Comparative Proximate Composition and Hypoglycemic Properties of Three Medicinal Plants (Verononia amygdalina, Azadirachta indica and Moringa oleifera).

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INTRODUCTION

Verononia amygdalina, Azadirachta indica and Moringa oleifera are edible and among the readily available medicinal plants grown in the South-Eastern Nigeria. V. amygdalina grows throughout tropical Africa and has been domesticated in some parts of West Africa, particularly, South Eastern Nigeria, where it is locally known as bitter leaf.¹ A. indica belongs to the family-Meliaceae and has a long history of use in folk medicine for treatment of various ailments.² The utilization of M. oleifera in the treatment of several ailments such as diarrhea, epilepsy, hysteria and as diuretics has been reported.³

ABSTRACT: The present study sought to investigate proximate composition in relation to the short and long term hypoglycemic effects of ethanol/water leaf extracts of Verononia amygdalina, Azadirachta indica and Moringa oleifera on hyperglycemic rats. Hyperglycemia was induced by a single intra-peritoneal injection of 0.1 mol/L alloxan monohydrate in phosphate buffer saline (PBS) solution (pH = 7.4) dosage = 140 mg/kg; b/w. Individual hyperglycemic rats received separate doses of either 400 mg/kg bw/12 h of V. amygdalina, A. indica or M. oleifera for 14 days. Blood samples were drawn from the rats at regular intervals of 12 h for 48h duration; for short-term study, and subsequently, on days 3, 7, 10 and 14; for long term study, and measured for fasting blood glucose (FBG). Determination of FBG was by the glucose oxidase method. V. amygdalina, A. indica and M. oleifera showed wide variation in crude protein content (CPC), which was within the range of 13.42–37.01%. Total carbohydrate content (TCC) of V. amygdalina and A. indica was the single highest component with values: TCC<sub>V. amygdalina</sub> = 40.65% and TCC<sub>A. indica</sub> = 50.14%. In contrast, the CPC content was higher than the TCC content for M. oleifera (TCC<sub>M. oleifera</sub> = 36.65%; CPC<sub>M. oleifera</sub> = 37.01%). The administration of V. amygdalina, A. indica and M. oleifera to the rats (0 h “ t “ 48 h) did not cause significant (p > 0.05) reduction in FBG of hyperglycemic rats compared to the control group. Conversely, the administration of the three leaf extracts caused reduction in FBG in a time dependent manner in the order: H<sub>M. oleifera</sub> = 400 mg/kg; bw = 2.73 folds >H<sub>V. amygdalina</sub> = 400 mg/kg; bw = 2.15 folds >H<sub>A. indica</sub> = 400 mg/kg; bw = 1.75 folds; p < 0.05. Comparative proximate composition of V. amygdalina, A. indica and M. oleifera and corresponding capacity of the three leaf extracts to reduce FBG appears to suggest that biomolecules responsible for promoting FBG lowering effects are for the most part the TCC and CPC elements.

KEYWORDS: Proximate composition, fasting blood glucose, Verononia amygdalina, Azadirachta indica and Moringa oleifera.
gестационный диабет меллиус (GDM).

The IDDM or juvenile-onset DM is more common amongst children/adolescence, arising from absolute absence or low circulating levels of insulin. Insulin insufficiency could be elicited by autoimmune/idiopathic disease, viral infection, pancreatic carcinoma and other complex interactions of both genetic and environmental factors. Type II/NIDDM is characterized by resistance or insensitivity of peripheral cells to insulin typified by hyperinsulinism. In addition to genetic factors, insulin resistance can be caused by acquired factors such as obesity, sedentary life style, pregnancy and hormone exchange. GDM often develops during pregnancy and may improve or disappear after delivery. The majority of the complications of DM are linked to oxidative stress, induced by hyperglycemia, which overweighs the antioxidant defense systems. In the later stage of the disease condition, lipid metabolism is compromised engendering hyperlipidemia. Hypercholesterolemia is particularly pronounced, which is a risk factor in artherosclerosis. Furthermore, distortions in metabolism of protein and amino acids are affected. Derangements in general metabolism engender several anatomic distortions, among which are weight loss, ketosis, polyuria, polydipsia, gangrene, cataract, neuropathy and renal dysfunction. However, distortions in metabolism are restored to normalcy after good glycemic control is obtained.

The global prevalence of DM is on the rise and was estimated to be over 171 million in 2000 with a projection of 366 million in 2030. Type II DM is rapidly emerging as a major public health challenge in Nigeria. In 2003, the International Diabetes Federation (IDF) reported a prevalence of 3.4% for 24 million Nigerians between the ages of 20 and 79 years with a projection of 3.9% by 2025.

Available drugs and insulin injection used for the management of hyperglycemia associated with the diabetic state come with undesirable side effects. The undesirable side effects and high cost of anti-diabetic drugs have led to the search for plants with hypoglycemic properties for the management of DM. Several plant species of medicinal values have been previously investigated for hypoglycemic properties. Therefore, the present study sought to investigate the short and long term hypoglycemic effects of ethanolic extracts of V. amygdalina, A. indica and M. oleifera on alloxan induced hyperglycemic rats.

MATERIALS AND METHODS

Collection of plant samples and preparation of extract

Fresh leaves of V. amygdalina, A. indica and M. oleifera were harvested in October, 2012 from the native community of Uke, Idemimiri-North Local Government Area, Anambra State, Nigeria. The plant species were authenticated by Mr. P.C. Nwaiwu, Department of Crop Science, Federal University of Technology, Owerri, Nigeria. The leaves were washed under a continuous current of distilled water for 15 min and air-dried at room temperature (25 ± 5°C) for 2 weeks. The dried leaves were ground separately with a ceramic mortar and pestle. Fifty grams (50 g) of each specimen was suspended in 1.0 L of ethanol/ water mixture (2:1; v/v) in stoppered flasks and allowed to stand in a water bath at thermostatically controlled temperature of 25 ± 5°C under continuous agitation for 48 h. The suspensions were filtered with Whatman No 24 filter paper. The separate filtrates were concentrated in a rotary evaporator at 50°C and dried in vacuum desiccator. The yields were calculated to be V. amygdalina (2.90%; w/w), A. indica (3.40%; w/w) and M. oleifera (3.19%; w/w). The extracts were finally suspended in phosphate buffered saline (PBS) solution osmotically equivalent to 0.9 g/100 mL NaCl {NaCl (9.0 g), Na2HPO4·2H2O (1.71 g) and NaH2PO4·2H2O (0.243 g)/100 mL, pH = 7.4} and stored at 4°C for 24 h before being administered to the rats.

Proximate composition of plant specimen

Dried samples (5 g) of the leaves were measured for moisture, ash, crude fat, crude protein, crude fiber and soluble sugar contents. The determinations were in accordance with the official methods of the Association of Official Analytical Chemists. Measurement of moisture content (MC) was by weighing the samples in crucible and drying in oven at 60°C until constant weight was obtained. The ash content (AC) was measured by incinerating the samples in oven at 105°C for 3 h and the weight of the residue was determined after cooling in a desiccator. The crude fat content (CFC) was measured by the soxhlet extraction methods, whereas crude protein content (CPC) was by the Kjedahl methods. Crude fiber content (CFBC) was determined by digestion methods. Total carbohydrate content (TCC) was calculated by difference of the sum of all the other proximate composition from 100%.

Experimental animals

Male albino Wister rats weighting between 170-220g were obtained from the Animal Unit of the Faculty of Pharmaceutical Science, University of Nigeria, Nsukka, Nigeria. The rats were fed with standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) and water, in a well-ventilated stainless steel cages. After randomization on weight bases, the rats were allowed to acclimatize for 7 days at an ambient room temperature of 25 ± 5°C, 30–55% of relative humidity on a 12-h light/12-h dark cycle. Animal handling was in accordance with the stan-
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Induction of hyperglycemia and study design

Hyperglycemia was induced in 12 h fasted animals by intra-peritoneal injection of 0.1 mol/L alloxaon monohydrate (Sigma, St. Louis, USA) in phosphate buffer saline (PBS) solution (pH = 7.4) at a dose of 140 mg/kg; bw. Hyperglycemia was confirmed in rats 72 h after alloxaon treatment. The rats with fasting blood glucose (FBG) >11.0 mmol/L were considered to be hyperglycemic and selected for the study.

A total of thirty (30) rats were divided into five (5) groups of six (n = 6) each as follows:

- **Group C1; Control-Normal (C/N):** Normal rats received only PBS + SCF (Vehicle; 1.0 mL/kg; bw/12 h, i. p.) for 14 days.
- **Group C2; Control-Hyperglycemic (C/H):** Hyperglycemic rats received PBS + SCF (Vehicle; 1.0 mL/kg; bw/12 h, i. p.) for 14 days.
- **Group T1; H** [A. indica] = 400 mg/kg; bw: Hyperglycemic rats received A. indica + SCF (400 mg/kg; bw/12 h, i. p.) for 14 days.
- **Group T2; H** [V. amygdalina] = 400 mg/kg; bw: Hyperglycemic rats received V. amygdalina + SCF (400 mg/kg; bw/12 h, i. p.) for 14 days.
- **Group T3; H** [M. oleifera] = 400 mg/kg; bw: Hyperglycemic rats received M. oleifera + SCF (400 mg/kg; bw/12 h, i. p.) for 14 days.

Measurement of fasting blood glucose

Blood samples were drawn from the tip of the tail of the rats at regular intervals of 12 h for 48h duration; for short-term study, and subsequently, on days 3, 7, 10 and 14; for long term study, and measured for fasting blood glucose (FBG). Determination of FBG was by the glucose oxidase method according to the Randox® kit manufacturer’s procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

Calculations

Cumulative FBS per h (%FBS h⁻¹) within the short- and long-term treatments were evaluated using the Simpson’s Rule. Thus:

\[
f(X_1)h_1 + f(X_2)h_2 + ... + f(X_n)h_n
\]

Area under the curve (AUC) of the plot of %FBS versus time (h) is given by:

\[
AUC(%FBS \ b^{-1}) = \frac{b}{2} (y_2 + 2y_{x-1} + 2y_{x+2} + 2y_{x+3} + ...)
\]

Where:

- \(h = \) time intervals in hours.
- \(y = \) %FBS at corresponding time interval.

Measurement of body weight

The weights of the rats in the various experimental groups were measured with electronic weighing balance at days 0, 3, 7, 10 and 14.

Statistical analyses

The data collected were expressed in means \((X) \pm SD\) and analyzed in one-way ANOVA and Least Significance Difference (LSD). The comparison was made between groups and significance was established by ANOVA at 95% confidence level. Difference of \(p < 0.05\) was considered statistically significant.

Results

Figure 1 showed the comparative proximate composition of dried samples of V. amygdalina, A. indica and M. oleifera. MC of V. amygdalina, A. indica and M. oleifera was within the range of 9.21–10.29% (\(p > 0.05\)). Leaf samples of A. indica gave the highest AC, representing 10.93% of dried matter. The lowest was AC_M. oleifera = 7.93%, whereas AC_A. indica = 10.93% gave the highest value among the three samples. CFC of the three plant samples were in the order: CFC_V. amygdalina = 9.05% > CFBC_A. indica = 5.17% > CFBC_M. oleifera = 2.11%. V. amygdalina, A. indica and M. oleifera showed wide variation in CPC, this was within the range of 13.42–37.01%. The CFBC of V. amygdalina was not significantly different (\(p > 0.05\)) from A. indica; CFBC_V. amygdalina = 12.08% and CFBC_A. indica = 10.11%. TCC of V. amygdalina and A. indica was calculated to be the single highest component with values: TCC_V. amygdalina = 40.65% and TCC_A. indica = 50.14%; except TCC_M. oleifera = 36.65%, which was approximately equal to CPC_M. oleifera = 37.01% (Figure 1).

Short-term investigation of FBG, i.e., within experimental time of 48 h (0 h ≤ t ≤ 48 h), showed that FBG of group C/N (C/N_FBG) was relatively constant (Table 1). Also, Table 1 showed that C/H_FBG was elevated and significantly different (\(p < 0.05\)) from C/N_FBG, with values within the range of 14.83 ± 3.00–16.95 ± 0.05 mmol/L. In addition, Table 1 indicates that C/H_FBG showed a marginal increment with progression of experimental time.
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Figure 1. Proximate composition (%) of *V. amygdalina*, *A. indica* and *M. oleifera*. MC: moisture content; AC: ash content; CFC: crude fat content; CPC: crude protein content; CFBC: crude fiber content; TCC: Total carbohydrate content.

Table 1: Short-term effect of *V. amygdalina*, *A. indica* and *M. oleifera* on FBG.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>C/N</th>
<th>C/H</th>
<th><em>H</em>&lt;sub&gt;V. amygdalina = 400 mg/kg; bw&lt;/sub&gt;</th>
<th><em>H</em>&lt;sub&gt;[A. indica] = 400 mg/kg; bw&lt;/sub&gt;</th>
<th><em>H</em>&lt;sub&gt;[M. oleifera] = 400 mg/kg; bw&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.00 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.83 ± 3.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.73 ± 1.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.98 ± 1.53&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>14.86 ± 0.83&lt;sup&gt;c,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>5.02 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.56 ± 2.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.32 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.03 ± 1.35&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>13.91 ± 0.44&lt;sup&gt;b,c,d,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>24</td>
<td>5.07 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.37 ± 3.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.99 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.23 ± 1.67&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>13.58 ± 0.46&lt;sup&gt;b,c,d,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>36</td>
<td>5.01 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.65 ± 2.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.52 ± 0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.39 ± 0.97&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>12.15 ± 0.70&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>48</td>
<td>5.00 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.95 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.42 ± 0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.97 ± 0.48&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>11.81 ± 0.51&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The results are means (X) ± SD of six (n = 6) determination; Means in the row with the same letter are not significantly different at p > 0.05 according to LSD.

(p > 0.05), representing 14.30% increase in FBG. The administration of *V. amygdalina*, *A. indica* and *M. oleifera* to the rats (0 h ≤ t ≤ 48 h) did not cause significant (p > 0.05) reduction in FBG of hyperglycemic rats compared to the group C/H. Thus, FBG of groups *H*<sub>[V. amygdalina] = 400 mg/kg; bw</sub> and *H*<sub>[M. oleifera] = 400 mg/kg; bw</sub> were significantly (p < 0.05) elevated compared to C/N<sub>FBG</sub>. It is noteworthy that within the experimental time (0 h ≤ t ≤ 48 h), FBG of groups C/H, *H*<sub>[M. oleifera] = 400 mg/kg; bw</sub> and *H*<sub>[V. amygdalina] = 400 mg/kg; bw</sub> were comparatively over 2 folds >C/N<sub>FBG</sub> (Table 1 and Figure 2). However, the capacities of the three leaf extracts to cause slight reduction in FBG, as shown in Figure 2, was in the order: *H*<sub>[M. oleifera] = 400 mg/kg; bw</sub> > *H*<sub>[A. indica] = 400 mg/kg; bw</sub> > *H*<sub>[V. amygdalina] = 400 mg/kg; bw</sub>; p > 0.05.

Figure 2 showed that at t = 48 h, C/H<sub>FBG</sub> increased by 14.30%, whereas hyperglycemic rats treated with extracts of *V. amygdalina*, *A. indica* and *M. oleifera* showed reduced FBG by 15.68%, 13.42% and 20.52% respectively, compared to their FBG at t = 0 h. Comparatively, the capacity of extracts of the three medicinal plants to lower FBS in hyperglycemic rats was in the order: AUC<sub>*H*<sub>[M. oleifera] = 400 mg/kg; bw</sub></sub> = 78.50 × 10<sup>2</sup>%FBS h<sup>−1</sup> > AUC<sub>*H*<sub>[A. indica] = 400 mg/kg; bw</sub></sub> = 87.14 × 10<sup>2</sup>%FBS h<sup>−1</sup> > AUC<sub>*H*<sub>[V. amygdalina] = 400 mg/kg; bw</sub></sub> = 89.08 × 10<sup>2</sup>%FBS h<sup>−1</sup>; p > 0.05, within the 48 h experimental time.
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Figure 2. Comparative FBG levels of hyperglycemic rats on short-term administration of V. amygdalina, A. indica and M. oleifera; (0 h ≤ t ≤ 48 h): AUC_{C/H} = 103.60 × 10^{2} %FBS h^{-1}; AUC_{H[V. amygdalina]} = 400 mg/kg; bw = 89.08 × 10^{2} %FBS h^{-1}; AUC_{H[A. indica]} = 400 mg/kg; bw = 87.14 × 10^{2} %FBS h^{-1}; AUC_{H[M. oleifera]} = 400 mg/kg; bw = 78.50 × 10^{2} %FBS h^{-1} (Equation 1).

Table 2: Long-term effect of V. amygdalina, A. indica and M. oleifera on FBG.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>C/N</th>
<th>C/H</th>
<th>H[V. amygdalina] = 400 mg/kg; bw</th>
<th>H[A. indica] = 400 mg/kg; bw</th>
<th>H[M. oleifera] = 400 mg/kg; bw</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.00 ± 0.25^{a}</td>
<td>14.83 ± 3.00^{b}</td>
<td>14.73 ± 1.49^{bc}</td>
<td>14.98 ± 1.53^{bcd}</td>
<td>14.86 ± 0.83^{bcde}</td>
</tr>
<tr>
<td>3</td>
<td>4.99 ± 0.40^{a}</td>
<td>18.58 ± 1.19^{b}</td>
<td>12.57 ± 0.53^{bc}</td>
<td>12.53 ± 0.50^{cde}</td>
<td>10.93 ± 0.78^{bcde}</td>
</tr>
<tr>
<td>7</td>
<td>5.00 ± 0.19^{a}</td>
<td>19.83 ± 0.36^{b}</td>
<td>10.46 ± 0.64^{c}</td>
<td>10.23 ± 0.22^{cde}</td>
<td>8.48 ± 0.54^{cde}</td>
</tr>
<tr>
<td>10</td>
<td>4.94 ± 0.13^{a}</td>
<td>21.42 ± 0.82^{b}</td>
<td>9.03 ± 0.71^{c}</td>
<td>9.74 ± 0.34^{cde}</td>
<td>6.96 ± 0.32^{cde}</td>
</tr>
<tr>
<td>14</td>
<td>4.99 ± 0.11^{a}</td>
<td>27.64 ± 0.94^{b}</td>
<td>6.86 ± 1.16^{c}</td>
<td>8.56 ± 1.04^{cde}</td>
<td>5.44 ± 0.20^{cde}</td>
</tr>
</tbody>
</table>

The results are means (X) ± SD of six (n = 6) determination; Means in the row with the same letter are not significantly different at p > 0.05 according to LSD.

Long-term investigation of FBG i.e., within experimental time of 14 days, showed that C/N_{FBG} was relatively stable with values between 4.94 ± 0.13–5.00 ± 0.25 mmol/L (Table 2). C/H_{FBG} was significantly (p < 0.05) elevated compared to C/N_{FBG}. Within the 14-day duration, C/H_{FBG} increased by 2 folds approx. Conversely, the administration of the three leaf extracts caused reduction in FBG in a time dependent manner, which was in the order: H[M. oleifera] = 400 mg/kg; bw = 2.73 folds > H[V. amygdalina] = 400 mg/kg; bw = 2.15 folds > H[A. indica] = 400 mg/kg; bw = 1.75 folds; p < 0.05 (Table 2). FBG of hyperglycemic rats was restored to values comparable to C/N_{FBG} in groups H[V. amygdalina] = 400 mg/kg; bw at t = 10^{th} and 14^{th} day (p < 0.05), whereas FBG of H[A. indica] = 400 mg/kg; bw was 71.5% above C/N_{FBG} at t = 14^{th} day (p < 0.05) (Figure 3).

Figure 4) showed that group C/N gained relatively maximum weight at the end of experimental time of 14 days by 6.58%. In contrast, group C/H exhibited weight loss by 6.74%. Hyperglycemic rats administered with the three leaf extracts exhibited an increase in relative body weight but with values lower than the group C/N. Notably, H[V. amygdalina] = 400 mg/kg; bw and H[A. indica] = 400 mg/kg; bw showed weight loss on day 10, after which maximum weight gain occurred on day 14. Specifically, the weight gained by hyperglycemic rats treated
Figure 3. Comparative FBG levels of hyperglycemic rats on long-term administration of *V. amygdalina*, *A. indica* and *M. oleifera*.

(0 h ≤ t ≤ 336 h): $AUC_{C/H} = 104.67 \times 10^3 \%FBS \ h^{-1}$; $AUC_{H[V. amygdalina]} = 400 \ mg/kg; \ bw = 37.69 \times 10^3 \%FBS \ h^{-1}$; $AUC_{H[A. indica]} = 400 \ mg/kg; \ bw = 40.25 \times 10^3 \%FBS \ h^{-1}$; $AUC_{H[M. oleifera]} = 400 \ mg/kg; \ bw = 26.81 \times 10^3 \%FBS \ h^{-1}$ (Equation 1).

Figure 4. Comparative body weights of normal and hyperglycemic rats on long-term administration of *V. amygdalina*, *A. indica* and *M. oleifera*.
with the three leaf extracts at the end of the experimental time were as follows: H$_{A. indica}$ = 400 mg/kg bw = 4.70% > H$_{M. oleifera}$ = 400 mg/kg bw = 2.59% > H$_{V. amygdalina}$ = 400 mg/kg bw = 1.38%.

**DISCUSSION**

Although certain plants contain noxious compounds, there are several therapeutic benefits derivable from the application of plant matter for medicinal purposes and general well-being. Previous reports have attributed the hypoglycemic properties of the leaf extracts to polysaccharides, proteins, alkaloids, flavonoids and related compounds.[36] Other nutritive compounds and phytochemicals which have been implicated in possessing hypoglycemic properties include terpenoids, phenolics, amino acids and related nitrogenous compounds.[37–40] The hypoglycemic properties of these compounds have been evaluated and demonstrated in animal models[41,42] as well as in humans.[43] For instance, the amino acid derivative, S-methyl cysteine sulphoxide and S-alkyl cysteine sulphoxide content in Allium sativum (Garlic)[43] stimulates insulin secretion, whereas Charantin (a peptide)[48] inhibits hepatic glycolysis. Lipid components such as phytosterols[47] stimulate insulin biosynthesis and secretion; β-sitosterol[46–48] suppresses glucose-6-phosphatase and promotes glycogen biosynthesis with reduced phosphofructokinase activity. Other phytochemicals such as tannins,[49,50] saponins glycosidic component[50] and pectin materials[51] act by suppressing gluconeogenic enzymes and stimulate insulin secretion. Proximate compositions of the three plant specimen (Figure 1) indicated that V. amygdalina, A. indica and M. oleifera are comparatively good sources of these bioactive agents that are capable of exerting glycemic control. Specifically, comparative analyses showed remarkable high TCC in V. amygdalina, A. indica and M. oleifera in combination with CPC in M. oleifera.

Another hypoglycemic potential of medicinal plants are attributable to trace elements present in them. Previous studies have shown that administration/intake of micronutrients are effective preventive and treatment measures for both type I and type II DM and common associated complications. Narendhirakannan et al.,[52] reported the roles of inorganic trace elements present in four traditional medicinal plants (Murraya koenigii, Mentha piperita, Ocimum sanctum, and Aegle marmelos) used in the treatment of DM. Also, Rajasekaran et al.,[53] noted that inorganic elements like vanadium, zinc, sodium, potassium, calcium, copper, manganese, and traces of chromium zinc and chromium are required to correct glucometabolic disorders associated with DM. Trace elements are integral components of enzymatic reactions where they act as cofactors. Notable enzymatic reactions are those involving chromium dependent activation of insulin receptor signaling and the cofactor antioxidant activity of selenium in glutathione peroxidase[54] involve in scavenging multiple reactive oxygen species associated with the diabetic states.[55–56] In addition, low magnesium levels have been associated with increased type II diabetes.[57–59] Results of comparative proximate composition of the three plants showed appreciable AC (Figure 1), which suggest that V. amygdalina, A. indica and M. oleifera contain relatively rich quantity of inorganic trace elements posited to possessing glycemic control property. In similar manner, CFBC of V. amygdalina, A. indica and M. oleifera advance the view that the plants products are agents of glycemic control. A high fiber content in diet engendered profound lowering of plasma glucose concentration in DM.[60] Likewise, dietary fiber increases viscosity of gastrointestinal tract, thereby suppressing carbohydrate absorption.[61] This mechanism is selectively advantageous in that the risk of hyperglycemia and repercussive hyperinsulinism is controlled. Based on the inferences of the present study, it is worthwhile to note that the mixtures and combinations of these bioactive principles and essential elements act in synergy to exert glycemic control.

Short-term administration of some herbal extracts have been previously reported to exhibit little or marginal efficacy in hyperglycemic rats.[53,62–68] In another study, a methanol/water extract of Phaleria macrocarpa fruits caused non-significant (p > 0.05) blood glucose lowering effect between 15 and 120 min, which was in contrast with the application of methanol extract of the same fruit.[69] This observation may not be unconnected with the type and nature of the bioactive principles present in the two extracts used in their study. Different bioactive principles in leaf extracts possess different mode of blood glucose lowering activity that determines the potency and duration required to restore therapeutic benefits. From the present study, the observed low efficacy (Table 1) following the administration of ethanol/water extracts of V. amygdalina, A. indica and M. oleifera to hyperglycemic rats for 48 h, was an obvious indication that the extracts were not fast acting anti-hyperglycemic preparations. However, the results as presented in Table 1 indicate that the three extracts elicited slight short-term efficacy in reducing elevated levels of FBG. Therefore, the capacity of extracts of V. amygdalina, A. indica and M. oleifera to induce reduction in levels of FBG was not comparable to the fast acting subcutaneous insulin injection and the oral sulfonylureas, biguanides and glinides.[64–69] The mechanisms of action of these drugs include: reduction in hepatic glucose release, enhancement of insulin secretion and sensitivity as well as inhibition of intestinal glucose absorp-
Although the present study has shown that extracts of *V. amygdalina*, *A. indica* and *M. oleifera* caused marginal short-term efficacy, oral administration of ethanolic extract of *Berberis lyceum* (Royle) has previously been demonstrated to possessing substantial short-term acting capacity to ameliorate elevated blood glucose in alloxan induced diabetic rats within 180 min.[67] They further noted that ethanol extracts of *B. lyceum* yielded slightly better results compared to equivalent aqueous extracts. Likewise, rats fed by gavage with 500 and 1000 mg/kg/day of *V. viscum album* extract for 72 h caused significant (*p < 0.05*) reduction in blood glucose and increased antioxidant power of alloxanized rats. [68] These findings indicate that the route and mode of drug administration alters the level of drug delivery and efficacy.

The present findings showed that long-term administration of extracts of *V. amygdalina*, *A. indica* and *M. oleifera*, in its present form, caused profound but variable capacity to reduce FBG in hyperglycemic rats at the end of 14 days treatment (Table 2), which agreed with the findings of Nagappa et al.,[50] on antidiabetic activity of *Terminalia catappa* Linn fruits. This was an indication that the leaf extracts acted by eliciting re-adjustments in the distorted haemostasis of hyperglycemic rats through mechanisms that are relatively of the delayed type. Such mechanisms include promoting biosynthesis of enzymes and structural molecules required for regeneration and restoration of the functional integrity of insulin secreting cells (Islet of Langerhans) in concert with quick acting mechanism of insulin mimicking.[40,69–70] The leaves of *Gymnema sylvestre* (Gumer)[71] and the fruits of *T. catappa* Linn[50] induced β-cell regeneration in simultaneous with β-cell stimulation and suppression of gluconeogenic enzymes[73] that corroborates the plausible mode of action of *V. amygdalina*, *A. indica* and *M. oleifera* extracts. From another perspective, Ugochukwu and Babady,[73] reported that the long-term hypoglycemic property of *Gongronema latifolium* extract was mediated by activation of hepatic hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase in diabetic rats. In furtherance to these findings, Ngozi et al.,[74] posited that the glycemic control mechanism of *G. latifolium* could be linked to the capability of the extract to increase cellular ratio of reduced glutathione to oxidized glutathione (GSH/GSSG). Thus, their findings were in concord with previously reported tendency of *G. latifolium* [75] and oil of *Eruca sativa* seeds[76] to ameliorate oxidative stress associated with the diabetic state.

Comparative proximate composition of *V. amygdalina*, *A. indica* and *M. oleifera* and corresponding capacity of the three leaf extracts to reduce FBG appears to suggest that biomolecules responsible for promoting FBG lowering effects are for the most part the TCC and CPC elements, which might have acted in synergy with other anti-diabetogenic components. The improvement in body weight of hyperglycemic rats treated with the leaf extracts, as against weight loss in the group C/H further confirmed nutraceutical benefits as previously reported.[14,22–77]


