AN ASSESSMENT OF BIOCHEMICAL, PHYTOCHEMICAL AND ANTI-NUTRITIONAL COMPOSITIONS OF A TROPICAL FERN: *Nephrolepis cordifolia* L.

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ABSTRACT

This study determined the biochemical, nutrients, phytochemical, ascorbic acid; anti-nutritional, elemental and toxicity levels of the leaflets of *Nephrolepis cordifolia* mainly to investigate its nutritive potentials. Ground sample of dried leaflets was used to determine proximate and elemental analyses, dry matter, ascorbic acid and cyanide; phytate and oxalate as anti-nutritional substances. $Na+$, $K+$ and $Ca^{2}+$ were determined using flame photometer while elemental composition was determined using X-ray fluorescence (XRF). The results of the proximate analysis showed that it contained 10.28% protein, 65.40% moisture, 1.27% ash, 1.58% crude fibre, 21.47% carbohydrate while fat was not detected; 34.59 g/100 g dry matter and 23.76 mg/100 g ascorbic acid. The mineral elements present included Sodium 45.500 + 0.000 mg/L, Potassium 141.200 ± 0.007 mg/L, Calcium 26.900 ± 0.003 mg/L, Chromium 0.135 ± 0.002 mg/L, Iron 7.486 ± 0.042 mg/L, Manganese 0.689 ± 0.004 mg/L, Copper 7.242 ± 0.002 mg/L, Cadmium, 0. 149 ± 0.00 1 mg/L, Lead 1.017 ± 0.002 mg/L, Nickel 0.027 ± 0.006 mg/L, Zinc1.075 ± 0.002 mg/L. Arsenium and Magnesium were not detected. Phytochemical screening showed that saponins, tannins, cardiac glycosides, reducing sugar and carbohydrates were present while flavonoids, philobatannins and alkaloids were not detected. The anti-nutritional compound found includes 0.06 mg/100 g oxalate but phytate was not detected and toxic component was 0.16/100 g hydrogen cyanide. Acid detergent fibre, neutral detergent fibre, hemi-cellulose and lignin were also determined. The absence of phytate and the extremely low levels of both oxalate and hydrogen cyanide in this plant are highly remarkable. Thus, the use of this plant as fodder and its extract as part of animals' diet may not pose any serious health problems to the ruminants eating it.

Key words: Anti-nutritional, Animal Feeds, Herbivore, *Nephrolepis cordifolia*, Toxicity Levels

INTRODUCTION

In Nigeria, a variety of unprocessed foodstuffs are eaten by different communities regardless of the nutritive value and potential toxic hazards posed to health by some of the constituents (Enechi and Odonwodo, 2008). The intake of *N. biserrata* L. by ruminants in the tropics is high and can be used as fodder for feeding West African dwarf goats Babayemi *et al.* (2006) due to its high nutritive potential, low oxalate and cyanide contents (Oloyede *et al.*, 2008). *Nephrolepis cordifolia* L. belongs to the genus *Nephrolepis*, Family Nephrolepidaceae, Order Filicales and its common name is Boston fern (David, 1987). It is a perennial, terrestrial, epiphyte or epilithic herbaceous plant with bright green fronds that are 40-80 cm long and 1-2 mm in diameter. It is an ornamental plant and extract from it is been used to cure cough, kidney, liver disorder, skin diseases, relieves pain and as contraceptives in India (Dhiman, 1998). It is anti-diuretic and anti-inflammatory agent (Rajasekaran and Sivakumar, 2009).

It has been reported that some herbivores consume ferns (Biplab and Subir, 2007) because ferns generally have high crude protein contents (Dahlah *et al.*, 1993).

Although, the nutrient constituent of commonly used leafy vegetable species in Nigeria have been studied to some extents (Taylor 1988), the lesser known regional and local species remain virtually neglected. Lack of information of the specific nutrients in a large number of natural vegetable species with which Nigeria is widely endowed is partly responsible for their under exploitation especially in areas beyond the traditional localities where they are found and consumed (Fashakin, 2004). Therefore the specific objectives of this work were to ascertain the presence or absence of some nutritious substances, investigate and document the elemental richness, toxicity potentials and some anti-nutritional substances of the leaflets of *N. cordifolia.*

MATERIALS AND METHODS

Matured leaflets of *Nephrolepis cordifolia* were

harvested from healthy plant at the Department of Botany, Obafemi Awolowo University, Ile-Ife, washed with water and identified using IFE herbarium specimens.

Proximate, Elemental, Dry Matter and Ascorbic Acid Analyses (AOAC, 2005)

Proximate analysis was done using dried leaflets for fat, crude protein, carbohydrate, ash, crude fibre and moisture contents. The moisture content was determined by drying 10 g of the sample in the oven at 80°C for 48 hours and expressed in percentage. The crude fibre was determined by digesting 5.0 g of the sample in 1.25% sulphuric acid and 1.25% sodium hydroxide. Crude protein content was calculated by using nitrogen values obtained by Kjeldahl method (N x 6.25). The lipid content was estimated using Soxhlet continuous extraction method. The ash content was obtained by digesting 5.0 g of ground sample using muffle furnace at 550°C for 24 hours. The difference in weight was converted to percentage and expressed as percentage ash content. The total carbohydrate was calculated by the difference. The dry matter content was estimated by subtracting moisture content from 100 and expressed in grams. Vitamin C content was determined by extracting 10.0 g of the sample using 90 ml of distilled water for one hour and dyestuff titration using 50 ml of the sample in a burrete. This is by titrating the extractant with 25 ml of 2, 6-dichloroindophenol until the light rose colour changed to pink colour and persisted for about 5-10 seconds. This standardization was done thrice and then the mean value that was obtained was used in calculating the ascorbic acid concentration.

Determination of Toxicant

Hydrogen cyanide was determined using alkaline titration with 0.02 M silver nitrate ($AgNO_3$) and 8 ml of 5% potassium iodide (KI) solution until light yellow colour was appeared. 1.0 m of 0.02 M of silver nitrate equals 1.08 mg of hydrogen cyanide (AOAC, 2005).

Determination of Some Anti-Nutritional Substances

The oxalate content was determined using high pressure liquid chromatography (HPLC) methods described by Wilson *et al.* (1982). Phytic acid was estimated using the method described by McCance (1955).

Elemental Analysis AOAC (2005)

Elemental analysis of Fe, Zn, Cd, Mg, Cu, Cr, Pb, Ni, As and Mn was estimated with 0.2 g of the sample using Atomic Absorption spectrophotometer (AAS) while Sodium, Potassium and Calcium ions were carried out using flame spectrophotometer (Perkinelmer).

Preparation of Extract for Preliminary Phytochemical Screening

The leaflets were air-dried and pulverized into powder, 40 g of the sample was extracted using 500 ml of 80% aqueous methanol for 48 hours; filtered and concentrated to dryness *in vacuo* using Heidolph rotary evaporator (Laborata 4010 digital, Germany). The residue was used for re-extracted for 48 hours, filtered and also concentrated to dryness. The two concentrated extracts were combined together and used for phytochemical screening (Harborne, 1998).

Phytochemical Screening of the Extracts (Sofowora, 2008)

Alkaloid content was determined using 1.0 ml of the filtrate of the sample treated with few drops of Mayer's reagent. For carbohydrate, 0.2 g of the extract was dissolved in 40 ml of distilled water and filtered; 2 ml of the filtrate was treated with 5 ml of Seliwanoff's reagent (resorcinol in 6 M HCI) and boiled. Cardiac glycoside was determined using Keller-Kiliani and Lieberman's test with 0.5 g of the extract dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution. 1.0 ml of concentrated tetraoxosulphate (VI) acid was carefully added. For flavonoid content, 0.5 g of the extract was added to 1% Aluminium chloride and dissolved in methanol. Few drops of concentrated HC1, magnesium turnings and potassium hydroxide solution were added. Phlobatannin content was determined with 4 ml of the aqueous extract boiled in 1% aqueous HCL. For reducing sugar, 0.5 g of the extract was dissolved in distilled water, 2 drops of Fehling's solution was added and warmed on a hot water-bath. Presence of saponin was confirmed by shaking 0.5 g of the extract with water in a test tube. Persistent frothing on warming was a preliminary evidence of the presence of saponin. For tannin content, 3.0 g of the extract was stirred with 10 ml of distilled water and

filtered. Freshly prepared ferric chloride solution was added to 5 ml of the filtrate. A blue-black, green or blue-green precipitate was an evidence for the presence of tannins.

Quantitative Estimation of Some Carbohydrate Fibres (AOAC, 2005)

Acid detergent fibre (ADF) was estimated by mixing 1.0 g of the extract with acid detergent solution at room temperature. 2.0 ml Dekalin was added, boiled and refluxed for six minutes. Neutral detergent fibre (NDF) was determined using 0.5 g of the extract in a beaker for fluxing. Hemicellulose was estimated quantitatively by subtracting ADF% from NDF% results. The quantity of lignin was determined by using 10-15 ml of ADF added to 72% sulphuric acid.

Reagents and Statistical Analysis

All the reagents and chemicals used were of analytical grades (BDH Chemicals). All the values were determined in triplicate; the results were expressed as mean ± standard deviation.

RESULTS

The result of proximate analysis showed the presence of protein, fibre, ash, moisture and carbohydrate but crude fat was not detected. Dry matter and vitamin C contents were also estimated. The oxalate was among the anti-nutritional substances present but in very small quantities while phytate was not detected. The mineral elements were qualitatively determined. The result of phytochemical screening of bioactive secondary metabolites showed the presence of tannins, reducing sugar, cardiac glycosides, carbohydrate and saponin while phlobatannins, alkaloids and flavonoids were not detected. Tannins, reducing sugar and cardiac glycosides were highly present (+++), followed by carbohydrate which was moderately high (++) and saponin was slightly present (+). ADF, hemicellulose and lignin were present in small quantities while NDF was slightly higher (Tables 1, 2, 3, 4 & 5). The toxic substance found includes very small quantities of hydrogen cyanide (0.16 mg/100 g).

DISCUSSION

Our observations showed that some ants, insects and small snails are feeding on the leaflets of *N. cordifolia* and this is similar to Oloyede *et al.* (2012) who reported that *Chorthippus brunneus* (common field grasshopper) and *Lirnicolaria oleira* (small snails) feed on the leaflets of *N. furcans*. Herbivores feeding on some species of pteridophytes have been reported (Babayemi *et al.*, 2006; Biplap and Subir, 2007; Oloyede *et al.,* 2008, 2010, 2012) as further evidence of nutritious value of some pteridophytes. The lethal dose for hydrogen cyanide for ruminants is 2.0 - 4.0 mg per kg body weight and the threshold of oxalate toxicity for sheep is 2.5 mg/l00 g (WHO, 2004). The oxalate (0.06 mg/l00 g) and HCN (0.16 mg/100 g) contents in this fern fall below these ranges and far below the toxic level; therefore it cannot pose any toxicity problem to herbivores feeding on its leaflets.The ascorbic acid (vitamin C) in *N. cordifolia* (23.76 mg / 100 g) was higher than in *N. undulata* (6.66 mg/100 g) Inamah (2011) and *N. bisserata* (4.79 mg/100 g) Oloyede *et al.* (2008) showing that *N. cordifolia* is a better source of dietary ascorbic acid. Calcium in *N. cordifolia* (26.900+0.003 mg/L) was higher than in *Jatropha tanjorensis* (1.14 mg per 100 g) Falade *et al.* (2004) hence calcium is preferred in *N. cordifolia.* It is however less than in *N. bisserrata, Ceratopteris cornuta* and *N.furcans* (Oloyede *et al.*, 2008, 2010, 2012). Protein level in *N. Cordifolia* (10.28%) was higher when compared with protein contents in *Persea grattissma* (3.15%), *N. biserrata* (6.13%) and *Dialium guineense* (8.30%) (Adepoju and Onasanya, 2008; Oloyede *et al.*, 2008).

Table 1: Proximate Composition, Dry Matter and Vitamin C contents of Fresh Leaflets of *Nephrolepis cordifolia*

Protein (%)	Fibre (%)	Fat (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Dry Matter (g/100 g)	Vitamin C (mg.100 g)
10.28	1.58	Nil	1.27	65.41	21.47	34.59	23.76

Table 2: The Anti-nutritional Substance in the leaflets of *Nephrolepis cordifolia*

Toxic substances	Content (mg/100 g)
Oxalate	0.06
Phytate	Nil

Table 3: Mineral contents of the leaflets of *Nephrolepis cordifolia*

Mineral elements	Content (mg/L)	±S. E
Na	45.500	0.002
K	141.200	0.004
Ca	26.900	0.002
Cr	0.135	0.001
Cu	7.242	0.001
Fe	7.486	0.024
Zn	1.075	0.001
Cd	0.149	0.001
Pb	1.017	0.001
Mn	0.689	0.002
Ni	0.027	0.003
As	Nil	Nil
Mg	Nil	Nil

Table 4: Phytochenmical screening of *Nephrolepis cordifolia*

Phytochemicals	Level of detection
Tannins	+++
Reducing sugar	+++
Cardiac glycosides	+++
Carbohydrates	++
Saponins	+
Phlobatannins	-
Alkaloids	-
Flavonoids	-

(+): slightly present, (+ +): moderately high, (+ + +): very high, (-): not detected.

Table 5: Values of NDF, ADF, Hemi-cellulose and Lignin in *Nephrolepis cordifolia*

Substances	%
NDF	44.61
ADF	29.84
Hemi-cellulose	14.77
Lignin	12.07

NDF – Neutral detergent fibre, ADF- Acid detergent fibre.

This study has shown that *N. cordifolia* is highly nutritious and it is a better source of ascorbic acid, carbohydrate, protein and some mineral elements when compared to others such as Duckweed and water hyacinth which are beneficial to small ruminants in the tropics due to their high nutritional properties (Tamang *et al*., 1992). Just as Fashakin (1999) reported that leaf protein of *Azolla africana* Desv. have high protein, low phytate and tannin contents. *N. biserrata* can be used as fodder to feed West African dwarf goats Babayemi *et al*. (2006) probably due to its high nutritional properties, as a good source of carbohydrate, proteins, Vitamin C and minera1s, low levels of oxalate and cyanide (Oloyede *et al*., 2008). Oloyede *et al*. (2012) also reported high nutrient, protein and cardiac glycosides contents and low toxicity potentials of *N. furcans*. The result of this work agrees with Auerback and Hendrix (1980) who reported that the assumption of less and underutilization of ferns by herbivores attributed to toxins and poor nutritional compositions were untrue and may be due to less documentation of herbivores and ferns relationship.

Saponins are secondary plant metabolites with potent antifungal, anti-bacteria, anti-inflammatory and phytoprotectant properties It forms barrier to microbial attack and in plant defense against herbivores (Papadopoulou *et al*., 1999). Saponins are present in the leaflets of *N. cordifolia* in small quantity (+). *N. cordifolia* was highly rich in Cardiac glycoside (+++) just as in *N. furcans* (Oloyede *et al*., 2012). Tannins are heterogeneous groups of complex compounds which make wood to be hard and durable, useful in manufacture of ink and having medicinal values Dutta (2009) and are present in high quantities in *N. cordifolia*. This result is similar to Oloyede *et al*. (2012) that *N furcans* is highly rich in tannins. The leaflets of *N. cordifolia* also contained high quantities of reducing sugar comparable to that of *N. furcans* (Oloyede *et al*., 2012). Both proximate analysis and phytochemical screening of the leaflets of this plant showed appreciable quantities of carbohydrate which is a high energy rich food substance.

ADF is smaller in *N. cordifolia* than in *N. furcans*, it consists of lignin, cellulose and silica, the higher the ADF, the lower the energy it contains and as it level increases, digestible energy level decreases (AOAC, 2005; Oloyede *et al*. 2012). NDF is the most common measure of fiber used for animal feed analysis but it does not represent a unique class of chemical compounds. Recent report on nutritional requirement of ruminants limits the NDF intake by animals. The level of NDF in the animal ration influences the time of rumination, although the concentration of NDF in feeds is negatively correlated with energy concentration (Van, 1991; AOAC, 2005). This result is similar to the results of Oloyede *et al*. (2012) who reported that NDF was moderately high in *N. furcans* but it is still slightly higher than in *N. cordifolia*. Hemi-cellulose is a mixture of different organic compounds that makes the cells to be elastic. It is stored as food reserves in some fruits (Dutta, 2009) and represented by the difference between NDF and ADF. Small quantity of it is found in *N. cordifolia* but it is smaller than in *N. furcans*. Lignin is responsible for thickening and strengthening of the secondary cell wall as in xylem elements. It is a hard, chemically complex substance; permeable to water and contribute to the rigidity of plant body Dutta (2009), it was present in small quantities in *N. cordifolia*. It is however, higher in *N. cordifolia* than in *N. furcans*.

The two major anti-nutritional substances commonly found in leafy green vegetables are oxalate and phytate Bello *et al*. (2008) but in *N. cordifolia*, the oxalate and HCN (toxic compound) contents were extremely low while phytate was not detected. HCN is a highly volatile, colourless and extremely poisonous compound (Website 1). This toxicant compound falls below the toxicity level in *N. cordifolia* and are within the safe and accepted limits as recommended by World Health Organization (Munro and Bassir 1969). Thus, it is safe for consumption without any serious injury; hence extract from it can be incorporated into livestock feeds.

CONCLUSION

In conclusion, this study has shown that *N. cordifolia* contains biochemical substances that are important in animal feeds. It is a good source of natural antioxidant, mineral elements and vitamin C that can provide health benefits to the herbivores. The presence of low quantity of lignin in this plant shows that ruminants would be able to

consume and digest it easily. It contains low level of anti- nutritional substances and toxicant such as oxalate and cyanide and it is a nutritious plant due to the presence of ascorbic acid, protein, carbohydrates and highly useful cardiac glycosides in higher quantities. Therefore, it is not likely to cause any serious health hazard especially at the level at which it is been consumed or utilized in animal diet. Thus, extract from it could be included in animal feeds to enhance animal production especially in peasant communities in developing countries.

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