Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2125-2131
Received: 07-05-2019
Accepted: 09-06-2019

Igbinidu GO
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Nimenibo Uadia R
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Correspondence
Igbinidu GO
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

GC-MS analysis, phytochemical screening and *In vitro* alpha amylase and alpha glucosidase inhibitory activities of *Vernonia amygdalina* root extract and fractions

Igbinidu GO and Nimenibo Uadia R

Abstract
The aim of this study was to evaluate the in vitro inhibitory activity of *Vernonia amygdalina* root methanol extract and fractions on Porcine alpha amylase and Baker's yeast alpha glucosidase at varying concentrations and to analyse the most potent of the methanol extract and its fractions through phytochemical screening and Gas Chromatography-Mass Spectroscopy analysis (GC-MS). Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia in which there is an elevated glucose level in the blood. Inhibitors of alpha amylase and alpha glucosidase are used to achieve good control over hyperglycemia in type 2 diabetes mellitus. The present study intends to screen novel alpha amylase and alpha glucosidase inhibitors from a natural source (plant) so as to reduce the toxicity and side effects of the synthetic inhibitors currently used to control hyperglycemia. The alpha amylase inhibition assay showed that the methanol crude extract of *Vernonia amygdalina* root (18.17µg/ml), the n-hexane fraction (71.41 µg/ml), the dichloromethane fraction (95.06µg/ml) and the aqueous fraction (165.51µg/ml) exhibited 50% alpha amylase inhibition activity at the mentioned concentrations. The alpha glucosidase IC_{50} for *Vernonia amygdalina* root methanol crude extract, n-hexane fraction, dichloromethane fraction and aqueous fraction was found to be 12.17µg/ml, 13.59µg/ml, 59.83 µg/ml and 12.93µg/ml respectively. The phytochemical screening and GC-MS analysis of the methanol crude extract (the most potent in both the alpha amylase and alpha glucosidase inhibition assays) revealed the presence of bioactive agents reported to have anti-diabetic activities. The results of this study therefore clearly support the traditional use of *Vernonia amygdalina* root extract to manage hyperglycemia.

Keywords: Methanol, aqueous, dichloromethane, n-hexane, alpha amylase, alpha glucosidase

Introduction
Diabetes mellitus is a chronic metabolic disorder that continues to present as a major health problem worldwide. It affects the metabolism of carbohydrate, fat, proteins, as well as water. It is characterized by chronic hyperglycemia, in which there is an elevated blood glucose level either as result of the pancreas not able to produce enough insulin or cells do not respond to the insulin produced [1]. Postprandial hyperglycemia is a characteristic of type 2 diabetes and results in the formation of advanced glycation end-products. These glycated products are the promoters of diabetes complications and aging [2]. These complications involve all the important organs of the body and are due to the abnormal metabolism in diabetes mellitus [3]. The management of diabetes has been a major problem in tropical Africa including Nigeria [4, 5]. One of the strategies for the management of diabetes mellitus is to decrease postprandial hyperglycemia by delaying glucose absorption. This may be achieved by reducing the rate of digestion of starch [6] via the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase [7]. Alpha amylase and alpha glucosidase are the two major enzymes involved in carbohydrates digestion. Alpha amylase breaks down long chain carbohydrates to monosaccharides and alpha glucosidase breaks down starch and disaccharides to monosaccharides. Alpha amylase and alpha glucosidase inhibitors are the potential targets in the development of therapeutic agents for the treatment of diabetes [8].
Many inhibitors (synthetic enzyme inhibitors) presently used in clinical practice for the management of diabetes are associated with some gastro-intestinal side effects such as abdominal bloating, Diarrhoea and flatulence [9-10]. It is necessary that alpha amylase and alpha glucosidase inhibitors with little or no side effects present in natural sources (dietary plants) are identified and explored in the management of diabetes mellitus. Since ancient time, natural products present in plants have been utilized in the management of diabetes mellitus especially in developing countries where there is the problem of limited resources, affordability as well

as access to modern treatment [11]. Many traditional plants have been known for their anti-diabetic effect and used for the treatment and management of diabetes mellitus [12]. Some of these plants have been validated through scientific studies to have anti-diabetic activity by means of biological actions against diabetes mellitus or its complications [13]. It has been reported that there are more than 400 of such traditional plant remedies but only a few of them have been scientifically and medically evaluated to assess their efficacy [14]. The medicinal properties of these plants have been ascribed to the biochemical or bioactive compounds present in the plant materials. It is reported that medicinal plants contain substances which could be used for treatment purposes or used to produce drugs [15]. Medicinal plants contain anti-diabetic agents which are very promising and these traditionally acclaimed medicinal plants are being investigated for their anti-diabetic potential by researchers [16-17].

Higher plants, animals and micro-organisms are known to produce a number of different protein inhibitors of alpha amylase and alpha glucosidase that control the activity of these two enzymes. Some of these enzyme inhibitors act by directly blocking the active centre of the enzyme at various local sites [18]. In animals alpha-amylase inhibitors decrease the high blood glucose levels that can occur after a meal by decreasing the rate at which alpha-amylase converts starch to simple sugars [19]. This is very important in diabetic people where low insulin levels prevent the fast clearing of glucose from the blood [20].

Alpha glucosidase inhibitors are used as oral anti-diabetic drugs for treating type 2 diabetes mellitus [11]. The intestinal alpha glucosidases breakdown complex carbohydrates to glucose and other monosaccharides in the small intestine which can easily be absorbed through the intestine. These inhibitors act as competitive inhibitors of alpha glucosidase enzymes, thus preventing the digestion of carbohydrates such as starch by alpha glucosidase and this can help reduce the rate of carbohydrates digestion [11].Therefore, a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia [21] which can be achieved by the inhibition of alpha amylase and alpha glucosidase [7].

Scientific and pharmacologic studies have shown the anti-hyperglycemic action of the roots [22] and leaves [23-24] of *Vernonia amygdalina* (bitter leaf). The present study was carried out with the aim of finding the best source of phytoconstituents from *Vernonia amygdalina* root with respect to various extraction procedures using solvents of increasing order of polarity. Furthermore, they were analysed for α-amylase and α-glucosidase inhibitory activities. The methanol crude extract of the root was also subjected to phytochemical screening and GC-MS analysis with a view of finding the phytoconstituent

Materials and Methods

Collection and identification of plant material

Vernonia amygdalina roots (bitter leaf root) were collected during the month of January, 2017, at Osasogie, Ugbowo, behind University of Benin, Benin City, Nigeria. Identification and authentication of the plant, *Vernonia amygdalina* (family: *Asteraceae*, voucher number; UBH_v342) was done by Dr. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

Preparation and sample extractions

The roots of *Vernonia amygdalina* were washed with running tap water for 5min to remove soil and chopped into small pieces using a sharp knife. The pieces were oven-dried at 60°C for 8hr till a constant weight was achieved. It was ground to powder, using a mechanical blender (Christy and Norris Ltd, England) and stored in an airtight container at 4°C. The sample was then subjected to extraction and fractionation. About 1000g of powdered plant materials were extracted with 5 litres of methanol for 24 hours and concentrated using water bath at 60°C. 80 percent of the methanol crude extract was fractionated sequentially with 400ml of different solvents (n-hexane Dichloromethane, and Distilled water) by using a separating funnel for 12hours. The fractions were concentrated using a water bath at 60°C. After extraction and fractionation, the samples were collected and stored in a vial for further studies.

In vitro inhibitory assay for the α-amylase activity

The *in vitro* alpha amylase inhibitory activity assay was carried out according to the method described by Nair *et al.*[11]. The assay mixture containing 200µl of 0.02M sodium phosphate buffer, 20µl of enzyme and the plant extracts in concentration range 20-100µg/ml were incubated for 10min at room temperature followed by addition of 200µl of starch in all test tubes. The reaction was terminated with the addition of 400µl DNS reagent and placed in boiling water bath for 5min, cooled and diluted with 15ml of distilled water and absorbance was measured at 540nm. The control samples were prepared without any plant extracts. The percentage inhibition was calculated according to the formula [25]:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{540}(\text{control}) - \text{Abs}_{540}(\text{extract})}{\text{Abs}_{540}(\text{control})} \times 100$$

The IC_{50} values were determined from plots of percentage inhibition versus inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

In vitro inhibitory assay for the α-glucosidase activity

The assay was carried out according to the method described by Nair *et al* [11].

The Yeast apha glucosidase was dissolved in 100mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant extracts were used in the concentration range 20-100µg/ml. Different concentrations of plant extracts were mixed with 320µl of 100mM phosphate buffer pH 6.8 at 30°C for 5min. 3ml of 50mM sodium hydroxide was added to the mixture and the absorbance was read at 410nm. The control samples were prepared without any plant extracts. The percentage inhibition was calculated according to the formula [25]:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{410}(\text{control}) - \text{Abs}_{410}(\text{extract})}{\text{Abs}_{410}(\text{control})} \times 100$$

The IC_{50} values were determined from plots of percentage inhibition versus inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

Qualitative estimation of phytoconstituents

Phytochemical screening of the methanol crude extract was carried out according to standard procedures [26-27] and the methods variously described by Trease and Evans [28], Sofowora [29] and Ayoola [30] to assess the qualitative chemical composition of the most potent crude extract for phytoconstituents such as alkaloids, anthraquinones, tannins, flavonoids, saponins etc.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis of the methanol crude extract of *V. amygalina* root was carried out using Gas Chromatography (Agilent technologies 7890, Germany) coupled with Mass spectrometer detector (Agilent technologies 5975, Germany): column used was HP5MS Agilent technologies, 30m in length and inner diameter of 0.32 mm and thickness 0.25µm. The initial column temperature was 80^0C and final temperature was 240^0C, with split less injectors and pressure of 8.267psi. The flow rate within the column was 2ml/minute. The detector temperature was 250 ^{0}C and helium was used as the gas carrier with mass spectrometric detector and the sample volume injected was 1µl. Compounds were identified by comparing mass spectra of the compounds with those of Wiley library.

Statistical analysis

Results expressed as mean ± SEM of the three individual experiments where applicable.

Results and Discussion

The inhibitory activity of *Vernonia amygdalina* root methanol extract and its fractions on porcine alpha amylase and Yeast alpha glucosidase were investigated in this study and the results are shown in Tables 1 and 2. The percentage alpha amylase and alpha glucosidase inhibition by the methanol extract and its three fractions were plotted as a function of concentration in comparison with Acarbose as shown in Figures 1 and 2. In the alpha amylase inhibition assay, methanol crude extract (18.17µg/ml), n-hexane fraction (71.41 µg/ml), dichloromethane fraction (95.06 µg/ml) and the aqueous fraction (165.51 µg/ml) exhibited 50% alpha amylase activity at the mentioned concentrations.

The results show that out of the methanol crude extract and its fractions, the methanol crude extract displayed a very good alpha amylase inhibitory activity, n-hexane fraction and dichloromethane fraction showed appreciable alpha amylase inhibitory activities while aqueous fraction exhibited the least alpha amylase inhibitory activity as justified by their respective IC_{50} values of 18.17µg/ml, 71.41µg/ml, 95.06µg/ml and 165.51µg/ml.

The results of the alpha glucosidase inhibition assay clearly show that the methanol crude extract efficiently inhibited alpha glucosidase enzyme *in vitro*, displaying an IC_{50} value of 12.17µg/ml. The n-hexane, dichloromethane and aqueous fractions of the methanol crude extract displayed an IC_{50} value of 13.59µg/ml, 59.83µg/ml and 12.93µg/ml respectively. The methanol crude extract exhibited the maximum % alpha glucosidase enzyme inhibition as justified by its IC_{50} value (12.17µg/ml) compared to the n-hexane fraction (IC_{50}=13.59µg/ml), dichloromethane fraction (IC_{50}=59.83µg/ml) and aqueous fraction (IC_{50}=12.93µg/ml). The methanol crude extract showed an increase in percentage alpha glucosidase inhibition as its concentration was increased from 20–60µg/ml. The highest percentage alpha glucosidase inhibition (88.24%) by the methanol crude extract was seen at a concentration of 60µg/ml. At concentrations of 80 – 100µg/ml, a gradual decrease in percentage inhibition was observed but was not statistically significant (p> 0.05) when compared to that at 60µg/ml. When the percentage inhibition by methanol crude extract was compared with % inhibition by Acarbose, the methanol crude extract displayed a better alpha glucosidase enzyme inhibition and it was statistically significant (p< 0.05) at concentrations of 20 – 80µg/ml. The methanol crude extract efficiently inhibited the alpha glucosidase enzyme more than the alpha amylase enzyme.

The phytochemical screening of the methanol extract revealed the presence of flavonoids, saponins, alkaloids, condensed tannins, phenolic compounds, glycosides, reducing sugars and steroids. The results are shown in Table 3. It has been reported that saponins, flavonoids and alkaloids are present in *Vernonia amygdalina* root bark extract [31]. Studies have shown that alkaloids, glycosides, phenolics, flavonoids and steroids are used in the management of diabetes mellitus [32]. Studies have also shown that alkaloids, flavonoids, saponins, carbohydrate, steroids, tannins and phenolic compounds are present in the ethanol and aqueous extracts of *Passiflora foetida* (L.) and these extracts exhibit alpha amylase and alpha glucosidase inhibitory activities [33]. Phenolic compounds are reported to be natural inhibitors of alpha amylase and alpha glucosidase with strong inhibitory effect on alpha glucosidase but mild inhibitory effect on alpha amylase enzyme and as such, can be used to prevent postprandial hyperglycemia with minimal side effects [34]. Glycosides are also reported to have anti diabetic properties [32]. The synergistic inhibition of intestinal alpha glucosidase and alpha amylase by cynidin-3-galactoside, a flavonoid, when combined with acarbose shows that flavonoids effectively inhibit alpha glucosidase and pancreatic alpha amylase, which is one of the strategies for the treatment of diabetes mellitus [35]. Studies have also shown that saponins can reduce hyperglycemia [36]. The synergistic action of these phytochemicals (identified in the methanol crude extract) in reducing blood glucose levels has been reported [32].

The GC-MS analysis of the methanol crude extract of *V. amygdalina* root revealed the presence of 15 compounds. The compounds with their retention time, molecular formula, molecular weight and peak area % are shown in Table 4. The major compounds identified were 9, 12-Octadecadienoic acid (Z,Z)- with 19.88% peak area, 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester with peak area12.90%, n-Hexadecanoic acid with 12.34% peak area, 9-Octadecenoic acid (Z)-, methyl ester with peak area 10.97% and Hexadecanoic acid, methyl ester with 10.37% peak area.

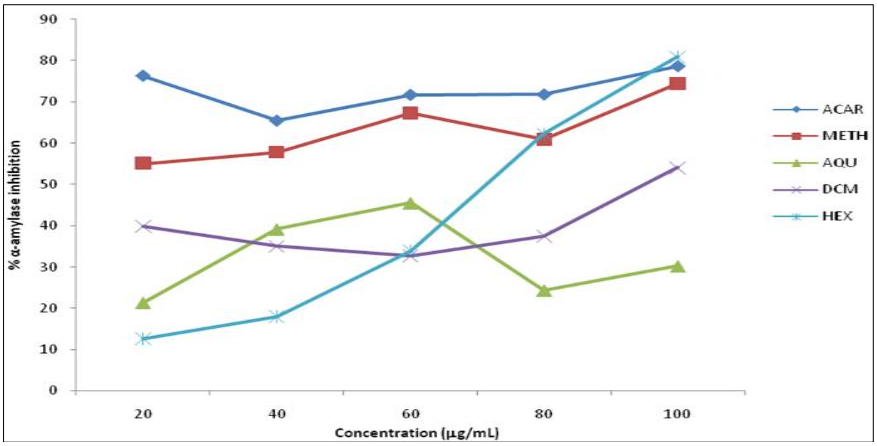
Studies have shown the anti-inflammatory and anti-cancer properties of 9, 12-Octadecadienoic acid (Z, Z) [37]. The anti-oxidant activity of this compound has also been reported [38]. Studies have also shown that anti-oxidants are beneficial in the treatment of type 2 diabetes mellitus [39]. Excess androgen is reported to be part of the constellation of symptoms of type 1 diabetes mellitus in women [40]. N-Hexadecanoic acid, a major compound identified in the GC-MS analysis in this study, is reported to have anti-androgenic activity, hypercholesterolemic activity, anti-bacterial activities [41], anti-oxidant activity [42] as well as anti-cancer and anti-diabetic activities [43]. Studies have shown that 9, 12-Octadecadienoic acid (Z, Z)-, n-Hexadecanoic acid and 9, 17-Octadecadienal, (Z) - identified in the GC-MS analysis have

anti-alpha amylase and anti-alpha glucosidase activity [33]. Evidence exists for the anti-oxidant, anti-cancer [44, 45] and anti-androgenic [46] properties of 9-Octadecenoic acid (Z)-, methyl ester. This compound is also reported to be present in the GC-MS analysis of the aqueous root extract of *Carica papaya* which exhibited hypoglycemic activity in alloxanum-induced diabetic experimental rats within the experimental period of 21 days [47].

Table 1: The percentage inhibition of porcine alpha amylase by methanol crude extract, n- hexane fraction, dichloromethane fraction and aqueous fraction at varying concentrations

Conc. (µg/ml)	% Inhibition by Meth. Crude extract	IC_{50} (µg/ml) Meth. extract	% Inhibition by n-Hex. fraction	IC_{50} (µg/ml) n-Hex. fraction	% Inhibition by Dichl. fraction	IC_{50} (µg/ml) Dichl. fraction	% Inhibition By Aque. fraction	IC_{50} (µg/ml) Aque. fraction
20	55.04 ± 2.34a		12.57 ± 25.68a		39.89 ± 11.46a		21.33 ± 15.92a	
40	57.78 ± 2.88a		17.95 ± 1.73ab		35.07 ± 2.64a		39.13 ± 6.78a	
60	67.24 ± 2.38bd	18.17	33.85 ± 12.23ab	71.41	32.73 ± 1.58a	95.06	45.53 ± 20.04a	165.51
80	60.88 ± 1.42ab		62.15 ± 1.37bc		37.55 ± 0.30a		24.34 ± 47.29a	
100	74.41 ± 0.09cd		80.91 ± 0.17c		54.08 ± 25.65a		30.21 ± 17.10a	

Data expressed as mean ± SEM. Mean values with different alphabets within group are significantly different (p< 0.05).



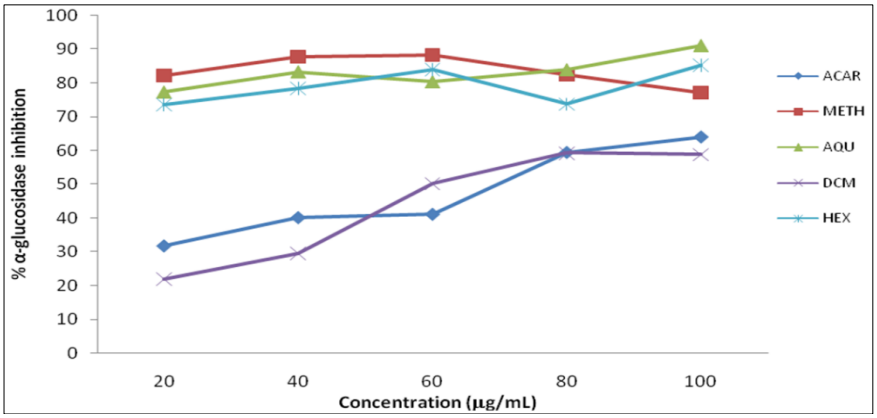
Key: acar = Acarbose, meth= methanol crude extract, aqu= Aqueous fraction, DCM= dichloromethane fraction, hex= n-Hexane fraction

Fig 1: % inhibition of porcine alpha amylase by Acarbose, methanol crude extract, aqueous fraction, dichloromethane fraction, n-hexane fraction and Acarbose

Table 2: The percentage inhibition of Yeast alpha glucosidase by methanol crude extract, n- hexane fraction, dichloromethane fraction and aqueous fraction at varying concentrations

Conc. (µg/ml)	% Inhibition by Meth. crude extract	IC_{50} (µg/ml) Meth. extract	% Inhibition by n-Hex. fraction	IC_{50} (µg/ml) n-Hex. fraction	% Inhibition by Dichl. fraction	IC_{50} (µg/ml) Dichl. fraction	% Inhibition by Aque. fraction	IC_{50} (µg/ml) Aque. fraction
20	82.16 ± 2.16a		73.60 ± 0.88a		21.77 ± 1.77a		77.33 ± 0.86ad	
40	87.68 ± 1.41a		78.43 ± 0.00ab		29.41 ± 5.88a		83.33 ± 2.94ab	
60	88.24 ± 3.93a	12.17	83.98 ± 0.34b	13.59	50.18 ± 4.73b	59.83	80.39 ± 1.96ab	12.93
80	82.35 ± 1.96a		73.77 ± 4.67a		59.27 ± 4.37b		83.98 ± 0.34b	
100	77.19 ± 4.64a		85.29 ± 0.98b		58.79 ± 7.88b		91.18 ± 0.98c	

Data expressed as mean ± SEM. Mean values with different alphabets within group are significantly different (p< 0.05).



Key: acar= Acarbose, meth= methanol crude extract, aqu= aqueous fraction, DCM= dichloromethane fraction, hex= n-hexane fraction.

Fig 2: % inhibition of Yeast alpha glucosidase by Acarbose, methanol crude extract, aqueous fraction, dichloromethane fraction, n-hexane fraction and Acarbose

Table 3: Results of phytochemical screening of the methanol crude extract of *Vernonia amygdalina* root.

Phytochemical type	Inference
Alkaloids	+
Anthraquinones	–
Cardiac glycosides	–
Tannins (condensed)	+
Flavonoids	+
Glycosides	+
Hydrolysable tannins	–
Phenolic compounds	+
Polysaccharides (starch)	–
Reducing sugars	+
Saponins	+
Steroids	+

(+) = present and (–) = not present

Table 4: Results of Gas Chromatography- Mass Spectrometry (GC-MS) analysis of the methanol crude extract of *Vernonia amygdalina* root.

0	Retention Time	Molecular Formula	Name of Compound	Molecular Weight	Peak Area (%)
1	4.552	C6 H12O	Oxirane, butyl-	100	1.22
2	14.439	C13 H26O2	Dodecanoic acid, methyl ester 214	0.87	
3	20.585	C17H34O2	Hexadecanoic acid, methyl ester 270	10.37	
4	21.483	C5H5N3O4	1-Methyl-5-nitro-4-pyrazolecarboxylic acid	171	5.67
5	21.763	C16 H32 O2	n-Hexadecanoic acid	256	12.34
6	23.532	C19 H34 O2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	12.90
7	23.629	C19 H36 O2	9-Octadecenoic acid (Z)-, methyl ester	296	10.97
8	23.949	C19 H38 O2	Methyl stearate	298	1.50
9	24.750	C18 H32 O2 \	9,12-Octadecadienoic acid (Z,Z)-	280	19.88
10	24.813	C19 H32 O2	9,12-Octadecadienoic acid (Z,Z)-	280	6.04
11	24.842	C18 H32 O	9,17-Octadecadienal, (Z)-	264	4.77
12	24.973	C20 H40 O4	Hexadecanoic acid,1,1-dimethyl ethyl ester	344	2.88
13	27.331	C21 H40 O2	n-Propyl 9-octadecenoate	324	8.26
14	27.371	C18 H30 O2	9,12,15-Octadecatrienoic acid, (Z Z,Z)-	278	1.11
15	29.402	C24 H38 O4	Diisooctyl phthalate	390	1.21

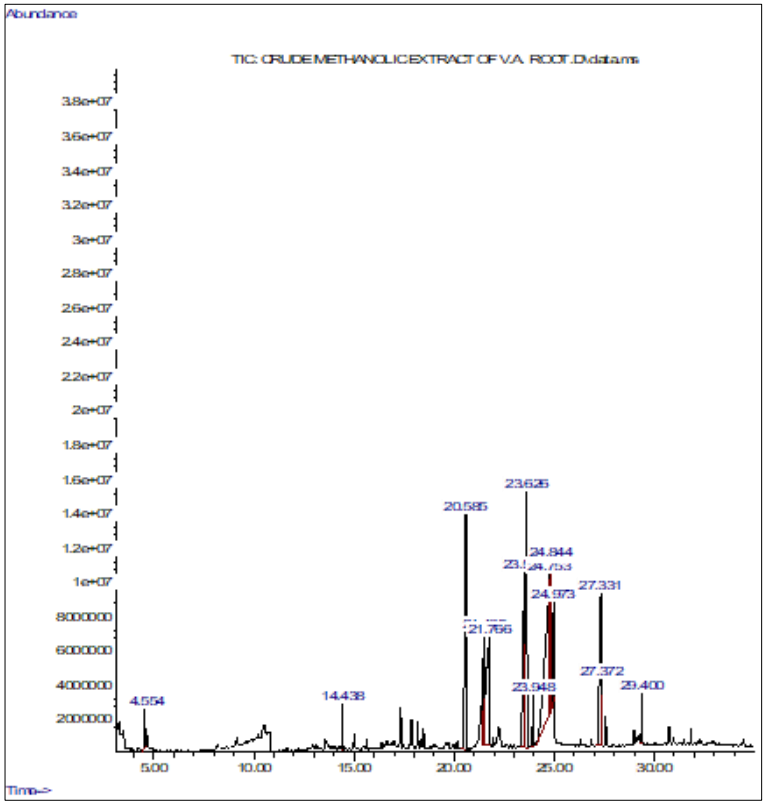


Fig 3: The GC-MS Chromatogram of the methanol crude extract of *Vernonia amygdalina* root

Conclusion

This study shows that the methanol crude extract was more potent than its fractions in both the alpha amylase and alpha glucosidase inhibition assays, as justified by its IC_{50} values of 18.17µg/ml and 12.17µg/ml respectively. The qualitative phytochemical screening of the methanol crude extract

revealed the presence of alkaloids, flavonoids, tannins (condensed), phenolic compounds, Glycosides, reducing sugars, saponins and steroids. All these phytochemicals are reported to have anti-diabetic activity. The GC-MS analysis of the methanol crude extract of *V. amygdalina* root also revealed the presence of bio-active compounds which have been shown to have anti-diabetic activity. These compounds may be the active agents in the methanol crude extract of *Vernonia amygdalina* root which may have acted synergistically to inhibit the alpha amylase and alpha glucosidase enzymes *in vitro*. This study therefore suggests that *Vernonia amygdalina* root may be useful in the management of diabetes mellitus and one of the mechanisms of the anti-diabetic activity of *Vernonia amygdalina* root may be through inhibition of alpha amylase and alpha glucosidase enzymes. This study also justifies the report from scientific and pharmacological studies that *Vernonia amygdalina* root possesses anti-hyperglycemic activity, hence, supporting the traditional use of *Vernonia amygdalina* root extract in the management of hyperglycemia. Furthermore, this study shows that methanol is capable of extracting the hypoglycemic agents present in *Vernonia amygdalina* root.

Acknowledgement
The authors wish to thank Prof. B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria for his immense contribution to the success of this research. Sincere gratitude also goes to Prof. A.C. Igboanugo of the Department of Production Engineering, Faculty of Engineering, of the same University, for his contribution to the success of this research.

References

1. West IC. Radicals and oxidative stress in diabetes. Diabetic Medicine. 2000; 17(3):171-180.
2. Ashok KT, Madham S, Shaik AB, Amtul ZS, Sachin BA, Kuncha M *et al.* Identification of proglycemic and anti-hyperglycemic activity in anti-oxidant rich fractions of some common food grains. International Food Research Journal b. 2011; 18 (3):883-891.
3. Wadham KA, Magdum CS, Patil S, Naikwade NS. Anti-diabetic potential and Indian medicinal plants. Journal of Herbal Medicine and Toxicology. 2008; 2(1):45-50.
4. Gill G. Practical management of diabetes in the tropics. Practical Diabetes Digest. 1990; 1:75-78.
5. Naidu R. Dietary advice for diabetics in Africa. International Diabetes Digest. 1992; 3(4):144-118.
6. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugan AT. *In vitro* anti-diabetic activity of *Caesalpina digyna* (R.) methanol root extract. Asian Journal of Plant Science and Research. 2011; 1(2):101-106.
7. Bhosale UP, Hallale BV. Gamma radiation-induced mutation in black grain (*Vigna mungo* (L.) Hepper). Asian Journal of Plant Science and Research. 2011; 1:96-100.
8. Subramanian R, Asmawi MZ, Sadikun A. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract, Andrographolide. Acta Biochemica Polonica. 2008; 55:391-398.
9. Bhutkar MA, Bhise SB. *In vitro* assay of alpha-amylase inhibitory activity of some indigenous plants. International Journal of Chemical Sciences. 2012; 10(1):457-462.
10. Bray GA, Greenway FL. Current and potential drugs for treatment of Obesity. Endocrine Review. 1999; 20:805-879.
11. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha-amylase and alpha-glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology. 2013; 3(1):128-132.
12. Akhtar FM, Ali MR. Study of anti-diabetic effect of a compound medicinal plant prescription in normal and diabetic rabbits. Journal of Pakistan Medical Association. 1984; 34:239-244.
13. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE *et al.* Comparative chemical compositions of leaf of some anti-diabetic plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. African Journal of Biotechnology. 2009; 8(18):4685-4689.
14. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care. 1989; 12(8):553-564.
15. Sofowora NA. Medicinal plants and Traditional medicine in Africa (3rd edn.). Spectrum Books Ltd., Ibadan, 1999, 172-188.
16. Babu V, Gangadevi T, Subramanian. Anti-hyperglycemic effect of *Cassia keinii* leaf extract in glucose fed normal rats and alloxan-induced diabetic rats. Journal of Pharmacology. 2002; 34(6):409-415.
17. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Anti-diabetic activity of *Thespesia populnea* bark and leaf extract against streptozotocin-induced diabetic rats. International Journal of Pharmtech Research. 2009; 1(4):1069-1072.
18. Sama K, Murugesan K, Siviraj R. *In vitro* alpha-amylase and alpha-glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. Asian Journal of Plant Science and Research. 2012; 2(4):550-553.
19. Boivin M, Zinsmeister AR, Go VL, Dimagno EP. Effect of a purified alpha-amylase inhibitor on carbohydrate metabolism after a mixed meal in healthy humans. Majo Clinical Proceedings. 1987; 62:249-255.
20. Mohammed A, Adelaiye AB, Bakari AG, Mabrouk MA. Anti- diabetic and some haematological effects of ethylacetate and n-butanol fractions of Ganoderma lucidum aqueous extract in alloxan-induced diabetic wistar rats. International Journal of Medical Sciences. 2009; 1(12):530-535.
21. Chakrabarti R, Rajagopalan R. Diabetes and insulin resistant associated disorders: disease and the therapy. Current Science. 2002; 83(12):1533-1538.
22. Nimenibo-Uadia, R. Effect of Vernonia amygdalina in alloxan-induced diabetic albino rats. Journal of Medical Laboratory Science. 2003; 12(1):25-31.
23. Akah PA, Okafor CI. Hypoglycemic effect of Vernonia amygdalina on experimental rabbits. Journal of Medicinal Plants Research. 1992; 1:6-10.
24. Akah, Peter, Njoku, Obioma, Nwanguma, Ada, Akunyili Dorathy *et al.* Effect of aqueous leaf extract of *Vernonia amygdalina* on blood glucose and triglyceride levels of alloxan –induced diabetic rats (*Rattus rattus*). Animal Research International. 2004; 1(2):90-94.
25. Jung M, Park M, Chul HL, Kang Y, Seo-Kang E, Ki-kim S *et al.* Anti-diabetic agents from medicinal plants. Current Medicinal Chemistry. 2006; 13:1-16.

26. Khandelwa KR. Practical Pharmacognosy Techniques and Experiments. 9th Edition, Nirali Prakashan, Pune, Indian, 2003.
27. Kokate CK. A Textbook of practical pharmacognosy. Vallabh Prakashan Edition. 2005; 5:105-111.
28. Trease GE, Evans WC. Pharmacognosy. 4th edn. USA, WB Saunders, 1996, b243-283.
29. Sofowora A. Medical plants and traditional medicine in Africa. Rep. Ibadan, Spectrum Books Ltd, 2006, 191-289.
30. Ayoola GA, Coker HB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC *et al.* Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in south-western Nigeria. Tropical Journal of Pharmaceutical Research. 2008; 7:1019-1024.
31. Eyong EU, Agiang MA, Atangwho IJ, Iwara IA, Odey MO, Ebong PE *et al.* Phytochemical and micronutrient composition of root and stem bark extracts of *Vernonia amygdaina* Del. Journal of Medicine and Medical Science. 2011; 2(6):900-903.
32. Gaikwad SB, Mohan GK, Rani MS. Phytochemicals for diabetes management. Pharmaceutical Crops. 2014; 5(1):11-28.
33. Paulraj JA, Subharamanian H, Suriyamoorthy P, Kanakasabapathi D. Phytochemical screening, GC-MS analysis and enzyme inhibitory activity of *Passiflora foetida* (L.) Indo American Journal of Phamaceutical Research. 2014; 4(8):3526-3533.
34. Martin TJ, Guillaume J, Kossa A. Condensed tannins in horse bean seeds: chemical structure and effects on Poultry. Journal of The Science of Food and Agriculture. 1977; 28:757-765.
35. Adisakwattana S, Charoenlertkul P, Yibchok-Anun S. Alpha- Glucosidase inhibitory activity of Cyanidin 3-galactoside and synergistic effect with acarbose. Journal of Enzyme Inhibition and Medicinal Chemistry. 2009; 24(1):65-69.
36. Rao AV, Gurfinkel DM. The bioactivity of saponins: triterpenoid and Steroidal glycosides. Drug Metabolism and Drug Interaction. 2000; 17(1-4):211-235.
37. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem and tuber parts of *Solena amplexicaulis* (lam.) *Gandhi* using GC-MS. International scholarly Research Notices. 2014, 1-13.
38. Oloyede GK. Anti-oxidant activities of methyl ricinoleate and ricinoleic acid dominated *Ricinus communis* seeds extract using lipid peroxidation and free radical scavenging methods. Research Journal of Medicinal Plants. 2012, 6:511-520.
39. Poh ZX, Goh KP. A current update on the use of alpha lipoic acid in the management of type 2 diabetes mellitus. Endcrine, Metabolism & Immune Disorder-Drug Targets. 2009; 9(4):392-398.
40. Hector F, Escobar-Morreale, Roldan B, Barrio R, Alonso, Sancho J, De la calle H *et al.* High prevalence of polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism. 2000; 85(11):4182-4187.
41. Dineshkumar G, Rajakumar R. Evaluation of bioactive molecules from the methanol leaf extract of *Azadirachta indica* (A. Juss). Asian Journal of Pharmaceutical Science and Technology. 2015; 5(2):64-69.
42. Lalitharani S, Mohan VR, Regini GS, Kalidas C. GC-MS analysis of ethanol extract of *Pothos scandens* (L.) leaf. Journal of Herbal Medicine and Toxicology. 2009; 3:159-160.
43. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanol extract of *Evolvulus alsinoides* (L.). Journal of Food Science and Technology. 2015; 52:1212-1217.
44. Syeda FA, Habib-ur-Rehman, Choudahry MI, Atta-ur-Rahman. GC-MS analysis of petroleum ether extract (oil) and bio-assay of crude extract of *Iris germanica.* International Journal of Genetics and Molecular Biology. 2011; 3(7):95-100.
45. Hema R, Kumaravel S, Alagusundaram K. GC-MS determination of bioactive components of *Murraya koenigii.* The Journal of American Science. 2011; 7(1):80-83.
46. Surender S, Vinod N, Sweety J, Gupta YK. Evaluation of anti-inflammatory activity of plant lipids containing α-linolenic acid. Indian Journal of Experimental Biology. 2008; 46(6):453-456.
47. Ezekwe SA, Chikezie PC. 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 metabolism. 2017; 8:740.