et al*., 2015; Matsuda *et al*., 2018). Among these phytochemicals, phytol is the most abundant (38.730%) and by implication, will be the major contributor of whatever effect exhibited by *Rauvolfia vomitoria* leaf. This was corroborated by the results of the effects of phytol on blood glucose (Table 3) levels.

The results from this study (Table 3) showed that 28 days treatment of diabetic rats with phytol caused significant ($P<0.05$) decrease in blood glucose level (95.73%), while the hydromethanolic extract of *Rauvolfia vomitoria* leaf had a 99.5%. The standard anti-diabetic drug, glyburide-treated group shows a 101.9% decrease in the blood glucose levels of diabetic rats. This is an indication that prolonged treatment with glyburide might cause hypoglycemia, which is one of the shortcomings of synthetic anti-diabetic drugs (Moller, 2001; DeRuiter, 2003).

The results from this study also showed that, there was added benefit in favor of the extract versus phytol based on statistically significant difference between the two groups (Table 3) which could be attributed to the other constituents of the extract. This finding has been corroborated by previous studies (Jananie *et al*., 2011; Govindappa *et al*., 2014; Ezekwe and Chikezie, 2017). For example, Govindappa *et al*. (2014) showed that hexadecenoic and octadecenoic acids are strong anti-diabetic compounds, Ezekwe and Chikezie (2017) implicated 10-octadecenoic acid, methyl ester and hexadecenoic acid, methyl ester for anti-oxidant and anti-diabetic actions.

Previous studies have shown various mechanisms through which phytol effect its anti-diabetic action. Elmazar *et al*. (2013) showed that phytol is metabolized in the liver into

phytanic acid which has high affinity to interact with peroxisome proliferator-activated receptors (PPARs) in a pattern similar to that of the thiazolidinediones (TZDs) agonists. Govindappa *et al*. (2014) implicated phytol and Hexadecanoic acid, methyl ester as major phytoconstituents of methanol extracts of Loranthus micranthus which showed strong α-amylase, α-glucosidase (anti-diabetic) inhibitory activities. The research study of Matsuda *et al*. (2018) supported the insulin sensitizing/anti-diabetic mechanism of action of phytol and also showed that phytol/phytanic acid act directly on pancreatic β-cells causing increase insulin secretion.

V. CONCLUSION

This study which is the first to investigate the anti-diabetic phytoconstituent(s) of *Rauvolfia vomitoria* leaf has shown that *Rauvolfia vomitoria* leaf is rich of phytol which is known for strong anti-diabetic actions. The efficacy of medicinal plants is usually a product of the synergic effects of several phyto-compounds and therefore, the more potent anti-diabetic activities of the hydromethanolic extract of *Rauvolfia vomitoria* leaf in comparison with phytol, can be attributed to the presence of other phytoconstituents.

REFERENCES

[1] Ajayi I.A., Ojelere O.O. (2013). Phytochemical analysis and mineral element composition of ten medicinal plant seeds from South-west Nigeria. New York Science Journal, 6(9): 1-7.
[2] Akpojotor P., Kagbo H.D. (2016). Histomorphological and biochemical effects of ethanolic extract of Monodora myristica seed (African nutmeg) on some liver function parameters using albino wistar rats. British Journal of Medicine and Medical Research, 18(7): 1-9.
[3] Amita P., Shalini T. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5): 115-119.
[4] Amole O.O. (2003). Blood pressure responses to aqueous extract of Rauvolfia vomitoria (Afzel). Nig. J. Health. Bio. Sci., 2: 50-51.
[5] Campbell-Tofte J.A., Mølgaard P., Josefsen K., Abdallah Z., Hansen S.H., Cornett C., Mu H., Richter E.A., Petersen H.W., Nørregaard J.C., Winther K. (2011). Randomized and double-blinded pilot clinical study of the safety and anti-diabetic efficacy of the Rauvolfia-Citrus tea, as used in Nigerian Traditional Medicine. Journal of Ethnopharmacology; 133: 402–411.
[6] Canadian Diabetes Association (2003). Canadian Diabetes Association 2003 clinical practice guidelines for the prevention and management of diabetes in Canada. Canadian Journal of Diabetes, 27: S1-S2.
[7] Davidson E.P., Coppey L.J., Dake B., Yorek M.A. (2011). Treatment of streptozotocin-induced diabetic rats with Alogliptin: effect on vascular and neural complications. Experimental Diabetes Research, 2011: 7.
[8] DeRuiter J. (2003). Overview of the antidiabetic agents. Endocrine Pharmacotherapy Module, Spring; 1-33.
[9] Elmazar M.M., El-Abhar H.S., Schaalan M.F., Farag N.A. (2013). Phytol/Phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS One, 8(1): e45638.
[10] Ezejindu D.N., Nwokoro T., Obaji N.N. (2014) Evaluation of Hepatoprotective Effects of Rauwolfia Vomitoria Leaf Extract on the Kidneys of Adult Wistar Rats. International Invention Journal of Medicine and Medical Sciences, 1(2): 21-25.
[11] Ezejindu D.N., Okafor I.A., Anibeze C.I.P. (2013). Histological effects of Rauwolfia vomitoria extract on carbon tetrachloride induced hepatotoxicity in adult wistar rats. Global Journal of Biology, Agriculture and Health Sciences, 2(2): 73-77.
[12] Ezekwe S.A., Chikezie P.C. (2017). GC-MS Analysis, Hypoglycemic Activity of Aqueous Root Extract of Carica papaya and Its Effects on Blood Lipid Profile and Hepatorenal Tissues Biomarkers of Diabetic Rats. Journal of Diabetes and Metabolism, 8(5): 1-9.
[13] Ezuruike U.F., Prieto J.M. (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. Journal of Ethnopharmacology, 155: 857-924.
[14] Gomathi D., Kalaiselvi M., Ravikumar G., Devaki K., Uma C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides L. Journal of Food Science and Technology, 52: 1212-1217.
[15] Govindappa M., Channabasava C., Chandrappa C.P., Sadananda T.S. (2014). In Vitro Antidiabetic Activity of Three Fractions of Methanol Extracts of Loranthus Micranthus, Identification of Phytoconstituents by GC-MS and Possible Mechanism Identified by GEMDOCK Method. Asian Journal of Biomedical and Pharmaceutical Sciences, 04 (34): 34-41.
[16] International Diabetes Federation (2014). Annual report. Retrieved 13 July, 2016.
[17] Jananie R.K., Priya V., Vijayalakshmi K. (2011). Phytoconstituents evaluation by GC-MS and anti-hyperglycemic activity of Cynodon dactylon on streptozotocin induced diabetes in rats. Journal of Chemical and Pharmaceutical Research, 3(4): 460-466.
[18] Koffi N., Beugre K., Guede N.Z., Laurent A.A. (2009). Phytochemical Screening of Some Ivorian Medicinal Plants used by the Krobou People (Agboville, Côte-d'Ivoire). Sciences Nature, 6: 1-15.
[19] Kumari R., Rathi B., Rani A., Bhatnagar S. (2013). Rauvolfia serpentine L. Benth. ex kuz.: Phytochemical, Pharmacological and Therapeutic Aspects. International Journal of Pharmaceutical Sciences Review and Research, 23(2): 348-355.
[20] Matsuda H., Suzuki D., Asakura M., Ooi S., Saitoh R., Otokozawa R., Takaaki Shirai T. (2018). Effects of Dietary Phytol on Glucose Uptake and Insulin Secretion in Vitro and in Vivo. Food & Nutrition Current Research, 1(1): 29-37.

[21] Melander A. (1988). Non-insulin dependent diabetes mellitus treatment with sulphonylureas in: clinical endocrinology and metabolism. Eds Natrass M. and Hale P., Balliare- Tindall, London: pp 443-453.
[22] Moller D.E. (2001). New drug targets for type 2 diabetes and the metabolic syndrome. Nature, 414: 821–827.
[23] N'doua L.A.R., Abo K.J.C., Aoussi S., Gbogbo M., Yapo A.P., Ehile E.E. (2015). Hypoglycemic and anti-hyperglycemic extract ethanolic 70% of roots of Rauvolfia vomitoria afzel (apocynaceae). European Scientific Journal, 11(6): 176-190.
[24] N'doua L.A.R., Abo K.J.C., Aoussic S., Kouakoud L.K., Ehile E.E. (2016). Aqueous Extract of Rauwolfia Vomitoria Afzel (Apocynaceae) Roots Effect on Blood Glucose Level of Normoglycemic and Hyperglycemic Rats. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS), 20(1): 66-77.
[25] National Research Council. Guide for the Care and Use of Laboratory Animals. 8th Edition, 2011. National Academies Press, Washington D.C. ISBN: 978-0-309-15401-7.
[26] Nguyen H.P., Kimaru I.W. (2014). GC-MS analysis of an herbal medicinal remedy to identify potential toxic compounds. Adv Pharmaceut Anal., 12:14-18.
[27] Ogbe F.D., Eruogun O.L., Uwagboe M. (2009). Plant used for female reproductive health care in Oredo local government area, Nig. Sci. Res. Essay, 4(3): 120-130.
[28] Ojo O.O., Ajayi S.S., Owolabi L.O. (2012). Phytochemical screening, anti-nutrient composition, proximate analyses and the antimicrobial activities of the aqueous and organic extracts of bark of Rauvolfia vomitoria and leaves of Peperomia pellucida. International Research Journal of Biochemistry and Bioinformatics, 2(6): 127-134.
[29] Okereke N.C., Iroka F.C., Chukwuma O.M., Kenneth U.E., Clement U.O. (2015). The effect of boiling on the phytochemical and nutritional content of Rauvolfia vomitoria. Journal of Global Biosciences, 4(6): 2561-2568.
[30] Okpuzor J., Ogbunugafor H.A., Kareem G.K. (2009). Hepatic and Hematological Effects of Fractions of Globimetula braunii in Normal Albino Rats. Experimental and Clinical Sciences, 8: 182-189.
[31] Olatokunboh A.O., Kayode Y.O., Adeola O.K. (2009). Anticonvulsant activity of Rauvolfia Vomitoria (Afzel). African Journal of Pharmacy and Pharmacology, 3(6): 319-322.
[32] Piero M.N., Nzaro G.M., Njagi J.M. (2014). Diabetes mellitus – a devastating metabolic disorder. Asian Journal of Biomedical and Pharmaceutical Sciences, 40(40): 1-7.
[33] Rajasekaran S., Sivagnanam K., Subramanian S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacological Reports, 57: 90-96.
[34] Rajeswari G., Murugan M., Mohan V.R. (2012). GC-MS analysis of bioactive components of Hugonia Mystax L. Research Journal of Pharmaceutical Biology and Chemical Science, 3: 301-308.
[35] Rossini A.A., Like A.A., Chick W.L., Appel M.C., Cahill G.F. (1977). Studies of streptozotocin-induced insulitis and diabetes. Proceedings of the National Academy of Sciences USA, 74: 2485–2489.
[36] Sermakkani M., Thangapandian V. (2012). GC-MS analysis of Cassia italica leaf methanol extract. Asian Journal of Pharmacology and Clinical Research, 5: 90-94.
[37] Shi Y., Hu F.B. (2014). The global implications of diabetes and cancer. The Lancet 383(9933): 1947–1948.
[38] Shukla R., Sharma S.B., Puri D., Prabhu K.M., Murthy P.S. (2000). Medicinal plants for treatment of diabetes mellitus. Indian Journal of Clinical Biochemistry, 15: 160-177.
[39] Sofowora A. (2008). Medicinal Plants and Traditional Medicine in Africa. Third Edition, Spectrum Books Limited Ibadan. ISBN: 978-978-029-881-4.
[40] WHO Expert Committee (1980). Diabetes mellitus 2nd rep. Geneva, World Health Organization Technical Report Series No. 646.
[41] World Health Organization (2016). Global Report on Diabetes. Geneva. Available at https://www.who.int/diabetes/global-report/en/.
[42] World Health Organization diabetes programme (2008). Geneva. Available at http://www.who.int/ diabetes/facts/world_figures/en/index2.html.