Antioxidant activities and nutritional compositions of *Vernonia amygdalina*

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**ABSTRACT**

The leaf of *Vernonia amygdalina* was harvested in the author’s compound and the high demand for its use in the area necessitated the evaluation of the proximate, antinutrients, the mineral compositions and the antioxidant activities of the leaf. The proximate analysis indicated ash content of 4.20% ± 0.21, dried weight moisture content of 10.56% ± 0.09, crude protein 18.75% ± 0.00, fat content 5.07% ± 0.12, fiber content of 8.78% ± 0.17 and carbohydrate by difference indicated 52.56% ± 0.17. The antinutrients indicated tannin value of 3.33mg/100g ± 0.01, phenol 1.76mg/100g ± 0.01, phylate 19.73mg/100g ± 0.04, oxalate 3.70mg/100g ± 0.01, saponin 3.27mg/100g ± 0.49, alkaloids 3.57mg/100g ± 0.02, flavonoid 4.05mg/100g ± 0.04. The results of mineral analysis showed the presence of the mineral contents in this progression (mg/kg); K > Na > Ca > Mg > Fe > Zn > Cu > Mn. However the leaf is highly rich in K, Na and Ca having K: Na: Ca of 5.80: 5.16: 1.00. The antioxidant of the leaf at different concentrations showed that at 0.4mg/ml, the percentage inhibition was 76.08% and least at 0.1mg/ml having 22.96% inhibition.

**Keywords:** *Vernonia amygdalina*, proximate, antinutrients, mineral contents and antioxidant.

**INTRODUCTION**

The botanical name of bitter leaf is *Vernonia amygdalina*, the leaves of the plant is highly medicinal and grows in the humid tropical secondary forests of Africa. The leaf is used traditionally to treat antimalarial, antihelminth and a laxative herb. Phytochemical screening of the leaves revealed the presence of tannins, phlobatannins, flavonoids, steroids, terpenoids, saponins and cardiac glycosides, which are the most important bioactive constituents of medicinal plants. Also, the leaves are used as a leafy vegetable for preparing the popular bitter-leaf soup and the juice or extract serves as a tonic drink. The various pharmacological usefulness of the leaf necessitated this research work to examine the proximate, antinutrients, antioxidant activities and the mineral compositions of the leaves.

**MATERIALS AND METHODS**

The leaves of *V. amygdalina* were harvested from the private compound of the author and they were washed to remove dirtiness and air dried for five days. The leaves were grinded into powdered form using a Thomas Wiley milling machine and kept in a clean sterilized bottle to avoid contamination.

The proximate analysis of the sample; moisture content, crude fat, crude protein, total ash and carbohydrate were all determined using the standard procedure of Association of Analytical Chemist (AOAC, 1990). The formula used for each determined parameters are:

\[
(a) \text{Moisture content} = \frac{W_1 - W_2}{W_1 - W_2} \times 100 \%
\]

Where: \(W_1 = \text{Weight of Petri dish}\)
W₂ = Weight of Petri dish + sample
W₃ = Weight of Petri dish + sample after drying

(b) crude fat = \( \frac{W₃ - W₁}{W₂ - W₁} \times 100 \% \)

Where: 
W₁ = Weight of the thimble
W₂ = Weight of the thimble + sample
W₃ = Weight of round bottom flask + residual oil
W₄ = Weight of round bottom flask + residual oil + sample

(c)\% Nitrogen = \( \frac{\text{tiratval ure } \times M \times 0.014}{\text{weight of sample}} \times 100 \% \)

Crude Protein = % Nitrogen \times 6.25
Where N is the total Nitrogen and 6.25 is the conversion factor

(d) total ash content = \( \frac{W₄ - W₁}{W₂ - W₁} \times 100 \% \)

Where: 
W₁ = Weight of crucible
W₂ = Weight of crucible + sample
W₃ = Weight of crucible + sample after drying

% organic matter = 100 - % Ash

(e) crude fibre = \( \frac{W₂ - W₃}{W₂ - W₁} \times 100 \% \)

Where: 
W₁ = Weight of sample
W₂ = Weight of sample + dish
W₃ = Sample weighted + dish after ignition

(f) Carbohydrate content was determined by subtracting the values of the analyzed components from 100%.

carbohydrate content = 100 - (cp + ash + crude fibre + crude fat)

Evaluation of antinutrients

Preparation of fat free sample: 2 g of the sample was defatted with 100 ml of diethyl ether using Soxhlet apparatus for 2 h.

(i) Determination of total phenol by spectrophotometric method: The free fat sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five (5) ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. Two (2) ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

(ii) Determination of alkaloid using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to the quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

(iii) Determination of tannin using Van Burden and Robinson (1981) method: 500 mg of the sample was weighed into a 50 ml plastic bottle. Fifty (50) ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Five (5) ml of the filtered was pipette into a test tube and mixed with 2 ml of 0.13 FeCl₃, 0.11 HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Flavonoid determination using the method of Boham and Kocipal (1974): 10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

(iv) Saponin determination: Obadoni and Ochuko (2001) method was used: 20 g of the sample was put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty (60) ml of n-hutanol was added and this was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as percentage.

(v) Determination of Percentage Phytalate (Rahila et al., 1994): A known weight of the grounded sample was soaked into 100 ml of 2% HCl for 5 h and filtered. Twenty five (25) cm³ of the filtrate was taken into a conical flask and 5 cm³ of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of FeCl₃ until a brownish yellow colour persisted for 5 min.
The concentration of the FeCl$_3$ was 1.04% W/V. 
Calculation mole ratio of Fe to phyta late = 1:1

Concentration of phyta late phosphorous = \( \text{Titre value} \times 0.064 \)

Weight of sample

(vi) Oxalate content determination: The total oxalic acid was determined by the modified method of Abeza et al. (1968). Two (2) g aliquot of the plant materials was weighed into a 250 ml flask, 190 ml, distilled water and 10 ml of 6 M hydrochloric acid were added. The mixture was digested for 1 h on boiling water bath, cooled, transferred into a 250 ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was heated to 100°C, allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled; 10 ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through Whatman No. 40 filter paper. The precipitate was washed several times with distilled water. The precipitate was transferred quantitatively to a beaker and 5 ml of 25% H$_2$SO$_4$ was added to dissolve the precipitate. The resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate used the oxalate content of the unknown sample was calculated:

\[
1 \text{ ml of potassium permanganate} = 2.24 \text{ mg oxalate}
\]

Antioxidant Determination Using DPPH method

The leaves of V. amygdalina was harvested and air dried at room temperature and 5 g were soaked in methanol of 10ml for five days, the extract was concentrated using rotary evaporator and the yield was found to be 1.2 g and designated as methanolic extract of V. amygdalina.

Procedure: (2, 2-Diphenyl 1-picrylhydrazyl) DPPH Solution: Stock solution of DPPH was prepared by dissolving 40 mg of DPPH in 100 ml of methanol. The methanolic solution of DPPH was further diluted with 100 ml of deionized water to obtain a DPPH stock solution of having a 50:50 v/v. The working solution of DPPH having an absorbance value of 0.75-0.80 at 517 nm was prepared by diluting 200 ml of stock DPPH. Solution with approximately 800 ml of water/methanol [50:50v/v] mixture. One (1) ml of the following concentrations; 0.4, 0.3, 0.2 and 0.1 mg/ml of the extract was measured, 2 ml of working solution of DPPH was added and incubated in the dark room temperature for 30 min (Figure 1). After incubation, the absorbance of the solution was measured at 517 nm. The control was treated by measuring 1 ml of methanol and 2 ml of DPPH and treated as above (Brand Williams, 1995).

\[
\% \text{ inhibition} = \frac{Abs_{\text{sample}} - Abs_{\text{control}}}{Abs_{\text{control}}} \times 100
\]

Mineral content analysis

The mineral contents were analyzed using Atomic Absorption Spectrophotometer (AAS).

Statistical analysis

Descriptive statistical method of analysis was used for proximate, mineral analysis and antinutrients.

RESULTS AND DISCUSSION

Table 1 shows the anti-oxidant activities at different concentrations. At 0.4 mg/ml, the % inhibition was found to be 76.08%, at 0.3 mg/ml, % inhibition of 55.86%, at 0.2 mg/ml, % inhibition 39.09% and at 0.1 mg/ml, the % inhibition was 22.96%. The anti-oxidant properties exhibited at different concentrations indicated that the plant leaf may provide anti-oxidant benefits as many chronic diseases and causes of food spoilage are linked to pro-oxidants. Antioxidant principles are therefore useful in food preservation and drug formulation.
Table 1. Results of Absorbance using UV visible spectrophotometer and % inhibition.

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Absorbance</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.146</td>
<td>76.08</td>
</tr>
<tr>
<td>0.3</td>
<td>0.271</td>
<td>55.86</td>
</tr>
<tr>
<td>0.2</td>
<td>0.374</td>
<td>39.09</td>
</tr>
<tr>
<td>0.1</td>
<td>0.473</td>
<td>22.96</td>
</tr>
<tr>
<td>Control</td>
<td>0.614</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Proximate analysis of V. amygdalina.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Statistic</th>
<th>Maximum Statistic</th>
<th>Mean Statistic</th>
<th>Std. Error Statistic</th>
<th>Std. Deviation Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Ash</td>
<td>4.07</td>
<td>4.49</td>
<td>4.2800</td>
<td>0.2100</td>
<td>0.29698</td>
</tr>
<tr>
<td>% MC</td>
<td>10.46</td>
<td>10.65</td>
<td>10.5550</td>
<td>0.0950</td>
<td>0.13435</td>
</tr>
<tr>
<td>% CP</td>
<td>18.75</td>
<td>18.75</td>
<td>18.7500</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>% Fat</td>
<td>4.95</td>
<td>5.19</td>
<td>5.0700</td>
<td>0.1200</td>
<td>0.16971</td>
</tr>
<tr>
<td>% Fibre</td>
<td>8.61</td>
<td>8.96</td>
<td>8.7850</td>
<td>0.1750</td>
<td>0.24749</td>
</tr>
<tr>
<td>% CHO</td>
<td>52.39</td>
<td>52.73</td>
<td>52.5600</td>
<td>0.1700</td>
<td>0.24042</td>
</tr>
</tbody>
</table>

Table 3. Results of anti-nutrients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum Statistic</th>
<th>Maximum Statistic</th>
<th>Mean Statistic</th>
<th>Std. Error Statistic</th>
<th>Std. Deviation Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin (mg/100g)</td>
<td>3.32</td>
<td>3.34</td>
<td>3.3300</td>
<td>0.0100</td>
<td>0.01414</td>
</tr>
<tr>
<td>Phenol (mg/100g)</td>
<td>1.75</td>
<td>1.78</td>
<td>1.7650</td>
<td>0.0150</td>
<td>0.02121</td>
</tr>
<tr>
<td>Phytalate (mg/100g)</td>
<td>19.69</td>
<td>19.78</td>
<td>19.7350</td>
<td>0.0450</td>
<td>0.06364</td>
</tr>
<tr>
<td>Oxalate (mg/100g)</td>
<td>3.76</td>
<td>3.78</td>
<td>3.7700</td>
<td>0.0100</td>
<td>0.01414</td>
</tr>
<tr>
<td>Saponin (mg/100g)</td>
<td>2.78</td>
<td>3.76</td>
<td>3.2700</td>
<td>0.4900</td>
<td>0.69296</td>
</tr>
<tr>
<td>Alkaloids (mg/100g)</td>
<td>3.55</td>
<td>3.59</td>
<td>3.5700</td>
<td>0.0200</td>
<td>0.02828</td>
</tr>
<tr>
<td>Flavonoids (mg/100g)</td>
<td>4.01</td>
<td>4.09</td>
<td>4.0500</td>
<td>0.0400</td>
<td>0.05657</td>
</tr>
</tbody>
</table>

The proximate analysis is shown in Table 2. The percentage moisture content was 10.55%±0.09. This value was higher than that reported (10.02%) by Asaolu et al. (2012). This variation may be due to soil nutrients and environmental factors which have effects on the nutrients availabilities for plants. However, the high moisture content provides greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these leafy vegetables (Iheanacho and Udebuani, 2009). Also, the ash content indicated 4.28% ± 0.21. This was however lower than that reported by Asaolu et al. (2012) as 9.56%. For bitter leaf and 13.01% for Scent leaf. The presence of ash content in bitter leaf is a confirmation of availabilities of mineral elements. The crude protein showed 18.75%±0.00. This high protein content was higher than that reported for some leafy vegetables such as Momordica balsamina (11.29%). It is noted that food plants provide more than 12% of their calorific value from protein (Ali, 2009). This indicated that bitter leaf is a good source of protein. The crude fibre content was found to be 8.78% ± 0.17, this value fell within the ranged (8.50-20.90%) for some Nigerian vegetables (Iheanacho and Idiong, 1997). Dietary fibre helps to prevent bowel problem, piles and constipation. The fat content (5.07% ±0.12) indicated the presence of oil in bitter leaf. This value was higher than that reported for some other leafy vegetable such as ocimum viride (Udosen, 1995). The carbohydrate content (52.56% ± 0.17) was higher than that reported by Asaolu et al. (2012) for the same plant (8.65%).

Table 3 shows the mineral contents of bitter leaf indicated this progression, K>Na>Ca > Mg > Fe >Zn > Cu >Mn. Sodium and Potassium are very essential intracellular and extracellular cations. Sodium is involved in the regulation of plasma volume, acid base balance, nerve and muscle contraction (Akpanyung, 2005). The Sodium content of bitter leaf (8833.02 mg/kg ±638.33) is higher than that reported by Asaolu et al. (2012) for the same plant. However, the Na, K, Ca mineral contents are higher
than the recommended daily allowance (RDA) of 400 mg/day for men 19-30 years and 310 mg/day for women 19-39 years old (FNB, 1997). The Zinc content (39.24 mg/kg ± 5.01) was higher than that reported by Asaolu et al. (2010) and Ayoola et al. (2010). Zinc is an important micronutrient essential for human development and immune functions (Black, 2003). The Iron content is also higher (69.26 mg/kg ± 2.89) and this plays an important function in haemopoiesis, control of infection and cell mediated immunity (Bhaskaran, 2011).

Table 4 shows the results of antinutrients. Anti-nutrient factors (ANF) are compounds which act to reduce nutrients utilization and or food intake (Osagie, 1998). They play very useful role in limiting the wider use of many plants. However, these anti-nutrients have found wide applications in the areas of medicine, food industries and pharmacy as pharmacologically active ingredients (Schopke and Hiller, 1990). The tannin content was 3.33 mg/100g±0.01, saponin content was (3.27 mg/100 g ± 0.49), alkaloids (3.57 mg/100 g ± 0.02) and flavonoid (4.05 mg/100 g ±0.04). Saponin and flavonoids have been found useful in the formulation of drugs, in food, drinks and beverage industries as foaming agents (Fenwick et al., 1983), as antioxidants, preservatives and flavouring agents (You et al., 1993; Fenwick et al., 1983). In addition, saponins have been reported to have antibiotic activities (Tchesche and Wulff, 1963; Zimber et al., 1965; Soetan et al., 2006), antifungal activities (Jun et al., 1989) and antiviral activity (Okubo et al., 1994). Powers (1964) reported that several flavonoids including phenolic acids showed inhibitory activity towards one or more of the bacteria studied. Moreover, alkaloids have been known to be the most essential potent anti-inflammatory agents of naturally occurring products of secondary metabolism (Igile, 1995).

### Conclusion

This research work has extensively revealed the important of bitter leaf both for human consumption, as traditional herbs and also as active principle in the formulation of drugs.

### ACKNOWLEDGEMENT

The author sincerely appreciates the efforts of Mr Oso, the Laboratory Technologist, in the Chemistry Laboratory, Afe Babalola University, Ado Ekiti, Nigeria for the active involvement in carrying out the antioxidant evaluation.

### REFERENCES


### Table 4. Mineral analysis of the Vernonia amygdalina.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (mg/kg)</td>
<td>66.37</td>
<td>72.15</td>
<td>69.26</td>
<td>2.89000</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>25.21</td>
<td>33.65</td>
<td>29.43</td>
<td>4.22000</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>34.23</td>
<td>44.25</td>
<td>39.24</td>
<td>5.01000</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>4.01</td>
<td>6.21</td>
<td>5.1100</td>
<td>1.10000</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>196.04</td>
<td>225.66</td>
<td>210.85</td>
<td>14.81000</td>
</tr>
<tr>
<td>K (mg/kg)</td>
<td>9734.51</td>
<td>10096.92</td>
<td>9915.71</td>
<td>181.20500</td>
</tr>
<tr>
<td>Ca (mg/kg)</td>
<td>1663.72</td>
<td>1762.11</td>
<td>1712.91</td>
<td>49.19500</td>
</tr>
<tr>
<td>Na (mg/kg)</td>
<td>8194.69</td>
<td>9471.36</td>
<td>8833.02</td>
<td>638.33500</td>
</tr>
</tbody>
</table>

Statistic, Maximum, Mean, Std. Error, Statistic

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