Proximate Composition of Five Accessions Of *Vernonia amygdalina* (Del) in South Eastern Nigeria

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Abstract
The proximate analyses of five accessions of bitter leaf collected from five different locations in South Eastern Nigeria, were investigated. Vernonia amygdalina, popularly known as "bitter leaf", is one natural product in high demand both as an edible vegetable and a herbal drug, hence, the need for this comparative study to identify the richest accession of the therapeutic herb in the South Eastern region of the country. Standard procedures for proximate analyses were employed in this study. The proximate content analyzed included ash(A), crude fibre(CF), crude protein(CP), ether extract(EE) and nitrogen free extract (NFE). The results obtained showed 21.11% CP, 25.61% EE, 16.47% CF, 17.64%A and 21.83% NFE for the CBL accession; 21.11% CP, 25.61% EE, 17.64% CF, 17.64% A and 21.29% NFE for the UBL accession; 25.37%CP, 8.0%EE, 24.0%CF, 12.0%A and 30.63%NFE for Onitsha accession; 26.69%CP, 8.5%EE, 18.50%CF, 11.0%A and 35.31%NFE for Eket accession; 29.31%CP, 8.5%EE, 13.50%CF, 10.0%A and 38.69% NFE for Ugep accession. These results present the percentage proximate composition of the five accessions of V. amygdalina.

Keywords: Vernonia amygdalina, Accessions, Proximate composition, bioactive, Phytocomponents.

Introduction

Vernonia amygdalina (Fig. 1) commonly known as "bitter leaf" is a house-hold herb in Nigeria. It is locally called 'ewuro'(Yoruba), 'etidot' (Efik/Ibibio), 'onugbu' (Igbo), 'Ityuna'(Tiv), 'oriwo' (Bini) and 'Chusardoki,'(Hausa). [1] *Vernonia amygdalina* is a shrub or small tree of 2.5m tall(much taller varieties have been seen in home gardens in Calabar), much branched; trunk up to 40cm in diameter; bark grey to brown, smooth becoming fissured; young branches, densely pubescent, leaves alternate, simple, stipules absent; petiole 6mm in diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste. The bitterness is caused by sesquiterpene lactones (e.g. *vernodalin, vernolepin* and *vernomygdin*) and steroid (glycosides) vernoniosides, [2]. It is propagated by seed but most farmers use stem cuttings. Cuttings used for propagation from mature stems are selected on the basis of attributes such as degree of bitterness, leaf size and growth characteristics. Cuttings may be planted erect or slanting at an angle of 45^0C to obtain more side shoots, and cuttings grow faster than seedlings [3]. *V. amygdalina* has been domesticated in some parts of West Africa, Nigeria inclusive [4]. The plant has a wide ethnobotany presence within Africa - Togo, Kenya, Tanzania, Cameroon, Ghana, etc. [1] and in other countries like America, Madagascar and Asia [5]

Besides its use as a vegetable in the popular bitter leaf soup, all parts of the plant has found usefulness in folk medicine [4; 6; 7; 8; 9]. Leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough. It is also used as a laxative and as fertility inducer.

In Nigeria, the leaves are placed on wounds serving as substitute for iodine. One of the most common medicinal uses of *Vernonia amygdalina* is as a treatment against intestinal worms including nematodes. Not only humans but also Chimpanzees ingest the pith of *Vernonia amygdalina* for the control of intestinal nematode infections [10]. Bark infusions are also taken to treat fever and diarrhoea, and dried flowers as a cure against stomach disorders. *Vernonia amygdalina* is also useful as a control agent against disease in plants. The ash from burnt branches is used to control seed-borne fungi (*Curvularia, Aspergillus, Fusarium* and *Penicillum spp*); thus ameliorating seed viability and germination capacity [11].

With the recent tilt towards ethno medicine in the country, it becomes necessary to identify and quantify the bioactive components of some of the most commonly consumed accessions of *Vernonia amygdalina* and to carryout comparative studies on these accessions in an attempt to present the most valuable accessions for inclusion into the tradomedicine database in the country.

Materials and Methods:

Five accessions of *Vernonia amygdalina* were collected from five different locations in the South Eastern part of Nigeria.. The freshly plucked leaves were collected from Calabar (CBL), Uyo (UBL), Eket (EBL), Onitsha (OBL) and Ugep (UGBL). They were wrapped in a newspaper to avoid decomposition from the site of collection to the experimental laboratory. The identification and authentication was done by Mr. Frank of the Department of Botany, University of Calabar, Calabar.

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Fig. 1: *Vernonia amygdalina plant*

Preparation of Samples: The leaves of each of the five samples of *V. amygdalina* were washed to remove contaminants and air-dried for three (3) days at room temperature. The dried samples were pulverized using a blender to obtain a fine powder. 20g of each sample was kept in air-tight containers and stored at 4^0C for later analyses.

Proximate Analysis: Standard procedures were used for the proximate analysis. The proximate content of the different plant accessions were determined according to [12] method.

Determination of Crude Protein Content (Kjedhal method): 2g of the plant powders were weighed in a Kjedhal digestion flask. The sample was acid digested by adding 20ml Conc. H_2SO_4, Kjedhal catalyst and a pinch of anti-bumping chips. The mixture was incinerated to gentle boiling and then heated strongly to clear the digest. The samples were then removed, cooled and transferred to a 700ml volumetric flask and made up to mark. The sample digest was introduced into the dumps sample tube and steam heated. 40% NaOH solution was added to the digest and the digested steam distilled into the Erlenmeyer flask and titrated with 0.1NHCL to a pink end point and the nitrogen content calculated. The nitrogen value was multiplied by 6.25(constant) to obtain the crude protein content.

Determination of Ash Content: A crucible was ignited at 550^0C for 3 hours, cooled in a desiccator and weighed. 7g of each sample was placed in the crucible, lid replaced and weighed. It was then ignited at 550^0C for 24 hours, cooled and weighed again. The ash was expressed as a percentage of the original dry weight.

***Estimation of Crude Fat (ether extract method)*:** 10g of each sample was weighed into the fat extraction thimble. 120ml of petroleum ether (bp 40^0-60^0C) was poured into a previously dried and weighed round bottom flask. The soxhlet extractor into which the thimble with its content has been introduced was then filtered into the round bottom flask and extraction apparatus set up with flask sitting on the space provided on the bath. The contents of the flask were heated. As the ether evaporated, it condensed and dropped into the thimble where it extracts the ether soluble constituents into the round bottom flask. The thimble was then removed and dried in the oven at 50^0C. The petroleum ether contained in the round bottom flask was distilled off and the lipid extract left in the flask. The round bottom flask with the lipid extract was then dried finally in an oven at 100^0C and weighed. This was to ensure total eradication of the petroleum ether. The amount of lipid extracted was obtained from the difference between the weight of the flask before and after extraction.

Determination of crude Fibre

Acid digestion: The fat free material (10g) was weighed and quantitatively transferred into a 400ml beaker which had been previously marked at 200ml. 50ml of 1.25% sulphuric acid was added and the mixture was made up to the 200ml mark with distilled water. The contents of the beaker were heated to boiling point for 30 mins.The content of the beaker were filtered through a Buchner funnel with acid of a suction pump. The residue was washed with hot water until it was acid free.

Base digestion: The residual left after acid digestion was quantitatively transferred into the 400ml beaker; 50ml of 1.25% (W/V) NaOH were added and made up to the 200 ml mark with distilled water.

The mixture was again heated for 30 minutes with constant stirring. The content of the beaker were filtered through the Buchner funnel and washed several times with hot water until it was washed twice with 95% methanol, quantitatively transferred into a porcelain crucible and dried at 100%.

Ignition: The weight of the oven dried residual was noted and the residual ignited in a furnace at 550^0C. The weight of the ash left after ignition was also noted.

Calculation: The crude fibre content was determined from the loss in weight of crucible and its content after ignition.

Determination of Nitrogen Free Extract (NFE): This was obtained by the difference obtained after subtracting the percentage crude protein, lipid, ash and fibre from 100g dry matter.

Statistical Analyses: All data collected were based on bioactive components of the herb. Percentage compositions were calculated using mean values. RCBD was used to analyse these results with the replicates as blocks and the accessions as treatment. Statistical analysis involved univariate Analysis of variance (ANOVA) using PASW ver. 18. LSD was used to compare significant means.

Results and Discussion

Results for the proximate analyses of bioactive components of five accessions – CBL,UBL, OBL, EBL and UGBL of *Vernonia amygdalina* are presented on Table 1 and Fig.2. Table 1 shows the mean values for proximate components thus: crude protein (CP) ranging from 25.37-29.75; Ether extract (EE) ranging from 7.0 - 8.5; crude fibre (CF) from 13.50-24.0; Ash (A) from 9.0-12.0 and Nitrogen free extract (NFE) (carbohydrate) from 30.63 - 40.25. Fig 2 gives the percentage (%) composition of bioactive components to be 21.11%, 21.11%, 18.0%,

Table 1: Proximate analysis of five accessions of *V. amygdalina* ($\bar{x} \pm SE$)

Bioactive Components	CBL	UBL	OBL	EBL	UGBL
Ether extract	$7.0^{a} \pm 0.53$	$7.0^{a} \pm 0.087$	$8.0^{b} \pm 0.015$	$8.5^{c} \pm 0.038$	$8.5^{c} \pm 0.0098$
Crude fibre	$14.0^{d} \pm 0.24$	$15.0^{c} \pm 0.02$	$24.0^{a} \pm 0.28$	$18.50^{b} \pm 0.04$	$13.50^{e} \pm 0.05$
Ash	$9.0^{d} \pm 0.07$	$9.0^{d} \pm 0.04$	$12.0^{a} \pm 0.07$	$11.0^{b} \pm 0.21$	$10.0^{c} \pm 0.03$
NFE	$40.25^{a} \pm 0.44$	$39.25^{b} \pm 0.32$	$30.63^{e} \pm 0.82$	$35.31^{d} \pm 0.43$	$38.69^{c} \pm 0.18$
Crude protein	$29.75^{a} \pm 0.03$	$29.75^{a} \pm 0.05$	$25.37^{d} \pm 0.03$	$26.69^{c} \pm 0.17$	$29.31^{b} \pm 0.29$

18.94% and 20.8% for CP; 25.61%, 25.61%, 29.27%, 31.1% and 31.1% for EE; 16.47%, 17.64%, 28.23%, 21.76% and 15.88% for CF; 17.64%, 17.64%, 23.52%, 21.56% and 19.6% for Ash and 21.83%, 21.29%, 16.61%, 19.17% and 21.01% for NFE for the five accessions studied respectively.

Figure 2 is a bar chart representation of the percentage compositions, comparing the proximate components of the five accessions of *Vernonia amygdalina.* Analysis of variance (ANOVA) results showed that the proximate composition of the five accessions of *Vernonia amygdalina* were highly significantly different ($P<0.01, 0.05$). This could be attributed to different growth conditions in the different environments they grow under, which may include different land uses (as home gardens, farmlands, crop fields or fallows) [13], soil type, manuring system and even mode of propagation. From the results obtained, the CBL and UBL accessions both had the highest crude protein contents, at 21.11% (Table 1). This is a slightly lower result than that reported by [5] on the crude protein level of *Vernonia amygdalina* which was 33.3% (Imo accession). On the other hand, the values are higher than crude protein values (23%) reported by Atangwho and colleagues[10] for the Calabar accession. [14] recorded 21%, a score lower than any recorded in our study on the five accessions. The lowest crude protein value obtained was that from OBL accession which was 25.37%. It would appear that if the purpose of consumption is protein related, either the CBL or UBL accessions would be recommended.

Values from the ether extracts ranged between 7.0% and 8.5%. The ether extract content is an indication of the crude fat content. Results from this study were lower than results recorded by Anyasor et al. [15]. The higher fat content recorded could be as a result of the ecological differences between their *Vernonia amygdalina* samples collected from Southwestern Nigeria and the samples used in this study. From our results (Table 1) *Vernonia amygdalina* has a low fat content and therefore does not pose any fat related health risks when consumed. Again CBL and UBL accessions scored the lowest fat content (7%) which gives them an edge over the other accessions. [16] recorded 4.24% for ether extract. This score is lower than that recorded in this study and also much lower than results reported by Anyasor et al. [15] obtained from another Southwest accession.

Crude fibre scores ranged from 13.50 (UBL) to 24% (OBL). [17] recorded a higher crude fibre content of 15.30% . Since fiber as a dietary requirement helps in the digestibility of food, the OBL accession will be the choice leaf for easy digestibility requirements.

Ash contents scored ranged between 9% - 12%; with the lowest being CBL and UBL accessions and the highest being OBL accession. The results are slightly higher than those scored by Anyasor et al. [15] . Ash contents scored

by previous investigators [18] were as high as 12.48% total ash and gives an idea of the amount of mineral elements present in the sample being

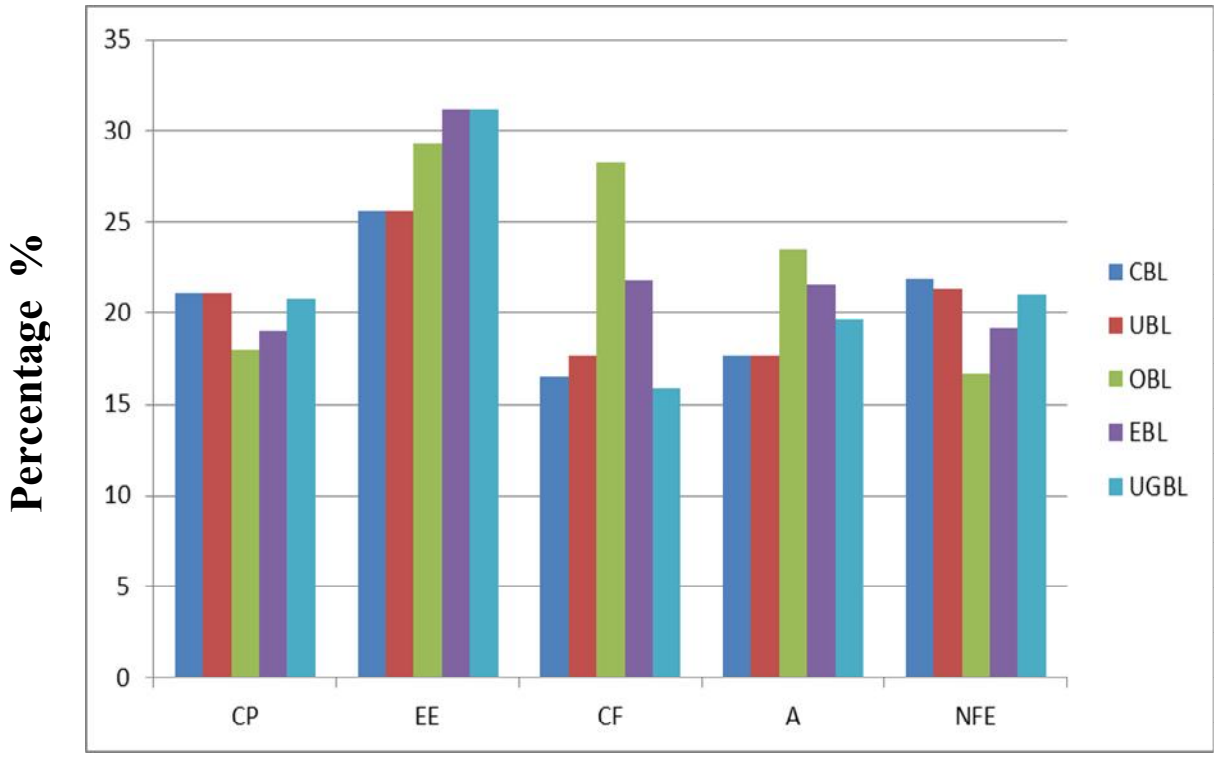

Fig. 2 : % Proximate composition of nutritional components in *V. amygdalina*

investigated [12]. The nitrogen free extract (NFE) which indicates the carbohydrate content of sample, ranged between 30.63% - 40.25% (Table 1) with the OBL accession scoring the lowest and the CBL accession, the highest. The NFE scores recorded in this study (Table 1) were lower than that reported for the Abeokuta accession [15]. However, Atangwho and colleagues [10] had earlier reported a lower score (51.37%) from another Southwestern Nigerian accession. The five accessions under investigation are rich energy-giving vegetables. The CBL accession also presented as the richest carbohydrate source among the 5 accessions studied.

From this comparative study, the CBL and UBL accessions had the highest scores for crude protein and carbohydrate. The UBL accessions however had the lowest ether extract and ash contents. Though these accessions are rich in protein and carbohydrate, and low in fat content, they will not be a good source of minerals. On the other hand, OBL accession, with the highest ash and crude fibre contents will present with the highest mineral content and will also prevent the uptake of excess starchy foods better than the other accessions.

Analyses of variance test showed highly significant differences ($P<0.05$) between the five accessions. Apart from its rich medicinal value, *V. amygdalina* is also a rich source of nutrients that will meet dietary needs of consumers for therapeutic and general well-being. . Further genetic and molecular analysis of this rich herb using genetic tools such as random amplified polymorphic DNA (RAPD) markers is recommended in order to establish the source of the observed differences between these accessions of the herb, *V. amygdalina*. .

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