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Nutritional and Antimicrobial Properties of *Vernonia amygdalina* Leaves.

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ABSTRACT: Fresh green *Vernonia amygdalina* leaves were anaysed for protein content, moisture, ash, minerals and antimicrobial activity. Leaves had a moisture content of 83.0% (dry matter, 17.02%), a protein content of 1.30% and ash content of 0.50%. Mineral content was as follows: phosphorus, 61.55 µg g^{-1}; selenium, $8.2x10^{-3}$µg g^{-1} ; iron, 4.71µg g^{-1} and zinc, 1.13µg g^{-1}, based on fresh weight of leaves. The aqueous extract of the leaves inhibited the growth of the gram +ve bacterium *Staphylococcus aureus* and the gram –ve bacterium *Escherichia coli*. The nutritional and food processing implications of the results are discussed.

Key words: *Vernonia amygdalina* leaves, protein ,moisture, ash, minerals, antimicrobial, *Staphococcus aureus*, *Escherichia coli.*

Introduction

Vernonia amygdalina, variously known as bitter leaf (English), oriwo (Edo), ewuro (Yoruba), shikawa (Hausa), and olubu (Igbo), is a tropical shrub, 1-3m in height with petiole leaf of about 6mm in diameter, and elliptic in shape (Igile *et al*, 1995). The leaves are dark green coloured with a characteristic odour and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub-Saharan Africa (Bosch *et al*, 2005).

The leaves are eaten, after crushing and washing thoroughly to remove the bitterness (Mayhew and Penny, 1998). All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others (Gill, 1992, Hamoiona and Saffaf, 1994). Antihelmitic and antimalarial properties (Abosi and Raserika, 2003) as well as antitumourigenic properties (Izevbigie *et al*, 2004), have also been reported for extracts from the plant. Other studies have demonstrated hypoglycaemic and hypolipidaemic effects of the leaf extract in experimental animals (Akah and Okafor, 1992; Nwanjo, 2005).

This paper reports the moisture, protein, ash and mineral content of *Vernonia amygdalina* leaves as well as the effect of their cold water extract on the bacteria *Stapylococcus aureus* and *Escherichia coli*.

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Materials and Methods

Materials

Vegetables.

Leaves of *Vernonia amygdalina were* purchased from Oba Market in Benin City in the month of May 2008. They were fresh and dark green in colour.

Reagents.

All reagents were of analytical grade.

Analytical Methods

Moisture.

Shredded fresh vegetable (10g) was dried in a thermostatically controlled ventilated oven at 105^{o}C until constant weight was obtained. The loss in weight was recorded as moisture content (AOAC, 1984).
Sample Preparation for Protein Ash and Mineral Analysis.

Leaves were cut into tiny pieces and dried in a ventilated oven at 60^{o}C for 5 days to constant weight. The dried vegetable was ground into powder and stored in airtight bottles for analysis.

Crude Protein.

Crude protein was determined by the Kjeldahl method. Dried and pulverised leaf (0.2g) was digested in 2ml concentrated H_2SO_4 in the presence of selenium catalyst, until a clear digest was obtained (AOAC, 1984). The nitrogen content of diluted digest was determined colourimetrically at 630nm according to Charlot (1964). Protein was calculated as: Nitrogen content x 6.25.

Ash.

For the determination of ash content, dried pulverised vegetable was ashed at 550^{o}C in a muffle furnace.

Minerals.

Minerals were obtained by ashing 2.0g dried and ground sample in a muffle furnace at 550^{o}C. The ash was dissolved in 10ml, 20% nitric acid and filtered through acid washed Whatman No. 541 filter paper into a 100ml volumetric flask. The filtrate was made up to the mark with deionised water and the resulting solution was used for the analysis of phosphorus, zinc and iron. Phosphorus was determined colourimetrically by the vanadomolybdate method [9]. Zinc and iron were determined by atomic absorption spectrophotometry at 630nm [10]. Selenium was determined titrimetrically [11]. Analyses were done in triplicate.

Antimicrobial Activity

Preparation of crude water extract of leaves.

Aqueous extract of *V. amygdalina* leaves was prepared by blending 10g fresh leaves in 100ml distilled water. The water extract was filtered through Whatman No. 1 filter paper and assayed for antimicrobial activity.

Preparation of Crude Antibiotic Discs.

Sterile Whatman No. 1 paper was punched into 5mm diameter disc sizes. The Whatman discs were placed in MacCartney bottles and sterilised in an autoclave at 120^{o}C for 15 min. The bottle was transferred into a hot air oven at 60^{o}C to dry for 30min. An aqueous extract of the leaves (1.0ml) was transferred into a sterile Bijou bottle containing sterile discs. The sterile crude discs were allowed to soak in extract for 6hr for proper absorption, after which they were removed and allowed to dry (Cheesebrough, 2000).

Antimicrobial Assay of Extract.

Aqueous extract of *V.* amygdalina leaves was evaluated in vitro for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

Nutrient agar (7g) was added to 250ml distilled water in a flask. This was stirred and autoclaved at 115^{o}C and then cooled to 50^{o}C. A portion of the medium (20ml) was poured into a sterile Petri dish and allowed to solidify. The sterility of the medium was confirmed by allowing it to stay for 8hr and observing no contamination.

An isolate colony of each test organism was subcultured on nutrient broth and incubated at 37^{o}C for 8hr. This was then spread on the entire plate medium to ensure uniform growth. The crude extract discs were incubated immediately for 24hr at 37 ^{o}C (Okwu and Iruabuchi, 2004). Antimicrobial assays were carried out in quadruplicate. Zones of inhibition were observed using a hand lens for proper magnification, and the zones measured.

Results and Discussion

Bitter leaves were purchased freshly cut and dark green in colour. The moisture, protein and ash content of the leaves are given in Table 1. Included for comparison are data for some locally available leafy vegetables-cassava leaves, cabbage, cowpea leaves and sweet potato leaves.

Table 1. Moisture, protein and ash content (g/100g) of *V. amygdalina* leaves.

Characteristics	*V. amygdalina*[a]	Cassava[b]	Cabbage[b]	Cowpea[c]	Sweet potato[b]
Moisture	82.0	80.0	79.0	85.0	83.0
Protein	1.3	6.0	1.4	4.7	4.6
Ash	0.5	-	-	-	-
Colour	Dark green	Dark green	Dark green	Dark green	Dark green

[a]This study. [b]van Gastel and van den Wijingaart (1997). [c]Madamba *et al* (2006).

The dominant constituent of fresh *V.amygdalina* leaves was water, which accounted for 83.0% of their weight. Fresh leaves had a protein content of 1.3% and an ash content of 0.5%. The leaves had moisture content similar to published values for cassava, cabbage, cowpea and sweet potato leaves. Protein content of leaves was low and similar to the published value for cabbage, but was lower than published values for cassava, cowpea and sweet potato leaves (van Gastel and van den Wijingaart, 1997; Madamba, 2006).

Overall, foods with a high water and adequate dietary fibre content provide a low energy density contribution to the meal and create a feeling of satiety. Generally, fresh leafy vegetables have low protein content. This protein is mostly in the form of enzymes, rather than acting as a storage pool, as in grains and nuts (Wills *et al*, 1998). The leaves of cassava, cowpea and sweet potato, and cabbage appear to be better sources of dietary protein than *V. amygdalina* leaves. Thus fresh *V. amygdalina* leaves may not be an important source of dietary protein.

Table 2 shows the mineral content of *V. amygdalina* leaves. Included for comparison are the mineral content of cassava, cabbage and cowpea leaves, and the recommended daily allowances (RDA) for the minerals.

Table 2. Mineral content (µg g^{-1}) of *V. amygdalina* leaves.

Mineral	*V. amygdalina*[a]	Cabbage[b]	Cowpea[c]	RDA[d] (% of RDA[f])
Phosphorus	61.55	540.0	90.0	1300 (0.47)
Selenium	$8.2x10^{-3}$	-	-	0.055[e] (1.50)
Iron	4.71	6.0	19.0	10-15 (3.1-4.7)
Zinc	1.13	3.0	3.0	15 (0.75)

[a]This study. [b]Eyabi (2001). [c]Madamba *et al* [10]. [d]RDA (Recommended Daily Allowance) in mg (Eyabi, 2001). [e]Wardlaw and Kessel (2002). [f]Percentage of RDA for element that was present in 100g fresh [leaves].

Phosphorus constituted 61.55µg g-^{1} of fresh *V. amygdalina* leaves. This was lower than the published values for cabbage (540.0µg g^{-1}), and for cowpea (90.0µg g^{-1}). One hundred g of fresh leaves contained 0.47% of the recommended daily allowance (RDA) for this element. Thus *V. amygdalina* leaves contain a modest amount of phosphorus.

Selenium constituted 0.0082µg g^{-1} of fresh leaves. Thus 1.5% of the RDA for this element (0.82µg) was present in 100g leaves, about one-tenth of its content in foods considered good sources of the mineral - one boiled egg (11.0µg), half a cup of oatmeal (10.0µg), a slice of whole wheat bread (10.0µg), or a slice of white bread (8.0µg) (Wardlaw and Kessel, 2002). Iron content was 4.71µg g^{-1} (3.1-4.7% of the RDA for this mineral was present in 100g of fresh leaves). This was lower than the published values for cabbage (6.0µg g^{-1}), for cowpea leaves (19.0µg g^{-1}) (Eyabi, 2001; Madamba, 2006). Zinc constituted 1.13µg g^{-1} of fresh leaves (i.e. 0.75% of the RDA for this element was present in 100g of fresh leaves), less than its content in cabbage (0.3µg g^{-1}), and cowpea leaves (0.3µg g^{-1}) and milk (0.35µg g^{-1}) (Eyabi, 2001; Madamba *et al*, 2006).

The contributions of the minerals studied to their total dietary requirements appear to be small. Thus *V. amygdalina* leaves appear to be a minor dietary source of these minerals. It is important to note however, that the nutritional value of vegetables depends not only on the concentration of nutrients in the produce, but also on the amount consumed in the diet. Since vegetables are usually eaten in combination with other dietary components, some of which may be better sources of the minerals under consideration, this vegetable could be of value in supplementing the minerals available from these. Also the leaves have medicinal properties, making them an important addition to the diet.

The effect of an aqueous extract of *V. amygdalina* on the gram positive bacterium *Staphylococcus aureus* and the gram negative bacterium *Escherichia coli* is presented in Table 3.

Table 3. Zones of inhibition (cm) of bacteria by aqueous extract of V.*amygdalina* leaves.

Microorganism	**Zone of inhibition**
Staphylococcus aureus	0.8
Escherichia coli	0.8

Staphylococcus aureus and *Escherichia coli* exhibited sensitivity to the extract, each giving a zone of inhibition of 0.8cm. Leafy vegetables are high moisture, low acid produce, which support the growth of a wide range of microorganisms. Thus great care is needed when processing them, in order to minimize the

risk of contamination by bacteria that cause food poisoning, especially those, for example *S. aureus* and *E. coli* which produce heat stable toxins that may not be destroyed by heat treatment such as cooking (James and Kuipers, 2003; Eyabi, 2001; Schmidt, 1985). *S. aureus* and *E. coli* are common food poisoning bacteria. The antimicrobial activity of *V. amygdalina* leaves could inhibit the growth of these bacteria on the vegetable itself and (by extraction during cooking, if the antimicrobial principle is heat stable) in food, e.g. sauces and stews in which it is an ingredient, thus protecting the consumer from their harmful effect.

Conclusion

The protein, ash, mineral content and antimicrobial properties of *Vernonia amygdalina* leaves were determined. Fresh *V. amygdalina* leaves had high water and low protein and ash with modest phosphorus content. The other elements studied in this work occurred in appreciable concentrations in *Vernonia amygdalina* leaves. However the potential contribution of the elements studied –phosphorus, selenium, iron and zinc to their RDAs appears to be minor. The cold aqueous extract of the leaves inhibited the growth of *S. aureus* and *E. coli*.

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