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Phytochemical Composition of *Brachstegia eurycoma* and *Mucuna flagellipes* Seeds

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ABSTRACT

Brachystegia eurycoma and *Mucuna flagellipes* seeds, which are commonly used as soup condiment and flavoring agents in South Eastern Nigeria were analyzed for their chemical composition. Phytochemical studies revealed the presence of bioactive compounds comprising flavoniods (2.24-6.23 mg.100 g^{-1}), alkaloids (0.50.77 mg.100 g^{-1}), phenolic compounds (0.05-0.07 mg.100 g^{-1}), saponins (0.46-0.79 mg.100 g^{-1}) and tannins (0.13-0.16 mg.100 g^{-1}). The protein, carbohydrate, lipid and fiber content were 7.0-10.50%, 71.74-72.02%, 4.20-8.34% and 3.76-6.60%, respectively. The seeds are a good source of water-soluble vitamins, ascorbic acid (4.84-10.12 mg.100 g^{-1}), thiamine (0.05-0.13 mg.100 g^{-1}), riboflavin (0.13-0.22 mg.100 g^{-1}) and niacin (0.19-0.36 mg.100 g^{-1}). Both plant samples are good sources of minerals such as Ca, P, K, Mg, Na, Zn, Fe and Cu, while Pb and Co were not detected.

Keywords: chemical composition, flavonoids, Leguminosae, nutraceutical, saponins, tannins

INTRODUCTION

Nigeria is blessed with indigenous plants of medicinal and nutritional importance. Some of these are used as condiments, flavoring agents and as a soup thickener. Apart from their thickening and flavoring properties in soup, these plants exhibit a wide range of biological and physiological activities, namely anti-hypertensive, anti-inflammatory, anti-microbial, anti-diabetic and anti-carcinogenic. Among these plants are *Mucuna flagellipes* and *Brachystegia eurycoma* Harms, both Leguminosae.

B. eurycoma and *M. flagellipes* are Nigerian food crops used as flavoring agents and as a soup thickener (Ene-obong and Carnovale 1990; Enwere 1998; Ene-Obong 2001).

B. eurycoma grows mainly along riverbanks or swamps but also on well-drained soils. It is a large tree with an irregular bole and huge-twisted, spreading branches, which forms a generous canopy (Enwere 1998). It also possesses a rough fibrous bark, which peels off in patches and often gives out brownish buttery exudates (Keay 1998). It flowers between April and May. The fruit occurs as broad leathery dark purplish brown pods containing between four and six brown shiny flat disc-like seeds (Keay 1989; Enwere 1998; **Fig. 1A**). Despite the fact that it exudes a gum, it is also used for its timber. The seeds have thin testa, which does not withstand long soaking in water (Burkik 1995). The seed is processed into flour and used as a soup thickener and stabilizer. The decorticated seeds are ground into a powder, dried and stored if not used immediately (Enwere 1998). The leaves make an excellent browse material for cattle, sheep and goats. In agro-forestry, the tree is suitable as a shade tree and ornamental plant especially in the dry season when the tree produce masses of colored young foliage. The fruit pods make good fuel wood (Keay 1989).

Timber, known on the international timber market is produced from *B. eurycoma*. It is brown in color, very dense, moderately strong, fairly durable and used to produce plywood cones and faces, flooring frames, stairway tool handles, furniture and cabinet works, roof trusses, rafters and shuttering (Keay 1989).

M. flagellipes is a tropical forest climbing perennial herb. It produces long, fairly longitudinal ribbed pods, which are covered with brownish yellow hairs. The pods are green when immature but black when dry and mature. Each pod contains one to four seeds, which are white when immature, but black when mature and dry (Enwere 1998; **Fig. 1B**). The seeds can be used either when they are fresh and tender or when dry. The dry mature seeds are broken to remove the testa. The cotyledon is cooked sufficiently and ground into flour, which is used as a soup thickener. A major set back in food is its tendency to darken in color after food preparation and exposure to air ambient temperature (Enwere 1998). Soups thickened with *M. flagellipes* flour darken after preparation. It is among the thickeners consumed in South Eastern Nigeria (Ene-obong and Carnovale 1990; Ene-obong 2001). *Mucuna* has also been used as a uterine stimulant and as an aphorodisiac (Ezueh 1997).

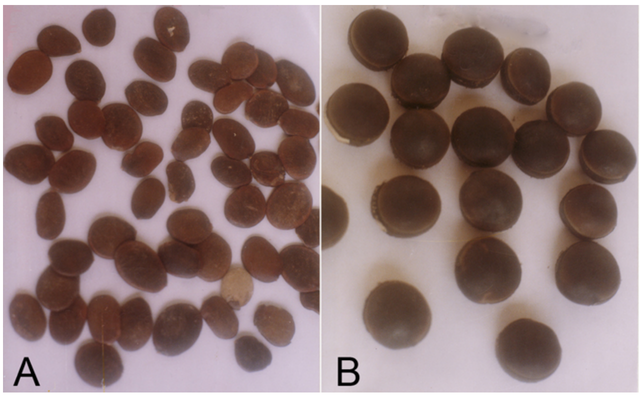

Fig. 1 (A) *Brachystegia eurycoma* and (B) *Mucuna flagellipes*.

In Nigeria, other varieties like *M. sloaneri* have been cooked for pregnant women to avoid miscarriages (Ezueh 1997). Odoh *et al*. (2005) reported on the blood sugar-lowering effect of gum extract of *M. sloaneri* seed, which is widely used in Nigeria for the management of diabetes

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mellitus (Odoh *et al*. 2005).

A variety of phenolic compounds have been isolated from *M. flagellipes*. The most important are catechins, cinamic acid ester, 3,4-dihydroxyphenolalamine (DOPA), and tyrosine (Sapers 1993), aromatic acids, quinones and cinamic acids (Walker 1976, 1995). In spite of the various uses of *B. eurycoma* and *M. flagellipes* as food additives, food thickener and condiments in Nigeria, their constituents have not been fully documented with respect to their phytochemical composition. The present study was undertaken to evaluate the chemical constituents of *B. eurycoma* and *M. flagellipes* and to consequently assess their potential usefulness as food supplements and pharmaceutical raw materials for drug formation.

MATERIALS AND METHODS

Seeds were purchased from Ndoro market, Ikwuano, Abia State, Nigeria. The plant material were identified and authenticated by Dr. A. Meregini of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike. Voucher specimens were deposited in the Forestry Department, Herbarium of Michael Okpara University of Agriculture, Umudike.

Mature seeds of *M. flagellipes* and *B. eurycoma* were each weighed (400 g). The testa of mature seeds of *M. flagellipes* were broken with a hammer, and the flattened cotyledons (400 g) were sufficiently cooked to tenderize them, then ground into uniform flour using a Thomas-Wiley mill machine (model Ed-5, USA). The flour was then dried and stored for up to three months in airtight bottles for chemical analysis; yield (260.10 g). The seeds of *B. eurycoma* were soaked in water overnight. The loosened testa were then peeled off and the decorticated seeds were ground into a uniform powder using the Thomas-Wiley mill machine, dried and stored for up to three months in airtight bottles (yield = 298.95 g).

Chemical analysis

Total nitrogen (N) content was determined by the use of a Micro-kjeldahl MD 55 (Singapore) apparatus. The protein content was calculated as N X 6.25. Crude fat (ether extract), crude fiber and ash content were determined according to the methods of AOAC (1984). Total carbohydrates were estimated as the remainder after accounting for ash, crude fiber, protein and fats (Muller and Tobin 1980). The gross food energy was estimated according to the methods of Osborne and Voogt (1978), by using the equation:

$$FE = (\%CP \times 4) + (\%CHO \times 4) = (\%Fat \times 9)$$

where: FE = food energy (in g calories), Cp = crude protein and CHO = carbohydrates.

The major elements were comprised of calcium, phosphorus, sodium, potassium, magnesium and trace elements (iron, copper, zinc and lead), all of which were determined according to the method of Shahidi *et al*. (1999).

Phosphorus content of the digest was determined calorimetrically according to the method described by Nahapetain and Bassiri (1995).

Alkaloids and phenols were determined according to the method of Harborne (1973) while tannin was determined using the method of van Burden and Robinson (1981). Saponin was determined using the method of Obadoni and Ochuko (2001). Flavonoids were determined according to the method of Boham and Kocipia (1994).

The B-complex vitamins (thiamine, riboflavin and niacin were determined according to the methods of SKALAR Analyzers (2000) while ascorbic acid (Vitamin C) was determined using the method of Baraket *et al*. (1993).

Statistical analysis

All measurements were replicated three times and standard deviations determined. The Turkey's student *t*-test at $P<0.05$ was applied to assess the difference between the means (Steel and Torrie 1980).

RESULTS AND DISCUSSION

The phytochemical content of *B. eurycoma* and *M. flagellipes* is shown in **Table 1**. The flavonoid content was very high in *M. flagellipes* (6.23 mg.100 g^{-1}) but lower in *B. eurycoma* (2.24 mg.100 g^{-1}). Flavonoids are a widely distributed group of polyphenolic compounds, characterized by a common benzopyrone ring structure that has been reported to act as an antioxidant in many biological systems. The various biological functions of flavonoids, apart from their antioxidant property, include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu and Okwu 2004; Okwu and Omodamiro 2005; Okwu and Ndu 2006). Another secondary metabolic constituent of *B. eurycoma* and *M. flagellipes* seeds that was detected was saponin. Saponin was found in *M. flagellipes* seeds at 0.79 mg.100 g^{-1} while 0.46 mg.100 g^{-1} was detected in those of *B. eurycoma*. Some of the general characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*. 2000).

Table 1 Phytochemical composition of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds on a dry weight basis.

Phytochemical	*Brachystegia eurycoma*	*Mucuna flagellipes*
Alkaloids	0.50 ± 0.10^a	0.77 ± 0.30^a
Flavonoids	2.24 ± 0.20^a	6.23 ± 0.20^a
Tannis	0.13 ± 0.11^a	0.16 ± 0.10^a
Saponins	0.46 ± 0.20^a	0.79 ± 0.10^a

Data are means ± standard deviation of triplicate determinations on a dry weight basis. Means followed by the same superscript in each row are not significant ($P<0.05$).

B. eurycoma has more phenol (0.7 mg.100 g^{-1}) than *M. flagellipes* (0.05 mg.100 g^{-1}). Phenolic compounds from plant extracts act as an antimicrobial agent (Ofokansis *et al*. 2005). The darkening of soup is due to the oxidation of phenolic constituents, especially *o*-hydroxy or trihydroxy phenolics, by a phenol oxidase present in the tissue of *M. flagellipes*. The presence of polyphenols such as L-Dopa (3,4 dihydroxyphenylalanine) and other micro-chemicals available in *M. flagellipes* may be responsible for its uses in herbal medicine and its ability to cause soup darkening. The presence of phenols indicates that *B. eurycoma* and *M. flagellipes* could act as anti-inflammatory, anti-clotting, anti-oxidant, immune enhancement and hormone modulators (Okwu and Omodamiro 2005).

A good amount of alkaloids were found in *M. flagellipes* (0.77 mg.100 g^{-1}) while 0.50 mg.100 g^{-1} of alkaloids were available in *B. eurycoma*. Pure, isolated plant alkaloids and their synthetic derivatives are used as a basic medicinal agent for its analgesic, antispasmodic and bacterial effects (Okwu and Okwu 2004; Okwu 2005). They exhibit marked physiological activity when administered to animals. Most plant parts used in the cure of diseases have been reported to contain traces of alkaloids. For instance *Azadirachta indica* used in the cure of malaria contains alkaloids (Stray 1998). Quinine, isolated from *Cinchona* bark is the oldest known effective anti-malarial agent (Vollhard and Schone 1994). The content of these phytochemicals are fairly similar to those of *Glycine max* (soybean) (Iwe 2003) and Nigerian legume seeds namely bambara groundnut, kidney bean, lima bean, pigeon pea, jack bean and *Vigna unguiculata* (cowpea) (Apata and Ologhobo 1994).

The seeds of *B. eurycoma* and *M. flagellipes* have a high content of protein, carbohydrates, lipids and minerals (**Tables 2, 3**). The highest amount of crude protein (10.50%) was found in *M. flagellipes* while *B. eurycoma* contained 7%. Plant protein may be consumed as whole plants or leaves, raw, dried or cooked (Okwu 2006). These flavoring agents are not only rich in protein but also calories. *B. eurycoma* had the highest food energy of 391.82 g.cal^{-1}. The high-energy value in *B. eurycoma* is due to its high lipid content of 8.54%. The total carbohydrates available in the

Table 2 Proximate composition and energy content of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds on a dry weight basis (mg.100 g^{-1}).

Constituents	*Brachystegia eurycoma*	*Mucuna flagellipes*
Crude protein N X 6.25%	7.00 ± 0.10[a]	10.50 ± 0.11[a]
Crude fibre %	3.76 ± 0.20[a]	6.60 ± 0.20[a]
Lipids %	8.54 ± 0.11[a]	4.20 ± 0.02[a]
Ash %	8.96 ± 0.20[a]	6.68 ± 0.03[a]
Carbohydrates %	71.74 ± 0.10[a]	72.02 ± 0.20[a]
Food energy (g/calories)	391.82 ± 0.10[a]	367.88 ± 0.11[a]

Data are means ± standard deviation of triplicate determinations on a dry weight basis. Means followed by the same superscript in each row are not significant (P<0.05).

Table 3 Mineral composition of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds on a dry weight basis (mg.100 g^{-1}).

Macro elements (%)	*Brachystegia eurycoma*	*Mucuna flagellipes*
Calcium	1.61 ± 0.20[a]	1.81 ± 0.20[a]
Magnesium	0.94 ± 0.11[a]	1.25 ± 0.13[a]
Potassium	0.83 ± 0.10[a]	0.62 ± 0.11[a]
Phosphorus	0.52 ± 0.01[a]	0.49 ± 0.20[a]
Sodium	0.14 ± 0.20[a]	0.23 ± 0.20[a]
Micro elements (mg.100 g^{-1})		
Iron	125.850 ± 0.10[a]	92.100 ± 0.20[a]
Copper	0.072 ± 0.21[a]	0.084 ± 0.10[a]
Zinc	1.200 ± 0.20[a]	1.700 ± 0.30[a]
Pb	<0.001	<0.001
Co	0.000	0.000

Data are means ± standard deviation of triplicate determinations on a dry weight basis. Means followed by the same superscript in each row are not significant (P<0.05).

Table 4 Vitamin content of *Brachystegia eurycoma* and *Mucuna flagellipes* seeds on a dry weight basis (mg.100 g^{-1}).

Vitamins (mg.100 g^{-1})	*Brachystegia eurycoma*	*Mucuna flagellipes*
Ascorbic acid (Vitamin C)	10.12 ± 0.10[a]	4.84 ± 0.20[a]
Niacin (Nicotinic acid)	0.36 ± 0.01[a]	0.19 ± 0.01[a]
Riboflavin (Vitamin B_2)	0.23 ± 0.20[a]	0.13 ± 0.10[a]
Thiamin (Vitamin B_1)	0.13 ± 0.11[a]	0.05 ± 0.20[a]

Data are means ± standard deviation of triplicate determinations on a dry weight basis. Means followed by the same superscript in each row are not significant (P<0.05).

seeds are very high, with *M. flagellipes* and *B. eurycoma* recording 72.02% and 71.74%, respectively. The amount of crude fiber was high with *M. flagellipes* having 6.60% fiber content. *M. flagellipes* therefore not only acts as food thickener but also as a dietary fiber, which, among other things and enhances frequent waste elimination including bile acids, sterols and fats (Akobundu 1999). The mineral content of both seeds is shown in **Table 3**. Calcium was the most abundant macro element present. Iron was present at 125.85 mg.100 g^{-1} in *B. eurycoma*, while *M. flagellipes* contains 92.10 mg.100 g^{-1} of iron. Zinc content was 1.70 and 1.20 mg.100 g^{-1} in *B. eurycoma* and *M. flagellipes*, respectively. The zinc content could mean that the seeds can play a valuable role in the management of diabetes, which results from insulin malfunctioning. Zinc is vital for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body (Okwu 2005). A high content of iron (125.85 mg.100 g^{-1}) was observed in *B. eurycoma* while *M. flagellipes* contained 92.10 mg.100 g^{-1}. Iron is a component of hemoglobin. It helps oxygen transport. Iron, together with hemoglobin and ferrodixin plays an important role in a man's metabolism by transporting oxygen within the body (Okwu 2006). However, the lower sodium content in these seeds might be an added advantage due to the direct relationship of sodium intake with hypertension in humans (Dahl 1974).

Lead and cobalt were not detected in the two seeds. Calcium was the most abundant microelement in the seeds with *B. eurycoma* having 1.61 mg.100 g^{-1} and *M. flagellapes* contained 1.81 mg.100 g^{-1}. Normal extra-cellular calcium concentrations are necessary for blood coagulation (Okaka and Okaka 2001). Thus the potential of *M. flagellapes* and *B. eurycoma* to stop bleeding and their use in treating osteoporosis in herbal medicine could be as a result of high calcium content. Results of the analysis of *B. eurycoma* and *M. flagelippes* showed that the seeds are rich in vitamins (**Table 4**). Riboflavin, thiamin and niacin were also detected in both seeds.

The use of high performance liquid chromatography (HPLC), gas chromatography (GC) and mass spectrophotometry (MS) allows for identification and quantification of relevant secondary metabolites. These equipments allow the development of micro separation techniques. HPLC and GC-MS permits the combination of powerful separation techniques with sophisticated structural elucidation and isolation devices (Seger *et al*. 2005). However, this equipment is very expensive and can not be afforded in many analytical laboratories. We have adopted and adapted alternative methods that are cheap and affordable to analyze medicinal plants and plant-derived phytopharmaceuticals. These methods also permit the identification and quantification of relevant secondary metabolites but could not produce structural elucidation of the compounds as HPLC and GC-MS.

Using the proximate, phytochemical, mineral and vitamin composition as approximate indices for the expression of the nutritional quality, it would appear that *B. eurycoma* and *M. flagelippes* lay in the range between most legumes such as *Glycine max* (soybeans), (Iwe 2003), *Vigna unguiculata* (cowpea), bambara groundnut, kidney bean, lima bean (Apata and Ologhobo 1994).

The outcome of this investigation has greatly elucidated the nutritional composition of the seeds of *M. flagellipes* and *B. eurycoma* as quality food with good medicinal properties. The nutritional and health benefits of *M. flagellipes* and *B. eurycoma* place these crops in an excellent position for utilization as nutraceuticals. The pharmaceutical and nutraceutical industries should undertake innovative research into the potential use of these plants for food and drug formulation.

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