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PHYTOCHEMICAL, TRACE AND MINERAL COMPOSITION OF *VERNONIA AMYGDALINA* LEAVES

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ABSTRACT

This study investigated the phytochemicals, trace and mineral contents of leaves of *Vernonia amygdalina* obtained from Benin City, Edo state, Nigeria. The mineral analysis was done using Atomic absorption spectrophotometric analysis method while phytochemical composition was determined using standard methods. The results of the phytochemicals analyses are as follows: saponins (2.50%), alkaloids (1.03%), flavonoids (11.50%), tannins (0.17%), ascorbic acids (46.64mg/100g), beta-carotene (9.05mg/100g), and reducing sugars (45.60%) while the mineral analysis reveals the following: sodium (483.06mg/kg), potassium (627.98mg/kg), magnesium (6,813mg/kg), calcium (12641.76mg/kg), zinc (14.23mg/kg), iron (322mg/kg), phosphate (33.25mg/kg), copper (19.50mg/kg), chromium (3.75mg/kg), and cadmium (4.99mg/kg). Our findings provides evidence that leaves of *Vernonia amygdalina* contain medicinally important bioactive compounds, shows its inert potentials for use as possible supplement in animal nutrition and it justifies their use in traditional medicines for the treatment of different diseases.

Key Words: *Vernonia amygdalina*, Leaves, Minerals, Quantitative, Phytochemicals.

INTRODUCTION

Leafy vegetables are important items of diet in many Nigerian homes. Apart from the variety which they add to the menu (Mepha and Eboh, 2007), they are valuable sources of nutrients especially in rural areas where they contributes substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed & Sharif, 2011). In many developing countries the supply of minerals is inadequate to meet the mineral requirements of farm animals and rapidly growing population.

Vernonia amygdalina commonly called bitter leaf because of its bitter taste is a member of the Asteraceae family, ethnomedically, consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various illnesses (Igile *et al.*, 1995). In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Huffman *et al.*, 1993). *Vernonia amygdalina* extracts have also been reported to help suppress, delay, or kill cancerous cells (Kupchan *et al.*, 1969). 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, Hamowia 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

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and Okafor, 1992; Nwanjo, 2005). We therefore in this study quantitatively analyzed the phytochemical constituents, trace and mineral composition of the leaves of *Vernonia amygdalina.*

MATERIALS AND METHODS

Collection and Preparation of the plant leaves

Fresh leaves of *Vernonia amygdalina* were purchased from a local market in Benin City, Edo state, Nigeria. The leaves were identified by Dr. Chris Akoma, a Botanist in the Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin city, Edo State. The *Vernonia amygdalina* leaves were separated from the stalk, washed and air-dried at room temperature (24^{o}C) and then pulverized, crushed into fine powder and weighed. Aliquot portions of the powdered leaves were weighed and used for mineral and quantitative phytochemical analysis.

Methods for quantitative phytochemical analysis

Determination of alkaloids

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered and allowed to stand for 4 hours. It was then filtered and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise 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 (2M) and then filtered. The residue which represents the alkaloids was then dried and weighed (Harbone, 1973).

Determination of tannin

5g of the sample was weighed into a 50 ml conical flask. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 395 nm within 10 minutes (Van–burden and Robinson, 1981).

Determination of saponins

20g of the sample was weighed into a conical flask followed by the addition of 100 ml of 20% aqueous ethanol and thereafter heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re – extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at about 90^{o}C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether added and shaken vigorously. 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 extracts were 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. Saponin content was calculated as percentage (Obadoni and Ochuko, 2001).

Determination of flavonoids

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 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight (Boham and Kocipai-abyazan, 1994).

Determination of beta-carotene

1g of the sample was weighed into a test tube and macerated with 10ml of acetone and hexane in the ratio of 1:1 and filtered. 10ml of 50% $(NH_4)_2SO_4$ was added and allowed to settle. Then the upper layer was collected and the absorbance read in the spectrophotometer at 450nm using hexane as blank. Beta-carotene content was subsequently estimated using a calibration curve of beta-carotene (1mg/ml) as standard (Alexander and Griffiths, 1993).

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Determination of Vitamin-C content

0.5g of the sample was dissolved in 20ml distilled water and filtered. 2ml of the filtrate was placed into a conical flask and 5ml of 20% metaphosphoric acid added and thereafter made up to 10ml with distilled water. The 10ml was titrated against dye solution (standard indophenols solution) until a faint pink colour persists for 15seconds. Standard indophenols solution was prepared by dissolving 0.05g 2,6-dichloroindophenol in water diluted to 100ml and filtered. To standardize, 0.053g of ascorbic was dissolved in 90ml of 20% metaphosphoric acid and diluted with water to 100ml. 10ml of this solution was pipette into a small conical flask and titrated with indophenols solution until a faint pink colour persists for 15seconds. The vitamin content in the samples was calculated (Adebayo, 2010).

$$\text{Vitamin C in mg/100g} = \frac{\text{Titre value x dye factor x volume made x 100}}{\text{Volume of aliquot x Weight of sample}}$$

Determination of reducing sugar

The DNSA reagent was prepared by dissolving 1.8g of 3,5-DNSA in 20ml of 1.0M NaOH and 60ml of distilled water. Potassium sodium tartarate (60g) was added and the solution was diluted to 200ml with distilled water. 0.5g of the powdered leaf sample in 20ml distilled water was shaken for 15minutes and thereafter filtered and the reducing sugar content was assayed by adding 2ml of

the 3,5-DNSA reagent to 1ml of the sample. The mixture was heated in boiling water for 5min and then cooled under running tap water. The absorbance of the resulting solution was read at 540nm in a spectrophotometer against a blank (distilled water). The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations (Bertrand *et al.*, 2003).

Mineral analysis using Atomic absorption spectrophotometer (AAS)

An acid digest of the powdered leaf was prepared by oxidizing 0.2g of the sample with conc. HCl/nitric acid followed by kjeldahl heating at 70^{o}C until brown fumes disappeared. The digest was diluted with distilled water and heated again (until colourless solution is obtained) to a certain volume and thereafter filtered with Whatmann filter paper No. 1 (110mm). The filtrate was then made up to 100ml with distilled water. Aliquots were thereafter used for mineral analysis using the Atomic Absorption Spectrophotometer (AAS). The blank and working standards were first run followed by the samples. Each sample was analysed twice, and the data reported as a mean of the analysed samples in mg/kg.

Statistical Analysis

Results obtained were expressed as mean ± standard deviation.

RESULTS

The quantitative results of the phytochemicals shown in Table 1 revealed that the leaf of *Vernonia amygdalina* is rich in flavonoids (11.50%), Beta-carotene (9.05mg/100g), ascorbic acid (46.64mg/100g), saponins (2.50%), alkaloids (1.03%), reducing sugars (45.60%) and tannins (0.17%).

The result of mineral elements found in the leaf of *Vernonia amygdalina* are shown in Table 2 with calcium found to have the highest concentration and chromium the least in concentration. The mineral elementa are as follows: potassium (627.98), calcium (12641.76), zinc (14.23), iron (322), copper (19.50), sodium (483.06), Magnesium (6813.60), cadmium (4.99) all in mg/kg.

Table 1. Phytochemical analysis (quantitative) of *Vernonia amygdalina* leaves

Phytochemicals	***Vernonia amygdalina***
Flavonoids	11.50 ± 1.01%
Saponins	2.50 ± 0.41%
Alkaloids	1.03 ± 0.04%
Beta-carotene	9.05 ± 0.3mg/100g
Ascorbic acid	46.64 ± 2.10mg/100g
Reducing sugars	45.60 ± 1.52%
Tannins	0.17 ±0.04%

Values are means ± SD for 3 determinations.

Table 2. Trace and Mineral composition of *Vernonia amygdalina* leaves (mg/kg)

Metals	***Vernonia amygdalina* (mg/kg)**
Sodium	483.06±6
Potassium	627.98±7.81
Magnesium	6813.6±400
Calcium	12641.76±1458
Zinc	14.23±0.89
Iron	322±67
Phosphate	33.25±2
Copper	19.50±0.50
Chromium	3.75±0.25
Cadmium	4.99±0.49

Values are means ± SD for 2 determinations.

DISCUSSION

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants (Ugwu *et al*., 2013) which have been found to be beneficial to human health as well as possessing antioxidant activity (Rafat *et al*., 2008). The quantitative phytochemical analysis shows the richness of *Vernonia amygdalina* leaves in flavonoids, saponins, alkaloids, tannins, betacarotene, ascorbic acid and reducing sugars. Atangwho *et al* (2009) reported *Vernonia amygdalina* to contain 0.87% flavonoids, 0.37% tannins, 2.15% saponins and 2.13% alkaloids while Ndukwe *et al* (2013) reported 0.47% flavonoids, 2.78% Alkaloids, 0.64% Saponins and 0.74% tannins.

It have been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). They also lower the risk of heart disease and provide anti-inflammatory activities attributable to their ability to neutralize and quench free radicals (Pourmorad *et al*., 2006; Omale and Okafor 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995). These observations support the usefulness of *Vernonia amygdalina* in folklore remedies. The highly antioxidant property of flavonoids present in the *Vernonia amygdalina* may act in synergy with other phytochemicals present to produce the medicinal benefits.

Tannins are polyphenols which occur in vascular plant tissues and exist in two forms; condensed and hydrolysable forms. Condensed tannins exist as oligomers and polymers of anthocyanidins, while the hydrolysable tannins consist of gallic acids which are bound to carbohydrates, forming esters (Geissman, 1963). Anti-microbial, as well as many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions have been attributed to tannins (Haslam, 1996). Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells (Adegboye *et al*., 2008). Tannins have been found to form reversible complexes with proline-rich proteins, resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001). Tannins are also known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu and Emineke, 2006; Li *et. al*., 2003; Adegboye *et al*., 2008). Thus, the presence of tannins in *Vernonia amygdalina* supports the traditional medicinal use of this plant in the treatment of ailments caused by microorganisms.

Several alkaloids containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich *et al*., 2005; Gurib-Fakin, 2005). Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity.

Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis *et al*., 2002) and as such may be used as anti-cholesterol agents or cholesterol lowering agent. Studies have it that saponins could possess anti-tumor and anti-mutagenic activities and can lower the risk of human cancer by preventing cancer cells from growing (Forester, 2006). Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Rao *et al*., 1995). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo, *et al*., 2000; Okwu 2004). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al*., 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002). Saponins present in *Vernonia amygdalina* leaves thus supports the usefulness of the plant in managing inflammation.

Vitamin C is a powerful and well-known antioxidant responsible for scavenging free radicals and suppression of peroxidation in both aqueous and lipid region of the cell (Gora *et al*., 2006). It helps to promote healthy tissue growth and repair and also maintain body's natural defense system (Hemila 1999). Ascorbic acid acting as a chain breaking antioxidant interferes with the formation of free radicals during the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine (Beyer, 1994). Vitamin C helps to boosts immunity, fights against infection and improves iron absorption from food. Vitamin C prevent scurvy, a generalized syndrome of tissue damage including disruption of collagen bundles, failure of wound healing, swollen and bleeding gums, weakening of the artery walls. It traps peroxyl radicals before they can initiate lipid peroxidation and helps in the regeneration of Vitamin E (Chatterjee and Nandhi, 1991).

It is well known that enzymatic activities as well as electrolyte balance of the blood fluid are related to adequacy of Na, K, and Mg. Deficiencies of trace elements like Zn, Cu and Mg have been implicated in various reproductive events like infertility, pregnancy wastage, congenital anomalies, pregnancy-induced hypertension, placental abruption, premature rupture of membranes, still births and low birth weight (Pathak and Kapil, 2004). Trace elements and anti-oxidants have also been shown to influence host cellular and humoral immunological functions. The immune system utilizes these essential minerals and factors to meet the demands of challenges by infectious agents (Spallholz *et al*., 1990; Sherman, 1990). Cu and Zn are an integral part of Cu-ZnSOD. Fe is an integral part of catalase, Mn is an integral part of Mn-SOD (Arinola *et al*., 2008). SOD (superoxide dismutase) catalyses dismutation of superoxides to H_2O_2, which must be removed by catalase or glutathione peroxidase. Deficiencies of Zn and Cu decrease activities of SOD (Ellis and Salt, 2003).

Zinc is very important for nerve function and normal sexual development especially for the development

of testes and ovaries. Zinc stimulates the activity of vitamins, formation of red and white corpuscles (Claude and Paule, 1979), healthy functioning of the heart and normal growth. Zinc is involved in normal function of immune system and is a component of over 50 enzymes in the body (Okaka *et al*., 2006). An estimated 20% of the world population is reported to be at risk of inadequate zinc intake (Hotz and Brown, 2004). Studies on Nigerian shows that zinc deficiency affects 20% of children less than five years; 28.1% of mothers and 43.9% of pregnant women (Dioxin and Haris, 2004).

Iron plays crucial roles in haemopoiesis, control of infection and cell mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Dioxin and Haris, 2004).

Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005). Deficiency of phosphorus-calcium balance result in osteoporosis, arthritis, pyorrhea, rickets and tooth decay. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth (Babatunde, 2012). Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na^+/K^+-ATPase. Deficiency disease or symptoms occurs secondary to illness, functional and structural abnormalities including impaired neuromuscular functions of skeletal, smooth, and cardiac muscle, muscular weakness, paralysis, mental confusion (Hays and Swenson, 1985; Murray *et al*., 2000). Others are cardiac arrythmias, impaired carbohydrate tolerance, altered electrocardiogram. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, and also causes alterations of gastric secretions and intestinal motility (Streeten and Williams, 1952).

Copper functions in the utilization of iron in an early stage of haemopoiesis. Copper deficiency results in an increase in iron in the liver, whereas an excess of copper results in a decrease in iron content of the liver, thus reflecting the role of copper in iron utilization. Copper is present in blood plasma as a copper-carrying plasma protein called erythrocuprin. Erythrocuprin provides a link between copper and iron metabolism and mediates the release of iron from ferritin and haemosiderin (Hays and Swenson, 1985). Copper is important for cellular defense and protection of the mucous membranes, anti-anemic and essential for the formation of haemoglobin (Claude and Paule, 1979).

Magnesium is a component of chlorophyll and it is an important mineral element in connection with ischemic heart disease and calcium metabolism in bones (Ishida *et al*., 2000). Magnesium is one of the major minerals involved in carbohydrate and fat metabolism. Hypomagnesium has been postulated as a possible risk factor in the development of diabetic retinopathy. Magnesium may also play a role in the release of insulin as studies in experimental animals have demonstrated that magnesium supplementation could retard or prevent the induction of insulin resistance and diabetes mellitus, while a magnesium deficit can predispose to hyperglycaemia (Chetan, 2002). A Study by Kao *et al* (1999) suggests that magnesium supplement may improve the action of insulin and decrease blood sugar levels, particularly in the elderly. It is likely that the reported magnesium in this study contributes to *Vernonia amygdalina* reduction of blood sugar levels in diabetic rats as reported by Gbolade (2009), Osinubi, (2007), Akah *et al*., (2004), Nwanjo, (2005), Taiwo *et al*., (2009), Nimenibo-Uadia, (2003), Igbakin and Oloyede, (2009), Atangwho *et al*., (2007).

Studies have shown improved blood sugar control for diabetes that have doses of chromium picolinate ranging from 200-1000µg/day (Anderson *et al*., 2001). Chromium affects the action of insulin in protein metabolism, as indicated by rats fed chromium-deficient diets repleted by chromium (Roginski and Mertz, 1969). Evidence of a role for chromium in lipid metabolism and chromium deficiency in the development of atherosclerosis is accumulating from animal and human studies (Frieden, 1984). In experimental animals, chromium is needed for growth of rats and its deficiency leads to a reduced life span, corneal lesions and interference with insulin action producing a diabetic state and this causes removal of glucose from the blood at a rate that is one-half that of animals on a chromium-containing diet (Juturu and Komorowski, 2003).

The results of this study shows that the leaves of *Vernonia amygdalina* contain appreciable amount of phytochemicals, trace and mineral elements and thus provide a basic rationale for its use as a tonic, appetizer and ipecacuanha in folk medicine. Thus, it can be concluded that *Vernonia amygdalina* leaves can contribute significantly to the nutrient requirements of man for normal growth and adequate protection against diseases arising from malnutrition as well as a source of nutrients to supplement other major sources.

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