# ELEMENTAL, PROXIMATE AND ANTINUTRIENT ANALYSIS OF THE CROZIERS OF *CYCLOSORUS AFER* (CHRIST.) CHING

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***ABSTRACT***
*Croziers of Cyclosorus afer were collected from their natural habitat at Elefosan, Ipetu-Ijesa, Osun State, Nigeria. The objective was to investigate the elemental, proximate and anti-nutritional properties of Cyclosorus afer. Atomic absorption spectrometer was used for the elemental analysis. The proximate analysis was carried out following standard methods of the Association of Official Analytical Chemists. Anti-nutritional content including oxalate and phytate was determined following standard techniques. The results of the elemental analysis showed the presence of sodium, calcium, potassium, magnesium, lead, chromium, copper, zinc, nickel, cobalt, arsenic, cadmium and manganese with sodium (1.040 ± 0.040 ppm) showing the highest proportion followed by potassium (0.420 ± 0.005 ppm) and arsenic (0.001 ± 0.000 ppm) was present in the least amount. The proximate analysis showed the presence of protein (5.067 ± 0.061%), moisture (10.540 ± 0.228%), fat (1.140 ± 0.057%), ash (9.470 ± 0.200%), crude fibre (1.227 ± 0.024%) and carbohydrate (72.550 ± 0.448%). The anti-nutritional compounds found included oxalate (26.700 ± 1.150 mg/g) and phytate (1071 ± 0.252 mg/g). The findings from this study indicated that although anti-nutritional components were high, croziers from Cyclosorus afer could serve as source of nutrients for animals and humans.*
**KEY WORDS:** *fern, croziers, health, nutritional composition*

## INTRODUCTION

Pteridophytes (ferns and fern allies) due to their wide distribution and fascinating foliage, have drawn the attention and admiration of horticulturists and plant lovers (Kumar, 2018). They are represented by approximately 165 species distributed in 64 genera and 27 families (Akinsoji *et al*., 2016). They are seedless vascular cryptogamic plants which occupy a position between the lower non-seed bearing and higher seed bearing plants. They grow luxuriantly in most tropical and temperate forests and their occurrence in different geographical threatened regions from sea level to high mountains have been reported (Dixit, 1984; Bir, 1992; Kumar, 2018).

Pteridophytes contain important phytonutrients which are beneficial for human health (Uzoekwe *et al.,* 2015). These phytonutrients include natural minerals (such as zinc, iron, calcium, copper and other elements) and vitamins (Bongoni *et al*., 2013; Uzoekwe *et al.,* 2015). More than 40 elements have been considered essential to animals and plants. An element is considered essential when it is required in certain amount by living organisms for important physiological functions (Armah *et al.,* 2001; Muhammad *et al.,* 2010). The nutritional composition of commonly used leafy vegetable species around the globe have been studied to some extent (Taylor, 1988), but the less familiar species remain neglected. Lack of information regarding the nutritional composition and benefits of a large number of vegetable species is partly responsible for their under-exploitation especially in areas beyond their natural habitats (Fashakin, 2004; Oloyede *et al*., 2013).

Pteridophytes are known to contain high amounts of vitamins, minerals, fatty acids and fibre (Gafar *et al*., 2011). They also contain other chemical compounds such as saponins, tannins, alkaloids, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides, which are known as secondary metabolites and are biologically active (Soetan *et al*., 2009; Kehinde *et al*., 2014). The economic importance of pteridophytes is well known since ancient times. According to Babayemi *et al*., (2006), the nutritional benefits of pteridophytes may be the reason for herbivores feeding on them. Theophrastus (327-287 BC) and Discorides (50 AD) had discussed the medicinal attributes of certain ferns (Singh *et al*., 2017). The pteridophytes play an important role in folklore as they have been successfully used in Ayurvedic, Unani and Homeopathic medicine (Singh *et* al., 2017). They are of immense economic importance and there is a need for their exploitation towards use in daily life (Benjamin *et* al., 2007).

Different parts of pteridophytes such as the rhizome, stem, fronds, pinnae and spores are reportedly used for different purposes such as medicine for treatment of various diseases (Singh *et* al., 2017). A number of research works have been published in different fields like taxonomy, ecology and cytology on pteridophytes (Akomolafe *et al*., 2017). Wild indigenous plants such as ferns (plants belonging to pteridophytes), canada lily (*Lilium canadense*), cattail (*Typha spp*), hyssop *(Hyssopus officinalis*), Indian pipe or Ghost plant (*Monotropa uniflora*) and Yellow cress (*Rorippa palustris*) have remarkably culinary, medicinal, agronomic, aesthetic and socio-cultural importance (Abbey, 2016). Crozier-forming pteridophytes are native plants and delicacy to many canadian communities (Abbey, 2016). Most of the genera (ca. 300) and species (ca. 11,000) of wild pteridophytes found worldwide are under-utilized (Abbey, 2016).

Croziers also called fiddleheads are circinately coiled leaf buds. Proximate analysis of a plant sample are noted for giving information about the gross components (protein, fat, carbohydrate, fibres, ash etc.) of the plant rather than individual nutrients such as amino acid, fatty acids, monosaccharides and minerals (Prohp *et al*., 2006). Akomolafe *et al*. (2017) had carried out a study on the proximate analysis of roots and fronds of *C. Afer*. However, little is known about the elemental and proximate composition of the croziers of *Cyclosorus afer*, which necessitates this study. In this study, elemental, proximate and antinutrient composition of croziers of *Cyclosorus afer* (Christ.) Ching were investigated.

## MATERIAL AND METHODS

**Plant Material.** Croziers of *Cyclosorus afer* (Christ.) Ching were used for this research work. The samples were collected from the natural habitat at Elefosan area (Longitude 7° 28' 14.71" N and Latitude 4° 53' 11.60"), Ipetu-Ijesa, Osun State, Nigeria. The collected samples were taken to the laboratory where they were washed, air dried at ambient temperature in the laboratory and stored for further laboratory studies.

**Digestion of Plant Sample.** The plant sample was dried in the oven at 45°C. The dried samples were ground into fine powder from which 0.25 g was weighed into a Teflon beaker. Twenty (20) ml of freshly prepared aqua-regia, $HCl:HNO_3$ (3:1) was added and then heated to near dryness. The heat source was removed and the medium was allowed to cool. Twenty (20) ml of distilled water was added and mixture was heated for 5 minutes. This was allowed to cool and filtered. The filtrate was made up in a 25 ml flask and kept refrigerated for elemental analysis using Atomic Absorption Spectrometer (AAS).

**Elemental Analysis.** The analysis of the plant sample for macronutrients {sodium (Na), calcium (Ca), potassium (K), magnesium (Mg)} and micronutrients {lead (Pb), chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), arsenic (As), cadmium (Cd), manganese (Mn)} was carried out at the Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife, Nigeria. The Atomic Absorption Spectrometer (PG990 AAS) was used for the analysis by flame atomization, utilizing air-acetylene flame and single element hollow cathode lamp following the equipment procedures.

**Calibration Curve Preparation for Elemental Analysis.** In preparation of calibration curve for the elemental analysis, different concentrations of each element were prepared from the stock solution (1000 ppm) for each cation. These were used in preparing a linear curve, passing through zero, for each of the elements.

**Proximate analysis of the sample.** The proximate composition analysis which involves the determination of moisture, crude fat , crude fibre, crude protein, ash and carbohydrate composition of the croziers of *C. afer* were carried out using standard procedures as described by the Association of Official Analytical Chemists (AOAC) 2012.

**Determination of Anti-Nutritional Substances.** The anti-nutritional substances determined include oxalate and phytate. The oxalate content was determined according to the method described by Falade (2004). Two (2) g of the sample was digested with 10 ml of 6M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with concentrated $NH_4OH$ solution until the colour of solution changed from salmon pink to faint yellow colour. Thereafter, the filtrate was treated with 10 ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate (left overnight). The suspension was then centrifuged at 2500 rpm, after which the supernatant was decanted and the precipitate was completely dissolved in 10 ml of 20% (v/v) $H_2SO_4$. The total filtrate resulting from the dissolution in $H_2SO_4$ is made up to 300 ml. An aliquot of 125 ml of the filtrate was heated until near boiling point and then titrated against 0.05 M of standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 s after which the burette reading was taken. The oxalate content was evaluated from the titre value.

The phytate content of the sample was evaluated using the method described by Iwuozor (2019). One (1) g of the sample was hydrated in 50 ml distilled water for 1 hour and filtered. Aliquots of 25 ml of the filtrate in a conical flask was added to 5.00 ml of 0.30% ammonium thiocynate. The mixture was titrated with standard iron (III) chloride solution to a persistent brownish yellow color that persisted for 4 minutes. The amount of phytate was calculated using the equation below:

$$Phytic\ acid = Titre\ value \times 0.00195 \times 1.9 \times 100$$

## RESULTS AND DISCUSSION

The results of the analysis of the croziers of *C. afer* for Na, Ca, K, Mg, Pb, Cr, Cu, Zn, Ni, Co, As, Cd and Mn contents showed that the macronutrients were present in higher concentrations than the micronutrients with Na (1.040 ± 0.040 ppm) showing the highest concentration (Table 1) and As (0.001 ± 0.000 ppm) had the least concentration (Table 2).
The results of the proximate analysis are shown in Table 3. Moisture, ash, fibre, fat, protein and carbohydrate contents of the croziers were expressed as percentage (%) of the dry sample per gram. Estimation of the antinutrient contents showed the presence of oxalate (26.700 ± 1.150 mg/g) and phytate (1071 ± 0.252 mg/g) as shown in Table 4.

**TABLE 1: Macronutrient Concentrations of the Croziers of *C. afer***

| Element | Na | Ca | K | Mg |
|---|---|---|---|---|
| Concentration (ppm) | 1.040 ± 0.040 | 0.243 ± 0.003 | 0.420 ± 0.005 | 0.154 ± 0.000 |

Results are expressed as mean (n = 3) ± standard error

**TABLE 2: Micronutrient Concentrations of the Croziers of *C. afer***

| Element | Pb | Cr | Cu | Zn | Ni | Co | As | Cd | Mn |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | 0.002 ± 0.000 | 0.022 ± 0.001 | 0.288 ± 0.003 | 0.072 ± 0.002 | 0.014 ± 0.000 | 0.009 ± 0.001 | 0.001 ± 0.000 | 0.007 ± 0.000 | 0.014 ± 0.001 |

Results are expressed as mean (n = 3) ± standard error

**TABLE 3: Proximate Composition of the Croziers of *C. afer***

| Moisture (%) | Ash (%) | Fibre (%) | Fat (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|
| 10.540 ± 0.228 | 9.470 ± 0.200 | 1.227 ± 0.024 | 1.140 ± 0.057 | 5.067 ± 0.061 | 72.550 ± 0.448 |

Results are expressed as mean (n = 3) ± standard error

**TABLE 4: Antinutrient Contents of the Croziers of *C. afer***

| Oxalate (mg/g) | Phytate (mg/g) |
|---|---|
| 26.700 ± 1.150 | 1071 ± 0.252 |

Results are expressed as mean (n = 3) ± standard error

The results of the elemental analysis are shown in Tables 1 and 2. Thirteen metals were detected and quantified. There is a great disparity in the elemental composition of *Cyclosorus afer* as compared to the studies of other edible ferns such as *Nephrolepis furcans* (Oloyede *et al*., 2012), *Diplazium sammatii* (Adebiyi *et al*., 2019), *Ceratopteris cornuta* (Oloyede *et al.*, 2010) and *Nephrolepis cordifolia* (Oloyede *et al*., 2013). The concentrations of the thirteen elements in this study are generally lower compared to other edible ferns; which is of advantage when it comes to the consideration of toxic/heavy elements (Pb, Ni, As, Cd). Metal ions are important requirements of humans, animals, plants and even microorganisms. They are responsible for numerous biological functions. They are often referred to as minerals in the classification of food. Essential minerals are required by the human body in different amounts and are classified into macronutrients and micronutrients. Macronutrients such as Na, Ca, K and Mg are required in bulk amounts while micronutrients such as Zn, Mn, Cu, Cr and Co are needed in trace quantities.

The recommended dietary intake (RDI) or adequate intake (AI) for humans are expressed in mg/day or ųg/day. The overloading of a particular mineral can lead to toxicity and deficiency of another element e.g. excess amount of zinc can lead to copper deficiency (Baker *et al.,* 1995). *C. afer* can supply sufficient amounts of Cr, Co and to some extent Cu, and thus can be considered as a supplementary source of micronutrients (Cu, Cr, Co) for humans. However, *C. afer* cannot be totally depended on as the sole source of macronutrients for humans because of the low composition of these elements. The essential elements required by animals include calcium, magnesium, sodium, potassium, phosphorus and sulphur. Zinc, magnesium, copper, chromium, cobalt and rarely nickel are required in trace quantities by animals (Underwood, 1977). The nutritional requirements of macrominerals in animal diets are usually expressed in percentages while the trace elements are expressed in milligrams per kilogram (mg/kg) or parts per million (ppm).

The potassium (K) content of *C. afer* (0.420 ± 0.005 ppm) is lower than in *Diplazium sammatii* (2,073.80 mg/100g) and *Nephrolepis furcans* (2,515.00 mg/100g) and thus disagrees with the work of Adebiyi *et al*., (2019) and Oloyede *et al*., (2012) respectively. Potassium is needed for proper functioning of the kidneys, heart, nerves and muscles in humans. Humans require an adequate intake (AI) of 2800 – 3800 mg/day of potassium (NMRC, 1991). Potassium works alongside sodium and chloride to maintain acid-base balance and osmotic pressure of humans and other mammals. It regulates tissue turgidity in plants. Underwood *et al*., (1999) suggested a minimum of 0.4% potassium for animal nutrition.

Sodium (Na) composition (1.040 ± 0.040 ppm) is lower than in *D. sammatii* (68.10 mg/100g) and *N. furcans* (803.00 mg/100g). An AI of 460 – 920 mg/day of sodium is needed for fluid homeostasis, muscle contraction and nerve transmission in humans. The result of this study does not meet up with the sodium dietary requirement for humans. Animals often need about 0.25 – 0.65% of sodium in their diet (Hummer, 1970).

Calcium (Ca) is required for buildup of healthy teeth and bones in humans and other mammals. It is also necessary for functioning of the heart, nerves and muscles (Weaver and Heaney 1999). Deficiency can result in osteoporosis in adults. The calcium content of *C. afer* (0.243 ± 0.003 ppm) is much lower than in *N. furcans* (13,041.00 mg/100g) and *D. sammatii* (1360.00 mg/100g) as reported by Oloyede *et al*., (2012) and Adebiyi *et al*., (2019) respectively. The calcium level does not meet up with the recommended dietary intake (RDI) of 1000 – 1300 mg/day for humans. A minimum of 0.5% calcium concentration is needed by animals (National Research Council, 1978). Abnormalities of bone and teeth as well as stunted growth due to calcium deficiency has been reported in some mammals (Underwood *et al*., 1999).

Magnesium (Mg) acts as a cofactor for many enzymatic reactions and required alongside calcium for functioning of bones, muscles, nerves and heart in humans and animals alike. The magnesium concentration (0.154 ± 0.000 ppm) is lower compared to *N. furcans* (1,426.50 mg/100g) and *D. sammatii* (105.30 mg/100mg). The RDI of magnesium for adults is an estimate of 310 – 420 mg/day. Cardiovascular, neurological and gastrointestinal disorders are signs of magnesium deficiency as reported by Shils (1999). An estimate of 0.04 – 0.074% magnesium is recommended for animal nutrition.

Zinc (Zn) is essential for the functioning of more than 100 enzymes. Zinc deficiency can lead to impaired sense of smell, delayed wound healing, suppression of immune system, malformations and abortions in mammals (King *et al*., 1999). The RDI for Zn is 8 – 14 mg/day for humans. The zinc content (0.072 ± 0.002 ppm) is lower compared to *D. sammatii* (8.30 mg/100g) as reported by

Manganese (Mn) concentration (0.014 ± 0.001 ppm) is lower than in *D. sammatii* (4.19 mg/100g) and *N. furcans* (6.00 mg/100g). Manganese is required for bone, cartilage and skin formation. It is also important in the activity of many enzymes (Nielsen, 1999). Humans require an adequate intake (AI) of 5.0 – 5.5 mg/day of manganese. However, the nutritional requirements of manganese vary largely for different mammals.

Copper (Cu) is essential for several enzymatic reactions and iron metabolism (Saari *et* al., 1999). Humans need an AI of 1.2 – 1.7 mg/day. The copper composition of *C. afer* (0.288 ± 0.003 ppm) is lower than in *D. sammatii* (0.51 mg/100g). It is a better source of copper compared to *N. furcans* in which copper was not detected. An estimate of 15 mg/kg of copper is estimated to be adequate for the dietary needs of animals.

Chromium (Cr) works with insulin to regulate blood glucose levels in humans and several mammals (Stoecker *et al*., 1999). An adequate intake of 25 – 35 μg/day is required for humans. *C. afer* has a Cr composition (0.022 ± 0.001 ppm) that meets up with NMRC requirements. The result is lower than in *N. furcans* (12.00 mg/100g). $Cr^{3+}$ is the common form used in animal nutrition while the hexavalent chromium ($Cr^{6+}$) has been found to be highly toxic for animals (National Research Council, 1997). Thus, nutritional requirement of animals for chromium has not yet been established.

Cobalt (Co) helps to activate biochemical reactions and helps in production of red blood cells. The average intake for adults is 5 – 8 μg/day. The cobalt level (0.009 ± 0.001 ppm) is lower than in *N. furcans* (244.50 mg/100g) but meets up with human dietary requirements. The nutritional need for cobalt in animals is yet to be demonstrated as it is incorporated into Vitamin B12 during microbial synthesis in the rumen.

Lead (Pb), Nickel (Ni), Arsenic (As) and Cadmium (Cd) are harmful to health and can lead to poisoning and even death of humans and animals. The detected levels of these elements in ferns indicates the need to exercise caution in their consideration as dietary food sources. The values of Pb (0.002 ± 0.000), Ni (0.014 ± 0.000) ppm, As (0.001 ± 0.000) ppm and Cd (0.007 ± 0.000) ppm are relatively low compared to the values (62.50 mg/100g Pb, 1070.25 mg/100g Ni, 1923.50 mg/100g As and 59.00 mg/100g) obtained in *N. furcans* as reported by Oloyede *et al*., (2012).

The proximate analysis showed the presence of carbohydrate in the highest percentage, followed by moisture content with the fat composition being the least. The protein content was found to be of moderate concentration. The high carbohydrate content recorded in this fern could serve as a source of energy to man and animals if consumed. Earlier studies have shown that consumption of carbohydrates provides the body with the necessary energy required to drive cellular metabolic activities (Mensah *et al*., 2008; Anyasor *et al*., 2014). The high moisture content of this plant part may be attributed to the succulent nature of the croziers. Appreciable moisture content in plants is noted for supporting enzymatic activities in plants (Iheanacho *et al*., 2009). The moisture content of the croziers of *C. afer* can also serve as a source of water to farm animals (Anyasor *et al*., 2014). The carbohydrate and moisture contents recorded for the croziers were higher than the value reported for the frond and root of the same plant (Akomolafe *et al*., 2017). The moisture content was lower than the values recorded for the fresh leaflets of *Nephrolepis furcans* (Oloyede *et al.*, 2012) and *Nephrolepis cordifolia* (Oloyede *et al*., 2013). These observed differences in values could be as a result of variations in maturity stages, season and geographical locations since the samples were collected from different locations and at different developmental stages.

The fibre composition of the croziers is an important dietary source. Dietary fibre helps to prevent constipation, aid bowel health and weight management (Rolfes et al. 2009, Bamigboye *et al*., 2020). Dietary fiber could also prevent the incidence of cardiovascular diseases and increase intestinal transport time (Mensah *et al*., 2008). Croziers of *C. afer* could be used as fodder in livestock production. The moderately high ash content recorded in this study indicates that the croziers of *C. afer* will be rich in mineral content, especially the macronutrient elements. Protein is essential for various body functions such as body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function (Emebu *et al.*, 2011). The low fat content recorded for the croziers was below the range (8.3% - 27.0%) reported for some leafy vegetables consumed in Nigeria as reported by Sena et al. (1998) and Achi *et al*., (2017). Leafy vegetables have also been reported to be poor sources of fats (Ifon *et al*., 1980; Ejoh *et al*., 1996), thus, consumption of the croziers from *C. afer* as vegetables could naturally reduce fat intake.

Oxalate and phytate are among the major anti-nutrient substances found in leafy green vegetables (Bello et al., 2008; Oloyede et al., 2013). Previous research studies have reported that anti-nutrient substances in plants have close negative relationship with the nutrient bioavailability in plants because high composition of anti-nutrient substances tend to reduce availability or absorption of minerals in plants, which could lead to nutrients deficiency or malnutrition (Bello *et al*., 2008; Oloyede *et al*., 2013; Jaybhaye *et al*., 2015; Handa *et al*., 2017; Mrinal *et al*., 2020). The beneficial aspect of oxalate and phytate detected in the croziers in this study had also been reported (Tanko *et al*., 2007; Bamigboye *et al*., 2020). Phytate was reported to have health benefits, as it tends to have antioxidant properties and protective against different types of cancer

(Tanko *et al*., 2007). Oxalate, when present in low concentration helps in maintaining the concentration of certain minerals in the body (Bamigboye *et al.*, 2020).

Anti-nutritional substances in plants have been reported as important factors that contribute to the reduction in nutritional value of some food crops. However the use of traditional food preparation methods such as fermentation, cooking, soaking and puffing as well as some other food processing techniques have been reported capable of reducing the effects of anti-nutritional compounds. This will support plant nutrient availability, increase protein digestibility and thus improve the biological value of crops (Jaybhaye *et al.*, 2015; Handa *et al.*, 2017; Mrinal *et al*., 2020).

## CONCLUSIONS

Croziers of *C. afer* have appreciable concentrations of carbohydrate, crude protein and mineral nutrients. The croziers could be considered as a potential source of carbohydrate when the carbohydrate content is compared to conventional sources of food like cereals, where 72% - 90% carbohydrate contents have been recorded (Isong *et al*., 1999). The heavy metal component of the croziers is present at permissible level as stipulated by World Health Organisation, which indicates that their usage in food preparation and consumption on daily basis is safe with no risk of toxicity. The carbohydrate and protein contents of the croziers suggest that *C. afer* could be an important source of dietary carbohydrate and protein feed supplements for humans. This study has therefore revealed that croziers of *Cyclosorus afer* have the potential of contributing to the nutritional and health needs of man and animals. The utilization as foliage feed for ruminant animals is also a good way to explore the nutritional benefits.

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