An Evaluation of the Phytochemical and Nutritional Compositions of Fresh Leaves of Cnidoscolus Aconitifolius [Miller] I.M.Johnston

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Abstract: Fresh leaves of Cnidoscolus aconitifolius [Miller] I. M. Johnston were analysed for phytochemical and proximate compositions, vitamin and mineral constituents. The phytochemical screening carried out on the fresh leaves revealed the presence of the bioactive compounds; Saponin 3,900mg/100g, Flavonoid 1200mg/100g, Alkaloid 490mg/100g, Tannins 236mg/100g, Oxalate 873mg/100g, Phenol 11.6mg/100g, Anthraquniones 59mg/100g, Cyanogenic glycoside 24mg/100g and Phlobotannins 67mg/100g. The proximate analysis showed that the fresh leaves contained 1.80% crude fat, 2.50% crude fibre, 5.11% crude protein, 6.54% carbohydrate, 1.60% ash and 82.45% moisture content. The result of the vitamin analysis depicted/revealed that the fresh leaves contained carotene (Vit A) 131.10mg/100g, Ascorbic acid (Vit C) 142.11mg/100g, Pyridoxine (Vit B₆) 1.34mg/100g, Folic acid (Vit B₉) 1.13mg/100g and Cyanocobalamin (Vit B₁₂) 0.13mg/100g. The mineral analysis revealed the constituents to be Mg 23mg/100g, K 102mg/100g, Ca 30.9mg/100g, Fe 4.7mg/100g, Cu 0.5mg/100g, Mn 3.2mg/100g, Zn 0.3mg/100g, and Na 21mg/100g. These results show that Cnidoscolus aconitifolius is a nutritious green vegetable which can serve as a food supplement and as a medicinal plant.

Keyword: Cnidoscolus aconitifolius, phytochemical screening, proximate analysis, mineral and vitamin constituents, nutritional composition, bioactive compounds.

1. INTRODUCTION

Cnidoscolus aconitifolius [Miller]I.M.Johnston is a tropical shrub, 3 – 5m tall, distributed throughout most of the Yucatán Penninsula (Abdala – Roberts and Parra – Tabla, 2005). It belongs to the family, Euphorbiaceae, It is commonly known as Chaya or Tree Spinach. The crop originated as a domesticated, leafy green vegetable in the Maya region of Guatemala, Belize and South – east Mexico during pre – Cambrian period (Ross – Ibarra and Molina – Cruz, 2002). It has continued to be used as food, medicine and ornamental plant till date.

C. aconitifolius belongs to a group of arbrescent shrubs. It is an evergreen,drought deciduous shrub, up to 6m in height with alternative pinnate lobed leaves, milky sap and small flowers on dichotomously branched cymes. The leaves are large, 32cm long and 30cm wide on chartacious and succulent petioles (Mordi and Akanji, 2012). This species is monoecious with flowers arranged in inflorescences, with dichotomic ramification, and stalks 15 – 40cm in length (as in *C. spinosus*: see Bullock, 1982). *C. aconitifolius* presents glandular trichomes on most of its aerial structures, which produce stinging compounds (L. Abdala – Roberts, pers. obs.). These compounds (i.e. serotonin) have been shown to confer resistance against herbivores (Pollard and Briggs, 1984), and are found in other species of the same genus (e.g. *C. texanus*, Lookadoo and Pollard., 1991). It is cultivated in domestic gardens rather than in agricultural fields and as such can be used throughout the year (Adeniran *et al.*, 2013).

Due to its ease of cultivation, potential productivity and substantial nutritional value, the plant has spread all over the world, including the tropics. Colloquially the plant is referred to as Chaya (Donkoh

et al., 1990). In the western part of Nigeria, it is called different names such as Efo Iyana Ipaja and Efo Jerusalem, while in the Niger Delta of Nigeria; it has been nick-named "Hospital Too Far" because of its numerous traditional claims (Mordi and Akanji, 2012), and is also called ''Blood''.

Although, the plant is mainly cultivated as food, it has continued to be an important medicinal plant. Much of its recent spread into new areas may likely be attributed to its medicinal value. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from its ability to strengthen fingernails and darken grey hair, to cure for alcoholism, insomnia, gout, scorpion stings, brain and vision improvement (Jensen, 1997; Atuahene *et al.*, 1999). It has certain antibacterial properties, as well as a contraceptive effect (Dong *et al.*, 2010). It has been observed in use as diuretic, circulation, and lactation stimulant and has also been recommended for diabetes, obesity, acne, kidney stones and eye problems (Rowe, 1994). Research has shown that *C. aconitifolius* is rich in natural antioxidant (Kuti and Konoru., 2004), which scavenges free radicals. Many chronic diseases and causes of food spoilage are linked to pro-oxidation. Antioxidant components are therefore used in food preservation and drug formulation.(Loliger,1991).

Kuti and Torres (1996) studied the potential nutritional and health benefits of Tree Spinach. Abdala – Roberts and Parra – Tabla (2005) showed that artificial defoliation induces trichome production in the tropical shrub *Cnidoscolus aconitifolius* (Euphorbiacea). Awoyinka *et al.*, (2007) reported on the phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitifolius*. Mordi and Akanji (2012) reported the phytochemical screening of the dried leaf extracts of *Cnidoscolus aconitifolius* and associated changes in liver enzymes, induced by its administration to wistar rats. Adeniran *et al.*, (2013) studied the phytochemical constituents and antimicrobial and antioxidant potentials of Tree Spinach (*Cnidoscolus aconitifolius* (Miller) I:M. Johnst). The phytochemical screening revealed the presence of secondary metabolites of both preventive and curative importance in medicine, and the presence of compounds with antioxidant activity.

Given its reputed medicinal efficacies and nutritional constituents, the present study evaluates the phytochemical and nutritional compositions of fresh leaves of *Cnidoscolus aconitifolius* variety found in the locality covered by the research.

2. MATERIALS AND METHODS

2.1. Plant Material

The fresh leaf samples of *Cnidoscolus aconitifolius* used for this study were obtained from a home garden at Federal Housing Estate, Umuguma, New Owerri in Owerri – west Local Governmet Area, Imo State, Nigeria. The leaves were identified by a taxonomist in the Department of Biology, Federal University of Technology Owerri, Imo State, Nigeria.

2.2. Phytochemical Analysis

Phytochemical screening on the sample and subsequent quantification was carried out using the methods described by AOAC (1984), AOAC (1990), Lewis (1974) and Agomuo *et al.*, (2002). Saponins, steroids, phenols, cyanogenic glycosides and phlobotannins were screened for using the methods described by AOAC (1984). Flavonoids and alkaloids were screened using the method of Harborne (1973), as contained in AOAC (1990). The method of Pearson (1976), as described in AOAC (1990), was used for the determination of tannins. Anthraquinones were analysed using the method of Lewis (1974), while oxalate was analysed using the method of Munro and Bassir (1969), as described in Agomuo *et al.*, (2002).

2.3. Proximate Analysis

The proximate analysis was carried out on the sample using the methods described by AOAC (1990). Protein content was determined by Kjeldahl method and ash content by ignition at $575 \pm 25^{\circ}$ C using muffle furnace until sample was carbonized (about 4hrs). Moisture content was determined by drying to constant weight ay 105° C in the oven. The crude fat content was determined by soxhlet extraction with petroleum ether as solvent, and crude fibre content by the acid and alkaline digestive method. The carbohydrate content was estimated by difference, subtracting the sum of water, protein, crude fibre and ash percentages from one hundred (percentage).

2.4. Mineral Analysis

The AOAC (1990) method was used for the determination of minerals in the test sample. Calcium and magnesium were determined by complexiometric method using EDTA. Potassium and sodium were

determined by flame photometric method, while copper, zinc, manganese and iron were determined by atomic absorption spectrophotometer (AAS) method. Phosphorus was determined using Spectrophotometer.

2.5. Vitamin Analysis

The carotene (Vitamin A) content was determined by the method of the Association of Vitamin Chemists (Kirk and Sawyer, 1998). Pyridoxine (B_6), Folic acid (B9), and cyanocobalamine (B_{12}) were analysed using the method described in AOAC (2005). Ascorbic acid (Vitamin C) was determined by the Barakate Titrimetric method (Barakate *et al.*, 1955).

3. RESULTS AND DISCUSSION

The results obtained from this study show that the fresh leaves of *Cnidoscolus aconitifolius* contain some bioactive compounds/phytochemicals and have high protein content. They also contain appreciable amounts of essential minerals and vitamins.

3.1. Phytochemical Analysis

The result of the phytochemical screening of the extracts of fresh leaves of *Cnidoscolus aconitifolus* is presented in Table 1. It reveals the presence of saponins, flavonoids, alkaloids, phlobotannins, steroids, anthraquinones and phenols in the ethanolic extract, and the presence of cyanogenic glycosides, tannins and oxalate in the water extract. (Table 1). Similar reports were made by Mordi and Akanji (2012) and Adenirian *et al.*, (2013). Peixoto Sobrinho *et al.*, (2012) reported the presence of high frequency of flavonoids, especially in the aerial parts of *Cnidoscolus* species and Yuan *et al.*, (2007) reported isolation of fifteen flavonoids from *Cnidoscolus texanus*. Flavonoids are antioxidants that neutralize free radicals - unstable, disease-causing molecules - and have the potential to also protect against the development of heart disease. They have been shown to have antifungal activity *in vitro* (Galeotti *et al.*, 2008). The potent antioxidant activity of flavonoids reveals the ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals, this may be the most important function of flavonoids (Alan and Miller, 1996). They also induce mechanisms that may kill cancer cells and inhibit tumour invasion (Williams *et al.*, 2004). The sole greenish color of this leafy vegetable substantiates the fact that flavonoids contribute to the brilliant multicolor for most plants (Sofowora, 1993).

A large amount of saponins were also detected in the ethanolic extract of *Cnidoscolus aconitifolius* leaves. Saponins have been shown to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic; permeabilization of the intestines) properties. (Price et al., 1987; Oakenful and Sidha, 1989).

Phytochemical	Ethanolic extract	Water extract
Constituents	[mg/100g fresh leaves]	[mg/100g fresh leaves]
Phlobotannins	67	
Steroids	38	
Phenols	11.6	
Anthraquinones	59	
Cyanogenic glycosides		24
Saponins	3900	
Flavonoids	1200	
Alkaloid	490	
Tannin		236
Oxalate		873

Table1. Phytochemical compositions of fresh leaves of Cnidoscolus aconitifolius

Price *et al.*, (1989) and Trease and Evans (1989) have shown saponins to have immense significance as antihypercholesterol, hypotensive and cardiac depressant agent, suggesting the suitability of the plant in this respect. Studies have illustrated the beneficial effect on blood cholesterol levels, cancer bone health and stimulation of the immune system.

An appreciable amount of alkaloids was obtained from the ethanolic extract. Alkaloids have been associated with medicinal uses for centuries, and one of the common biological properties is their cytotoxicity[Nobori *et al.*,1994]. Alkaloids are naturally occurring compounds commonly found to

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have antimicrobial properties, due to their ability to intercalate with DNA of the micro-organism (Kasolo *et al.*, 2010). This could be responsible for their much acclaimed medicinal value. Alkaloids have been implicated for its detoxifying and antihypertensive properties (Trease and Evans, 1989; Zee-cheng, 1997). Several workers have reported the analgesic (Antherden, 1969), antispasmodic and antibacterial (Stray, 1998) properties of alkaloids. Phlobotannins were also detected in the ethanolic extract. This report is however, in contrast with the report of Awoyinka *et al.*, (2007). The presence of phlobotannins suggests the diuretic property of the plant [Okuda,1990]. The foregoing would suggest the possible utilization of *C. aconitifolius* as diuretic agent. Anthraquinones were found present in the ethanolic extract, though the therapeutic applications of these metabolites are vaguely understood (Trease and Evans, 1997; Sofowora, 1993).

Tannins were detected in the water extract (Table 1). Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent (Asquith and Butler, 1986). The healing and anti-inflammatory activities popularly attributed to *Cnidoscolus spp.* are strongly associated with its tannins content (Araujo *et al.*, 2008). A little amount of phenols were found in the ethanolic extract. This is in agreement with the report of Mordi and Akanji (2011). Phenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular diseases. Plant phenols may interfere with all stages of their cancer process, potentially resulting in a reduction of cancer risk (Hollman, 2011).

Cyanogenic glycosides were detected in the water extract of fresh leaves of *Cnidoscolus aconitifolius* in this study. Kuti and Torres (1996) reported the presence of toxic hydrocyanic glycosides in the raw leaves of tree spinach; however, cooking which is essential inactivates the toxic compound. Arthur (2012) also reported that uncooked Chaya leaves contain Cyanogenic glucosides (linamarin) that produce hydrogen cyanide upon tissue damage. The cooking time required to lower HCN to safe level is about 15minutes. The presence of steroids in the ethanolic extract is of great significance, due to their relationship with such compounds as sex hormones (Okwu, 2001). Steroids have been reported to have antibacterial properties (Raquel, 2007). Some plant steroids are also useful for their effects when consumed by human beings, because their presence reduce the amount of cholesterol in the bloodstream (Wisegeek, 2013).

Oxalates were also found present in the water extract(Table 1). Oxalate occurs in many plants where it is synthesized via incomplete oxidation of carbohydrates. Many metal ions form insoluble precipitate with oxalates, a prominent example being calcium oxalate, the principal constituents of the most common kind of kidney stone (Wikipedia, 2014). The biological roles of calcium oxalate crystal formation in plant growth and development include high-capacity calcium regulation, protection against herbivores and tolerance to heavy metals, Nakata, (2012). Oxalates are toxic to the kidneys, because they form oxalic and crystals that do not dissolve and can be precipitated in many parts of the body including the brain. They cause damage to the kidney, arteries, stomach, etc (Botanical Online, 2014). The role of oxalates in the human body is not clearly understood.

3.2. Proximate Analysis

The result of the proximate analysis of fresh leaves of *Cnidoscolus aconitifolius* is presented in Table 2. It reveals that the fresh leaves contain high (5.11%) crude protein (82.45%) moisture (2.50%) crude fibre (1.80%), crude fat and 6.54% carbohydrate. The result is within the range reported by Kuti and Torres (1996), Ross-Ibarra and Molina-Cruz (2002), and Jansen (2014). It shows that the fresh leaves are good source of protein, thus supporting previous reports by Kuti and Konoru (2004).

Parameter.	Composition (%)
Crude fat content	1.80
Crude fibre content	2.50
Crude protein content	5.11
Ash content	1.60
Moisture content	82.45
Carbohydrate content	6.54

Table2. Proximate composition of fresh leaves of Cnidoscolus aconitifolius

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3.3. Mineral and Vitamin Analyses

The results of the mineral and vitamin analyses, as presented in Tables 3 and 4, show that the fresh leaves of *Cnidoscolus aconitifolius* contain appreciable amounts of minerals and vitamins essential for human growth and maintenance. They are relatively high in potassium (102mg/100g), Calcium (30.9mg/100g), Iron (4.7mg/100g), Ascorbic acid (142.11mg/100g) and also contain Vit A (Carotene) (13.10mg/100g), B6 (pyridoxine) (1.34mg/100g), B9 (Folic acid) (1.06mg/100g), B12 (Cyanocobalamin) (0.13mg/100g).

Kuti and Torres (1996) reported that the levels of Chaya leaf nutrients are two to threefold greater than most edible leafy-green vegetables. In terms of the average nutritive value (ANV), Chaya leaf (14.9) is by far superior to other leafy, green vegetables such as spinach (6.4), Amaranth (1.3), Chinese cabbage (7.0) and Lettuce (5.4) (Grubben, 1978). While some edible, leafy green vegetables are usually good sources of mineral macronutrients (Lavender, 1990), Chaya leaf furnishes appreciable quantities of several of the essential mineral macronutrients of several of the essential mineral macronutrients of several of the sential mineral macronutrients of several of the sential mineral macronutrients of several of risks of stroke (National Research Council, 1989). Calcium is important for ossification and iron is necessary for normal hematopoiesis (Hodges *et al.*, 1978).

Vitamins	Composition [mg/100g fresh leaves]
Vitamin A (Carotene)	13.10
Vitamin B ₆ (Pyridoxine)	1.34
Vitamin B ₉ (Folic acid)	1.06
Vitamin B ₁₂ (Cyanocobalamin)	0.13
Vitamin C (Ascorbic acid)	142.11

Table3. Vitamin compositions of fresh leaves of Cnidoscolus aconitifolius

Minerals.	Composition [mg/100g fresh leaves]	
Magnesium	23	
Potassium	102	
Calcium	30.9	
Phosphorus	22	
Iron	4.7	
Copper	0.5	
Manganese	3.2	
Zinc	0.3	
Sodium	21	

Table4. Mineral compositions of fresh leaves of Cnidoscolus aconitifolius

Other authors (Booth *et al.*, 1992; Jansen, 2004, and Arthur, 2012), have also reported on the rich, nutritional compositions of *Cnidoscolus aconitifolus*. The presence of Vitamins B_6 , B_9 , and B_{12} , as well as iron in the fresh leaves of *Cnidoscolus aconitifolius*, as reported in this study (Tables 3 and 4) support the use of these leaves as a blood builder in some parts of South – East Nigeria, where it has also been nick-named "Blood". Folic acid (B_9) and cyanocobalamin (B_{12}) are the two essential vitamins required for optimum red blood cell formation and this proper maturation. Vitamin B_{12} works closely with Vitamin B_9 , also folate or folic acid, to help make red blood cells; and to help iron work better in the body (Ehrlich, 2011).

Vitamin B_{12} is an especially important vitamin air maintaining healthy nerve cells, and it helps in the production of DNA and RNA, the body's genetic material. Vitamins B_{12} , B_6 and B_9 work together to control blood levels of the amino acid "homocysteine". High levels of homocysteine are associated with heart diseases (Ehrlich, 2011).

Other vitamins detected in the leaf samples in this study are Vitamins A and C[Table 3].Vitamin A has several potential preventive and therapeutic uses. Vitamin A is an important "medicine" for the immune system. It keeps skin and mucous membrane cells healthy. This vitamin is particularly helpful in diseases caused by viruses. Measles, respiratory viruses and even Human Immune Deficiency Virus (HIV), the virus that causes AIDS, may retreat in the presence of vitamin A. Vitamin A fights cancer by inhibiting the production of DNA in cancerous cells. It slows down

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tumour growth in established cancer and may keep leukaemia cells from dividing (Brett, 2013).Vitamin C (or ascorbic acid) is essential to a healthy diet, as well as being a highly effective antioxidant acting to lessen oxidative stress (Wikipedia, 2014). There is an interesting ability of ascorbic acid, as an antioxidant, to prevent or at least minimize the formation of carcinogenic substances from dietary material (Hunt *et al.*, 1980). This vitamin can also be used for the treatment of common cold and other diseases like prostrate cancer (Okwu and Okwu, 2004; Okwu and Okeke, 2003).

4. CONCLUSION

The phytochemical analysis of the fresh leaves of *Cnidiscolus aconitifolius* revealed the presence of saponins, flavonoids, alkaloids, phlobotannins, steroids, anthraquinone and phenols in the ethanolic extract and the presence of oxalate, tannins and cynanogenic glycosides in the water extract. These bioactive compounds may contribute to the reputed medicinal efficacy of the plant. The proximate analysis depicted/showed that the fresh leaves have high protein content. The mineral and vitamin analyses revealed that the fresh leaves of *Cnidoscolus aconitifolius* contain appreciable amounts of essential minerals/ macronutrients including magnesium, potassium, calcium, phosphorus, iron, copper, manganese, zinc and sodium and vitamins A, B₆, B₉, B₁₂, and C. The presence of vitamins B₆, B₉, B₁₂, and Iron, suggest the use of the leaves as a blood builder.

Cyanogenic glycosides that produce toxic hydrogen cyanide upon tissue damage were also detected in the fresh leaves. However, cooking which is essential before consumption inactivates the toxic compounds.

Thus, results from this study show that *Cnidoscolus aconitifolius* is a nutritive, green vegetable and can be used as a medicinal plant. Being underexploited its commercial cultivation and use is strongly encouraged.

Further studies are recommended to isolate, characteristics are elucidate the structures of bioactive compounds contained in the leaves to elucidate their mechanism of action and use in industrial drug formulations.

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