Phytochemical analysis and mineral element composition of ten medicinal plant seeds from South-west Nigeria

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Abstract: The phytochemical, proximate and mineral element composition of ten different medicinal plant seeds were assessed and compared. The medicinal plant seeds investigated are *Canna bidentata, Ceasalpinia bunduc, Cola millenii, Hunteria umbellata, Hydrocotyle asiata, Megaphrynium macrostarchyum, Perinari excelsa, Rauwolfia vomitoria, Solanum dasyphyllum and Sphenocentrum jollyanum.* The result of the phytochemical analysis showed that all the selected plant seeds contain alkaloids and saponin except *Megaphrynium macrostarchyu* while phenolic group is present in *Perinari excelsa only*. The moisture content of the samples ranged between (12.51-26.7%), crude protein (8.65-48.09%), crude fibre (2.69-12.66%), crude fat (2.65-18.10%), ash content (3.26-11.45%) and carbohydrate (16.79-59.38%). Mineral element analysis showed that the selected plant seeds contained low levels of potassium (2.14-8.12 mg/L), zinc (1.38-5.53 mg/L), iron (0.22-1.90 mg/L) and manganese (0.14-1.40 mg/L) and high level of calcium (3.25-68.55mg/L). All the selected plant seeds have potential of serving as supplementary sources of antimicrobial drugs and essential nutrients to man and livestock.

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Introduction

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners. Medicinal plants are the source of synthetic and traditional herbal medicine (Satheesh et al., 2012). Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance. From ancient times, different parts of medicinal plants have been used to cure specific ailments (Abubakar et al., 2010). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Ciocan and Bara, 2007; Iwu et al., 1999). Thousands of rural communities still depend mainly on folklore medicine to cure diseases in developing countries. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. Medicinal plants are cheap for most of the populations around the globe. As a result of proximity, reliability and age long practice, people still depend largely on traditional medicine for their

health care. Medicinal plants play significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world.

Canna bidentata beloinging to the family of Cannaceae is a tree of about 11-19m height, and it is commonly called Ido among Yorubas in the Western part of Nigeria. The plant has lots of medicinal application as described by traditional healers and good in the treatment of some infectious diseases. Caesalpinia bonduc commonly known as Gray Nicker nut or Fever nut in English and Avóo in Yoruba is a prickly shrub with grey, hard, globular shaped seeds with a smooth shining surface (Nadkarni, 1954). It is a medicinal plant predominantly distributed in the tropical and sub-tropical regions of Africa, Asia and the Caribbean (Gupta et al., 2003). It has a lot of applications in folk medicine. Cola millenni belong to the legume family called Sterculiaceae and commonly known as Monkey cola in English and Obi-edun in yoruba. In Nigeria, people eat C. millenni as food and in combination with other plants for medicinal use.

Hunteria umbellate, a tree of about 15-22 m in height is found in west and central Africa. In Nigeria, it is known as Osu (Edo), erin (Yoruba) and nkpokiri (Ibo). The leaves have been described as broad, abruptly acuminate and broadly lineate. Hydrocotyle asiata, as it is commonly called, is known in the world as memory nut because it enhances the memory. It acts as cleaner of the blood, facilitates learning ability and strengthens the nervous

system, and also effective in the treatment of menstrual problems. Megaphrynium macrostachyum, of the family Marantaceae, is found in the rainforest of West and Central Africa (Jennings et al., 2001). The leaves are harvested from the forest and used fresh in wrapping food in order to preserve the food. Perinari excels, commonly known as Grey plum or rough skin plum in English and Abere in Yoruba, is an evergreen tree found growing up abundantly in the humid rain forest and less in the Guinean forest. They are widely distributed in tropical Africa and occur gregariously at elevations between 3,000 and 6,000 ft (Keay, 1964). The bark of the tree, pounded or macerated is traditionally applied as treatment to fresh wounds especially in circumcision, while the bark decoction is taken to relieve stomach ache (Stephen and Joseph, 2011). Rauvolfia vomitoria, an Apocynaceae, is a medicinal plant widely distributed all over the world especially in Asia and West-African countries. It is a tree that grows to a height of about 15 m and is found in most lowland forest. In Nigeria, especially in Yoruba speaking region, the plant is popularly known as "Asofeyeje" meaning bearing fruits for the birds. It is known as "Akanta" in Ibo and "Penpe" in Ashanti, Twi and Wassaw and poison devil's pepper in English. The plant has been used extensively for various ailments; it is useful in the lowering of blood pressure (Amole, 2003). Solanum dasyphyllum also known as Bamoni in Yoruba, brown, soft, globular shaped seeds with a rough surface (Nadkarni, 1954). It belongs to the family of Sterculiaceae and has been described useful in traditional medicine for the treatment of caugh and other infectious diseases. Sphenocentrum jollyanum commonly called Akerejupon by the Yorubas is a small erect sparsely branched shrub, growing up to 1.5 m in height with very few branches. All morphological parts of the plant are prominent ingredients in several recipes for the management of sickle cell disease (Abbiw, 1990). The root hair is used with other anti-malaria plants as remedies against fevers, body pains and rheumatism while leafy twigs and fruit have been reportedly used for their aphrodisiac activity (Burkill, 1985 and Iwu, 1993).

This research work looks into the fundamental scientific bases for the use of these medicinal plant seeds by determining the phytochemical, proximate and mineral composition of these seeds in order to evaluate their pharmacological and nutritional values.

MATERIALS AND METHODS

Collection and Identification of Plant

Fresh ten different plant seeds viz., Canna bidentata, Ceasalpinia bunduc, Cola millenii, Hunteria umbellata, Hydrocotyle asiata, Megaphrynium macrostarchyum, Perinari excelsa, Rauwolfia vomitoria, Solanum dasyphyllum and Sphenocentrum jollyanum free from disease were purchased from Ojee market in Ibadan North-East local Government and Obada market, Tapa in Ibarapa North local Government both in Oyo state, Nigeria during the month of March, 2013. The plant seeds were identified and authenticated at Herbarium Unit of Botany Department, University of Ibadan, Oyo state, Nigeria. The seeds were sun-dried and screened to remove undesirable materials such as stones and other impurities, after which they were dehulled, milled into powder and the powder kept in an air-tight polythene bags until needed for analysis.

Proximate analysis

The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of the samples were determined using methods of the Association of Official Analytical Chemists (AOAC, 1984). All determinations were done in triplicates. The proximate values were reported in percentage. Determination of moisture content was done by weighing the sample in crucible and drying in oven at 105°C, until a constant weight was obtained, determination of ash content was done by ashing at 550°C for about 3h. The kieldah method was used to determine the protein content by multiplication of the nitrogen value with a conversion factor of 6.25. The crude fibre content of the samples was determined by digestion method and the crude fat was done by Soxhlet extraction method. Total soluble carbohydrate was determined by the difference of the sum of all the proximate composition from 100%. The calorific energy value was obtained according to the methods of Akinyeye et al. (2010, 2011). This was done by multiplying the value of carbohydrate, protein and crude fat by the Atwater factors of 17, 17 and 37 respectively (Akinyeye et al., 2011 and Kilgour, 1987).

Mineral element analysis

The mineral contents of the selected plant seeds: potassium and sodium were determined using flame photometer, while calcium, magnesium, iron, zinc and manganese were determined using atomic absorption spectrophotometer as described the methods of the Association of Official Analytical Chemists (AOAC, 1990) after appropriate digestion by acids. All the determinations were done in triplicates.

Phytochemical analysis

Qualitative phytochemical analyses of the selected plant seeds were determined using the methods of Sofowora (1993) and Harborne (1999). All determinations were done in triplicates.

Test for tannins

1 g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride was added and observed for any formation of precipitates and any colour change. The reaction mixture was observed for a brownish green or blue-black colouration for the confirmation of the presence of tannins.

Test for saponins

1 g of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out

- (a) Demonstration of frothing: 2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2mins, formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate
- (b) Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

Test for steroids

- (a) About 0.2g of each portion of the powdered sample was dissolved in 2 ml of chloroform.0.2 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown colour at the interface between the layer indicates the deoxy-sugar characteristics of cadenolides which indicates the presence of steroid
- (b) 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml of concentrated H₂SO₄. The colour change from violet to blue or green in some samples is an indication of the presence of steroids.

Test for alkaloids

1 g of each powdered sample was separately boiled with water and acidified with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

Test for cardiac glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed

Test for free anthraquinones

5 ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

Test for combined anthraquinones

1 g of powdered sample of each sample was boiled with 2 ml of 10 % hydrochloric acid for 5mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

Test for flavonoids

1 g of powdered sample of each sample was separately boiled in 20 ml of water and then filtered. 5 ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4 . A yellow coloration was indicative of the presence of flavonoids.

Test for terpenoids

5~ml of each extract was mixed in 2~ml of chloroform. 3~ml of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

Test for carotenoids

1 g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Phenolics

0.5 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5mins and filtered while hot. Then 1ml of ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

Test for reducing sugars

To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5mins. The mixture was filtered while hot and the cooled; 5ml of mixture of equal volumes of Fehling's solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2mins. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.

Statistical analysis

All data generated were analyzed using descriptive statistic (Olawuyi, 1996). Statistical values that were calculated include mean and standard deviation.

RESULTS AND DISCUSSION

Presented on Table 1 is the scientific, family, English and local names of the plant seeds that were screened.

Proximate Analysis

The protein content determined for the selected seeds as presented in Table 2 shows that crude protein content was significantly higher in S. jollyanum (48.09%) and lower in *R. vomitoria* (8.65%). Others are C. bidentata (9.0%), C. bunduc (19.67%), C. millenii (8.52%), H. umbellata (21.31%), H. asiata (17.51%), M. macrostarchyum (10.78%), P. excelsa (11.76%) and S. dasyphyllum (10.25%). The protein contents was reported by Bello et al. (2008) for C. millenii as (9.19%) which falls to the same range with our findings. The little variation in the values could be as a result of different locations in which the plants are collected. Availability of such high contents of protein are helpful in maintaining proper growth and development in adults, children, and pregnant which require good quantity of protein daily (Aletor and Adeogun, 1995). Crude fibers of these samples varied from (2.69-12.66%) being lowest in P. excelsa and highest in C. bidentata. As a nutritive value of food,

fibers in the diet are necessary for digestion and effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida et al., 2000). Thus these medicinal plants can be considered as a valuable source of dietary fiber in human nutrition. The moisture content of the selected seeds revealed that C. bunduc and C. bidentata contained the highest and lowest moisture content (26.70%) and (12.51%) respectively among the ten selected medicinal plants. Hussain et al. (2010) suggested a strong correlation between moisture contents and fiber, which could be of interest to human health as the fibrous are easily digested and disintegrated. The difference in moisture content between different plants is directly depended to the plant physiological setup and climatic changes (Goss, 1980). The results of the fat analysis indicated that C. bunduc (18.10%) and H.umbellata (17.60%) have higher concentration of fats as compared to the other species. The crude fat contents in some of the plant species such as P. excelsa and C. millenii (6.95 and 8.72%) are low compared to reported values by Stephen and Joseph (2011) for P. excelsa (7.50%) and Bello et al. (2008) for C. millenii (40.0%). Ash value turned out to be high in C. bidentata (11.45%), and low in S. jollyanum (3.26%). The ash content of C. millenii reported by Bello et al. (2008) was 3.0%; this is significantly lower than the present findings of 8.31% for the same plant species. The carbohydrate values obtained for the selected seeds ranged from 16.79-59.38% for S. jollyanum and P. excelsa respectively. Carbohydrates are known to be important components in many foods, and the digestible carbohydrates are considered as an important source of energy. Our findings revealed that the selected seeds are very good sources of carbohydrate with high energy values which gives the needed energy for good living of human and livestock.

Minerals composition

All the selected plant seeds that were used in this study contained appreciable amount of minerals (Table 3). In this study, plants with higher mineral compositions are *S. dasyphyllum* containing Mn (130.63±2.65), Ca (60.40±2.97) and *H. umbellata* containing Ca (68.55±2.62) while values obtained for *C. millenii* was lower than the one reported by Bello *et al.* (2008). The differences in the composition may be due to the differences in the locality of their growth. Minerals are required for normal growth, activities of muscles and skeletal development (calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium). Iron is useful in prevention

of anemia and other related diseases (Oluyemi *et al.*, 2006). Manganese plays a role in energy production and in supporting the immune system, (Muhammad *et al.*, 2011). Deficiency of these nutrients and minerals are known to affect the performance and health in both humans and livestocks (Merck, 2005).

Phytochemical screening

Phytochemical screening of the selected plant seeds shows that all the selected plant seeds contained alkaloids; all contained saponin except *M. macrostarchyu*; flavonoids is present in *C. bunduc*, *H. umbellata*, *H. asiata*, *S. dasyphyllum* and *S. jollyanum*; terpenoids is present in *C. millenii* and phenolic group is present in *P. excelsa* only. Tannins, steroids,

reducing sugar, cardiac glycoside are present interchangeably while phylobatannin and free anthraquinone are absent in all the selected plants. These compounds have been shown to be active against potentially significant pathogens including those that are responsible for enteric infections (Owolabi et al., 2007). Apart from their potential antibacterial activity, compounds present in this study such as alkaloids are known as antimalarial agents, analgesics and can act as stimulants. Glycoside moieties such as saponins, anthraquinones, cardiac glycosides and flavonoids can inhibit tumor growth, act as an antiparasitic agent, and can be used as an antidepressant.

Table 1: Scientific, Family, English and Local names of the seeds investigated

Scientific Name	Family Name	English Name	Local Name
Canna bidentata	Cannaceae	-	Ido
Ceasalpinia bunduc	Fabaceae	Gray Nicker Nut	Ayo
Cola millenii	Sterculiaceae	Kola nut	Obi Edun
Hunteria umbellata	Apocynaceae	-	Erin
Hydrocotyle asiata	Sterculiaceae	Wonderful kola (memory nut)	Obi Awogba arun
Megaphrynium macrostarchyum	Marantaceae	-	Gbodogi
Perinari excelsa	Chrysobalanaceae	Grey plum	Abere
Rauwolfia vomitoria	Apocynaceae	Poison devil's pepper	Asofeyeje
Solanum dasyphyllum	Sterculiaceae	-	Bamoni
Sphenocentrum jollyanum	Menispermaceae	-	Akerejupon

Table 2: Proximate (%) results of the selected plant seeds

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PLANT SEEDS	MOISTURE	CRUDE	CRUDE	ASH	FIBRE	CARBOHYDRATE	Energy value			
		FAT	PROTEIN				(Kcal/100kg)			
C. bidentata	12.51±0.010	3.25±0.000	9.00±0.800	11.45±0.007	12.66±0.040	51.13±0.120	1142.26			
C. bonduc	26.70±0.010	18.10±0.002	19.67±0.460	7.21±0.010	7.74±0.017	20.58±0.071	1353.95			
C. millenii	19.00±0.000	8.72±0.010	12.52±0.400	8.31±0.012	5.28±0.020	51.54±0.018	1411.66			
H. umbellate	23.30±0.010	17.60±0.002	21.31±0.470	5.56±0.010	5.95±0.010	26.58±0.031	1468.9			
H. asiata	18.41±0.007	8.15±0.001	17.51±0.300	5.12±0.010	3.26±0.060	47.55±0.107	1407.57			
М.	16.60±0.000	6.30±0.000	10.78±0.010	10.38±0.010	8.26±0.002	47.68±0.010	1226.92			
macrostarchyum										
P. excels	13.31±0.007	6.95±0.007	11.76±0.290	5.91±0.007	2.69±0.040	59.38±0.020	1466.53			
R. vomitoria	20.00±0.010	7.50±0.010	8.65±0.330	8.97±0.010	7.40±0.020	47.48±0.101	1231.71			
S. dasyphyllum	19.40±0.010	2.65±0.002	10.25±0.090	9.63±0.007	7.04±0.200	51.03±0.080	1139.81			
S. jollyanum	16.70±0.000	9.65±0.007	48.09±0.440	3.26±0.010	5.51±0.010	16.79±0.230	1460.01			

Table 3: Mineral element composition of selected plant seeds (mg/L)

Table 3. While at element composition of selected plant seeds (mg/L)										
PLANT SEEDS	Calcium	Magnesium	Potassium	Iron	Manganese	Zinc	Sodium			
C. bidentata	3.25±0.02	2.19±0.20	2.13±0.11	0.58±0.10	1.40±0.05	5.50±0.08	7.75±0.07			
C. bonduc	9.81±0.33	0.589±0.12	4.50±0.07	0.53±0.27	0.52±0.01	3.19±0.03	7.96±0.14			
C. millenii	23.8.±3.50	2.16±0.09	4.34±0.35	0.36±0.09	0.50±0.04	3.05±0.50	4.80±0.14			
H. umbellata	68.55±2.62	1.82±0.22	4.53±0.14	0.45±0.25	0.14±0.03	2.12±0.39	20.0±1.41			
H. asiata	3.98±0.04	0.456±0.05	4.25±0.04	0.29±0.50	0.22±0.01	1.94±0.07	6.88±0.25			
M. macrostarchyum	37.95±1.62	1.34±0.02	6.44±0.28	0.40 ± 0.08	6.86±0.04	2.67±0.01	5.85±0.21			
P. excelsa	30.45±0.21	0.35±0.00	4.29±0.01	0.35±0.06	0.47±0.01	2.28±0.30	3.90±0.14			
R. vomitoria	10.68±0.34	22.41±1.66	8.12±0.12	1.90±0.02	1.06±0.03	2.90±0.06	36.0±1.41			
S. dasyphyllum	60.40±2.97	130.63±2.65	4.15±0.00	0.33±0.30	1.12±0.01	5.53±0.07	8.90±014			
S. jollyanum	8.92±0.24	0.44±0.02	4.26±0.10	0.22±0.03	0.19±0.09	1.38±0.09	4.70±0.14			

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PLANT SEEDS						C.	R.	P.			COM.	F.	
	SAP	TERP	FLAV	TAN	ALK	GLY	SUG	GROUP	STER	PHL	ANTH	ANTH	
R. vomitoria	+	_	_	+	+	+	+	-	_	-	_	-	
C. bonduc	+	-	+	-	+	+	+	-	+	_		_	
P. excelsa	+	-	_	+	+	+	_	+	_	_		+	
H. umbellata	+	-	+	+	+	+	+	=	_	-	-	-	
M. macrostarchyum	_	-	_	+	+	-	+	-	_	_		_	
C. bidentata	+	-	-	+	+	-	+	-	_	-		-	
H. asiata	+	_	+	_	+	-	+	-	+	-	_	-	
S. jollyanum	+	-	+	+	+	-	_	-	_	_		+	
S. dasyphyllum	+	_	+	_	+	+	+	-	_	-	_	+	
C. millenii	+	+	_	+	+	+	_	_	+	_	_	_	

Table 4: Phytochemical analysis of selected medicinal plant seeds

CONCLUSION

In conclusion, the result of this research work showed that all the selected plant seeds contained appreciable amounts of phytochemicals like alkaloids, glycoside, reducing sugar and flavonoids which have good pharmacological effect and also carbohydrate, protein and minerals which are nutritional requirements of both humans and livestock. Possibly, the seeds from these plants could be useful as feed supplement and as medicine to improve health and growth performance in humans and livestock.

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