Antioxidants and hypoglycemic effect of some medicinal plants

Abubakar Asmau Niwoye *, Saidu Abubakar Ndaman, Akanya Helmina Olufunmilayo and Egwim Evans Chidi

Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria.

Publication history: Received on 07 June 2019; revised on 23 July 2019; accepted on 26 July 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.8.2.0124

Abstract
There is an increasing trend to replace synthetic drugs, which are of safety concern, with the natural remedies available from plant extracts. This study investigated the phytoconstituents as well as antioxidants and hypoglycemic effect of Anacardium occidentales, Hunteria umbellata, Parkia biglobosa, Psidium guajava and Vitellaria paradoxa. Phytochemical compositions were assayed using standard procedures. Antioxidant assays employed were ferric reducing antioxidant power and scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl radicals. Oral glucose tolerance test (OGTT) and amylase inhibitory assays were employed for hypoglycemic study. The concentration of phenols ranges from 184.368±0.23 mg/g (Anacardium occidentales) to 120.241±0.01 mg/g (Hunteria umbellata). However, Hunteria umbellata extract had the highest alkaloids (76.76±0.01 mg/g), Parkia biglobosa extract had the highest tanins (137.55±0.05 mg/g) while Vitellaria paradoxa extract had higher saponins (188.50±0.01 mg/g). Flavonoids was higher in Parkia biglobosa (458.06±0.06 mg/g) while Psidium guajava had the least. In DPPH assay A. occidentales extract had the lowest IC50 (217.78±3.45 µg/ml) comparable with the standards while H. umbellata had the highest (IC50=311.72±3.45 µg/ml) and thus lowest activity. Anarcadium occidentales had higher α-amylase inhibitory activity (IC50 =171.13±0.14µg/ml) while Psidium guajava (IC50 =304.64 ±0.14µg/ml) had the least activity. Oral glucose tolerance revealed the activities of the extracts in order of Anarcadium occidentales>Hunteria umbellata>Vitellaria paradoxa>Parkia biglobosa>Psidium guajava. In conclusion, five medicinal plants investigated in this study possesses some antioxidants and hypoglycemic properties with Anarcadium occidentales extract being the most potent, which may be attributed to their polyphenolic constituents.

Keywords: Phytochemicals; In vitro; Antioxidants; Hypoglycemic; Medicinal plants

1. Introduction
Diabetes mellitus (DM) is a group of metabolic disorder characterized by the alteration in metabolism of the major macromolecules (fat, proteins and carbohydrate) in response to loss of insulin sensitivity or insulin deficiency [1]. According to the recent update by World Health Organization, a total of about 150 million people are diabetic globally, and this is likely to scale up to 300 million by the end of 2022. Also, about 9% of adult’s population in 2014 were diabetic, of which 1.6 million cases were associated with deaths in 2016 [2]. The dilemma of diabetes complications also takes a great burden on global expenditure. The current drug therapies including biguanides, α-glucosidase inhibitors, sulfonylureas and glinides are synthetic and are besieged with limitations in terms of cost, safety and efficacy [3].

Oxidative stress is very important because it unites the pathogenesis of all diabetic complications and indeed plays a role in all other aspects of diabetes pathology [4].According to the study carried out by Dokken et al., [5],a reactive oxygen species such as hydrogen peroxide, can cause a marked decrease in the insulin stimulation of the insulin signaling proteins like insulin receptor substrate (IRS) and protein kinase B (PKB) as well as glucose transport activity
thereby leading to insulin resistance. Another study also suggested that stress-activated serine kinases are involved in the pathogenesis of oxidant-induced insulin resistance and that excess mitochondrial H$_2$O$_2$ productions play a pivotal role in causing insulin resistance in the skeletal muscle of individuals with excess energy [6].

Since time immemorial, African medicinal plants have been used in folk medicine for the treatment of different diseases, which has been scientifically validated by different in vitro and in vivo studies [7-8]. Medicinal plants are known sources that have rich, yet unexploited potentials for anti-diabetic drugs. Thus, many of the synthetic drugs were discovered directly or indirectly from plant sources [9]. As a result, some natural products like herbs have been approved as new antioxidants and hypoglycemic agents, Although, there is need to identify novel substances that are safe, inexpensive and active towards free radicals and diabetes since most of the conventional drugs have undesirable side effect, expensive and loss of efficacy [10]. The present study therefore was aimed at evaluating the phytochemical constituents, antioxidants and hypoglycemic effect of Anacardium occidentales, Hunteria umbellata, Parkia biglobosa, Psidium guajava and Vitellaria paradoxa.

2. Material and methods

2.1. Sample collection

Fresh leaves of Anacardium occidentales, Hunteria umbellata, Parkia biglobosa, Psidium guajava and Vitellaria paradoxa were collected between March and December, 2017 from Minna, Niger State, Nigeria. Authentication of the plants was done at the Herbarium Department of National Institute of Pharmaceutical Research and Development (NIPRD), Abuja Nigeria with the voucher numbers Anarcadium occidentaess (NIPRD/H/6870/) Parkia biglobosa (NIPRD/H/6868/) Vitellaria paradoxa (NIPRD/H/6867/) Psidium guaja (NIPRD/H/6869/) Huntaria umbellata(NIPRD/H/6871/).

2.2. Experimental animal

Healthy albino rats of average weight (134.87±3.23) grams were obtained from animal holding unit, Federal University of Technology, Minna, Niger State Nigeria. The rats were maintained under standard laboratory condition. They were allowed access to rat pellets and water ad-libitum.

2.3. Chemicals and reagent

Alpha-amylase from Aspergillus oryzae was a product of Sigma-Adrich Co., St Louis, USA, while methanol was a product of Merck, Germany. Other chemicals and reagents were of analytical.

2.4. Sample preparation and extraction

The plants were thoroughly washed under running tap water and dried for 2 weeks at room temperature 37°C±1 and finally grinded by a milling machine. An aliquot 50g of each of the plant material was extracted separately in 200ml of methanol using Soxhlet apparatus and the resulting extract was concentrated using a rotary evaporator. The extracts were weighed and the percentage yield were calculated by using the formula below

\[
\% \text{ Yield} = \frac{\text{Weight of the Crude Extract (g)}}{\text{Weight of Dried powdered sample (g) \times 100}}
\]

Tsado et al.,[11]

2.5. Phytochemical analysis

Qualitative and quantitative phytochemical analysis of crude methanol extract of the plants were carried out by standard methods [12-16].

2.6. Antioxidant study

Radical Scavenging ability of the crude extracts at varying concentrations was assayed by DPPH and FRAP methods described by Blois, [17] and Oyaizu et al., [18] respectively. Ascorbic acid and Gallic acid were used as the reference drug.
2.7. Alpha amylase inhibition assay

Alpha-amylase inhibitory activities of the extract was determined at varying concentrations of the extract (62.5-500 µg/mL) using potato starch solution substrate as described by Nickavar and Yousefian [19]. The α-amylase inhibitory activity of the extract was calculated using the formula below [20].

\[
\text{The } \alpha - \text{amylase inhibitory activity} = \left( \frac{(Ac+)-(Ac-)}{(Ac+)-(Ac-)} \right) \times 100
\]

Where, Ac+ = Absorbance of 100 % enzyme activity (only solvent with enzyme).

Ac- = Absorbance at Zero % (0 %) enzyme activity (only solvent without enzyme).

As= Absorbance of test sample (with enzyme).

Ab= Absorbance of blank (a test sample without enzyme).

2.8. Oral glucose tolerance test (OGTT)

Animals were fasted overnight for 12 hours and divided into groups of four rats each. The control group received 0.5 ml normal saline. The crude extracts groups was given 200 mg kg/bw while the standard drug group received glibenclamide (2.5 mg/kg). All drugs were diluted with normal saline (0.9%) Nacl and administered orally by a garage. Glucose levels were measured before the rats received the treatment (zero time) and 30 min after glucose was administrated (2g/kg). Blood samples were collected from the tail vein at 30, 60, 90 and 120 minutes after glucose loading, and the glucose level (mg/dl) was assayed by a glucometer (Accu-Check®Performa).

2.9. Statistical analysis

Data were analyzed using Statistical analysis system (SAS) and presented as means ± SEM. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). The level of significance was set at \( P < 0.05 \) [21].

3. Results

3.1. Extracts yield

The yield of the crude methanol extract of *Anacardium occidentales*, *Hunteria umbellata* *Parkia biglobosa*, *Psidium guajava* and *Vitellaria paradoxa* leaves are presented in Table 1. *Hunteria umbellata* leaf extract had the highest yield (31.50%) while *Parkia biglobosa* leaf extract had the lowest yield (0.69%).

<table>
<thead>
<tr>
<th>Plant samples</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anacardium occidentales</em></td>
<td>25.33</td>
</tr>
<tr>
<td><em>Hunteria umbellata</em></td>
<td>31.50</td>
</tr>
<tr>
<td><em>Parkia biglobosa</em></td>
<td>20.69</td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>26.60</td>
</tr>
<tr>
<td><em>Vitellaria paradoxa</em></td>
<td>27.77</td>
</tr>
</tbody>
</table>

3.2. Phytochemical composition of the selected plants

The qualitative phytochemical constituents of the methanol leaf extracts of the selected plants are shown in Table 2. Anthraquinones, flavonoids, tannins, steroids, alkaloids, glycosides, phenols, and saponins were detected. All the plants also had reducing sugars except in the leaf extract of *Hunteria umbellata*. Phlobatannins was not detected in all the extracts. The concentration of phenols ranges from 184.368±0.23 mg/g to 120.241±0.01 mg/g in *Anacardium occidentales* extract and *Hunteria umbellata* extract respectively (Table 3). Among the selected plants, *Hunteria umbellata* extract had the highest concentration of Alkaloids (76.76±0.01 mg/g) *Parkia biglobosa* extract had the highest
tanins content (137.55±0.05 mg/g) and *Vitellaria paradoxa* extract contains more saponins (188.50±0.01 mg/g) as compared to other plant extracts. Flavonoids concentration was higher in *Parkia biglobosa* extract (458.06±0.06 mg/g) while *Psidium guajava* extracts had the lowest flavonoids content (84.28±0.02 mg/g).

**Table 2** Qualitative phytochemical constituents of crude methanol leaf extract of some medicinal plant

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Anacardium occidentales</th>
<th>Hunteria umbellata</th>
<th>Parkia biglobosa</th>
<th>Psidium guajava</th>
<th>Vitellaria paradoxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: - = Not Detected  + = Detected

**Table 3** Quantitative phytochemical constituents of crude methanol leaf extract some medicinal plant

<table>
<thead>
<tr>
<th>Plants</th>
<th>Phenols</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavonoids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anacardium occidentales</em></td>
<td>184.36±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.50±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>110.20±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.00±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.50±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Hunteria umbellata</em></td>
<td>120.24±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.76±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>101.53±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.32±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.59±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Parkia biglobosa</em></td>
<td>151.20±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.65±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>137.55±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.24±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>458.06±0.06&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>167.33±0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>52.34±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114.28±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.55±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.28±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Vitellaria paradoxa</em></td>
<td>156.31±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.59±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>188.50±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125.84±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 determinations. Values with different alphabets along a column are significantly different at p < 0.05

### 3.3. Alpha amylase inhibition

The α- amylase inhibition of the extracts and their corresponding half maximal inhibitory concentration (IC<sub>50</sub>) values are presented in Figures 1 and 2. The ability to inhibit α- amylase activity by the standard drug (acarbose) and the extracts increased with concentrations (62.5–500 μg/mL). At concentrations 62.5, 125, and 250 μg/mL, acarbose had a significant (p<0.05) higher activity than all the extracts of the same concentration. But at 500 μg/mL, the α-amylase inhibitory activity of *Parkia biglobosa* extract was the same with acarbose. Amongst the extracts, the α-amylase inhibitory activity of *Parkia biglobosa*, *Hunteria umbellata* and *Anacardium occidentales* extracts were significantly (p<0.05) higher at various concentrations when compared to that *Psidium guajava* and *Vitellaria paradoxa* extracts.

Acarbose had a significantly (p<0.05) higher activity and thus lower IC<sub>50</sub> of (76.34±0.12 μg/mL) when compared to all the extracts. *Parkia biglobosa* (178.64±0.98 μg/mL), *Hunteria umbellata* (199.13±0.18 μg/mL) and *Anacardium occidentales* (171.13±0.14 μg/mL) extracts had comparable IC<sub>50</sub> values but significantly lower and thus better activity than IC<sub>50</sub> value of *Psidium guajava* (304.64±0.14 μg/mL) and *Vitellaria paradoxa* (224.95±0.14 μg/mL) extracts.
Figure 1 Effect of the some medicinal plant extracts on α-amylase inhibition (AOC- Anacardium occidentales; HU- Huntaria umbellate; PB- Parkia biglobosa, PG- Psidium guajava and VP: Vitellaria paradoxa).

Figure 2 IC_{50} of the plant Extracts on α-amylase inhibition (AOC- Anacardium occidentales; HU- Huntaria umbellate; PB- Parkia biglobosa, PG- Psidium guajava and VP: Vitellaria paradoxa).

3.4. Antioxidant / DPPH radical scavenging activity of the crude methanol leaf extract of the selected plants.

Table 4 DPPH Radical scavenging activity of the crude methanol extracts of the selected plants

<table>
<thead>
<tr>
<th>Conc (µg/mL)</th>
<th>Gallic acid</th>
<th>Ascorbic acid</th>
<th>Anacardium occidentals</th>
<th>Hunteria umbellata</th>
<th>Parkia biglobosa</th>
<th>Psidium Guajava</th>
<th>Vitellaria paradoxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>19.45±0.45^a</td>
<td>17.34±0.34^a</td>
<td>20.45±0.54^a</td>
<td>6.97±0.06^a</td>
<td>16.67±0.46^a</td>
<td>19.23±0.4^a</td>
<td>14.56±0.05^a</td>
</tr>
<tr>
<td>100</td>
<td>32.76±0.36^b</td>
<td>30.34±0.32^b</td>
<td>35.56±0.21^ab</td>
<td>19.24±0.31^b</td>
<td>29.34±0.52^b</td>
<td>34.56±0.3^b</td>
<td>19.45±0.14^a</td>
</tr>
<tr>
<td>200</td>
<td>57.34±0.67^c</td>
<td>61.23±0.98^c</td>
<td>59.43±0.53^b</td>
<td>40.56±0.32^c</td>
<td>54.56±0.67^c</td>
<td>51.23±0.7^c</td>
<td>48.56±0.14^b</td>
</tr>
<tr>
<td>400</td>
<td>75.89±0.29^d</td>
<td>75.66±1.04^d</td>
<td>69.45±0.21^b</td>
<td>52.34±0.45^c</td>
<td>77.34±0.38^d</td>
<td>74.56±0.3^d</td>
<td>69.34±0.21^bc</td>
</tr>
<tr>
<td>500</td>
<td>87.46±0.56^e</td>
<td>88.56±0.45^d</td>
<td>86.75±0.36^c</td>
<td>84.56±0.54^d</td>
<td>85.14±0.62^d</td>
<td>85.67±1.4^d</td>
<td>83.66±2.45^c</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different (p < 0.05).
All the extracts and the standard antioxidants (Gallic acid and Ascorbic acid) at varying concentrations (50-500 μg/ml) demonstrated DPPH radical scavenging activities. They inhibited DPPH radical with increasing concentrations. *A. occidentales* extracts at concentrations 50, 100, and 200 μg/ml exhibited more potent DPPH radical scavenging activity than other extracts but at 500 μg/ml, all the extracts and standard antioxidants had comparable activities (Table 4). The concentration of the extracts that produce 50% inhibition (IC₅₀) of DPPH radicals of *A. occidentales* (217.78±3.45 μg/ml) extracts are lower than the values of ascorbic acid and gallic acid (218.01±0.57 μg/ml and 219.66±0.97 μg/ml) respectively but not significantly (p<0.05) different. However, *H. umbellate* extract had a significantly higher (P<0.05) IC₅₀ values (311.72±3.45 μg/ml) and consequently lower activity as compared to other extracts tested (Figure 3).

**Figure 3** Inhibitory Concentration (IC₅₀) of methanol extract of the selected plants on DPPH radical scavenging activity

### 3.5. Ferric reducing power activity (FRAP) of the crude methanol extracts

The ferric reducing power activity of gallic acid and ascorbic acid increases steadily with increasing concentrations, however a dose independent activity was observed for all the extracts. *Anacardium occidentales* extract (1.73±0.02) and *Parkia biglobosa* extract (1.58±0.01) at 1000 μg/ml had significantly (p< 0.05) higher % inhibition than that of standards gallic acid (1.23±0.01), ascorbic acid (1.27±0.01) and the remaining extracts.

**Figure 4** Ferric reducing power activity of the crude methanol extracts of the selected plants (AOC- *Anacardium occidentales*; HU- *Huntaria umbellata*; PB- *Parkia biglobosa*, PG- *Psidium guajava* and VP: *Vitellaria paradoxa*).
3.6. Oral glucose tolerance

The plasma glucose concentration of the treated and normal rats reached hyperglycemic peak at 30 minutes after the oral administration of glucose and gradually decreased to the pre-prandial level. *Anacardium occidentales* extract produced plasma glucose levels significantly (*p < 0.05*) lower than the group administered glibenclamide at 60, 90 and 120 minutes after the glucose administration. The percentage glucose reduction of the extracts was in this order *Anacardium occidentales* > *Hunteria umbellata* > *Vitellaria paradoxa* > *Parkia biglobosa* > *Psidium guajava*. Plasma glucose concentration of the normoglycemic rats showed the lowest as well as a time dependent decrease when compared with the groups administered extracts and glibenclamide. The rats not treated (negative control) had the highest plasma glucose level when compared to normal and extract treated groups (Table 5).

Table 5 Effect of methanol leaf extracts of the selected plants on glucose tolerance in rats

<table>
<thead>
<tr>
<th>Extracts</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>% glucose reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOC</td>
<td>100.67±0.88b</td>
<td>189.00±6.81d</td>
<td>108.00±2.89a</td>
<td>106.00±1.73a</td>
<td>99.00±3.46b</td>
<td>47.61%</td>
</tr>
<tr>
<td>HU</td>
<td>89.67±2.40a</td>
<td>178.33±7.13c</td>
<td>130.67±4.33b</td>
<td>105.00±4.04a</td>
<td>100.67±2.60a</td>
<td>43.54%</td>
</tr>
<tr>
<td>PB</td>
<td>101.67±2.02b</td>
<td>129.67±9.53ab</td>
<td>139.00±8.08b</td>
<td>102.00±1.16a</td>
<td>96.00±2.31a</td>
<td>25.58%</td>
</tr>
<tr>
<td>PG</td>
<td>102.67±0.88a</td>
<td>163.00±9.23bc</td>
<td>127.00±0.58bc</td>
<td>107.67±1.45a</td>
<td>104.67±3.17ab</td>
<td>19.29%</td>
</tr>
<tr>
<td>VP</td>
<td>97.00±5.77ab</td>
<td>165.00±5.20bc</td>
<td>134.00±4.04b</td>
<td>107.00±2.89a</td>
<td>107.67±2.60a</td>
<td>34.74%</td>
</tr>
<tr>
<td>NC</td>
<td>105.50±5.50b</td>
<td>157.00±12.00b</td>
<td>174.50±2.50c</td>
<td>167.86±2.00b</td>
<td>165.00±3.00c</td>
<td>5.09%</td>
</tr>
<tr>
<td>STD</td>
<td>92.67±0.89ab</td>
<td>169.00±3.46bc</td>
<td>139.00±6.35b</td>
<td>117.00±1.15b</td>
<td>104.67±3.76ab</td>
<td>38.06%</td>
</tr>
<tr>
<td>Normal</td>
<td>98.00±1.53ab</td>
<td>95.67±2.96a</td>
<td>96.33±3.17a</td>
<td>95.67±2.03a</td>
<td>94.33±2.03a</td>
<td>1.40%</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM of triplicate determinations. Values with different alphabets along a column are significantly different at *p < 0.05*. AOC- *Anacardium occidentales*; HU- *Hunteria umbellata*; PB- *Parkia biglobosa*; PG- *Psidium guajava*; VP- *Vitellaria paradoxa*; NC- Negative Control and STD- Glibenclamide.

4. Discussion

The medicinal importance of plants is due to the presence of some chemical substances in them. The presence of flavonoids in these plant extracts may have contributed to their hypoglycemic effect, many flavonoids due to their phenolic structure are antioxidants, and are known to be involved in the healing process of free radical-mediated diseases including diabetes [22-23]. The result obtained in the phytochemical screening of *P. biglobosa* seed as presented in Table 3, seems to justify the use of its seed for cardiac diseases as high number of flavonoids was found in them. This result is in agreement with the reports of Enujiugha, [24] that *P. biglobosa* plant are recognized to be very rich in phenolic compounds. However, the present study disagrees with the work of Ajaiyeoba [25] which indicated the absence of saponins and anthraquinones in the leaf extract of *P. biglobosa*. The differences in the phytochemical components of medicinal plant may account for the part of the plant used and solvent used in the extraction process.

Phenolic compounds have received considerable attention as protective factors against cancer and heart diseases because of their antioxidant potentials [26]. The higher concentration of phenols in *Anacardium occidentales* (184.368±0.23 mg/g) may be the rationale behind the use of *Anacardium occidentales* extract as antioxidants and anticancer agent as reported in different literatures. This is further buttressed by ability to protect the oxidation of low-density lipoprotein cholesterol, a major step in developing atherosclerosis and enhancement of antioxidant [27].

The presence of alkaloids in the crude methanol extracts of the plant under study probably gives credence to their hypoglycemic activity. *Hunteria umbellata*, *parkia biglobosa* and *anarcadium occidentales* extracts contained more alkaloids than *vitellaria paradoxa* and *psidium guajava* extracts. This finding correlate with the reports of [28] that *Hunteria umbellata* seed extracts had appreciable amount of alkaloids. Several other studies have also reported the presence of alkaloids in the stem bark and leaves of *Parkia biglobosa* and *Anacardium occidentales* extracts [29]. It has
been shown that medicinal plants with hypoglycemic and antidiabetic effect usually contain high concentration of alkaloids and flavonoids [30].

This present study has provided an empirical basis for the use of these plants in traditional medicinal practices. It is well documented in the literature that medicinal plants with hypoglycemic and antidiabetic effects usually contain high concentrations of alkaloids, flavonoids steroid glycosides [30-32]. Therefore, various composition of these important phytoconstients in these plants could justify their hypoglycemic action.

Alpha amylases are the carbohydrate metabolizing enzymes. The inhibition of this pancreatic alpha-amyrase is an important therapeutic targets for delaying oligosaccharide digestion to absorbable monosaccharides in the intestinal brush border, resulting in reduced postprandial hyperglycemia [33]. The significant inhibition of this enzyme by the five plant extracts evaluated in this study will therefore cause an increase in carbohydrate digestion time and reduces the glucose absorption [34]. By indication, this finding show that the plant extracts will be very useful in the control of glucose level by diabetic patient and also serve as alternative to chemical hypoglycemic agent such as acarbose and viglibose which are associated with various side effects like as diarrhoea, bloating dizziness and vomiting [35].

Psidium guajava efficiently inhibits alpha amyrase enzyme in vitro in a dose dependent manner. This is in line with the finding of Manikannd et al. [36] who reported a dose-dependent increase in percentage inhibitory activity of alpha amyrase enzyme by methanol seed of Psidium guajava, also Karthic et al. [37] reported that the aqueous extracts from Syzygium cumini seeds and Psidium guajava leaves also showed a dose dependent inhibitory effect on alpha-amylase activity. However, the higher IC50 of Psidium guajava when compared with standard drug (acarbose) and the remaining plants investigated in this study indicate its low alpha amyrase inhibition potency as compared to other plants. However, this finding disagrees with earlier findings by Balasubramanian et al., [38], who reported that the ethyl acetate extract of Psidium guajava seed showed better alpha amyrase inhibition than acarbose at higher concentration. Ramasamy et al., [39] also reported that the ethanolic extracts of P. guajava leaf extract have a high inhibitory activity on alpha amyrase and alpha glucosidase enzymes than the aqueous extract of the same plant. The difference in activities reported could be attributed to the differences in the extracting solvents, as it has been affirmed that the phytochemical and bioactive agent in medicinal plant vary with the polarity of the solvent used in the extraction process.

As shown in Table 5, the significant (p<0.05) time related attenuation of the elevated post-absorptive blood glucose concentrations in the glibenclamide- and plant (Anacardium occidentales, Hunteria umbellata, Vitellaria paradoxa, Parkia biglobosa, Psidium guajava)-pretreated rats is suggestive that glibenclamide and plant extracts could be mediating their hypoglycaemic action via inhibition of intestinal glucose uptake, a mechanism which is similar to those of α-glucosidase inhibitors (e.g. acarbose). The 60th-120th minutes postprandial hypoglycaemia recorded in the distilled water-pretreated, glucose loaded rats could be due to the secondary effect of hyper insulinaemia following the glucose load. This is a well-established physiological response to hyperglycaemia following a high glucose load or intake. The 25.58% decrease in blood glucose level of glucose-loaded rats following 2hrs (120 minutes) pre-treatment with P. biglobosa extract correspond adequately with the previous study by Fred-Jaiyesimi and Abo [40] who reported that P. biglobosa seed methanol extract administered to the glucose-loaded diabetic rats exhibited a decrease in the blood glucose levels of the animals throughout the period of the study, with a peak decrease in blood glucose level of 64% at 5 hr. The higher activity demonstrated by the Anacardium occidentales as compared to the remaining plant could be justified by its significant (p<0.05) higher contents of phenol (184.368±0.23 mg/g) and saponins (85.00±0.01 mg/g). In the same trend the lower activities of Psidium guajava also correspond with it significant (p<0.05) lower saponin contents (2.55±0.00 mg/g). Literatures has shown that extracts with high triterpenoid saponins and phenol content mediate their hypoglycaemic effect via inhibition of intestinal glucose uptake, increased hepatic glucose deposition and enhanced hyper insulinaemia [27]. It is therefore reasonable to assume that the saponins contained in these plant extracts is responsible for the observed attenuation in the post absorptive glucose concentration.

There is increasing interest in deleterious role of free radicals and preventive/ protective properties of natural products against oxidation processes [26]. In the present study, methanol extract of the plant investigated, particularly, the A. occidentales promoted an inhibition of DPPH radical and the transformation of FeIII to FeII in a similar trend to that of the standard drugs. The Lower IC50 recorded for A. occidentalis is an indication of it higher antioxidants activities compared with other plants, thus this plant could be considered as a natural source of antioxidants. Thetotal phenolic and total flavonoid contents of the extracts recorded in this study could be responsible for the observed free radical scavenging activity of the plant extract. The IC50 reported for the five medicinal plants in this study were however, higher than 52.45±0.05 μg/mL reported for X. aethiopica [41], but lower than 263.53 mg/mL reported for Phyllanthus [42], 263.53±3.24 mg/mL reported for Senna ocidentalis [43], C. adansonii (1562.52 mg/mL) and N. laevis (155.17 mg/mL [44]. This differences could be explained by the findings from previous studies, who reported that bioactive metabolites...
in medicinal plants varies with the type of plants, part of the plants, duration of sample collections and also the differences in the solvents uses in extraction process [45]. This is also supported by the absence of phlobatannins in the plants investigated in this study.

5. Conclusion
The medicinal plants investigated in this study had antioxidants and hypoglycemic properties with Anacardium occidentales being the most potent, this could be attributed to their polyphenolic constituents.

Compliance with ethical standards
Acknowledgments
The authors wish to thank Tertiary Education Trust Fund (Tetfund) Nigeria for funding this research, Centre for Genetic engineering and Biotechnology and Department of Biochemistry Federal University of Technology Minna for providing the enabling environment.

Disclosure of conflict of interest
The authors declare that they have no conflict of interest.

Statement of ethical approval
The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

References


How to cite this article