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Estimation of tannins, alkaloids, saponins and proximate composition of *Vernonia amygdalina* (Del) root

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Abstract
Quantitative chemical compositions of *Vernonia amygdalina* Del. root were assessed by determining phytochemical and proximate compositions using standard procedures. Phytochemical analysis showed levels of total tannins in root (33.90 ± 0.06 mg %) were less than those in the leaf (69.33 ± 0.67 mg %) and stem (50.70 ± 0.37 mg %); total alkaloids in the leaf was 4.407 ± 0.079 g%, root (1.951 ± 0.037 g%) and stem (1.505 ± 0.045 g%); saponin content in the leaf was highest at 7.92 ± 0.46 g%, the level in the root was 4.00 ± 0.50 g% while the stem recorded the least value of 0.85 ± 0.20 g%. Proximate composition revealed high contents of crude fibre (22.50 ± 1.44) and total ash (13.50 ± 0.87); crude protein content was 2.42 ± 0.23, crude fat (1.95 ± 0.05) in g% dry weight. Moisture content was 81.08 ± 0.72 g% wet weight. Considering the adverse effects of high tannins, alkaloids and saponins, *Vernonia amygdalina* root may be a better alternative to the leaf in the treatment of diabetes mellitus and the high fibre content in the root may play a role in decreasing blood cholesterol and glucose uptake.

Keywords: *Vernonia amygdalina*, tannins, alkaloids, saponins, proximate composition.

1. Introduction

In the practice of traditional medicine, people use plants as medicine to cure diseases. Plants are sources of a variety of bioactive chemical compounds which elicit pharmacological effects. These bioactive compounds are usually referred to as secondary metabolites which are known to be agents of plant therapies [1]. They are classified based on the precursor, the basic structure and biosynthetic pathway, into four categories namely: phenols, alkaloids, terpenoids and non-protein amino acids [2, 3]. These compounds are diverse in distribution, function and chemistry [2] and some of them are capable of precipitating deleterious effects in man and animals, acting to reduce nutrient utilization and/or food intake [4].

Vernonia amygdalina Del. (Family: Compositae) commonly called ‘bitter leaf’ is a common vegetable that is used regularly in many homes. The plant grows without much care in the tropical rain forest and is drought tolerant [5]. It has also been found in Madagascar, Asia and tropical America [6]. *Vernonia amygdalina* is well known as a medicinal plant, with several uses attributed to it [5, 7-10].

The root of the plant is used for diabetic treatment in Nigeria. Scientific investigations have confirmed its efficacy in animal models [11]. Others have reported the leaf having antihyperglycaemic [12], hypolipidaemic and antihyperlipidaemic [13] properties. Also reported to possess antihyperlipidaemic activity is the aqueous root extract [14]. However, there is a dearth of information on the antidiabetic mode of action of diabetic plants.

Our previous study had earlier reported the presence of some secondary metabolites: tannins, alkaloids, saponins among others in the aqueous extract of *V. amygdalina* root, noting the absence of flavonoids [11]. Research has shown that the action of medicinal plants is due to a relatively small number of chemical constituents known as the active principles. Tannins, alkaloids, flavonoids and saponins have been implicated as active components of certain hypoglycaemic plants. For example, three of the alkaloids isolated from *Catharanthus roseus*: Leurosine, vindoline and vindolinine administered in doses of 100 mg/kg to rats with fasting hyperglycaemia exerted a more potent action than tolbutamide at equivalent doses [7]. As first line studies to gain an insight into the mode of anti-diabetic action of *Vernonia amygdalina* root we thought it expedient to estimate the levels of tannins, alkaloids and saponins in the root, leaf and stem for comparison and proximate composition of the root.

2. Materials and Methods

2.1 Chemicals: All reagents used in this study were of analytical grade.

2.2 Plant Material: The leaves, stems and roots (with the barks) were collected as one batch from *Vernonia amygdalina* (Del) growing in a garden at the University of Benin, Benin City, Nigeria and identified at the Plant Biology and Biotechnology Department Herbarium of the same University. The plant parts (except the leaves) were broken into small bits, all were sun-dried for 6 h and then oven-dried at 40 ^{0}C till a constant dry weight was recorded. Dried pieces of the plant parts were subsequently milled (Thomas-Wiley machine, England) into powder and stored in air-tight containers until required for analysis.

2.3 Phytochemical Analysis

The presence of tannins, alkaloids and saponins in the aqueous and ethanolic extracts of the leaf, stem and root of *V. amygdalina* were detected by the methods described by Harborne; Odebiyi and Sofowora; Sofowora [15-17].

2.3.1 Estimation of Tannins: Total and condensed tannins were estimated in the leaf, stem and root of *V. amygdalina* using the spectrophotometric methods of Okuda *et al*. [18] and Broadhurst and Jones [19] respectively. Tannins are generally extracted using aqueous organic solvents, mainly methanol and acetone.

2.3.1.1 Condensed tannins: The corrected Vanillin – HCl assay for condensed tannins is an exo-type reaction in which vanillin reacts with the meta-substitute A-ring of tannins (flavanols) to form a chromophore. The number of flavanols is proportional to the absorbance of the solution. The test samples were run along with standard tannic acid solution (10 mg/ml) in distilled water. The absorbance at 500 nm was read against a reagent blank. To allow for the presence of anthocyanins, a parallel measurement was made where methanol replaced the vanillin solution and the absorbance due to anthocyanins was subtracted from the first measurement.

2.3.1.2 Total tannins: The method developed by Okuda *et al*. [18] for total tannins is based on the relative affinity of tannins to methylene blue. Test samples were run along with standard tannic acid (100 mg/ml) in water and the absorbance read at 660 nm.

2.3.2 Estimation of Alkaloids: Alkaloids were determined in the leaf, stem and root by the gravimetric method of Harborne [15]. 200 g of the milled sample was weighed into 500 ml conical flask and 1000 ml of 10% acetic acid in ethanol added, covered, allowed to stand for 4 hours and then filtered. The filtrate was concenrated on a water bath at 60 ^{0}C to one quarter of its original volume and concentrated aqueous ammonium hydroxide solution added drop-wise to the extract in order to precipitate the alkaloids. The whole solution was centrifuged (B. Bran, England) and the pellet (precipitate) collected and washed with 15% ammonium hydroxide solution. The precipitate, which is the alkaloid, was dried in an oven (Gallenkamp, England) at 60 ^{0}C for 30 min and weighed. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

2.3.3 Estimation of Saponins: Saponins were determined using the method of Obadoni and Ochuko [20]. 20 g of each milled sample (leaf, stem and root) was put into different conical flasks and 100 ml of 20% aqueous ethanol added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{0}C. The mixture was then filtered and the residue again extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over a water bath at about 90 ^{0}C. The concentrate was transferred to a 250 ml separatory funnel and 20 ml diethyl ether added with vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extract 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 (Gallenkamp, England) to a constant weight, and saponin content calculated as a percentage.

2.4 Proximate Analysis: Proximate composition to determine moisture, crude fibre, crude lipid, crude protein and total ash were carried out by the methods of AOAC [21].

2.5 Statistical Analysis: All quantitative analyses were carried out in triplicates and results expressed as Mean ± SEM.

3. Results and Discussion

Qualitative analyses of tannins, alkaloids and saponins in aqueous and ethanol extracts from *V. amygdalina* leaf, stem and root are presented in Table 1. The presence of phytochemicals suggests both physiological and medicinal activities [17]. From the general screening (Table 1) it was observed that alcohol is a better solvent for extracting tannins, alkaloids and saponins. This is in agreement with the report of Trease and Evans [22] and also El-Astal *et al*. [23] who compared the antimicrobial activity between the aqueous extract and alcoholic extract of *V. amygdalina* leaves. They observed that the alcoholic extract had higher antimicrobial activity than the aqueous extract.

The quantitative analyses showed the leaf and stem were rich in tannins with the root recording the lowest value. This agrees with the general knowledge that secondary metabolites are mostly concentrated in the leaf. Our results fall within the range for roots and tubers, from 20.00 mg% for *Dioscorea rotundata* (white yam) to 75.00 % for *Dioscorea alata* (water yam) [24]. The level of total tannins obtained in this study for leaf and stem is comparable to the 59% of Lima beans (*Phaseolus lunatus*) reported by Egbe and Akinyele [25] and the 57.5% reported for *Dioscorea esculenta* (cassava) by Udoessien and Ifon [26]. The concentration of condensed and total tannins were in the order of leaf > stem > root. The present study is in contrast with that of Chakraborty and Eka [27] who reported the absence of condensed tannins in the stem and root of *V. amygdalina*. Wide variability in published data may be due to the methods of analysis. Deshpande *et al*. [28] reviewed the chemistry of phenolic compounds and the methods of analysis, including the vanillin-assay, oxidation – reduction methods, and protein precipitation methods. They discussed the extraction procedures, the effects of storage time, the choice of the standard and stated a preference for the corrected – vanillin - HCl assay (which was used in this study) over the redox assays [24]. A hypoglycaemic drug based on the seeds of Syzygium (which contains galli- and elagi-tannins) was put on the market in France [7]. Thus the presence of tannins in *V. amygdalina* lends credence to its antidiabetic properties. However, tannins are known to form complexes with proteins [29] and these complexes can precipitate dietary protein thus rendering them unavailable for digestion. Apart from proteins, tannins also complex divalent metals, cellulose, hemicellulose, pectin and other carbohydrates [30]. Consumption of large amounts of tannins is therefore deleterious to health. The tannin content of the root of *V. amygdalina* was the lowest (Table 2) and therefore appears to be safest for treating diabetic persons, when compared to the leaf and stem.

Results obtained from the quantitative evaluation of alkaloids (Table 2) showed the leaf has the highest percentage of alkaloids, the root and the stem having lower levels. Alkaloids possess antimalarial activity and this may be the reason why the extracts of leaf and root of *V. amygdalina* are given to persons ill with malaria to cure the illness [19]. Plant families such as Leguminosae, Amaryllidaceae and Compositae (of which *V. amygdalina* is one) are noted for higher levels of these compounds [31]. Although some alkaloids are among the most powerful poisons known, a good number can be taken in fairly large doses without danger. Many derived drugs are alkaloids, well known examples include morphine, codeine, cocaine, caffeine, nicotine, emetine, atropine and quinine [32]. Fenugreek seed (*Trigonella foenum - graecum)* contains an alkaloid compound trigonelline which is thought responsible for some of its action relating to lowering of blood sugar. It has also been effective in lowering overall cholesterol and triacylglycerols [33]. The presence of alkaloids in *V. amygdalina* thus supports its use in diabetes treatment where blood cholesterol levels are elevated apart from high blood glucose levels. The root having a lower content of alkaloids thus appears to be a better choice in treating diabetics compared to the leaf. This is because many alkaloids interfere with liver and kidney functions [34].

Our results (Table 2) revealed the leaf of *V. amygdalina* had the highest concentration of saponins followed by the root, while the stem recorded the least concentration. The presence of saponins in the leaf, stem and root of *V. amygdalina* agree with the report of Gill [8]. The values recorded in this study are much higher than those reported by Osagie *et al.* [35] for *Cajanus cajan* (pigeon pea) and *Manihot utillisima* (cassava tuber).

Table 1: Qualitative analysis of some secondary metabolites of *Vernonia amygdalina* in aqueous and alcohol extracts of leaf, stem and root.

Sample	Tannins		Alkaloids				Saponins	
	$FeCl_3$ Test		Mayer's Test		Dragendorff's Test		Frothing Test	
	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract
Leaf	+	+	+	+	+	+	+	+
Stem	+	+	+	+	+	+	+	+
Root	+	+	+	+	+	+	+	+

Table 2: Quantitative analysis of some secondary metabolites in the leaf, stem and root of *Vernonia amygdalina*

Plant Metabolite	Concentration		
	Leaf	Stem	Root
Tannins: Condensed	24.40 ± 0.92	2.30 ± 0.13	0.37 ± 0.03
(mg/100g) Total	69.33 ± 0.67	50.70 ± 0.39	33.97 ± 0.06
Alkaloids (g%)	4.407 ± 0.079	1.505 ± 0.045	1.95 ± 0.037
Saponins (g%)	7.92 ± 0.46	0.85 ± 0.20	4.00 ± 0.50

Values are Means ± SEM of triplicate analysis

Saponins possess both beneficial and deleterious properties depending on its concentration in the sample [36]. The nutritional significance of saponins stems largely from their hypocholesterolaemic action, suggesting they may prove useful in the control of human cardiovascular disease [36] and also diabetes mellitus where blood cholesterol levels are raised along with elevated glucose levels. The biological properties of saponins suggest that they may also have some anti-carcinogenic effect [37], through a number of mechanisms. Saponins are present in a number of herbal remedies e.g. ginseng, liquorice, sarsaparilla, which appear to have expectorant and anti-inflammatory effects [38]. Apart from their use as expectorants, saponins are generally used as mild laxatives and diuretics. Thus, the presence of saponins explains the use of *V. amygdalina* as a laxative and diuretic. The search for saponins in plants has been awakened by the need for readily available sources which can be of therapeutic value. *V. amygdalina* plant can be a ready source of saponins since it grows well with little or no care. A well-known toxic effect of saponins, though, is its ability to lyse erythrocytes (haemolysis). A high dose of 300 mg/kg body weight of saponins given orally to rats caused diarrhoea, restlessness and histopathological changes in liver and kidney, ultimately leading to death [39]. Again, the root with its lower saponin content compared to the leaf seems a safer option for long term use in diabetic treatment.

Proximate composition of *V. amygdalina* root is presented in Table 3. The lipid content was the lowest while moisture content was the highest among the components analysed (Figure 1). The high moisture content suggests it would be liable to microbial spoilage if not properly dried or dehydrated before storage. The lipid content of 0.2% of *D. esculenta* (cassava) [40] is less than that reported in this study for the root. A low value for crude protein was also recorded for *V. amygdalina* root in the present study. The value falls slightly outside the range of 0.55% of *D. esculenta* to 2.2% of *D. alata* reported for some edible roots and tubers [40]. Total ash content for *V. amygdalina* root was moderate and higher than that reported for *D. esculenta*, an edible root. The value reported in our study is comparable to some legumes [41]. Ash content is a reflection of the concentration of minerals in the plant sample. The crude fibre recorded in this study for *V. amygdalina* root is the only proximate component higher than those of the leaf reported by other researchers [42, 43].

Table 3: Proximate composition of *Vernonia amygdalina* root

Component	Value (% composition)
Moisture	81.08 ± 0.72
Crude protein	2.42 ± 0.23
Crude fat	1.95 ± 0.05
Crude fibre	22.50 ± 1.44
Total Ash	13.50 ± 0.87

Values (except for moisture) are expressed on dry weight basis and are Means ± SEM of triplicate analysis.

The crude fibre is the plant polysaccharide and lignin, which are resistant to hydrolysis by digestion with acid and alkali [21]. *V. amygdalina* root is very woody hence the high fibre value recorded. Epidemiological studies suggest that increased fibre consumption may contribute to a reduction in the incidence of certain diseases of the digestive tract such as constipation and colon cancer.

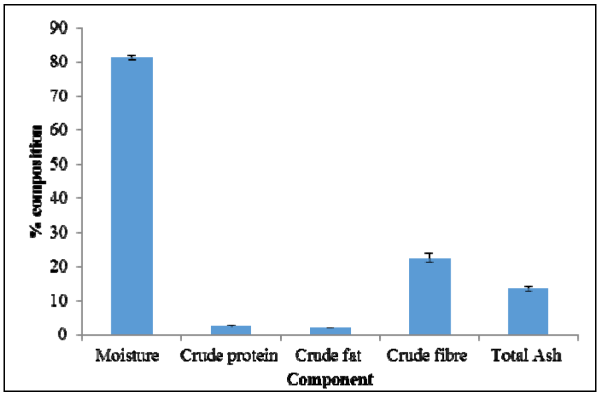

Fig 1: Proximate composition of *Vernonia amygdalina*

Fibre also plays a role in obesity, hypertension, coronary heart disease and diabetes [44, 45]. In terms of diabetes mellitus, fibre (lignins) binds cholesterol thus decreasing blood cholesterol. Also, fibres decrease the rate of gastric emptying, hence slowing the rate of absorption of many nutrients including carbohydrates. Thus, both rise in blood sugar and the subsequent rise in insulin levels are significantly decreased due to the slow rate at which carbohydrates are digested and absorbed [46].

4. Conclusion

Both the leaf and root of *Vernonia amygdalina* Del. are used in treating diabetes mellitus as well as other ailments in Nigeria. The present study has shown that the leaf, stem and root of *V. amygdalina* contain tannins, alkaloids and saponins, known hypoglycaemic agents, with the leaf having the highest concentrations. *V. amygdalina* is thus a potential source of novel drugs. The lower levels of these phytochemicals recorded in the root makes it a better alternative compared to the leaf in diabetes treatment, where cumulative doses may prove to be deleterious, considering the adverse effects of high concentrations of tannins, alkaloids and saponins. The high fibre content of the root may play a role in the mode of antidiabetic activity of the root by binding cholesterol and decreasing rate of sugar uptake. Further studies are warranted in order to isolate and characterize the bioactive principles.

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