



Proximate and elemental composition of *Bauhinia rufescens* Lam (Leguminosae : Caesalpinioideae)

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ABSTRACT

This study is aimed at investigating the proximate contents, concentrations of some micro/macroelements and anions of the stem bark and leaves of *B. rufescens* with the view of validating its used as forage and medicinally by man in the treatment of diarrhoea, dysentery and in wound healing. The stem bark and leaves of *Bauhinia rufescens* were collected from Gathla-Gwoza, Borno State, Nigeria. Proximate analysis was done following methods of Association of Official Analytical Chemists and the results showed that the stem bark had higher dry matter (94.50%), carbohydrate (82.03%), nitrogen free extract (54.53%), crude fibre (33.00%) and ash (3%), while crude proteins (15.58%), fats (5%) and moisture content (5.7%) were estimated to be higher in the leaves of this plant. The levels of 10 elements (Ca, Mg, Na, K, Fe, Cu, Zn, Ni, Cr, Co) were determined using atomic absorption spectrophotometry, the anions (nitrates, phosphates and sulphates) were estimated using the Smart Spectro Spectrophotometer. Results revealed higher concentrations of macroelements and microelements in the leaves sample except for Cr concentration which was higher (0.0247 mg/g) in the stem bark than in the leaves (0.0050 mg/g). The concentrations of nitrates and phosphates were higher in the leaves; on the other hand, sulphates concentration was higher in stem bark (12.0 mg/g) than found in the leaves (10.8 mg/g). In conclusion, both the proximate, elemental and anions concentrations for these parts of *B. rufescens* were mostly found within the permissible region set by World Health Organization.

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INTRODUCTION

Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world (Prajapati and Prajapati, 2002; Latif et al., 2003; Shinwari et al., 2006, Adnan et al., 2010). The chemical constituents of plants,

including metal ions, are particularly responsible for medicinal and nutritional properties, as well as the toxicity. The cumulative levels of these metals in both the roots and other tissues of plant above the ground can be transferred from soils into the food chain; thus causing phytotoxicity in plants and having potential harmful effects on

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animals and humans (Hussain et al., 2006). WHO (1998) recommends that medicinal plants, which form the raw materials for the finished products, may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination.

B. rufescens has wide array of medicinal uses – the stem bark is astringent and also used to cure diarrhoea, dysentery, leprosy (Burkill, 1995; TopTropicals, 2009) and to reduce fever (Vogt 1996).

It is therefore worthy to note that, whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals (Prasad, 1976). The excesses of these essential nutrient and trace of toxic metals may cause serious effects on human health (Underwood, 1997; Khan et al., 2008).

Till now, no report is available on the proximate, elemental and anions contents of *B. rufescens*; and thus, the aim to investigate the levels in the stem bark and leaves, with the view to validating the traditional use of this plant in Borno State – Nigeria, as forage and remedy against diarrhoea, dysentery, for wound healing among others.

MATERIALS AND METHODS

Collection sites of medicinal plant

The study was carried out on the stem bark and leaves of *B. rufescens* collected in June 2008 from Gathla Musa bakin kogi; 11° 00.562' N; 13° 31.369' E; Gwoza – Borno State, Nigeria. The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S. S. Sanusi, Department of Biological Sciences, while the voucher specimen - #003/2008 was deposited at the Post Graduate Research Laboratory, Department of Chemistry, University of Maiduguri, Nigeria.

Sample preparation and determination of proximal composition

The air-dried stem bark and leaves were manually crushed using wooden mortar

and pestle and then treated onwards. 2.0 g of the powdered sample were processed for various parameters according to the Association of Official Analytical Chemists methods (AOAC, 1990; AOCS, 2000).

Proximate analysis

The proximate analysis (carbohydrates, fats (ether extract), crude proteins, moisture, dry matter, crude fibre, nitrogen free extracts and ash) of stem bark and leaves samples were determined using AOAC methods. Briefly, the moisture and ash were determined using weight difference method. Fibre content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrate was determined when the sum of the percentages of moisture, ash, crude protein and fats were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method, involving digestion, distillation and finally titration of the sample (AOCS, 2000). The nitrogen value was converted to protein by multiplying with a factor of 6.25. The determination of crude lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 – 60 °C). The nitrogen free extract (NFE) was calculated indirectly by difference as the sum of crude protein, crude fibre, ether extract and ash subtracted from 100. All the proximate values are presented in percentage (AOAC, 1990; AOCS, 2000).

Elemental analysis of the plant samples

The macro and microelements were determined using Perkin-Elmer Analyst 300 single beam Atomic Absorption Spectrophotometry and the data was obtained in parts per million (ppm) which was then converted to mg/g. Calibration curve was established using working standards for each element. Laboratory procedures for the preparation and determination of macro- and micronutrients were used as outlined by

Radojević and Bashkin (1999) for plant samples.

Sample digestion and preparation for analysis

The air-dried plant samples were pulverised manually in wooden mortar and pestle into coarse powder; 0.5 g of each sample was independently packed into an acid-washed porcelain crucible and then placed in a muffle furnace for four hours at 550 °C. The crucibles were removed from the furnace and cooled. Ten ml of 6 M HCl were added and then covered, this content, was heated on a steam bath for 15 minutes. One ml of HNO₃ was later added and evaporated to dryness by continuous heating for one hour so as to dehydrate silica and completely digest organic substances. Lastly, 5 ml of 6 M HCl and 10 ml of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask and then made up to the mark with distilled water (Radojević and Bashkin, 1999).

Anions analysis in plant samples

Determination of nitrate

The concentration of nitrate in the leaves and stem bark was carried out by standard cadmium reduction method using Smart Spectro Spectrophotometer (La Motte, 2000). Plant samples solutions were prepared by chopping each sample into smaller sizes. About 0.5 g of the samples was transferred into 100 ml flask and soaked with 50 ml of distilled water. The flask was corked and shaken for 30 min, then filtered into another 100 ml volumetric flask and the volume made to the mark with distilled water (Radojević and Bashkin, 1999).

Determination of phosphate

The air-dried and ground powdered samples of the stem bark and leaves of *B. rufescens* were used. About 0.5 g was weighed into crucibles; this was then

underlaid with 5 ml of (20%) (w/v) magnesium acetate and thereafter evaporated to dryness. The content of the crucible was then transferred into the muffle furnace and heated to 500 °C. Furthermore, the crucible contents were ashed at 500 °C for 4 hours and then removed and cooled in desiccators. Ten ml of 6 M HCl were then added to each of the crucible and covered and then heated on a water bath for 15 minutes. The contents of the crucible were completely transferred into different evaporating basins and 1 ml of concentrated HNO₃ was added. The heating was continued for 1 hour to dehydrate silica. One ml of 6 M HCl further added, swirled and then followed by the addition of 10 ml distilled water and again heated on the water bath for complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No. 1 filter paper into 100 ml volumetric flasks and the volumes made up to the marks with distilled water (Radojević and Bashkin, 1999). Phosphate was determined using batch direct reading 2000 spectrophotometer.

Determination of sulphate

Sulphate was determined using smart spectro spectrophotometer (La Motte, 2000). The samples were prepared as follows: 5 ml of magnesium nitrate solutions were added to each of the ground samples in the crucibles which were then heated to 180 °C on a hot plate. The heating process was allowed to continue until the colour of the samples changed from brown to yellow (AOAC, 1990). The samples were then transferred to the furnace at a temperature of 500 °C for four hours. Magnesium nitrate was added to prevent loss of sulphur. The contents of each crucible were carefully transferred to different evaporating basins; 10 ml of concentrated HCl were added to each and covered with watch glass. The content of each crucible above was boiled on a steam bath for 3 minutes. On cooling, 10 ml of distilled water were added to each of the basins and the contents filtered into 100 ml volumetric flask and the volume

made up to the mark with distilled water (Radojević and Bashkin, 1999).

RESULTS AND DISCUSSION

Proximate composition

The result of proximate analysis shows variant proportions of nutrients and their contents. The data for the proximate contents of the stem bark and leaves are presented on Figure 1. The observed value for carbohydrates (82.03%) in the stem bark was higher than that in the leaves (71.72%). There are certain plants like *Croton tiglium* that can yield carbohydrates up to a low amount of 15.51% (Shah et al., 2009). These comparisons showed that *B. rufescens* is relatively a good source of carbohydrates. The crude protein concentration in leaves sample of this plant showed 15.58%, while that of the stem bark was 6.47% which is almost 2.5-fold less than that produced by the leaves; this trend is similar to the results reported for *F. tenacissima* compared to *Croton tiglium* by Shah et al. (2009). The moisture contents was obtained as 5.50% and 5.70% in the stem bark and leaves respectively, which was higher than that found in *O. limbata* which had a lower moisture contents of 1% (Shah et al., 2009). The fats content was found in the stem bark and leaves as 3.00% and 5.00% respectively, notably because of the lipophilic substances mostly present in the leaves than the stem bark (Pomeranz and Clifton, 1981). Our data for ash content in the stem bark and leaves were found as 3.00% and 2.00% respectively. This was in line with the standard recommended range of 1.5 - 2.5% for ash content of nuts and seeds suitable for animal feeds (Pomeranz and Clifton, 1981); hence, the use of this leaves as fodder for animal in ethno-veterinary practices as well as medicine for humans (Burkill, 1995). The crude fibre contents was higher in the stem bark (33.0%) than leaves (27%); also, the nitrogen free extract was more in the stem bark (54.53%) than that found in the leaves (50.42%) . The highest proximate contents

were presented by percentage dry matter in both parts: the stem bark had 94.5% and the leaves had 94.3%. From these results, the increasing order of these nutrients among the plant parts is Dry matter > Carbohydrate > Nitrogen free extract > Crude fibre > Crude protein > Moisture > Fats > Ash.

Elemental analysis

The results of macro- and microelements concentrations reported in mg/g are presented in Table 1.

Macroelements

According to WHO recommendation, medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic (As), cadmium (Cd) and lead (Pb), which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1989, 1998). Although, macro- and microelements are valuable for diseases and disorders control in man (Iwalewa et al., 2009), they could however produce harmful effects in excessive amounts (Hussain et al., 2006).

The results of the macro- and microelements concentration in stem bark and leaves of *B. rufescens* are presented in Table 1. The macroelements analysed were calcium, magnesium, potassium and sodium. The concentration of these elements were higher in the leaves sample than in the stem bark sample of *B. rufescens*, the highest being Ca and the lowest was Mg in both cases. This pattern could be as a result of the fact that, the leaves store most of these macroelements in for photosynthesis and other vital processes than occurred in the stem bark.

Microelements

This study reports the concentrations of six microelements Cd, Cr, Cu, Fe, Ni and Zn. The results showed highest concentration of Fe in both plant parts as 1.5238 mg/g in leaves and 1.3721 mg/g in the stem bark sample. The concentration of cadmium (Cd) was very low in both the stem bark and leaves with values

of 0.0013 and 0.0012 mg/g respectively. This is in agreement with most medicinal plants studied as reported by several workers that cadmium was either very low in concentration or below the detection limit of the instruments used (Hussain et al., 2006; Khan et al., 2008; Khanzada et al., 2008; Hussain et al., 2009; Iwalewa et al., 2009; Adnan et al., 2010). Cadmium (Cd), which is known to be practically toxic (Khan et al., 2008), was found to be below the permissible limit of 0.3 mg/kg (WHO, 1998, 2007).

The concentration of Cr was found to be higher in the stem bark than in the leaves, although the concentration (0.0247 mg/g) falls within the permissible region in plant as reported by Adriano (1986), that most phytotoxic level of Cr in plant occur at 10 mg/kg. Copper (Cu) concentration has been found maximum in leaves (0.0454 mg/g) than those in the stem bark (0.0282 mg/g). Nickel (Ni) concentration was found to be higher in the leaves sample (0.0113 mg/g) than in the stem bark (0.0033 mg/g); these values fall within safety region, since the phytotoxic concentration of Ni ranges from 40 to 246 mg/kg (Gough et al., 1979) and also lower

than the Ni concentration range of 0.05 – 5 mg/kg for plant foods (FAO/WHO, 1993).

The concentrations of Zinc (Zn) in both samples were less than 0.1500 mg/g; the values fall within the normal range of 25 – 150 mg/kg (Jones, 1972). This element being essential, is utilised in the development of brain, bone formation and wound healing (Khan et al., 2008). The concentration of Zn which effect human health ranges between 100 to 500 mg/l (Macnicol and Beckett, 1985).

Anions

The levels of anions in the leaves and stem bark of *B. rufescens* are presented in Figure 2. The results revealed that the concentrations (mg/g) of nitrates and phosphates were higher in the leaves compared to those in the stem bark; the sulphates concentration on the other hand was higher in the stem bark (12.0 mg/g) than in the leaf samples (10.8 mg/g). According to Santamaria et al. (1999), nitrate content of various parts of a plant differs in the increasing order of leaf > stem > root > tuber > bulb > fruit > seed. However, our findings was not in agreement with this pattern.

Table 1: Macro- and microelements contents of the stem bark and leaves of *B. rufescens*.

Elements	Concentration (mg/g)		
	Stem bark	Leaves	
Macroelements	Ca	12.0058	45.5789
	Mg	0.1037	0.1199
	K	2.1752	2.8510
	Na	1.8743	2.1404
Microelements	Cd	0.0013	0.0012
	Cr	0.0247	0.0050
	Cu	0.0282	0.0454
	Fe	1.3721	1.5238
	Ni	0.0033	0.0113
	Zn	0.0578	0.1200

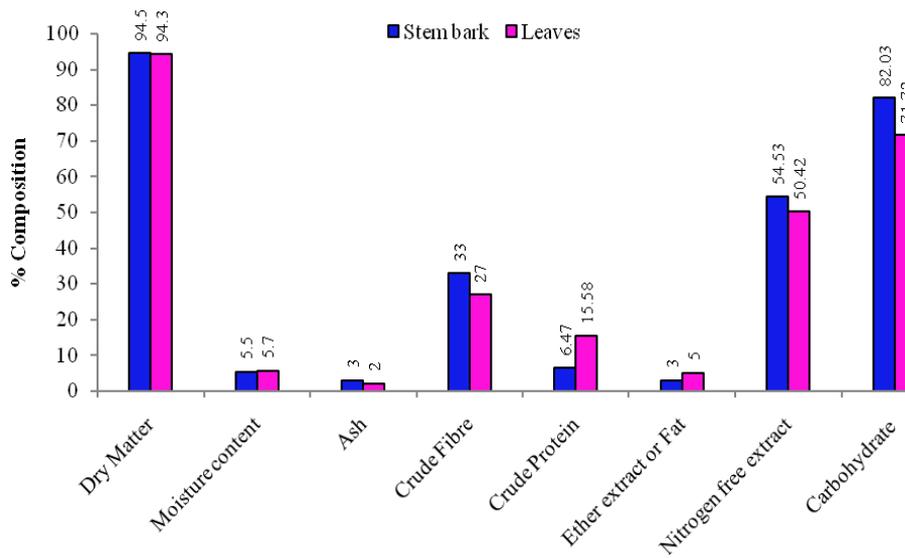


Figure 1: Proximate levels of the stem bark and leaves of *B. Rufescens*.

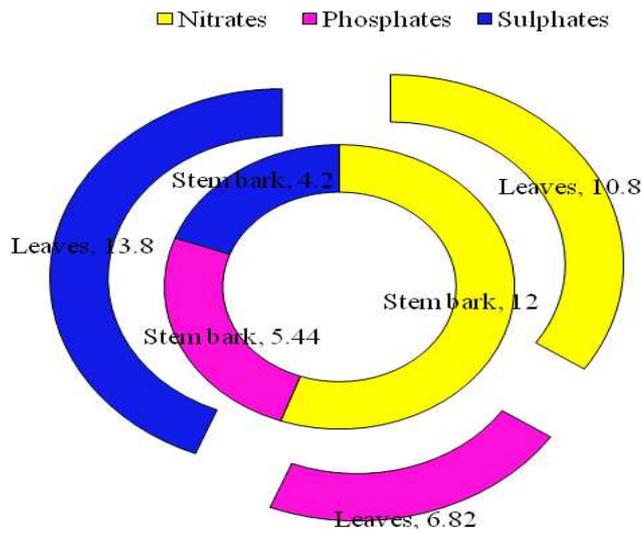


Figure 2: Concentrations of anions (mg/g) in the stem bark and leaves of *B. Rufescens*.

Conclusion

In conclusion, both the proximate, elemental and anions concentrations for these parts of *B. rufescens* were mostly found within the permissible region set by World Health Organization. This report further corroborates the use of this plant in some parts

of Borno State, Nigeria, in the treatment of diarrhoea, dysentery, among others.

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