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Full Length Research.

DISTRIBUTION OF NUTRIENTS, SOME ANTINUTRIENTS AND PHYTOCHEMICALS IN THE FRUIT OF *Adenopus breviflorus* Benth

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Adenopus breviflorus **fruits locally called tagiri in Yoruba language were sourced from Oba market in Akure, Ondo State, Nigeria. The wild leguminous plant fruit is nutritionally underutilized and was evaluated for some secondary metabolites, nutrients composition, anti-nutritional properties and minerals. The results showed the presence of saponin, tannin, steroid, cadiac glycoside and flavonoids in extracts of n-hexane, methanol and ethanol respectively. The distribution of the proximate, minerals, antinutritional properties and phytochemicals of the seed, fleshy part and whole fruit revealed that the seed contained high value of crude protein 27.33±0.39 than 7.12±0.06 for the whole fruit; 4.33±0.04 for the fleshy inner succulent part and crude fat 39.91±0.08 than 19.49±0.13 for the whole fruit; 18.37±0.33 for fleshy part. The oxalate and tannin contents are more concentrated in the fleshy part (9.301±0.15%); (0.21±0.001 mg/100mg) than the seed (2.37±0.06%); (0.02±0.01 mg/100mg) and the whole part (6.33±0.15%); (0.17±0.01 mg/100mg) on dry matter basis respectively, while phytate is more concentrated in the seed (0.52±0.01mg/100mg)than any other parts. Sodium, potassium, magnesium, calcium, iron and phosphorus were detected in all the parts of the fruit but copper, manganese, cobalt, chromium and lead were not detected in any part of the sample. Therefore the *Adenopus breviflorus* can be processed to improve the nutritional requirements of animals and as bioactive components of drugs.**

Keywords: *Adenopus breviflorus,* proximate, antinutrients, phytochemicals, minerals

INTRODUCTION

The family Cucurbitaceae is a tropical family with 110 genera and 640 species. In Nigeria it is represented by 24 genera 60 species, the family is economically important as the seeds of many cucurbits are extensively used in Nigerian diet (Gill and Karatela 1982).

These constituents of the fruit have shown to posses medicinal activities as well as exhibiting physiological activity (Sofowora, 2006). Saponin has been reported to posses the ability to inhibit the growth of cancer cells, to act as a cholesterol-reducing agent, to cause depletion of body cholesterol by preventing reabsorption, thus increasing its excretion in much the same way as other cholesterol-lowering drugs such as cholestyramine, it is also an immune boost and as natural antibiotic (Malinow et al.,1977; Topping et al., 1980; Malinow et al., 1985)

Many flavonoids are antioxidants and free radical scavengers, which prevent oxidative cell damage with strong anticancer activity (Salah et al., 1995). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes (Okwu, 2006). Many terpenoids and steroidal compounds are under investigation for their antibacterial, and other pharmaceutical effects due to their relationship to sex hormones (Okwu, 2001). Alkaloids have been reported (Stray, 1998) for use as medicinal agents to posses (analgesic, antispasmodic and bactericidal effects).

The fruit of *Adenopus breviflorus* is nutritionally underutilized although its use in traditional herbal medicine and has been reported to be cheaper, more accessible and more widely acceptable than orthodox medicine, it has been perceived to be unscientific, cultic and lack precise diagnosis and dosage as well as being unhygienic in preparation, traditionally, *Ademopus breviflorus* is widely used against measles by placing it at the corners of the room, mixing it with some other herbs in its treatment of chickenpox and its use to treat stomach disorder in women, gonorrhea in man etc. Though, there has not been scientific prove for this. Because of its abundance either as wild leguminous fruit or cultivated plant was initiated in the search for its economic use such as for feeding live stock (Sofowora,2006) and its medicinal importance. This work therefore aimed at investigating the distribution of phytochemical parameters in various solvents extracts, nutrients composition, minerals and antinutritional properties in the seeds and

whole pod of the fruit of *Ademopus breviflorus* to establish the nutritional and pharmaceutical importance of this fruit.

MATERIALS AND METHODS

The fruit of *Aderopis breviflorus* plant was obtained from Oba's Market in Akure Ondo State Nigeria. The sample identification was confirmed by staff of Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure Nigeria.

EXTRACTION AND CONCENTRATION OF FRESH FRUIT EXTRACT:

The fruits were cut into pieces, blended and extracted with ethanol, methanol, n-hexane and water 1:3 w/v of the blended sample to the respective solvent for 72 hours. The extracts obtained were concentrated in vacuum using rotary evaporation to determine the phytochemical constituents.

PHYTOCHEMICAL SCREENING

Chemical tests were carried out on each extract to screen for phytochemical constituents as described by Sofowora (2006), Trease and Evans (1989) and Harborne (1984).

Test for tannins

0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponin

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for alkaloids

5.0 mL of 1% aqueous hydrochloric acid was added to 2mg of the extract in a test tube, heated in a steam bath and filtered; 1mL of the filtrate was treated with 6-10 drops of Dragendoff's reagent. The presence of creamish precipitate or turbidity after addition was taken for the presence of alkaloid.

Anthraquinone Determination

5g of each plants extract was shaken with 10ml benzene, filtered and 5ml of 10 percent ammonia solution added to the filterate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicate the presence of free anthraquinones.

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 presence of phlobatinins.

Test for flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample (Sofowora, 2006; Harborne, 1984).

5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids.

The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Salkowski test for cardiac glycosides (steroidal ring or terpenoids)

5.0 mL of each extract was mixed in 2 mL of chloroform, and concentrated H_2S0_4 (3 mL) was carefully added to form a lower layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Keller-Killani test for cardiac glycosides (deoxysugar)

50mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1.0 mL of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Lieberman's Test for steroidal nucleus

2.0mL of acetic anhydride was added to 0.5 g of each solvent extract of sample with 2.0 mL H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

PROXIMATE ANALYSIS

Moisture content, ash, crude fibre, crude protein and crude fat were determined using standard methods (AOAC, 1990). The whole fruit, endocarp without seed and seed were washed, cut into piece oven dried and pulverized. Moisture was determined by oven dried method, ash through burning the organic matter at $550^{O}C$, crude fibre was determined by hydrolyzing definite sample with dilute acid and the dilute Alkali. Crude fat was determined through Soxhlet extraction method while carbohydrate obtained by difference. Nitrogen was determined using micro Kjeldahl method, K_2SO_4 plus CuSO4 mixture was used as digestion catalyst. The protein was obtained by multiplying percentage nitrogen with a factor of 6.25.

Mineral elements were determined from the ash obtained from the proximate analysis which was dissolve in 10mL of 10% HCl filtered and diluted to 100mL with 0.01% HCl and analyzed with Atomic absorptions spectrophotometer according to Perkin-Elmer (1982) method.

ANTINUTRIENTS ANALYSIS

Phytate was determined by extraction with 2% HCl and titrated with standard Iron II Chloride solution as described by Wheeler and Ferrel (1971).
Tannin was determined using spectrophotometer; tannic acid was used as standard where serial dilutions were prepared. Absorbance of samples and serial dilution were measured at 700nm wavelength. The value of tannins as tannic acid determined from standard curve as described by Makkar et al., (1993).
Oxalate was determined by titration using AOAC (1990) method, the samples were digested with H_2SO_4 and titrated against a 0.1MKM_nO_4 solution.

RESULTS

Table 1 shows the various extracts phytochemical screening of the sample with tannin present in all the extracts, n-hexane extract did not show any trace of saponin while only the ethanolic extract contained flavonoid ; none of the solvent showed extraction of alkaloid, anthraquinone and phlobatannin. Water could not extract any of the cardiac glycoside, but other solvents extracted them in varying degree.

Table 1: Phytochemical screening of *Adenopus breviflorus*

Extractant	Tannin	Saponin	Flovonoid	Alkaloid	Antraquinanine	Phlobatamin	Legal Test for cadinolides	Salkowski test for terpenoid or cardiac glycoside (steroidal ring)	Lieberman test for Steroidal nucleus	Keller-Kiliani test for cardiac glycoside (deoxy sugar)
n-Hexan	+	–	–	+	–	–	+	+	–	+
Metahnol	+	+	–	+	–	–	–	–	+	+
Ethanol	+	+	+	+	–	–	–	+	+	+
water	+	+	–	–	–	–	–	–	–	–

Key: + presence of constituent – absent of constituent

Table 2 shows the distribution of the nutrients in the whole fruit, the fleshy part and seed, the results showed that the oil is highly concentrated in the seed the seed also contained highest level of protein; the seed contained less amount of carbohydrate, crude fiber and ash as compared with other parts.

Table 2:Proximate composition of *Adenopus breviflorus*

	Whole fruit	Fleshly part	Whole seed
Moisture	5.77±0.02	7.37±0.06	7.96±0.53
Crude fat	19.49±0.13	18.37±0.3	39.91±0.08
Protein	7.12±0.06	4.33±0.04	27.33±0.39
Ash	11.49±0.00	9.18±0.01	2.59±0.01
Crude fibre	11.34±0.08	11.67±0.12	3.25±0.02
Carbohydrate	44.78±0.21	27.53±0.26	18.96±0.94

Apart from phytate that is relatively concentrated in the seed, the seed contained significantly lower amount of oxalate and tannin as seen in Table 3

Table 3:Antinutritional properties of *Adenopus breviflorus*

	Whole fruit	Fleshly part	Seed
Oxalate %	6.33±0.15	9.301±0.15	2.37±0.06
Phytate mg/100g	0.30±0.01	0.19±0.001	0.52±0.01
Tannin mg/100mg	0.17±0.01	0.21±0.001	0.02±0.01

The results of the mineral content from Table 4 showed the presence of nutritive valuable elements like Fe, Ca, Mg, Na and K as heavy metals like Pb, Cr, Co, Cu and Mn were not detected in the sample.

Table 4: Menerals analysis of *Adenopus breviflorus* in part per million (ppm)

	Whole fruit	Fleshly part	Seed
Fe	18.19	6.06	3.03
Ca	75.41	68.25	34.67
Mg	1080.00	1015.99	1120.52
Na	656.84	691.12	619.40
K	983.02	1029.94	1074.63
P	0.75	0.50	0.95
Zn	ND	ND	ND
Co	ND	ND	ND
Pb	ND	ND	ND
Cr	ND	ND	ND
Cu	ND	ND	ND
Mn	ND	ND	ND

ND: not detected

Discussion

The qualitative estimation of the chemical constituents of the *Adenopus breviflorus* fruit in various solvents as studied showed that the fruit contained tannin and saponin, flavonoids, terpenoid, cardiac glycoside, and steroidal nucleus which were known to show medicinal activity as well as exhibiting physiological activity as reported by (Sofowora,2006). On the other hand antraquinone and phlobatannin were not detected in the solvent extracts of *Adenopus breviflorus,* the methanol and ehanolic extracts of the samples showed the presence of steroidal nucleus which are of importance and interest in pharmacy due to their relationship to compounds like sex hormones as reported by (Edeoga *et al.,* 2005). As observed from the results, n-hexane and ethanol extracts of the sample contained terpenoids which has been reported by researchers to be medicinal (Hayashi et al., 1993).

The highest concentrations of crude protein and crude fat were distributed in the whole seed while crude fiber, ash and carbohydrate contents of the seed are the least, these results are however similar to that obtained by Akintayo and Bayer (2002) for crude protein and Amoo et al., (2009) for seed of *Mucuna* species, higher than that obtained by Oboh and Akindahunsi (2003) for cassava flour subjected to solid state fermentation but far less than that observed by Omotosho (2006) for *Cirina forda* and Alobo (2003) for papaya kernel flour.

The antinutritional contents of the sample are highly concentrated between the seed and the fleshy part of the fruit. Nevertheless, these antinutrient factors are far lesser than those reported by Amoo et al., (2009) for seed of *Mucuna* species, lower than phytate and oxalate reported by Omotosho (2006) for *Cirina forda*.

Apart from zinc, cobalt, lead, chromium, copper and manganese that were not detected in any part of the fruit, other elements like iron calcium, magnesium, sodium, potassium and phosphorus were well distributed within the fruit. The elemental composition are well compared with that obtained by Omotosho (2006) for *Cirina forda*; Oboh and Akindahunsi (2003) cassava flour subjected to solid state fermentation; Amoo et al., (2009)

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