Journal of Medicine and Medical Science Vol. 2(6) pp. 900-903, June 2011
Available online@http://www.interesjournals.org/JMMS

Full Length Research Paper

Phytochemicals and micronutrients composition of root and stem bark extracts of V*ernonia amygdalina* Del.

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Accepted 13 June, 2011

The root and stem barks of *Vernonia amygdalina* (VA) are used traditionally in the management of an array of ailments. The present study therefore evaluated their chemical constituents: proximate, phytochemicals, mineral elements and vitamins using standard methods. The proximate analysis revealed (%) the presence of crude protein (6.71±0.10 and 7.30±0.20), moisture content (18.50±0.02 and 12.00±0.20), ash (17.99±0.02 and 11.01±0.01), crude fat (34.03±0.05 and 30.15±0.20) in the stem and root bark extracts respectively. The phytochemicals determined in the stem and root bark extracts respectively were (%): saponins (13.21±21 and 28.52±0.03), flavonoids (1.02±0.04 and 0.51±0.05), alkaloid (7.02±0.04 and 6.11±0.02) and hydrocyanic acid (3.41±0.02 and1.18±0.05). Analysis of vitamin concentration indicated vitamins A (21.5±0.35 and 30.90±0.20 IU/100g), C (49.00±4.4 and 10.30±2.5mg/100g) and E (106.20 ±1.90 and 35.83±1.90 IU/100g); Thiamin (0.50±0.00 and 0.37±0.00mg/100g) and Niacin (0.03±0.005 and 0.05±0.002mg/100g) in stem and root bark extracts respectively. Mineral elements including selenium, chromium, copper, zinc, and iron were also present in trace amounts. The findings from this study besides validating the pharmacological action of this herb, hence its use in trado-medicine also show its inert potentials for use as possible supplement in animal nutrition.

Keywords: Medicinal plants, phytochemicals, micronutrients, vitamins and mineral elements

INTRODUCTION

Vernonia amygdalina is a medicinal plant use in folk medicine to manage several ailments (Allen, 1987). The leaves are characteristically bitter although the bitterness can be abated by boiling or by soaking in several changes of clean water (Burkill, 1985). The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are used to prepare the very popular bitter leaf soup in Nigeria, and are also reportedly consumed by goats in some parts of Nigeria (Aregheore et al., 1998). The roots and leaves are used in ethno medicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses (Burkill, 1985). Both aqueous and alcoholic stem, root and leaf extracts are reported to be extensively used as purgative and anti-malarials as well as in the treatment of eczema (Kupcham, 1971). The plant has acquired special relevance recently, having been shown in human medicine to possess potent anti-tumorigenic properties (Izevbigie et al., 2004) with an amazing anti-parasitic efficacy in zoo pharmacognosy, as it is easily recognized and used for self medication by parasitized chimpanzees (Huffman, 2003). Pharmacological studies have shown that the leaf extract has both hypoglycemic and hypolipidaemic properties in experimental animals and so could be potentially useful in the management of diabetes mellitus (Akah and Okafor, 1992; Ebong et al., 2008).

Nutritionally, *Vernonia amygdalina* is used mainly in soup making in the tropics and also as an appetizer and febrifuge (Ijeh et al., 1996; Iwu et al., 1996) and has successful been used as a supplement in weaning foods (Eleyinmi et al. 2005). In Nigeria, as in other tropical countries of Africa where the daily diet is dominated by

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Table1: Phytochemical composition of root and stem bark extracts of *V. amygdalina*

Elements	HCN (%)	Saponin (%)	Flavonoid (%)	Alkaloid (%)	Tannin (%)
Stem bark extract	3.41 ± 0.02	13.21 ± 0.02	1.02 ± 0.04	7.02 ± 0.04	Trace
Root bark extract	1.18 ± 0.05 [a]	28.52 ± 0.03 [a]	0.51 ± 0.05 [a]	6.11 ± 0.02 [a]	Trace

Result is expressed as mean of three determinations ± SEM, $p<0.05$ vs stem bark

starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Okafor, 1983) . An attempt on its importance in animal nutrition in Nigeria has also been documented (Onwuka et al., 1989; Aregheore et al., 1998).

In spite of the usefulness of various parts of the plant as a remedy against disease and its nutritional relevance in traditional settings, chemical composition reports in literature have concentrated on the leaves whereas both root and stem barks have been shown to be medically responsive. For instance the root and stem barks are astringent and found useful as febrifuge and in management of diarrhea in folk medicine. In fact an ethno botanical survey of herbs used by traditional health practitioners in Southwestern Nigeria reveals that 60-70% of the respondents use root rather than leaves extract as antidiabetic agent (Abo et al., 2000). Therefore, it was necessary to evaluate the chemical composition of the root and stem barks since such information is fundamental and vital to natural product production leading to drug discovery.

MATERIALS AND METHODS

Sample collection and preparation

Vernonia amygdalina plant was identified and authenticated by a botanist, Department of Botany, University of Calabar. Thereafter, fresh roots were excavated and stems of the plant harvested from the Endocrine Research Farm of the University of Calabar. The roots and stems were thoroughly washed to remove debris and the earth remains. From these the barks were divested and chopped into bits and allowed to dry under shade. They were blended into fine powder using a Q-link electrical blender Model QBL-18L40. Three hundred and ten point eight (310.8g) of the blended stem bark and Three hundred and sixty (360g) of the blended root barks were separately soaked in 1200ml of ethyl alcohol (80% BDH) each and agitated then allowed to stay in refrigerator for 48 hours at 4^{o}C. The mixtures were first filtered with cheese cloth, then with WhatMan No 1 filter paper (24cm). The filtrates were then separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37^{o} C - 40^{o} C. These were concentrated to complete dryness in water bath, yielding 37.1g (11.96%) of stem bark and 49.1g (13.6%) of root bark extracts. The extracts were stored in a refrigerator from where aliquots were used for the proximate, phytochemical and micronutrients analyses.

Phytochemical analysis

Quantitative phytochemical compositions of the leaves were determined using the methods variously described by Harbone (1973), replace with) and Sofowara (2008) including percentage composition of saponins, alkaloids, flavonoids, hydrocyanide and tannins (Atangwho et al., 2009).

Proximate analysis

Moisture content was determined using the gravimetric methods (AOAC, 2000) and crude protein determined by the Kjeldhal method describe adapted by Chang (2003). Crude fat was extracted using the solvent extraction gravimetric method and ash content determined using the incineration gravimetric method of the Association of Official Analytical Chemists (AOAC, 2000).

Vitamins and mineral elements determination

The Perkin Elmer Atomic Absorption spectrophotometer (Model 306 UK) was used for the determination of Se, Zn, Fe, Cu and Cr using the methods of AOAC (2002, James, 1995). Vitamin A, C and E content were determined spectrophotometrically, again using the standard methods of AOAC (2000). Thiamine, Niacin and Riboflavin content were determined using the colorimetric method (Okwu and Ndu, 1996).

RESULT

The qualitative phytochemical screening revealed the presence of tannins, saponins, alkaloids, Flavonoids and hydrocyanide (result not shown). Quantitative estimate in percent (%) shows saponins in the stem and root bark extracts as (13.21 ± 0.02 and 28.52 ± 0.03), alkaloids (7.02 ± 0.04 and 6.11±0.02), flavonoids (1.02 ± 02 and 0.51 ± 0.05), hydrocyanide (3.407 ± 0.01 and 1.185 ± 0.05) respectively and trace amounts of tannins (Table 2).

Table 2: Proximate composition of root and stem bark extracts of *V. amygdalina*

Plant material	Moisture content (%)	Crude protein (%)	Crude fat (%)	Ash (%)
Stem bark extract	18.50 ± 0.02	6.71 ± 0.10	34.03 ± 0.05	17.99 ± 0.02
Root bark extract	12.00 ± 0.02[a]	7.30 ± 0.20 [a]	30.15 ± 0.20 [a]	11.01 ± 0.01 [a]

Result is expressed as mean of three determinations ± SEM, $p<0.05$ vs stem bark

Table 3: Vitamin composition of root and stem bark extracts of *V. amygdalina*

Vitamins	Stem bark extract	Root bark extract
C (mg/100g)	49.0 ± 4.4	10.3 ± 2.5 [a]
A (IU/100g)	21.5 ± 0.35	30.90 ± 0.20 [a]
E (IU/100g)	106.20 ± 1.90	35.83 ± 1.90 [a]
Thiamin (mg/100g)	0.50 ± 0.00	0.37 ± 0.00 [a]
Riboflavin (mg/100g)	0.13 ± 0.0007	0.15 ± 0.0003 [a]
Niacin (mg/100g)	0.03 ± 0.0005	0.05 ± 0.002 [a]

Result is expressed as mean of three determinations ± SEM, $p<0.05$ vs stem bark

Table 4: Mineral composition of root and stem bark extracts of *V. amygdalina*

Elements	Cr (mg/100g)	Cu (mg/100g)	Se (mg/100g)	Zn (mg/100g)	Fe (mg/100g)
Stem bark extract	Trace	0.021	Trace	0.14	0.12
Root bark extract	Trace	0.022	0.016	0.26	0.09

The proximate composition is shown in Table 2 shows result of determined moisture content (%) of the stem and root bark extracts were (18.50 ± 0.02 and 12.00 ± 0.02), crude fat (%) (34.03 ± 0.05 and 30.05 ± 0.06), ash (%) (17.99 ± 0.02 and 11.01 ± 0.01), and crude protein (%) (6.71 ± 0.01 and 7.30 ± 0.02), respectively. Table 3 presents the result of vitamin composition of the stem and root bark extracts. The stem and root bark extracts respectively contained 49.01 ± 4.4 and 10.30 ± 2.50 mg/100g vitamin C, 21.50 ± 0.32 and 30.90 ± 0.20) IU/100g vitamin E, 0.50 ± 0.00 and 0.37 ± 0.06 mg/100g thiamin, and trace amounts of riboflavin and niacin (Table 4). The mineral content estimation of the stem and root barks of *V. amygdalina* revealed in trace concentrations Se, Cu, Fe, Zn and Cr.

DISSCUSION

The phytochemical screening of the stem and root bark extracts of *V. amygdalina* revealed the presence of alkaloids, tannins, flavonoids, saponins and hydrocyanide. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Plants used in the treatment of diseases are said to contain bioactive principles with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Evans, 2002). Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose at the gut through intra-lumenal physiochemical interaction (Price et al., 1987). Saponins as a class of natural products are also involved in complexation__ with cholesterol to form pores in cell membrane bilayers (Francis et al., 2002) as such may be used as anti-cholesterol agents or cholesterol lowering agent. Extract from the roots of this plant; *V. amygdalina* have shown hypolipidemic action in diabetic rats after two weeks of administration (Neminibo-Uadia, 2003; Asaolu, et al., 2010).Hence, a potential management for atherosclerosis and related disorders such as in obesity and diabetes.

Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. However, when ingested by animals, they affect glucagon, thyroid stimulating hormones and inhibit certain enzymatic activities (Okaka et al., 1992). Flavonoids were also determined in the two extracts and they in general serve as flavoring ingredients in plants. Besides their role as flavoring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity (Kujumgiere et al., 1999). Flavoniods have also been implicated as antioxidants both in physiological and diseased states. For instance tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides (Erdman, 2007).

Tannins in this study were indicated to be present but in low concentration in both plant parts. This bioactive compound is known to have potentials anti-viral activity (Cheng et al., 2003) as well as potential prophylactic and therapeutic effect against cancer cells, but via different mechanisms (Narayanan et al., 1999). Moisture content,

crude protein, crude fat and crude ash and Vitamin C, A, E, Riboflavin, Thiamine and Niacin were also reported in this study although in small concentrations.

The broad distribution of nutrients and phytochemicals in the extracts studied support, as well as provide a basic rationale for its use as a tonic, appetizer and ipecacuanha in folk medicine. The reported vitamins have variously been shown to posses anti oxidant activities particularly A, C and E (Yeh et al., 2003). This may also corroborate the report of Igile et al (1994) on the antioxidant properties of *V. amygdalina* and may also give an insight into the antioxidant properties of the plant. From the foregoing this work therefore indicates that the stem and root bark extracts, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition particularly that the components are bio-degradable compared to synthetics currently used, environmentally friendly, cost-effective and would meet the current demand to go green.

ACKNOWLEDGEMENT

This work was supported by a grant from World Bank Assisted STEP-B Project. It provided for the reagents and the equipment needed for this investigation.

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