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Proximate and mineral compositions of the leaves and stem bark of *Cassia nigricans* Vahl

Rachael Gbekele-Oluwa Ayo

Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria. E-mail: gbekeayo@yahoo.com Tel. +2348036371606

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The aim of the study was to determine proximate and elemental compositions of the leaves and stem bark of *Cassia nigricans* Vahl, used in phytomedicine. The result of the proximate composition showed that the leaves contained higher values of crude proteins, crude lipids, carbohydrates and energy than the stem bark. The stem bark was richer in crude fibre, ash and moisture than the leaves. The concentrations of potassium and sodium were greater in the leaves than in the stem bark, while those of calcium and magnesium were higher in the stem bark than in the leaves. The concentrations of the microelements in the leaves of *C. nigricans* in $\mu g/g$ were in the order of: Fe > Mn > Cr > Zn > Cu > Pb > Cd, while those of stem bark were in the order of: Fe > Mn > Zn > Cr > Pb > Cd > Cu. In conclusion, the leaves and stem bark of *C. nigricans* contain nutrients and mineral elements that are of high nutritional value. The leaves are richer in mineral elements than the stem bark.

Key words: Cassia nigricans, leaves, stem bark, proximate composition, minerals.

INTRODUCTION

Medicinal plants from which natural products are obtained play very important role in the discovery of modern day drugs. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. There are about 35,000 species of higher plants that are used for medicinal purposes (Boroomand and Grouh, 2012). Some of these species of plants contain nutrients that have therapeutic properties and are nutritionally important because of their high contents of minerals, essential fatty acids, fibres and proteins (Turan et al., 2003; Jabeen et al., 2010; Ghani et al., 2012). The efficacy of medicinal plants for therapeutic purposes is often based on their organic constituents such as flavonoids, tannins, alkaloids and essential oils. Of recent, several authors reported that the chronic accumulation of different elements caused various health problems as a result of prolonged ingestion or overdose of these medicinal plants (Sharma et al., 2009; Arceusz et al., 2010; Ghani et al., 2012). Mineral nutrients are usually present in plants in low concentrations which fluctuate greatly in both space and time due to environmental factors such as weather, climate and physico-chemical properties, including soil type, soil pH and erosion (Chaves et al., 2013; Maathuis and Diatloff, 2013). In this context, determination of the elemental composition of the medicinal plants is very important since some essential metals induce toxic effects, when their intake is in high concentration. Furthermore, the non-essential metals are toxic even in very low concentrations (Ajassa et al., 2004; Basgel and Erdemoglu, 2006; Jabeen et al., 2010). Cassia nigricans Vahl (Caesalpiniaceae) is a medicinal plant that is widely used across Africa for treating various ailments (Avo, 2010). The phytochemical screening of the leaves of the plants has been carried out (Ayo and Amupitan, 2007). The leaves possess analgesic, anti-ulcer, antimicrobial, antiplasmodial and anti-oedema activities, and they are beneficial in the treatment of gastro-intestinal disorders (Akah et al., 1998; Obodozie et al., 2004; Ayo, 2010). Although some studies have been carried out on the pharmacological properties of C. nigricans, there is paucity of information on the proximate and elemental compositions of the plant.

The aims of the present study were to evaluate the proximate and elemental compositions of the leaves and stem bark of *C. nigricans,* and also to determine whether the use of the plant is safe for consumers according to the World Health Organization (WHO) standard. Four macroelements (potassium, K; sodium, Na; calcium, Ca; and magnesium, Mg) and seven microelements (zinc, Zn;

manganese, Mn; chromium, Cr; iron, Fe; lead, Pb; copper, Cu; cadmium, Cd) were determined in the dry powder of *C. nigricans* leaves and stem bark.

MATERIALS AND METHODS

Sample Collection

The *C. nigricans* Vahl plant, growing in the wild and in its natural habitat, was harvested close to Jama'a village, near Ahmadu Bello University Dam, Zaria (11° 10′N, 07° 38′E), located in the Northern Guinea Savannah zone of Nigeria during the rainy season in September, 2011. The plant was authenticated by Mallam U. Gallah of the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria with Voucher Number 613. The leaves were cleaned and removed from the stem, and they were air-dried separately. The samples were then pulverised. The powdered plant samples were sieved with a 2-mm rubber sieve and then kept in an air-tight container until used.

Proximate Analysis

The samples of the leaves and the stem bark were analyzed for moisture content, crude protein, crude fibre, crude fat and nitrogen free extract, using standard methods as outlined by Association of Official Analytical Chemists (AOAC, 1995). Briefly, the moisture content was determined by oven-drying method, using 2.0 g each of the samples of the leaves and stem bark until constant weights were obtained. The ash content was analyzed using 2.0 g each of the samples and the result was expressed as percentage. The crude protein value was estimated by determining the nitrogen value according to Kjeldahl method. To calculate the percentage crude protein, percentage nitrogen obtained was multiplied by a factor of 6.25 (AOAC, 1995). Crude fibre was determined by AOAC (1995), using Gallenkhamp furnace at 550°C. Crude fat was evaluated by the continuous solvent extraction using the Soxhlet apparatus. The leaves and the stem bark were extracted continuously with petroleum ether (40 - 60°C) for 6 hours. The extractible lipid (crude fat) was calculated as the loss of weight of the content after the extraction. The carbohydrate content was calculated by the difference between the total sum of the moisture, fat, protein and ash content of each part of the sample from 100%:

Percentage (%) carbohydrate content = 100 - [% protein + % moisture + % lipids + % ash + % crude fibre] The sample calorific value was estimated (in Kcal/100 g) by multiplying the % crude protein, crude lipid and carbohydrate by factor of 4, 9 and 4, respectively. Determinations of all parameters were carried out in triplicate.

Mineral Analysis

The mineral contents of the samples were estimated by wet digestion method (AOAC, 1995). Briefly, 0.5 g of the powdered plant sample was mixed with a mixture of perchloric acid, HClO₄ (60%, 2 cm³), nitric acid, HNO₃ (69.7%, 5 cm³) in a Kjeldahl digestion tube. Digestion was initially carried out at low heat until the brown fumes had been given off, and heating continued until all the solids dissolved and the appearance of white fumes. After cooling, the digest was filtered and transferred into 100 cm³ volumetric flask and made up to the mark with distilled water. The solution was used for the estimation of minerals. The elements, K, Na and Ca were estimated by flame photometer (PFP76 Flame Photometer, Jenway), while Zn, Fe, Mg, Mn, Cr, Cd, Cu and Pb were determined using atomic absorption spectrophotometer (Model AA240FS, Varian).

Statistical Analysis

The data obtained were expressed as percentage and mean \pm SD. Values were subjected to statistical analysis, using Student's *t*-test. Values of P < 0.05 were considered significant.

RESULTS AND DISCUSSION

The results of the proximate composition and analysis of mineral elements of the leaves and stem bark of *C. nigricans* are summarized in Tables 1 and 2. The moisture content of the dry leaves was found to be 3.92%, and that of the stem bark was 4.65%. The values recorded were, however, relatively low because the leaves and the stem bark were air-dried before the analysis. The low-moisture content obtained in the plant is in agreement with the finding of Bamigboye et al. (2010), who obtained higher dry matter yield in sesame seeds, associated with long storage of the plant.

The crude fibre content of 41.36% obtained for the stem bark of C. nigricans was relatively very high, compared to that of the leaves which was 20.64%. The crude fibre content in the leaves of C. nigricans obtained in the present study compared very well with the value of 22.36%, recorded in the leaves of Cassia siamea by (Hassan and Ngaski, 2007). The consumption of most leafy vegetables, relatively high in crude fibre, has been shown to reduce serum cholesterol level, risk of coronary heart diseases and hypertension. It also increases glucose tolerance and insulin sensitivity (Araya et al., 2003). The crude lipid content of C. nigricans leaves (9.81%) obtained in the present study was higher than those of C. siamea (6.70%) and M. angolensis (3.12%) (Tairo et al., 2011); but lower than 18.4%, reported for the leaves of Adansonia digitata (Osman, 2004). Lipids are

Table 1. Proximate composition of the leaves and stem bark of Cassia nigricans (%).

Parameters	Leaves	Stem bark	
Moisture Content	3.92 ± 0.17	4.65 ± 0.15**	
Crude fibre	20.64 ± 0.14	41.36 ± 0.15***	
Crude lipid	9.81 ± 0.08	9.13 ± 0.10***	
Crude protein	20.83 ± 0.02	10.51 ± 0.01***	
NFE (Carbohydrates)	36.27 ± 0.08	22.37 ± 0.08***	
Ash content	8.53 ± 0.06	11.98 ± 0.01***	
Energy value, Kcal/100 g	316.69 ± 0.08	$213.69 \pm 0.12^{***}$	

NFE = Nitrogen-free extract = Carbohydrate content

Values are mean \pm SD of triplicate

** = P < 0.01

***= P < 0.001

essential nutrients since they provide the body with maximum energy. The percentage of crude protein in the leaves of C. nigricans (20.83%) was higher than that recorded in its stem bark (10.51%). The C. nigricans leaves contained moderate concentration of crude protein (20.83%), comparable with the reported values in some Nigerian leafy vegetables (Igbal et al., 2006). The ash content of the leaves of C. nigricans; that is, the total mineral content of the plant was considerably of high amount. This is particularly true when the ash content in the leaves of the plant is compared with the reported values in some medicinal plants such as C. siamea and Maerua angolensis, whose values are 10.61% and 12.90%, respectively (Hassan and Ngaski, 2007; Tairo et al., 2011). The ash content of *C. nigricans* leaves (8.53%) showed that the leaves contained lower amount of mineral elements than the stem bark (11.98%). The percentages of available carbohydrate in the leaves and stem bark were 36.27% and 22.37%, respectively, but the values were low and they compared very well with those obtained in some leafy vegetables in Northern Nigeria, including Corchorous olitorius (34.74%) and Hyptis suaveolenses (30.55%) (Mohammed and Mann, 2012). The energy value of the leaves (316.77 Kcal/100 q) was higher than that of the stem bark (213.69 Kcal/100 g). The values were higher than those recorded in Corchorus olitorious (177.56 Kcal/100 g) and Amaranthus hybridus (183.93 Kcal/100 g). Thus, the low-energy values obtained in C. nigricans are in agreement with the finding that vegetables have low-energy values (Mohammed and Mann, 2012).

The result of the present study showed that *C. nigricans* is a good source of mineral elements. The result also indicated that the leaves were richer in mineral elements than the stem bark (Table 2). The concentrations of the elements in the plant, *C. nigricans* are summarized in Table 2. The concentrations of K, Na, Ca and Mg in *C. nigricans* leaves were found to be 4660, 77.85, 4315.92 and 18.30 μ g/g, respectively; while the

corresponding contents in the stem bark were 1163.67, 47.09, 4390.24 and 28.39 µg/g, respectively. The concentration of potassium in the leaves, with the value of 4660 µg/g, was the highest. This value was in agreement with those recorded from previous studies on medicinal plants, which were within the range of 1511.26 and 9234.00 µg/g (Ozcan and Akbulut, 2008; Ghani et al., 2012). Potassium is a multi-functional nutrient, which is an essential part of many important enzymes. C. nigricans plant accumulated low sodium; that is, 77.85 ug/g in the leaves, and 47.09 ug/g in the stem bark. The concentration of sodium in C. nigricans leaves is comparable to those in Hyptis suaveolens and Solanum torvam, with the values of 72.5 and 78.7 µg/g, respectively (Ajassa et al., 2004). Sodium and potassium are closely related in the body fluids. They regulate the acid-base balance. Sodium remains one of the major electrolytes in the blood. The Na/K ratio of the leaves was found to be 0.02, and that of the stem was 0.04. According to Akinyeye et al. (2010), the recommended Na/K ratio is 0.6, and the values for the leaves and stem bark obtained in the present study were less than 0.6, considered good for effective health utilisation. A diet high in potassium and low in sodium content has added advantage because of the direct relationship of sodium intake with hypertension in humans (Njoku and Akumefula, 2007). The calcium concentration was found to be 4315.92 and 4390.24 µg/g in the leaves and stem bark of C. nigricans, respectively, and the values compared very well with those in the herb, B. campestris (4210.92 µg/g). High concentration of calcium in the body is very important because of its role in formation of bones and teeth, clotting of blood, muscle contraction and synaptic transmission of nerve impulses (Brody, 1994; Ghani et al., 2012). Calcium is an essential structural and functional element in living cells. It participates in cell division and the regulation of cell proliferation and differentiation (Whitfield et al., 1979). The intake of calcium has been found to be very important for cancer

Elements	Leaves	Stem bark
Zinc	21.3 ± 0.05	15.6 ± 0.05
Magnesium	18.3 <u>+</u> 0.09	28.39 <u>+</u> 0.01 ^{***}
Manganese	93.33 ± 0.53	$61.89 \pm 0.32^{***}$
Calcium	4315.92 ± 0.06	$4390.24 \pm 0.05^{***}$
Chromium	32.64 ± 0.08	$4.53 \pm 0.04^{***}$
Iron	98.2 ± 0.15	$62.73 \pm 0.07^{***}$
Lead	0.58 ± 0.002	$1.18 \pm 0.06^{***}$
Copper	6.35 ± 0.05	$0.16 \pm 0.06^{***}$
Cadmium	0.106 ± 0.03	$0.26 \pm 0.03^{**}$
Potassium	4660 ± 0.06	$1163.67 \pm 0.66^{***}$
Sodium	77.85 ± 0.05	$47.09 \pm 0.10^{***}$

Table 2. Composition of mineral elements in the leaves and stem bark of Cassia nigricans (µg/g).

Values are mean \pm SD of triplicate

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^{*} = P < 0.001

patients. It helps in building and maintaining bone mass, and strength because some chemotherapeutic agents cause osteopenia and osteoporosis (Lipkin and Newmark, 1999; Naga Raju et al., 2013). Thus, the high concentration of calcium contained in C. nigricans may be of high therapeutic value. The plant, C. nigricans showed low accumulation of magnesium. The concentration of Mg in the leaves was 18.30 µg/g and that of the stem bark was 28.39 µg/g. The values are very low, when compared with those obtained in the previous studies in medicinal plants (Ozcan and Akbulut, 2008; Ghani et al., 2012). It has been shown that K, Ca and Mg take part in neuromuscular transmission; and, together with Zn, Mn, Cu, Cd and Cr, are involved in biochemical reactions in the body. The elements also serve as constituents of biological molecules and co-factors for various metabolic processes (Mayer and Vyklicky, 1989; Brody, 1994). Deficiency or excess of these elements may cause many metabolic disorders. Zn, Mg and Cr have important roles in the metabolism of cholesterol and in heart diseases (Brody, 1994). The concentration of microelements in the leaves of C. nigricans are in the order of: Fe > Mn > Cr > Zn > Cu > Pb > Cd. In the stem bark, the order is as follows: Fe > Mn > Zn > Cr > Pb > Cd > Cu.

Concentration of iron in the powdered leaves was $98.20 \ \mu g/g$, while that of the stem bark was $62.73 \ \mu g/g$. The permissible limit set by Food and Agricultural Organization (FAO)/WHO (1984) in edible plants was 20 ppm. However for medicinal plants, it was found that all plants accumulated Fe above this permissible limit (FAO/WHO, 1984), including *C. nigricans*. Iron is very important in the formation of haemoglobin and in transporting oxygen in the body. The Pb concentration was found to be 0.58 $\mu g/g$ in leaves and 1.18 $\mu g/g$ in the stem bark. According to the WHO (1998), the permissible

limit for medicinal plants, based on Acceptable Daily Intake, for Pb is 10 mg/kg. Thus, C. nigricans accumulated Pb at a concentration appreciably below the permissible level. The Mn concentration of the leaves was 93.33 µg/g and that of the stem bark was found to be 61.89 µg/g. The permissible limit set by FAO/WHO (1984) in edible plant was 2 ppm and it was found that all plants accumulated Mn above this limit. However for medicinal plants, the WHO (2005) limits have not yet been established. Sheded et al. (2006) reported the range of Mn to be between 44.6 and 339 ppm in selective medium of plants in Egypt. Manganese acts as a catalyst and co-factor in many enzymatic processes, involved in the synthesis of fatty acids and cholesterol. It is an important co-factor in the enzymes, necessary for mucopolysaccharide and alvcoprotein syntheses (Shomar, 2012). The Zn concentrations in the leaves and stem bark were 21.30 µg/g and 15.6 µg/g, respectively. The permissible limit set by FAO/WHO (1984) in edible plants was 27.4 µg/g. According to Brody (1994), the concentration of Zn in agricultural produce should be between 15 and 200 ppm. Thus, Zn concentrations in the leaves and stem bark of C. nigricans, obtained in the present study were within this range. Zinc is essential for growth and development. It is essential for the function of the cells of the immune system. It is used in the prevention and treatment of diarrhoea, pneumonia, cold, respiratory infections and malaria (Ghani et al., 2012; Deshpande et al., 2013). Copper is an essential nutrient, required for a wide range of biological functions such as enzymatic and redox reactions (McLaughlin et al., 1999). The Cu concentration obtained in the leaves of C. *nigricans* was 6.35 μ g/g, and that of the stem bark was 0.16 µg/g. The permissible limit set by FAO/WHO (1984) for edible plants was 3.00 µg/g. Therefore, the plant accumulated Cu above this limit. In medicinal plants, permissible limits for Cu set by China and Singapore were 20 and 150 ppm, respectively (WHO, 2005). The WHO is yet to establish the limit for Cu in medicinal plants. The concentration of Cr in the plant was very high, when compared with the value obtained in medicinal plants studied by Ghani et al. (2012). The concentration of Cr in the leaves of C. nigricans was found to be 32.64 µg/g, and in the stem bark it was 4.53 µg/g. The high accumulation of Cr in the plant may result in liver, kidney and lung damages (Zayed and Terry, 2003). The permissible limit set by FAO/WHO (1984) in edible plants was 0.00 ppm. Cadmium concentration was lower in the leaves than in the stem bark, and the values were 0.106 µg/g and 0.26 µg/g, respectively. The permissible limit set by FAO/WHO (1984) in edible plants was 0.21 µg/g, and the limit for Cd set by Canada for medicinal plants was 0.3 µg/g in raw medicinal plant materials and 0.006 mg/day in finished products (WHO, 2005). Therefore, it was found that C. nigricans accumulated Cd below this limit. High concentration of Cd causes both acute and chronic poisoning, with adverse effects on the kidneys, liver, vascular and immune systems (Hayes, 1997). The results of the present study showed that both the leaves and stem bark of C. nigricans contained large amounts of nutrients, and were rich in K, Ca, Na, Mg, Fe and Zn. Therefore, the plant may be a source of biologically important elements. The mineral content of the plant may be of value in enhancing its medicinal properties.

The study of the mineral elements also revealed the accumulation of the metals in the plant, which is highly relevant for the quality assessment of its safe use as a potential herbal drug. Since chemical composition of *C. nigricans* may not be adequate to evaluate completely the nutritional value of the plant, further studies are required to determine the availability of the nutritive values of the plant. It is also pertinent to evaluate the contents of vitamins, acids and anti-nutrient factors that are present in the plant. Other factors that may affect the proximate and elemental compositions of the plant are season of harvest, soil and environmental properties. They require further investigations.

CONCLUSIONS

The leaves and stem bark of *C. nigricans* contain nutrients and mineral elements that are of high nutritional value. The leaves are richer in mineral elements than the stem bark. According to WHO standard, the consumption of the plant obtained in the zone is safe.

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