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DETERMINATION OF PROXIMATE ANALYSIS AND HEAVY METAL CONCENTRATION IN BITTER LEAF (*Vernonia amygdalina*).

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Abstract

This study examined the proximate composition and heavy metal concentration of *Vernonia amygdalina*. Bitter leaves were purchased from Towobowo market, sun-dried and grind for laboratory analysis. Proximate analysis was determined using standard procedure and heavy metal constituents were carried out using Atomic Absorption Spectrophotometer (AAS) following standard procedure. The result of analysis carried out on bitter leaf revealed the following result; Fat (1.66 g/100g), Carbohydrate (52.80g/100g), Moisture (10.12g/100g), Dry matter (39.88g/100g), Crude fibre (6.19g/100g), Ash Content (6.38g/100g) and Crude protein(22.86g/100g) respectively. Heavy metals contents includes; Zn (0.096mg/100g), Pb (0.041mg/100g), Cr (0.05mg/100g), Fe (0.31mg/100g), but Cadmium was below detection level in the samples. The bitter leaf examined had good proximate contents and all heavy metals observed were within the acceptable limits of WHO except Cadmium that was not detected and Lead that was above the recommended values of WHO. Therefore, the bitter leaf examined is good for consumption and could be used in drug development for diseases treatment and prevention.

Key words: AAS, *Vernonia amygdalina*, Proximate, Zn, Pb, Igboora.

Introduction

Medicinal plants are known to contain substances that could be used for treatment purposes or drug production. Many of such plants are primitively used to alleviate symptoms of illnesses screened for medicinal importance, some of which include: *Azadirachta indica* (Dogonyaro), *Vernonia amygdalina* (Bitter leaf), *Allium sativum* (Garlic), *Occimum gratissimum* (Scent leaf), and *Zingiber officinale* (Ginger). These plants are being repeatedly used in the treatment of ailments such as stomach disorder, fever symptoms and cough traditionally. Medicinal plants play vital roles in the health of individuals and the communities. The medicinal value of some plants lies in its ability to produce definite physiological actions in the human body. Some of these important bioactive constituents allowed these plants to serve the purpose mentioned include alkaloids, tannins, flavonoids and phenolic compound (Newman *et al.*, 2003).

The leaves of *Vernonia amygdalina* are green with a characteristic odour and bitter taste. *Vernonia amygdalina* is a valuable medicinal plant that is widespread in West Africa, known as bitter leaf due to its bitter taste and flavour, used as an active anticancer, antibacterial, antimalarial and anti-parasitic agent. This plant contains complex active components that are useful pharmacologically. In ethno-medicine, the roots and leaves are used to treat fever, hiccups, kidney problems and stomach discomfort. Many West African countries like Cameroon, Ghana and Nigeria use the stem and root as chewing sticks. It is also documented that *V. amygdalina* has been used traditionally in blood clotting and has elicited a substantial reduction in the level of glucose in the human blood. Oshoet *et al.* (2007) observed a close-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts.

Also, the author demonstrated *V. amygdalina* leaf extracts as a DNA-damaging of anticancer agent in the management of breast cancer. The wide use of antibiotics (drugs) in the treatment of bacterial infections has emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease. Recently, the antibacterial activity of *V. amygdalina*

and *O. gratissimum* leaves extract on selected food borne pathogens were assessed and the high zone of inhibition at low concentration proved the plants to be medically useful (Shinwari *et al.*, 2006). Therefore, this study intends to examine the proximate and heavy metal concentration of bitter leaves to ascertain the levels of metals and proximate contents in its composition. This is because there is paucity of information on the properties of bitter leaves, particularly the sample from Igboora.

Materials and methods

Study Area: The study was carried out in Ibarapa central local government area with about 440km² of savannah zone to the west of Ibadan, Ogun and Oyan rivers from Eastern and Western boundaries respectively. It is between approximately Latitude 7.53° E and Longitude 3.08 °N with the population of 102,979 according to the 2006 population CENSUS. The study area has an average annual rainfall of 1278mm and average annual temperature of 27°C (Elizabeth *et al.*, 2003 and Awojobi *et al.*, 2011).

Sample Collection and Preparation: The bitter leave was obtained at Towobowo Local Market Ibarapa Central Local Government, Igboora Oyo State Nigeria. Bitter leaves was washed with distilled water for the removal of sand and dust, sun dried for two days at room temperature and oven dried for 30mins at temperature of about 20°C. The bitter leaves were grinded using mortal and pestle into powdered form. The powdered samples were taken to the laboratory for proximate and heavy metals concentration analysis.

Determination of Proximate Analysis: The sample analyzed for proximate analysis were crude protein, Crude fat, Crude fibre, Moisture, Ash, Carbohydrate and Dry matter contents were determined using the method described by AOAC (2004).

Moisture Content: The Moisture of each sample was determined by weighing 5g of the sample into an aluminum moisture. The sample was then dried to constant weight at 105±2C.

$$\text{Moisture content} = \frac{(\text{Weight of can} + \text{sample}) - (\text{weight of empty can})}{\text{Weight of sample}} \times 100$$

Determination of Crude Protein: The Protein Content was determined using a Foss Tescator protein digester and KJECTEC 2200 distillation apparatus (Kjeldahl method) according to the procedure of AOAC (2004). Concentrated H₂SO₄ (12ml) and 2 tablets of catalyst were put into a Kjeldahl digestion flask containing 1g of the sample. The flask was placed in the digester in a fume cupboard and switched on and digestion was done for 45 minutes to obtained a clear colourless solution. The digest was distilled with 4% boric acid, 20% Sodium hydroxide solutions were automatically metered into the KJECTEC 2200 distillation equipment until distillation was completed. The distillate was then titrated with 0.1M HCl until a violet color formation indicating the end point. A blank was run under the same condition with the sample. Total nitrogen content was then calculated according to the formula:

$$\text{Crude Protein} = \frac{(\text{Titre value (of sample)} - \text{blank}) \times 0.01 \times 14.007 \times 6.25 \times 100}{1000 \times \text{Weight of sample}}$$

Determination of Crude Fat Content: Crude fat was extracted in a Soxhlet extractor with hexane and quantified gravimetrically. 1g of sample was weighed into an extraction thimble and then stopped with grease-free cotton. Before extraction commenced the round bottom cans was dried, cooled and weighed. The thimble was placed in extraction chamber and 80ml hexane was added to extract the fat. The extraction was carried out at 135°C, for 1hour 40minutes after which the fat was collected in the bottom cans and cooled in a desiccator.

$$\text{Crude Fat} = \frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$$

Determination of Crude Fiber: 2g of the sample was transferred into 1 litre conical flask. 100ml of sulphuric acid (12.5M) was heated to boiling and then introduced into the conical flask containing the sample. The contents were then boiled for 30 minutes ensuring that the level of the acid was maintained by addition of distilled water. After 30 minutes, the contents were then filtered through a muslin cloth held in a funnel. The residue was rinsed thoroughly until its washing was no longer acidic to litmus. The residue was then transferred into a conical flask. 100ml of sodium hydroxide (12.5M) was then boiled and introduced into the conical flask containing the sample. The contents were then boiled for 30 minutes ensuring that the level of the acid was maintained by addition of distilled water and filtered through a muslin cloth in a funnel. The residue was rinsed thoroughly until its washing was no longer alkali. The residue was then introduced into an already dried crucible and ashed at $600^{\circ}\text{C} \pm 200^{\circ}\text{C}$

$$\text{Crude Fiber} = \frac{\text{Final Weight of Crucible} - \text{Initial weight of crucible} \times 100}{\text{Weight of Sample}}$$

Determination of Ash Content: 2g of samples were weighed into well incinerated crucibles and burnt into ash in a muffle furnace at 600°C for 3 hours.

Determination of Heavy Metal Concentrations: Various heavy metals like Zn, Pb, Cd, Cr and Fe were analyzed from bitter leaves. The sample were analyzed for heavy metal content the zinc (Zn), lead (pb), cadmium (Cd), Chromium (Cr) and iron (Fe) were determined using Atomic Absorption Spectrophotometer (AAS) using the method described by (AOAC, 1995).

Results and discussion

Table 1: Proximate Composition of *Vernonia amygdalina* (Bitter Leaves)

Parameters	Value (g/100g)
Moisture	10.12
Dry Matter	39.88
Fat	1.66
Ash	6.38
Crude Fibre	6.19
Crude Protein	22.86
Carbohydrate	52.80

Table 2: Heavy Metal Concentrations of *Vernonia amygdalina* (Bitter Leaves) and WHO Limits

Heavy Metals	Value (mg/kg)	WHO (mg/kg)
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Zinc (Zn)	0.096	3.0
Lead (Pb)	0.041	0.0
Cadmium (Cd)	ND	0.03
Chromium (Cr)	0.005	0.05
Iron (Fe)	0.316	3.0

Keys: **ND= Not Detected**

From table 1, presented above, the proximate constituent obtained from the bitter leave had the highest value of carbohydrate content (52.80g/100g) which Indicated that the leaf protein concentration is not a good source of energy since food carbohydrate is an important source of energy and dietary fibre. The value is lower compared to other leafy vegetables like *Tribulus terrestris* which contains value of 55.67% and 54.20% reported for water spinach leaves (Asibey-Berko and Tayie, 1999). Fat content had the highest value of (1.66g/100g) compared to 0.3% reported for spinach leaves and 0.40% Chaya and 1.6% for *Amaranthus hybridus* leaves (Nwaogu *et al.*, 2007).

It was revealed that the heavy metal concentration present in bitter leaf (*Vernonia amygdalina*) had Zinc content that is less than the recommended limits, while lead recorded higher values more than the required value. This implied that the bitter leaves contain the Zn in right proportion. Iron content of 0.316 mg/100g was significantly lower compared with WHO allowable limit of (3.0 mg/100g) (Abimbola *et al.*, 2015). However, *V. amygdalina* was found to contain lower concentration of chromium (0.005mg/100g) than WHO allowable limit of (0.05mg/l). This result is in line with the findings of previous studies Yekeen *et al.* (2011) and Abimbola *et al.* (2015) who also reported lower concentration of heavy metal concentrations lower than the WHO limits in the vegetables studied. In the present study, the concentration of cadmium was not detected in the bitter leave sample. This may indicates that consumer of this food material may not be exposed to cadmium toxicity since it was not detected in the vegetable sample.

Conclusion

This study justified the use of bitter leaf in traditional medicine practice as a therapeutic agent due to the highest carbohydrate, dry matter and crude protein contents. The study also revealed the level of heavy metals in the bitter leaves and that the values were below the recommended daily intake of these metals, except lead (Pb) that had the highest concentration which was higher compared to the WHO standards. Lead is the predominant toxic heavy metals present in bitter leaf, this may indicated that the exhaust from the moving vehicles along roads may sink to the soil for uptake by plants. These can be hazardous if the bitter leaves are taken in large quantities but the detrimental impact becomes apparent only after decades of exposure.

Recommendation

From the study it is recommended that further work on proximate and heavy metals should be done on (*Vernonia amygdalina*) bark and root considering, age of the plants, different locations to ascertain proximate and heavy metal concentrations in them for further studies.

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