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Full Length Research Paper

Phytoconstituents, proximate and nutrient investigations of *Saba florida* (Benth.) from Ibaji forest

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Quantitative determination of chemical and nutritional composition of the leaf, fruit pulp, pericarp and seed of *Saba florida* (Apocynaceae*)*, an underexploited medicinal and food plant in Nigeria, was carried out using standard methods. The plant parts contain levels of alkaloids, tannins, saponins, flavonoids and other phytochemical. Proximate analysis (total protein, fats, carbohydrate, ash, and moisture contents) were carried out following methods of Association of official Analytical chemists. The order of increasing concentration of the proximate composition is protein —> moisture—>ash—>crude fibre—>fats —>carbohydrate in all plant parts. Elemental nutrients Ca, K, Na, Mg, Pb, Fe, Cu, Zn, Ni and Cd were analyzed using atomic absorption spectrometry. Results revealed higher concentration of macronutrients in all plant parts except K. In conclusion *S. florida* has high nutritional and medicinal value.

Key words: *Saba florida* (Apocynaceae*)* proximate analysis, phytoconstituents, macro and micronutrients, Ibaji.

INTRODUCTION

The quest for plants with medicinal properties continues to receive attention as scientist survey plants, particularly of ethnobotanical significance, for a complete range of biological activities, which range from antibiotic to antitumor. Thus, plants have provided western medicine with an abundance of drugs and treatment for a variety of health problems (Lewis and Elvin-Lewis, 1977; Bruneton, 1999).

Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, an increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers (Zhang, 1976). One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects (Mehta, 1982).

Saba florida (Benth) belongs to the family Apocynaceae. It grows on other tree in the riparian equatorial rain forest of Africa. The plant is very abundant in undisturbed forest, coastal areas and around Great Lake regions of Africa from sea level to 1250 m (Maundu et al., 1999) but rare in open areas. The plant regenerates naturally by seeds on fertile moist soil under partial and full shades. It can be propagated by cuttings and the vine can be copied. The seed germinates in about 12 days with a high germination rate in excess of 90% (FAO, 1983).

The plant is found in Ibaji and other parts of Kogi State, Nigeria. The fruit pulp is edible and it makes a refreshing sour drink. The fruit does not abscise and must be harvested when it turns yellow. The stem yields latex that is an inferior rubber. Traditionally, bark decoction is used to treat rheumatism. The leaves are utilized in Senegal to prepare sauces and condiments as salty appetizer. In Coted'Ivoire, the latex is prescribed as an adhesive for

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poison preparation for arrows as it hardens upon exposure. The inferior rubber produced from the latex is sometimes used to adulterate genuine rubber (FAO, 1993).

In Kogi state, the latex is used as trap for birds, rats and smaller rodents. The leaves are eaten as antidote against vomiting and the bark decoctions are administered for diarrhea and food poison. The leaves are also efficacious in the treatment of skin ulcer. The fruit is a special delicacy for the monkeys in the forest and humans are beginning to compete with monkeys for the fruit. It appears in the local market during fruiting season.

Proximate and nutrient analysis of medicinal plants, edible fruits and vegetables plays a crucial role in assessing their nutritional significance (Pandey et al., 2006). As various medicinal plant species are also consumed as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species (Pandey et al., 2006). To the best of our knowledge, literatures are scanty on the phytochemical constituents, proximate and nutrient composition of *S. florida* (Benth.) from Ibaji forest. This study therefore focuses on the phyto-chemical, proximate composition, mineral analysis of *S. florida* parts with a view to assess the nutritional potential of the plant in relation to its ethnomedicinal uses.

MATERIALS AND METHODS

Plant collection

The plant material was collected from Igboigbo-Unale in Ibaji Local Government Area, eastern part of Kogi State, Nigeria during dry season (November, 2009). Dirt was removed from the plant parts by rinsing in clean water. The leaves were air – dried for 3 weeks and pulverized using motorized blender into a fine powder of 60 mesh sieve size. The fruit pulp, seed and pericarp were dried in oven at 40°C. The dried samples were then used for the various analysis.

Plant identification

The plant was identified in the Botany unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *S. florida* (Benth).

Quantitative determination of the phytoconstituents

Saponins determination

The method employed was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was 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 on a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content were calculated as percentage.

Alkaloid determination

The determination of alkaloid was as described by Harborne (1973). A portion (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination

Tannin determination was done by Van – burden and Robinson (1981) method. A portion (500 mg) of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Flavonoid determination

This was done following the method of Boham and Kocipai – Abyazan (1994). A portion (10 g) of the plant 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 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of cyanogenic glycoside

The extraction was according to Wang and Filled method as described by Onwuka (2005). A portion (5 g) of sample was made into paste and the paste was dissolved into 50 ml distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1 ml of the sample filtrate, 4 ml of alkaline picrate was added and absorbance was recorded at 550 nm and cyanide content was extrapolated from a cyanide standard curve.

$$\text{Cyanide (mg/g)} = \frac{\text{Absorbance} \times GF \times DF}{\text{Sample weight}}$$

Where:
GF = gradient factor and
DF = dilution factor.

Determination of phytic acid

Phytic acid was determined using the procedure described by Lucas and Markakas (1975). A portion (2 g) of each sample was weighed into 250 ml conical flask; 100 ml of 2% concentrated hydrochloric acid was used to soak each sample for 3 h. The mixture

Table 1. Proximate compositions of *S. florida* parts.

Plant parts	Nutrients					
	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
Leaf	0.26 ± 0.008	11.50 ± 0.082	4.30 ± 0.082	8.10 ± 0.082	1160 ± 0.064	74.40 ± 0.064
Pulp	0.13 ± 0.009	10.50 ± 0.131	9.10 ± 0.186	7.30 ± 0.163	1.50 ± 0.122	72.40 ± 0.04
Pericarp	060 ±0.060	11.30 ± 0.131	7.20± 0.064	5.501 ± 0.184	1.50 ± 0.0082	73.22 ± 0.033
Seed	0.04 ± 0.064	12.50 ± 0.113	3.80 ± 0.063	2.40 ± 0.090	1.50 ± 0.081	79.56 ± 0.033

Mean values of three determinations, Mean ± S.D.

was filtered, and 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added to each solution as indicator and was titrated with standard iron chloride solution which contained 0.00195 g iron per ml.

% Phytic acid = y × 1.19 ×100

Where y = titre value × 0.00195.

Proximate analysis

The proximate analysis (carbohydrate, fats, protein, moisture and ash) of *S. florida* sample was determined by using AOAC (1990) methods. Carbohydrate was determined by difference method (100 – (protein + fat + moisture + ash)). The nitrogen value which is the precursor for protein of a substance was determined by micro – kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. The moisture and ash were determined using weight difference method while determination of crude lipid content of *S. florida* sample was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 – 60 °C). All the proximate values were reported in percentage (AOCS, 2000; Okwu and Morah, 2004).

Elemental assay

The sample was investigated for elemental composition by using atomic absorption spectrophotometer (AAS), Bulk Scientific model AVG 210. Appropriate working standard solution was prepared for each element. The calibration curves were obtained for concentration versus absorbance. The data were statistically analyzed by using fitting of straight line by least square method. All elements were determined in the medicinal plant (*S. florida*) under this investigation procedure. Laboratory procedures for the preparation and determination of macro and micronutrients were used as outlined by Shah et al. (2009) for plant samples.

Statistical analysis

All data were expressed as Mean ± S.D and Graph Pad Instat-[DataSet.1.ISD] was applied.

RESULTS AND DISCUSSION

Proximate analysis

The proximate composition of the different parts of *S. florida* are given in Table 1. Moisture content of the medicinal plant ranged from 1.10% in the leaf to 1.50% in the seed (Table 1). The moisture contents of each plant parts are different. Looking at the overall percentage of moisture composition, the pulp, pericarp and seed are the same. In the case of ash content, it was highest in the leaf and the seed had the lowest percentage. Total carbohydrate content ranged from 72.40% in the pulp to 79.56% in the seed. In all the parts investigated, carbohydrate is highest in quantity. This implies that the plant could be a good source of energy. The observed mean value for carbohydrate 79.56% in the seed was highest in all the plant parts. There are certain plants like *Croton tiglium* that can yield carbohydrates up to a low amount of 15.51% (Shah et al., 2009) these compositions showed that *S. florida* is relatively a good source of carbohydrates.

Table 1 presents the total crude fibre content of *S. florida* parts. The total dietary fibre content of pulp was maximum (9.10 ± 0.186%) while it was minimum in the seed (3.80 ± 0.0635). The pulp of *S. florida* is the main food eaten by man and animals. The leaf and other parts are traditionally applied as medicine in different ailments. The fibrous nature of the pulp could as well offer protection against certain diseases such as cancer of the colon and increase bowel content transit time. While analyzing the protein contents in the plant parts, the result showed that the pericarp had highest concentration of protein compared to other parts. The protein content was not very high in all the plant parts. A range of 0.04 to 0.60% of protein concentration between plant parts was observed, which is lower compared to other protein rich plants ranging between 23 - 33% (Shah et al., 2009). This trace of protein could be contributory to the nutritional value of this plant. Fat results demonstrated that the seed with 12.50 ± 0.1135 stand highest percentage compared to other parts of the plant investigated (Table 2). This plant parts are very rich in fatty acids and vitamins (Omale and Omajali, 2010).

Phytochemical composition

The results of the phytochemical composition (Table 2) indicated that the plant, S. *florida* is rich in Phytochemical such as alkaloids, flavonoids, tannins, saponins, cyanogeni

Table 2. Phytochemical compositions of *Saba florida*.

Plant part	Phytochemical					
	Alkaloid (%)	Cyanogenic glycoside (mg/g)	Flavonoid (%)	Tannin (%)	Saponin (%)	Phytate (%)
Leaf	3.33 ± 0.004	0.34 ± 0.006	0.062 ± 0.001	0.001 ± 0.047	0.60 ± 0.001	0.28 ± 0.001
Pulp	4.00 ± 0.131	0.80 ± 0.008	0.079 ± 0.001	0.001 ± 0.001	0.685 ± 0.001	0.163 ±0.001
Pericarp	2.00 ± 0.131	0.55 ± 0.003	0.041 ± 0.001	0.001 ± 0.002	0.68 ± 0.001	0.42 ± 0.001
Seed	2.00 ± 0.118	0.44 ± 0.013	0.0024 ± 0.001	0.002 ± 0.001	1.10 ± 0.001	0.14 ± 0.001

Mean values of three determinations, Mean ± S.D.

Table 3. Concentrations of elements detected in *S. florida*.

Plant parts	Elements (Mg/g)									
	Ca	K	Na	Mg	Pb	Fe	Cu	Zn	Ni	Cd
Pericarp	37.90±0.01	0.72±0.01	31.76±0.01	22.50±0.01	0.30±0.01	0.56±0.01^{a}	0.20±0.01ab	0.04±0.01ab	0.04±0.01ab	0.18±0.011^{a}
Seed	50.20±0.10	0.32±0.01^{a}	23.45±0.02	23.10±0.10	0.14±0.01	17.0±1.00	0.02±0.01^{ab}	0.06±0.10^{a}	0.02±0.01abc	0.16±0.09^{a}
Pulp	72.91±0.01	0.32±0.01^{a}	40.06±0.01	27.80±0.10	0.24±0.01	0.74±0.01^{a}	0.14±0.01^{a}	0.06±0.10^{a}	0.08±0.01^{abcd}	0.08±0.01
Leaf	22.87±0.01	11.20±0.10	36.91±0.01	31.6±0.10	0.04±0.01	11.00±1.00	0.16±0.01^{a}	0.20±0.1ab	0.20±0.01^{abc}	0.21±0.01

Mean values of three determinations, Mean ± S.D; Mean Values with superscript a = no significant difference; b = significant difference between pericarp and leaf (Zn) pericarp, seed, pulp and leaf (Ni); C = significant difference between seed and pulp (Ni); D = significance difference between pulp and leaf (Ni) ($p < 0.05$).

glycosides. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity (Sofowora, 1993). For instance, flavonoids have been shown to have antibacterial, anti – inflammatory, anti allergic, antiviral antineoplastic activity (Alan and Miller, 1996). Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chellators (Nakayama et al., 1993). Steroidal compounds are of importance in pharmacy because of their relationship with compounds used as sex hormones (Okwu, 2001). Saponins have been reported to show tumor inhibiting activity on experimental animals (*Rattus novergicus*) (Akindahunsi and Salawu, 2005). Saponins are more in the seed. Saponins may also enhance nutrient absorption and aid in animal digestion (Liener and Irvin, 1980). Alkaloid contributes to plant species fitness of survival. They often have pharmacological effects and are used as medication and recreational drugs (Roger and Wink, 1998). They produce bitter taste that repels insects from feeding on plant leaves. On the average, the alkaloid composition of the plant parts ranged from 2.00 ± 0.118 to 4.00 ± 0.131% with the highest level in the pulp. It could be contributory to the medicinal value of the plant leaf. Phytic acid is more abundant in the pericarp when compared to other parts. It has been reported that phytic acid chelating effect inhibits or even cure some cancers by depriving those cells of mineral (especially iron) they need. The deprivation of essential mineral like iron would much like other serve as broad treatment for cancers (Hunell, 2003). Phytic acid can also be used as preservative and also food additive (Malleshi and Desikachar, 1980).

The positive effects of glycoside and cyanogenic glycoside are not common but their toxic effects include decreased heart rate, symphatetic activity and systematic vascular resistance (Siegler, 1998). Cyanogenic glycoside concentration in *S. florida* parts studied appears verylow.Thepresenceofsomeoftheseanti-nutrients

can be reduced by various processing techniques (Siegler, 1998).

Macro and micronutrient composition

Results indicated that high concentrations of calcium (Ca), sodium (Na) and magnesium (Mg) have been found in *S. florida* parts investigated. The plant is not rich in potassium (K). In all the plant parts calcium (Ca) concentration is highest. On the average, the increasing order of the macronutrients among the investigated plant parts is K —>Mg—>Na —>Ca. The concentrations of the macronutrients are higher in the fruit pulp than other parts. The micronutrients analysis of the medicinal plant revealed significant variation among different micronutrients (Table 3). In case of Fe, it was highest in the seed followed by the leaf. The concentration of zinc which affects human health ranges from 100 to 500 mg/l (Macnicol and Beckett, 1985). The level of zinc is low in all the plant parts. Generally, all the studied plant parts have very lesser concentration of the micronutrients.

Conclusion

Our study showed that there is variation in phytochemical, proximate and elemental composition of *S. florida*. This plant could be well integrated into Nigerian food and medicine considering the results of this investigation. However, more work is needed to confirm this statement which includes feeding experiments utilizing various animal species. Isolation and characterization of the phytoconstituents to justify biological activities would be pursued vigorously.

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