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

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Abstract
The aim of this study was to evaluate the \textit{in vitro} inhibitory activity of \textit{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 phychochemical 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 \textit{Vernonia amygdalina} root (18.17\textmu g/ml), the n-hexane fraction (71.41 \textmu g/ml), the dichloromethane fraction (95.06 \textmu g/ml) and the aqueous fraction (165.51 \textmu g/ml) exhibited 50\% alpha amylase inhibition activity at the mentioned concentrations. The alpha glucosidase \textit{IC}_{50} for \textit{Vernonia amygdalina} root methanol crude extract, n-hexane fraction, dichloromethane fraction and aqueous fraction was found to be 12.17\textmu g/ml, 13.59\textmu g/ml, 59.83 \textmu g/ml and 12.93\textmu g/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 \textit{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 \cite{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 \cite{2}. These complications involve all the important organs of the body and are due to the abnormal metabolism in diabetes mellitus \cite{3}. The management of diabetes has been a major problem in tropical Africa including Nigeria \cite{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 \cite{6} via the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase \cite{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 \cite{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 \cite{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, Ughowo, behind University of Benin, Benin City, Nigeria. Identification and authentication of the plant, *Vernonia amygdalina* (family: Asteraceae, voucher number; UBH342) 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.* 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\textsubscript{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 alpha 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 50M 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\textsubscript{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. amygdalina 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°C and final temperature was 240°C, with split less injectors and pressure of 8.267psi. The flow rate within the column was 2ml/minute. The detector temperature was 250°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 (71.41µg/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 IC₅₀ values of 18.17µg/ml, 71.41µg/ml, 95.06µg/ml and 165.51µg/ml.

The results of the alpha glucosidase inhibitory activities while aqueous fraction exhibited the least inhibitory activity with peak area % are shown in Table 4. The major compounds identified were 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester with peak area 10.97%, Hexadecanoic acid, methyl ester with peak area 10.37% and Octadecenoic acid (Z), methyl ester with 12.34% peak area, 9-Octadecenoic 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.

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 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 bark extract (L.) and these extracts exhibit alpha amylase and alpha glucosidase inhibitory activities [13]. 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 cyanidin-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].

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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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% Inhibition by Meth. Crude extract</th>
<th>IC₅₀ (µg/ml) Meth. extract</th>
<th>% Inhibition by n-Hex. fraction</th>
<th>IC₅₀ (µg/ml) n-Hex. fraction</th>
<th>% Inhibition by Dichl. fraction</th>
<th>IC₅₀ (µg/ml) Dichl. fraction</th>
<th>% Inhibition By Aque. fraction</th>
<th>IC₅₀ (µg/ml) Aque. fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>55.04 ± 2.34a</td>
<td>18.17</td>
<td>12.57 ± 25.68a</td>
<td>71.41</td>
<td>95.06</td>
<td>21.33 ± 15.92a</td>
<td>165.51</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>57.78 ± 2.88a</td>
<td>17.95 ± 1.73ab</td>
<td>38.98 ± 11.46a</td>
<td>95.06</td>
<td>21.33 ± 15.92a</td>
<td>165.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>67.24 ± 2.38bd</td>
<td>33.85 ± 12.23ab</td>
<td>32.73 ± 1.58a</td>
<td>95.06</td>
<td>21.33 ± 15.92a</td>
<td>165.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>60.88 ± 1.42ab</td>
<td>62.15 ± 1.37bc</td>
<td>37.55 ± 0.30a</td>
<td>95.06</td>
<td>21.33 ± 15.92a</td>
<td>165.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>74.41 ± 0.09cd</td>
<td>80.91 ± 0.17c</td>
<td>54.08 ± 25.65a</td>
<td>95.06</td>
<td>21.33 ± 15.92a</td>
<td>165.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>% Inhibition by Meth. crude extract</th>
<th>IC₅₀ (µg/ml) Meth. extract</th>
<th>% Inhibition by n-Hex. fraction</th>
<th>IC₅₀ (µg/ml) n-Hex. fraction</th>
<th>% Inhibition by Dichl. fraction</th>
<th>IC₅₀ (µg/ml) Dichl. fraction</th>
<th>% Inhibition By Aque. fraction</th>
<th>IC₅₀ (µg/ml) Aque. fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>82.16 ± 2.16a</td>
<td>73.60 ± 0.88a</td>
<td>21.77 ± 1.77a</td>
<td>59.83</td>
<td>77.33 ± 0.86ad</td>
<td>83.33 ± 2.94ab</td>
<td>91.18 ± 0.98c</td>
<td>12.93</td>
</tr>
<tr>
<td>40</td>
<td>87.68 ± 1.41a</td>
<td>78.43 ± 0.00ab</td>
<td>29.41 ± 5.88a</td>
<td>59.83</td>
<td>77.33 ± 0.86ad</td>
<td>83.33 ± 2.94ab</td>
<td>91.18 ± 0.98c</td>
<td>12.93</td>
</tr>
<tr>
<td>60</td>
<td>88.24 ± 3.93a</td>
<td>83.98 ± 0.34b</td>
<td>50.18 ± 4.73b</td>
<td>59.83</td>
<td>77.33 ± 0.86ad</td>
<td>83.33 ± 2.94ab</td>
<td>91.18 ± 0.98c</td>
<td>12.93</td>
</tr>
<tr>
<td>80</td>
<td>82.35 ± 1.96a</td>
<td>73.77 ± 4.67a</td>
<td>59.27 ± 4.37b</td>
<td>59.83</td>
<td>77.33 ± 0.86ad</td>
<td>83.33 ± 2.94ab</td>
<td>91.18 ± 0.98c</td>
<td>12.93</td>
</tr>
<tr>
<td>100</td>
<td>77.19 ± 4.64a</td>
<td>85.29 ± 0.98b</td>
<td>58.79 ± 7.88b</td>
<td>59.83</td>
<td>77.33 ± 0.86ad</td>
<td>83.33 ± 2.94ab</td>
<td>91.18 ± 0.98c</td>
<td>12.93</td>
</tr>
</tbody>
</table>

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

\[2128\]
Table 3: Results of phytochemical screening of the methanol crude extract of *Vernonia amygdalina* root.

<table>
<thead>
<tr>
<th>Phytochemical type</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>–</td>
</tr>
<tr>
<td>Tannins (condensed)</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysable tannins</td>
<td>–</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
</tr>
<tr>
<td>Polysaccharides (starch)</td>
<td>–</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) = present and (−) = not present

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

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Molecular Formula</th>
<th>Name of Compound</th>
<th>Molecular Weight</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C6H12O</td>
<td>Oxirane, butyl</td>
<td>100</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>C13H26O2</td>
<td>Dodecanoic acid, methyl ester 214</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C17H34O2</td>
<td>Hexadecanoic acid, methyl ester 270</td>
<td>10.37</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C5H5N3O4</td>
<td>1-Methyl-5-nitro-4-pyrazolecarboxylic acid</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C16H32O2</td>
<td>n-Hexadecanoic acid</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C19H34O2</td>
<td>9,12-Octadecadienoic acid (Z,Z)-, methyl ester</td>
<td>294</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C19H36O2</td>
<td>9-Octadecenoic acid (Z)-, methyl ester</td>
<td>296</td>
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</tr>
<tr>
<td>8</td>
<td>C19H38O2</td>
<td>Methyl stearate</td>
<td>298</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>C18H32O2</td>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>C19H32O2</td>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>280</td>
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<tr>
<td>11</td>
<td>C18H32O</td>
<td>9,17-Octadecadienal, (Z)-</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>C20H40O4</td>
<td>Hexadecanoic acid,1,1-dimethyl ethyl ester</td>
<td>344</td>
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</tr>
<tr>
<td>13</td>
<td>C21H40O2</td>
<td>n-Propyl 9-octadecenoate</td>
<td>324</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>C18H30O2</td>
<td>9,12,15-Octadecatrienoic acid, (Z,Z,Z)-</td>
<td>278</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>C24H38O4</td>
<td>Disoctyl phthalate</td>
<td>390</td>
<td></td>
</tr>
</tbody>
</table>

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 IC50 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-hyperglycaemic 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.

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