East African Scholars Journal of Biotechnology and Genetics
Abbreviated Key Title: East African Scholars J Biotechnol Genet
ISSN: 2663-189X (Print) & ISSN : 2663-7286 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-.4 | July-Aug -2019 |

Research Article

Phytochemical and Nutritional Evaluation of *Nephrolepis cordifolia* (L) C. Presl., a Tropical Edible Fern

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Abstract: The fresh leaflets of *Nephrolepis cordifolia* was assessed for its active biochemical constituents, nutritive potentials and antinutrient content. Fresh leaflets of the edible fern were collected, air dried, ground into powdered form and analysed using standard methods. The results of the qualitative phytochemical screening of the dried leaflet sample revealed that the plant contained alkaloids, flavonoids, tannins, saponins, phlobatanins, steroids, anthraquinone and cyanogenic gylcosides. The quantitative phytochemical estimation revealed alkaloids (0.10±0.00mgATE/g), saponins (0.05±0.00mg/g), tannins (4.95±0.03mtTAE/g), flavonoids (5.63±0.13mgQE/g) and total phenol (24.44±0.02mgGAE/g). The results of the proximate composition showed considerable amount of crude fiber, crude protein, ash content, carbohydrate and energy content. The results of the mineral element content revealed that the fern contained high quantity of Potassium, Phosphorus, Calcium, Magnesium, Iron and moderate amount of Manganese and Zinc. Antinutrient analysis showed low concentration of cyanide (0.06±0.01mg/100g), phytate (0.25±0.01mg/100g) and oxalate (0.69±0.01g/100g). The rich health promoting phytochemical present in this fern supports its acclaimed medicinal importance and could also be a potent source of drug. The fern is equally rich in crude fiber, crude protein, ash, carbohydrate, vitamin C and essential mineral elements. Thus, the fern could be recommended as a good source of energy and nutritionally rich fodder for domestic animals.

Keywords: Fern, phytochemical, antinutrient, mineral, *Nephrolepis cordifolia*.

INTRODUCTION

Pteridophytes show great range of forms and are cosmopolitan in distribution, found from sea level to high mountain (Padey, 2005). They are about 13,500 species of fern and allies distributed throughout the world (Moran, 2006). Some are edible while some are ornamental. They are of economic importance to man in food, medicine, ornamental, fibers and cultural usages (Camus *et al.*, 1991). Edible ferns are some of the most common wild food plants collected at the times of food shortage by the people around the world (Nwosu, 2002). Edible pteridophytes are found in the east central, South and West Africa. The fern leaves, young fronds, stems, rhizomes and in some cases the whole parts of the plant are used for food (Liu *et al.*, 2012). The various parts of the leaves of *N. cordifolia* and *Diplaziun sammatti* are consumed by various people throughout the world (Igboh *et al.*, 2009). In Nigeria, seven species of ferns are utilized as human food (Nwiloh *et al.*, 2014).

There is high intake of fern, especially *N. biseratta* by domestic animals in the tropics. Babayemi et al. (2006) was the opinion that the plant can solve the problem of animal feeds as it can be used as excellent fodders for feeding animals due to its high nutritive value. Apart from the nutritive potential of ferns, they also have medicinal applications. Sathiyaray *et al.*, (2015) presumed that all the fern and fern allied have remarkable effective medicinal value. Singh and Singh (2012) documented 23 species of pteridophytes that are traditionally used in treating different gynaecological and reproductive health related diseases by the tribal women in India.

N. cordifolia L. C. Presl. belongs to the family Nephrolepidaceae and a native to northern Australia and Asia. It is an herbaceous plant that grows in a wide variety of habitats. It is widely cultivated in the tropical and subtropical regions for its ornamental foliage. It spreads by rhizomes, stolons, tubers and spores. It has

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjbg/

Article History
Received: 25.0.2019
Accepted: 15.08.2019
Published: 30.08.2019

bright green fronds that are from 40-80 cm long and 10 cm wide at the widest point. It grows to 2-3 feet tall usually as epiphytes on palm trees. Its leaflets are boiled and eaten as vegetables. The fronts are used for treating boils, blisters and sores of the skin (Christensen, 1997) Snails and some grasshoppers feed regularly on the leaflets of *N. cordifolia* leaving the rachis alone on the plant (Adams, 2010). The plant is used as diuretic, contraceptive and in liver disorder (Dhiman, 1998). In Indian, rural women use rhizoids extract once during the menstrual period to address the problem of sterility.

There has been an increase interest recently in the food uses of pteridophytes. Gauchan *et al.,* (2008) investigated the nutrient value of *N. cordifolia* in Dhulikhel, India. Oloyede *et al* (2013) reported phytochemical and proximate compositions of *N. cordifolia* in Nigeria. The preliminary work on the matured leaflets of *N. cordifolia* with respect to their proximate and phytochemical constituents was carried out by Adebiyi (2016). The use of this edible fern is still considered as underutilized in Nigeria hence, the need for this study.

Therefore, this study aims at investigating the phytochemical, proximate, vitamins and anti- nutrient contents of *N. cordifolia* in Ekiti State, Nigeria.

MATERIAL AND METHODS

The sample of *N. cordifolia* used in this study was collected from Ekiti State University campus, Ado Ekiti, Ekiti State, Nigeria. The fresh matured leaflets of the plant were harvested, rinsed with distilled water to remove the sand and other impurities. The plant material was air dried for three weeks. The dried leaflets were then ground into a powder using an electric blender (Binatone BLG-600SMK2). The powdered sample was used for phytochemical, proximate and mineral analyses.

Qualitative Phytochemical Screening

Test for flavonoids

Flavonoid was tested using 1 g of the extract dissolved in 1% Aluminum chloride in methanol. Few drops of concentrated HCL, magnesium turnings and potassium hydroxide solution were added. Orange to pink colour change indicates the presence of flavonoids.

Test for Alkaloids

Alkaloid was detected by taking 1 g of the powdered sample and stirred with 5ml of 1% HCL on a steam bath and filtered. 1ml of the filtrate was treated with a few drops of Dragendorff's reagent (Bismut nitrate + conc, HCl). A change in the colour of the sample to black indicates the presence of alkaloids.

Test for Saponins

A portion (2 g) of the dry powdered sample was taken and boiled in 20 cm^3 of distilled water in a water bath and then, filtered. The filtrate (5 cm^3) was mixed with 5 cm^3 distilled water and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Tannins

To test for tannins, 1 g of the sample was taken and boiled in 10 ml of distilled water in a test tube and filtered. A few drops of 5% ferric chloride were added. Black colouration or precipitation shows the presence of tannins (Banso and Adeyemo, 2006)

Test for Phenols

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of the sample extract in a test tube. Formation of deep bluish green solution shows the presence of phenol.

Test for Steroids

The presence of steroids was confirmed by taken 0.5 ml of sample extract and dissolved in 3 ml of chloroform, then filtered. A few drops of concentrated Tetraoxosulphate (VI) acid were added. Reddish brown colour at the lower layer of the tube indicated the presence of steroid.

Test for Phlobatannin

In test tube, 0.5 ml of the extract was taken, 3 ml of distilled water was added and shaken for a few minutes then 1 % aqueous hydrochloric acid (HCl) was added and boiled on water bath. The formation of red colour indicated the presence of phlobatanins.

Test for Terpenoids

One gram of the powdered sample was mixed with 2 ml of chloroform and 3 ml of conc. H_2SO_4 were carefully added to form layer. A reddish-brown colouration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides (Keller-Killiani test)

One gram of the powdered sample was shaken shaken with 5 ml of distilled water in a tube and 2 ml of glacial acetic acid containing a few drops of ferric chloride was added slowly along the side of the test tube. Formation of brown ring at the interface gives positive indication for cardiac glycoside. A violet ring may also appear below the brown ring (Ayoola *et al.,* 2008).

Quantitative Estimation of the Phytochemicals

The amounts of phytochemicals in the sample was further determined using the standard procedure of Harbone (1993), Boham and Kocipal (1994) and Obadoni and Ochukwo (2001).

Proximate analysis

The estimation of moisture, ash, crude fat and crude fibre were determined in accordance with the official method of AOAC (1990). The moisture content was determined by taken 2g of the dried sample and

drying at 105^0C in an ovum until a constant weight was reached. For total ash determination, the dried plant sample was weighed and converted to dry ash in a muffle furnace at 550^0C for incineration. The crude fat content was determined by extraction with hexane, using Soxhlet's apparatus. The crude protein content was calculated as percent of nitrogen value and were converted to protein by multiplying with a factor of 6.25 (AOAC, 1990) Crude lipid content of the sample was determined using Soxhlet type of direct solvent extraction method. The solvent used was petroleum ether. Carbohydrate was determined according to Onwuka (2005) by calculating the difference between the sums of all the proximate composition from 100 percentages i.e.

Available carbohydrates = (%moisture + %Ash + %Protein + %Fiber).

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Determination of some Anti-Nutrient Substances

The oxalate content was determined using high pressure liquid chromatograph (HPLC) methods described by Wilson *et al.*, (1982). Phytate and cyanide were determined according to methods described by Wheeler (1971) and AOAC (2010) respectively.

Determination of Vitamin A and C contents

Vitamin A was determined as β- carotene according to AOAC (2010) while vitamin C was determined by iodine titration (Helmenstine, 2006).

Determination of Energy

The estimated energy value in the sample in Kilocalorie (Kcal/100g) was determined by adding the multiplied values for crude protein, crude lipid and carbohydrate respectively, using the factor (4Kcal, 9Kcal and 4Kcal).

Energy value (Kcal/100g) = (Crude protein x 4) + (Crude fat x 9) + (Total carbohydrate x 4).

Determination of the mineral composition

Elemental analysis was carried out using atomic absorption spectrophotometer (Buck Scientific Model-210 VGP) for sodium, potassium, manganese, magnesium, iron, calcium, zinc and copper while phosphorus was determined calorimetrically according to method described by State *et al.*, (2011).

Statistical analysis of data

All experiments were carried out in triplicates and the data expressed as mean + SD.

RESULTS

Quantitative Phytochemical Screening of *N. cordifolia* Leaflets

The results of the phytochemical screening of the ground dried sample of the fern leaflets revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, anthraquinone and cyanogenic glycosides while terpenoids, phlobatanins and cardiac glycosides were not detected in the plant investigated (Table 1).

Table1. Quantitative Phytochemical Screening of *N. cordifolia* Leaflets

Phytochemical	Occurrence
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	++
Steroids	+
Terpenoids	-
Phlobatanins	-
Anthraquanone	+
Cardiac glycosides	-
Cyanogenic glycoside	+

Keys: - not detected, + detected in low concentration, ++ present in moderate concentration

Quantitative Phytochemical Estimation of *N. cordifolia* Leaflets

The results obtained as indicated in Table 2 revealed quantitative estimation of the bioactive constituents of the plant. The dried powdered sample contained low concentrations of alkaloids (0.10mgATE/g) and saponins (0.05mg/g) while tannins (4.95mgTAE/g), flavonoids (5.63mgQE/g) and total phenols (24.44mgGAE/g) were found in high concentrations.

Table2. Quantitative Phytochemical Estimation of *N. cordifolia* Leaflets

Phytochemical	Quantity
Alkaloids (mgATE/g)	0.103±0.00
Flavonoids (mgQE/g)	5.63±0.13
Saponins (mg/g)	0.050±0.00
Tannins (mgTAE/g)	4.95±0.03
Total phenol (mgGAE/mg)	24.44±0.02

Proximate and Mineral composition of *N. cordifolia* Leaflets

The results of the proximate composition showed low moisture content (15.76%), considerable amount of crude fiber (7.31%), high crude protein (13.60%), ash content (12.12±0.04%), carbohydrate (47.67%) and energy content (227.05kcal/100g) (Table3). The analysis of the mineral composition of *N. cordifolia* revealed that the plant contained high amount of potassium, phosphorus, calcium, magnesium, iron and moderate quantity of sodium, manganese and zinc (Table 4).

Table3. Proximate composition of *N. cordifolia* Leaflets

Parameters	Quantity
Moisture%	15.76±0.51
Crude Fiber%	7.31±0.03
Crude Protein%	13.60±0.26
Ash Content%	12.12±0.04
Carbohydrate%	47.67±0.03
Crude Fat%	3.54±0.14
Energy (kcal/100g)	227.05±0.01

Table4. Mineral composition of *N. cordifolia* Leaflets

Mineral element	Quantity (mg/100g)
Sodium	57.45±0.45
Potassium	1985.0±5.00
Phosphorus	207.80±2.20
Magnesium	142.0±2.00
Calcium	541.50±1.50
Iron	36.30±0.30
Zinc	6.15±0.50
Manganese	2.45±0.11
Copper	0.33±0.03

Vitamins and Antinutrient composition of *N. cordifolia* Leaflets

The vitamin estimation of the plant sample as shown in Table 5 revealed low quantity of vitamin A (0.25mg/100g) and high amount of C (15.85mg/100g). The antinutrient in the fern leaflets as illustrated in Table 6 revealed low concentration of cyanide (0.06mg/100g), phytate (0.25mg/100g) and oxalate (0.69mg/100g).

Table5. Vitamin Composition of *N. cordifolia* Leaflets

Vitamin	Quantity (mg/100g)
Vitamin A	0.25±0.01
Vitamin C	15.85±0.56

Table6. Antinutrient Composition of *N. cordifolia* Leaflets

Antinutrient	Quantity (mg/100g)
Cyanide	0.06±0.00
Phytate	0.25±0.01
Oxalate	0.69±0.01

DISCUSSION

Investigation of active secondary metabolites in plants is of great importance in identifying new sources of therapeutically and industrially valuable compounds which are of great medicinal use (Adeniyi *et al.,* 2014). Studies have shown that the medicinal importance of flowering and non-flowering plants lie in some bioactive chemical substances (phytochemical) that have definite physiological action on the human body (Edeoga *et al.*, 2005, Asekun *et al.*,2013). Phytochemicals act in numerous ways to help the body in combating diseases and health challenges. The results of the present study revealed that the edible fern investigated was rich in certain health promoting phytochemicals. The results obtained corroborate with the results of Adebiyi (2016) who reported the presence of tannin, alkaloid, flavonoid, phenols and saponin in *N. cordifolia*. Our findings equally agree with the presence of tannins and saponin, and absence of phlobatanins in *N. cordifolia* leaflets reported by Oloyede *et al.*, (2013). The later reported absence of alkaloids and flavonoids which negate our findings. Omotayo and Borokini (2010) equally affirmed that family pteridophytes represented by *Pteris togoensis* lack phlobatanins and cardiac glycosides.

The fern investigated revealed the presence of alkaloids. Alkaloids are one of the most effective therapeutic bioactive substances in plants. Analgesic, antispasmodic and antibacterial properties of plants containing alkaloids have been reported by Okwu and Okwu (2004). Flavonoids possess a wide range of biological activities such as effects on central vascular system, diuresis, spasmolytic, antiviral, anti-inflammatory properties (Okwu, 2004).

Tannins are moderately found in the fern investigated and have astringent property and possess antimicrobial and antioxidant activities (Riviere *et al.*, 2009). The saponins content is low and is in agreement with the work of Oloyede *et al.*, (2013) who reported low concentration of saponins in *N. cordifolia*. Saponins have been reported to have anti-fungi, anti-tumours and anti-viral activities as well as analgesics and healing of wound properties (Arawande *et al.*, 2013). Steroids are known to regulate carbohydrate and protein metabolism and also possess anti-inflammatory

properties (Nielson and Cox, 2005). The presence of phenolic compounds in the leaflets of the fern investigated indicated that it might have antimicrobial agent which makes it effective in the treatment of typhoid fever and other bacterial infections (Otofokansi *et al.*, 2005).

The presence of these phytochemicals supports the widely acclaimed medicinal attributes of *N. cordifolia* as well as its efficacies as antimicrobial, anti-diuretic, antioxidant and anti-fungi agent.

The nutritional composition of food is the estimation of the nutritive value of human food in its chemical form (Alli Smith, 2009). The proximate estimation of the *N. cordifolia* in this present study revealed the rich nutritive value with low moisture content, high ash content, high crude protein, high carbohydrate and high energy content and moderate crude fat. The moisture content obtained for *N. cordifolia* is lower when compared to the value reported for *Neptunia oleracea* (Saupi *et al.*, 2015). Low moisture content promotes shelf life of plant and hamper the growth of microorganisms. The low moisture content of the edible fern implies that the fern can be kept for some times without deterioration. The quantity of fiber obtained (7.13%) is comparably higher than 3.20% reported for *N. furcans* (Oloyede *et al.*, 2012) but lower to the value (37.90%) reported for *N. cordifolia* (Gauchan *et al.*, 2008). High crude fiber content in diets have been reported to help in increased removal of carcinogens, potential mutagens and xerobiotic by binding to dietary fiber components (Ayoola and Adeyeye, 2009). The available fiber in *N. cordifolia* in this study could contribute to the intake of dietary fiber through the consumption of the fern.

The result obtained in this study showed that the leaf contained appreciable amount of crude proteins (13.60%). The results obtained is higher when compared with 10.25% reported for *N cordifolia* (Oloyede *et al.*, 2013) but comparably lower to some vegetable consumed in Nigeria (Arowosegbe *et al.*, 2015). High protein content in this fern agrees with the work of Biplab and Subir (2007) who reported that herbivorous animals consume fern because ferns generally have high crude protein contents. Protein is an essential component of diet needed for growth and development in animals and human being. Protein supply adequate amount of required amino acids in nutrition.

The sample contained high ash content (12.12%) when compared to 1.07% for *N. oleracea* (Saupi *et al.*, 2015) and (2.50%) for *N. cordifolia* reported by Adebiyi (2016). The ash content is an index of inorganic minerals in plant (Hassan and Umar, 2006). High ash content in any food substance is an indication of high mineral content (Fagbohun *et al.*, 2012). The crude fat of the sample was 3.54%, fourteen times the value reported for *N. furcans* (Oloyede *et al.*, 2012). The carbohydrate content of *N. cordifolia* leaflets investigated was high (47.67%) and can be good sources of energy for domestic animals. The value was relatively higher than 11.35% (leaf), 9.31% (Rhizome) and 13.42% (Tuber) of *N. cordifolia* reported by Gauchan *et al.*, (2008), but lower than 65.5% and 83.9% reported for *Diplazium. esculentum* and *Diplazium sammanti* respectively (Alfred, 2014)

The energy value of the sample investigated is relatively higher than 109.78 kcal/100g for *Amaranthus dubius*, a wild vegetable (Mih *et al.*, 2017) but lower than the value 3413 kcal kg^{-1} for *D. esculentum* (Alfred, 2014).

Results of the minerals composition clearly shows that *N. cordifolia* leaflets constitute a rich source of mineral elements. The relatively high mineral content obtained in this study has proved the *N. cordifolia* as a cheap source of dietary mineral for man and ruminant animal, sodium is important in the maintenance of acid-base balance in the body (Adeyeye, 2002). Na and K are important in the transport of metabolites in the human body. The Na/K must be less than 1 to control blood pressure (FAO/WHO, 2016). The Na/K ratio in this study is less than 1 and could probably serve to reduce high blood pressure diseases in man.

The K content is high compared to other microelemenst in the fern. Plants generally have been reported to have higher amount of K contents (deMan, 1999). Calcium plays an important role in building strong bones and teethes. Iron helps in the formation of haemoglobin, metabolism of carbohydrate, protein and fats (Gupta, 2014).

Phosphorus play an important role in the formation of strong bone and teeth, heart function cell metabolism (Rolfe *et al.*, 2009), Other essential and micro elements could serve as source of mineral supplements needed for normal health growth.

The antinutrient factors interfere with metabolic process so that growth and bioavailability of nutrients are negatively influenced (Binita and Khetarpaul, 1997). The cyanide, phytate and oxalate are some factors that tends to inhibit or antagonize as well as interfere with the functionality and availability of nutrients if present in foods (Ogungbenle, 2009). The fern investigated contained low amounts of oxalate, cyanide and phytate, hence the edible fern is highly recommended for consumption. Vitamins are required in small quantity to ensure normal metabolism, growth and physical well-being. Our findings revealed high content of vitamin C (15.85mg/100g). Vitamin C plays an important role in many physiological processes in humans. Its deficiency leads to impaired wound healing, haemorrhage, mucous membrane, weakening of collagenous structures in bone, cartilage, teeth and

connective tissues (Carr and Fred, 1999; Chaudhry *et al.*, 2005).

CONCLUSION

The results of our findings revealed that *N. cordifolia* leaflets have an enormous nutritional potential and could make a significant contribution to food supplement. The plant could be favourably be considered as good sources of phytochemicals, crude fiber, crude protein, carbohydrate, vitamin C and essential minerals. The antinutritional factors are below the recommended toxic level which makes the fern to be safe. Hence, could be recommended for consumption of man or herbivores.

CONFLICT INTERESTS

The authors declared that they have no competing interests

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors.

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