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Nutritional and antioxidant potential of *Cnidoscolus aconitifolius* (Mill.) I. M. Johnst.

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ABSTRACT

Cnidoscolus aconitifolius (Mill.) I. M. Johnst. known as 'tree spinach' in the family Euphorbiaceae is a shrub widely used as medicine and food. The plant is native to Mexico but currently distributed in many countries and used mainly for its nutritional value. There are many plants around the globe with antiutritional profile comparable to or better than commonly used vegetables that remain underutilized. This is due to the lack of scientific data to validate their up-gradation to the category of vegetables. The analysis of the nutritional composition of the leaf of *C. aconitifolius* was carried out through standard methods in the present study. The results indicated that the leaves possess high amount of proteins (8.876 mg/g), carbohydrates (68.733 mg/g), lipids (33.2 %) and vitamins *viz* thiamine (757.916 mg/g), riboflavin (0.050 mg/g), niacin (3.006 mg/g) and ascorbic acid (2.201 mg/g). Antinutritional components such as oxalate (61.3 μg/g), phytates(333μg/g) and phenols (4.1 μg/g) were observed in meager amounts, which is a favorable property for edible plants. The chlorophyll and carotenoid pigments were also estimated. The result indicated that the consumption of *C. aconitifolius* in substantial quantity can provide several nutritients necessary for health. In DPPH radical scavenging assay, the leaves proved to have high free radical scavenging activity with an IC 50 value of 92.301 μg/ml. So the leaves of *C. aconitifolius* can function as a good dietary supplement that can neutralize the free radicals formed in human body.

Keywords: *Cnidoscolus aconitifolius,* leafy vegetable, nutritional, anti-nutritional, DPPH radical scavenging

INTRODUCTION

Leafy greens are consumed since time immemorial and plants are part of the cuisine all over the world. *Cnidoscolus aconitifolius* commonly called 'tree spinach', "Mayan Spinach", 'Chaya' etc., is an underutilized crop plant cultivated by Mayan people from pre-Hispanic era (Munguia-Rosas *et al.,* 2019). The plant is a perennial shrub with succulent stem. *C. aconitifolius* is a valued food source since its leaves are found to be a source of vitamins, proteins, iron, calcium, antioxidants etc. (Kuti and Konuru, 2004). The estimation of the nutritional parameters of the plant is significant for popularizing its consumption.

Healthy food is an inevitable component of a healthy lifestyle and a balanced diet with all the required nutrient supplements is important for the physical well-being of all. Vegetables form a major part of a well- balanced diet. They play a significant role in human nutrition, especially they are the sources of phytonutraceuticals such as different vitamins, minerals, dietary fiber, and phytochemicals. An unhealthy diet can cause deficiency-related diseases such as blindness, anemia and scurvy. As per the World Health Report 2007, an unbalanced diet with low vegetables, low carbohydrates and low dietary fiber are estimated to cause some 2-7 million deaths each year and were among the top 10 reasons that lead to mortality (Dias, 2011). *C. aconitifolius* can support nutritious food cultures of Mesoamerica due to the high nutritional quality, stress tolerance and minimal maintenance requirement for cultivation (http://www.nuscommunity.org/nus/neglected-underutilized-species/chaya/). The leaves of *C. aconitifolius* need to be cooked before consumption, due to the presence of hydrocyanic glycosides.

Green leafy vegetables have functions in the maintenance of health and prevention of various diseases too (Mohammed and Sharif, 2011). Oxidative stress triggered by the increase in free radicals causes many health issues like oxidation of macromolecules such as proteins, lipids, carbohydrates and DNA in the biological system. An antioxidant is a molecule that donates an election to rampaging free radicals and neutralize it. Vitamin C, vitamin A, vitamin E, Beta-carotene, Lycopene, etc are prominent antioxidants. Such molecules can safely interact with the free radicals and delay, scavenge or inhibit these radicals from their deleterious effects on the tissues. Plants are sources of innumerable phytochemicals with favorable therapeutic effects (Nagori and Solanki, 2011). The leaves of tree spinach are home for many natural antioxidants (Kuti and Konuru, 2004). So the antioxidant potential of *C. aconitifolius* needs to be investigated as part of discovering novel and potent free radical scavengers.The present study focuses on the estimation of nutritional and antinutritional factors as well as evaluation of the antioxidant potential of *C. aconitifolius*.

MATERIALS AND METHODS

Collection of Plant material

C. aconitifolius was collected from the Kariavattom campus, University of Kerala. Identification was done by evaluating the taxonomical characteristics of the plant material. Fresh leaves were taken for nutritional analysis.

Estimation of Nutritional parameters

Nutritional parameters like Moisture Content, Carbohydrate, protein, Lipid, Reducing Sugar, Chlorophyll and Carotenoids were analysed using standard procedures. Moisture content was analyzed as per ISTA protocol (ISTA, 1976). The amount of total carbohydrate present in the sample was estimated by Anthrone method (Hedge and Hofreiter, 1962) and total protein by Lowry's method (Lowry *et al*., 1951). The lipid content was analysed using the procedure of Bligh and Dyer (1959) and reducing sugar by Dinitrosalicylic acid method (Miller, 1972). chlorophyll and carotenoids were quantified by using Arnon's formula (Arnon, 1949). vitamins namely vitamin B_1 (Deepak, 2011), vitamin B_2 (Indian pharmacopeia, 1996), vitamin B_3 acid, vitamin C (Sadasivam and Manickam, 1996) were also estimated through standard procedures.

Estimation of Antinutritional Parameters

Antinutritional factors such as oxalate, phytate and phenol were estimated in fresh leaves of *C.aconitifolius*. Amount of oxalate in the sample was estimated by AOAC protocol (AOAC, 1984). The Phytate content was analysed using the procedure by Reddy *et al*. (1982) and phenol content by the method of Malick and Singh, (1980).

Antioxidant Activity Analysis

DPPH Free Radical Scavenging Assay was conducted on the methanolic extract of the leaf sample (Blois, 1958). Different concentrations of the extract ranging from 0.2 to 1.0 mg/ml were prepared and 1 ml each of various concentrations of extract was added to 2 ml of DPPH solution. The reaction mixture was incubated in dark at 37^0C for 20 minutes and the absorbance was read at 517 nm against blank. The activity is given as percentage of DPPH radical scavenging.

RESULT AND DISCUSSION

Estimation of Nutritional parameters

Nutritional quality analysis of leaves *C. aconitifolius* were carried out through the estimation of the total carbohydrate, protein, lipid, reducing sugar, moisture, vitamin B_1, vitamin B_2, Vitamin $B_3$and Vitamin C. The result substantiated that the leaf of *C. aconitifolius* is edible and has high nutrient content, with the presence of considerable amount of all the nutritional parameters tested. The results are represented in Table 1.

Proteins are complex, nitrogen- containing macromolecules that serve many functions like catalyzing biochemical reactions, transport of ions and molecules, muscle building, storage, regulation of metabolism and cellular functions etc. Human body acquires the essential amino acids through the breakdown of proteins in food, which is crucial for protein synthesis to occur. The amount of protein in *C. aconitifolius* was found to be 8.876 mg/g, whereas the protein content in the commonly used leafy vegetables *Brassica oleracea* and *Spinacia oleracea* were 13 mg/g and 20 mg/g respectively (Eskin, 1989; Tewani *et al*., 2016).

Carbohydrates are the major source of cellular energy and are important structural components of cell wall. The degradation of carbohydrate yields energy to run life processes (Bhaskaran and Agith, 2016). Carbohydrates were found to be high in *C. aconitifolius* (68.73 mg/g), whereas *Spinacia oleracea* (29 mg/g) and lettuce (21 mg / g) were reported to contain lesser carbohydrate (Eskin, 1989; Tewani *et al* ., 2016). Only a minor quantity of reducing sugar is present in the leaf of *C. aconitifolius* ie 0.0079 mg/g. Lipids are essential for the structural organization of cell membranes and some cell organelles, protection of internal organs from mechanical damage and also for the normal functioning of the cells. Lipids serve as electrical and heat insulators too (Bhaskaran and Agith, 2016). The amount of lipid was found to be highest in *C. aconitifolius* (33.2%) than commonly consumed spinach (1.17%) (Gupta and Wagle, 1988).

Vitamins are required in very small amounts by the body and still have important catalytic and regulatory functions. Hence they are essential components of the animal diet and are often defined as indispensable, accessory food factors (Jain, 2017). thiamine (vitamin B1) is a free form of the vitamin found in food and is manufactured as a dietary supplement. It is involved in glucose metabolism in the body and it supports nerve and heart health. The present study reported a higher quantity of thiamine in *C. aconitifolius* (757.91 mg/g) than *Spinacia oleracea* (1.1 µg/g) and lettuce (0.5 µg/g) (Eskin, 1989; Tewani*et al.*, 2016). Vitamin B_2 (Riboflavin) is commonly acquired from soybeans and green vegetables and is pivotal in ATP synthesis in the body. The riboflavin levels were found to be higher in *C. aconitifolius* (3.0 mg/g) than spinach (6 µg/g) and lettuce (1.9 µg/g) (Eskin, 1989; Tewani *et al.,* 2016).

Niacin (Vitamin B_3) is widely distributed in plants. Cereal grains, tomatoes, yeast, leafy vegetable are a rich source of this vitamin. It found as amide and forms the important constituents of coenzymes NAD and NADP (Jain, 2017). The quantity of Niacin in *C. aconitifolius* was 3.006 mg/g. Vitamin C is an essential nutrient required for the normal metabolic functions of the body. It functions in the synthesis of collagen and carnitine, strengthening the immune system, for healthy eyes, skin, etc. Vitamin C intake reduces the severity of cold and prevents secondary viral or bacterial infections. Also, it is reported to be effective in lowering the risk of developing cancers in the colon, lung, mouth, prostate, and stomach. Vitamin C values were found to be higher in *C. aconitifolius* (2.201 mg/g) than lettuce with 0.039 mg/g of vitamin C content (Michael, 1989).

Table 1
Amount of Nutritional parameters in the leaves of *Cnidoscolus aconitifolius*

Sl. No.	Parameters	Quantity
1	Lipid	33.2 %
2	Protein	8.876 mg/g
3	Reducing Sugar	0.007 mg/g
4	Carbohydrates	68.733mg/g
5	Moisture	3.09 %
6	Vitamin B1	757.916 mg/g
7	Vitamin B2	0.050 mg/g
8	Vitamin B3	3.006 mg/g
9	Vitamin C	2.201 mg/g

Estimation of antinutritional parameters

Antinutrients or antinutritional parameters are the factors that reduce nutrient intake, digestion, absorption, and utilization. The major antinutrients observed in plants are phytate, oxalate, phenol, lectin and cyanogenic glycoside. The concentration of antinutrients varies with plant species, cultivars and post-harvest treatments (Akande, 2010). In this study phytate, oxalate and phenols in the leaves of *C. aconitifolius* were measured and the results are represented in Table 2. Phenolic compounds are antinutrients involved in plant resistance, function in the regulation of seed germination, regulation of plant growth and defense responses during infection, excessive sun exposure, injuries, and heavy metal stress. A major feature of the phenolic compound is its antioxidant activity (Kulbat, 2016). The analysis indicated that only the meager amount of phenol (4.1 µg/g) was present in the leaves of *C. aconitifolius*, which is less compared to fresh samples of cabbage and coriander which ranged between 16.78 to 0.43 mg/g (Singh., 2018).

The antinutrient factor Oxalate can affect calcium and magnesium metabolism. They react with proteins form complexes that have an inhibitory effect in peptic digestion. Depending on the plant species, oxalates can occur as insoluble salts of magnesium, calcium, iron and soluble salts of potassium and sodium or as a combination of these two forms. The insoluble calcium oxalate in the crystal forms leads to kidney stones (Hemmige *et al.*, 2017). The amount of oxalate was found to be minimal in *C. aconitifolius* (61.3 µg/g) when compared with spinach 86.9 mg/g (Gupta and Wagle, 1988).

Phytate is a natural substance functioning as the major storage form of phosphorus in all green leafy vegetables. They are found in plant tissues as salts of cations such as magnesium, potassium, and calcium. Phytate has a negative impact on digestive enzymes (Ravindran *et al.*, 1999). Phytate content in *Moringa oleifera* is 11.00 + 0.23 µg/g and in *Amaranthus hybridus* it is 0.60 + 0.02 µg/g (Agbaire, 2012). The result indicated that a higher amount of phytate was present in the leaf of *C. aconitifolius* (333 µg/g) when compared to *Moringa oleifera* and *Amaranthus hybridus*.

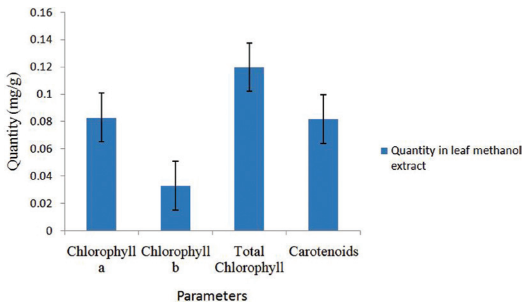
Table 2: Amount of atinutritional factors in the leaves of *Cnidoscolus aconitifolius*

Sl.no.	Parameters	Quantity (µg/g)
1	Oxalate	61.3
2	Phytate	333
3	Phenol	4.1

Estimation of chlorophyll and carotenoid pigments

Photosynthetic pigments *viz* chlorophylls and carotenoids are the light- sensitive molecules in thylakoid membranes of Chloroplast. Chlorophylls are magnesium porphyrin compounds (Jain, 2017). Chlorophyll- a is a principal photosynthetic pigment present in all phototrophs other than bacteria. Chlorophyll b is an accessory photosynthetic pigment found in all phototrophs, other than diatoms, cyanobacteria, red algae and brown algae (Bhaskaran and Ajith, 2016). Carotenoids are fat- soluble accessory photosynthetic pigments that absorbs blue-violet range of the visible spectrum and transfer energy to chlorophylls to be used in photosynthesis. They protect chlorophyll molecules from photooxidation as well (Bhaskaran and Ajith, 2016). The amount of chlorophyll a, chlorophyll b and carotenoids present in *C. aconitifolius* were estimated to be 0.083 mg/g, 0.033 mg/g and 0.082 mg/g respectively. The results are represented in Fig. 1. The amount of chlorophyll a and b were less, and carotenoids were higher in *C. aconitifolius* compared to lettuce fresh leaves. The amount of chlorophyll a, b and carotenoids reported to be present in lettuce were 17.21 mg/g, 1.76 mg/g, and 0.0426 mg/g respectively (Morna, 2015). The amount of chlorophyll-a and chlorophyll-b in *C. aconitifolius* is less when compared to *Coriandrum sativum* (0.091 mg/g and 0.121mg/g respectively). But the carotenoid content in the leaf of *C. aconitifolius* is high when compared to *Coriandrum sativum* (0.062 mg/g).

Fig. 1
Chlorophyll and carotenoid pigments present in the leaf of *Cnidoscolus aconitifolius*



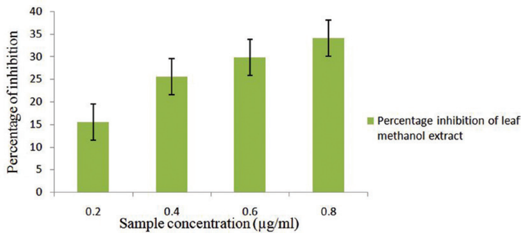
Evaluation of antioxidant property

Plants are the key components of folklore medicine due to the presence of diverse phytochemicals with therapeutic potential. They are the sources of potent antioxidant molecules (Johnson, 2001). Free radical formation occurs continuously in the body both through enzymatic and non-ezymatic reactions. Some internally generated sites of free radicals are mitochondria and peroxisomes (Lobo *et al.,* 2010). Also free radicals and other ROS are produced due to external sources such as cigarette smoking, air pollutants, industrial chemicals, exposure of X- ray and ozone. When the amount of reactive oxygen species increases, it causes damage to proteins, lipids, carbohydrates, DNA and ultimately results in the death of the cell (Foyer and Noctor, 2005). It also leads to diseases such as inflammation, arthritis, diabetes, cancer, neurological disorders such as Alzheimer's disease (Shukla *et al.,* 2009).

Antioxidants delay or inhibit the cellular damage mainly by their free radical scavenging property (Halliwell, 1995). They act through different mechanisms including electron donation, metal iron chelation, co-antioxidants or gene expression regulation (Krinsky, 1992). Antioxidants can reduce the risk of chronic diseases including cancer and heart diseases. Primary sources of natural antioxidants include whole grains, fruits, vegetables etc., and vitamin C, vitamin E, Carotenoids, Phenolic acid etc., are plant-derived antioxidants.

DPPH (2,2-diphenyl-1-picryl- hydrazyl hydrate) assay was used to measure the antioxidant activity of *C. aconitifolius*. This is an antioxidant assay based on electron transfer (Huang *et al.*, 2005). The plant extract showed concentration dependent scavenging activity of DPPH radicals (Fig. 2). Methanolic extract of the leaf at different concentrations from 0.2 to 1 µg/ml showed the inhibition percentage in the range of 15.49 to 62.44. The IC 50 value of the antioxidant activity of the leaf extract was 92.301 µg/ml. It is reported that in *Spinacia oleraceae* leaf methanolic extract , the IC 50 value of its antioxidant activity is 211.08 mg/ml (Sharif *et al.,* 2018). So the plant has considerable antioxidant activity that is higher when compared to *S. oleraceae*.

Fig.2
DPPH radical scavenging activity of methanolic extract of *Cnidoscolus aconitifolius* leaf



CONCLUSION

Health and nutrition are the most important factors for human civilization. The nutritional evaluation of fresh leaves of *C. aconitifolius* showed a rich amount of nutrients such as proteins, lipids, carbohydrates, vitamin B_1, vitamin B_2, vitamin B_3 and vitamin C. Antinutritional factors including phytate, oxalate and phenol are found in lesser amounts. So *C. aconitifolius* could be suggested as a highly suitable leafy vegetable for enhancing body health on the basis of the high nutrient level and low antinutrient level. Medicinal plants are considered as reliable source of molecules to be used in drug development, either pharmacopoeial, non pharmacopoeial or synthetic drugs. The phytochemical pharmacological research on medicinal plants yields an effective solution to certain diseases that synthetic drug industries fail to cure. Antioxidants play an important role in protecting the human body against damage caused by free radicals. The present study indicated that the methanolic leaf extract of *C. aconitifolius* has higher antioxidant potential. The plant can be exploited further for its free radical scavenging activity. The analysis successfully elucidated the health benefits of *C. aconitifolius* and recommends its consumption.

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