Asian Plant Research Journal

Volume 12, Issue 5, Page 91-97, 2024; Article no.APRJ.122751
ISSN: 2581-9992

Biophysicochemical and Antioxidant Properties of *Cnidoscolus aconitifolius* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author TAN conceptualized the study. Authors TAN and CUN did formal analysis of the study. Authors TAN and CUN participated in the literature searches, wrote and prepared the original draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/aprj/2024/v12i5273

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122751

Original Research Article

Received: 04/07/2024
Accepted: 08/09/2024
Published: 26/09/2024

ABSTRACT

Cnidoscolus aconitifolius (Chaya leaf or spinach tree) commonly found in West Africa especially Nigeria, was carefully investigated for its biophysicochemical properties, and in-vitro antioxidant potential. Well dried and pulverized sample of *C. aconitifolius* was assayed for its flavonoids, phenolics, alkaloids, nitrate, moisture, ash and crude content as well as peroxidase, superoxide dismutase (SOD) and its scavenging activity against 2,2-diphenyl-1-picryhydrazyl (DPPH) following the standard assay methodologies. The result showed that *C. aconitifolius* contains 79.12 ± 0.16% moisture content, 6.61 ± 0.27% ash and 13.35 ± 0.27% crude fiber. The result recorded flavonoid

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Cite as: *Nnadiukwu, Tochukwu A., and Chinonso U. Nnadiukwu. 2024. "Biophysicochemical and Antioxidant Properties of Cnidoscolus Aconitifolius Leaves". Asian Plant Research Journal 12 (5):91-97. https://doi.org/10.9734/aprj/2024/v12i5273.*

content, at 132.83±9.25 mg/100gQE, alkaloid (105.91±1.52 mg/100g), phenol (351.53±2.33 mg/100gCE), and nitrate concentration of 461.29 ± 5.83 mg/100g. The in-vitro antioxidant activity results revealed 0.442 ± 0.027 activity/min/unit/g for peroxidase, and 0.076 ± 0.004 units/g Units/g fresh weight for SOD. Meanwhile, the inhibitory concentration (IC_{50}) value of the sample against DPPH activity was 12.95 as against 0.13 recorded by the standard (ascorbic acid), indicating that the sample has a lower DPPH scavenging potential when compared to ascorbic acid. In conclusion, these findings highlight the nutritional abundance and antioxidant properties of *Cnidoscolus aconitifolius*, indicating its potential health benefits especially in countering the deleterious actions of reactive nitrogen and oxygen species, however the antioxidant activity should be examined in-vivo. Also, the high moisture content is an indication that the sample requires effective storage mechanism to inhibit or lower microbial growth, which can reduce its shelf life.

Keywords: Antioxidant; biophysicochemical; Cnidoscolus aconitifolius; chaya leaf; DPPH; flavonoid; and nitrate.

1. INTRODUCTION

The bioactive components of plants including vegetables are known to contribute significantly to the nutritional and therapeutic potentials and general wellbeing and survival of humans and animals. According to [1], *Cnidoscolus aconitifolius* (family Euphorbiaceae), commonly called chaya or spinach tree is a leafy perennial shrub native of Yucatan peninsula of Mexico in Central America. In Southwest Nigeria, it is commonly known as 'Efo Jerusalem' or 'Efo Iyana Ipaja'; in Niger Delta region (South-South) of Nigeria it is known as 'Hospital Too Far'. It is mostly consumed as vegetable in soups, salads and therapeutically used as anti-diabetic, hepatoprotective, anti-microbial, amongst others [1]. According to [2], it is used to treat rheumatism, gastrointestinal disorders and inflammatory diseases. The therapeutic potentials highlight the ethnobotanical significance of the plant within indigenous cultures, requiring the necessity for scientific investigation to elucidate and validate the bioactive compounds responsible for that effect. Chaya leaves is rich in protein, vitamins, calcium, and iron; and antioxidants [3]. According to [4], its shoot and leaves are used as laxative, diuretics, circulation aid, and lactation stimulants. Researches have shown that moisture content, ash, crude fiber, and nitrate levels contributes significantly in the variation of the bioactive and nutritional value of vegetables. The moisture content, indicating the water content in plant materials, impacts the overall quality and shelf life [5]; ash content, indicates the presence of inorganic elements [6]; crude fiber, consisting mainly of cellulose, hemicellulose, and lignin, adds to dietary fiber content, impacting digestive health and nutrient absorption [7]; while nitrate, though present in many leafy greens, can be lethargic due to their potential health implications when consumed in high amounts [8], but has been proven to have cardioprotective effects [9]. Antioxidant compounds such as cumarin, flavonoids, phenolics, tannin, antraquinine, triterpenoid, flabotanin, kaempherol, and cyanogenic glycosides, in addition to nutrients such as protein, carbohydrates, fiber, vitamins, calcium, iron, sodium, potassium, magnesium, zinc, and copper in Chaya leaves was reported by [10]. The anti-nutritional contents of Chaya leaves such as oxalate, phytate, hydrogen cyanide, saponin and tannins was reported by [11]. Although some previous studies have reported the nutritional and therapeutic value of *C. aconitifolius*, there are limited scientific information on the specimens harvested at Choba locality of Rivers State, Nigeria. Considering geographical area of previous studies, regional differences may contribute to variations in the plant's overall quality and potential in contributing to human health. Exploring and understanding the biophysicochemical composition and in-vitro antioxidant capacity of plants in their specific region is essential for providing accurate information on dietary planning and nutritional interventions to the local population, health practitioners, and policymakers. This study encompassed a detailed examination of the chemical composition, nutritional content, and in-vitro antioxidant capacity of *Cnidoscolus aconitifolius.*

2. MATERIALS AND METHODS

2.1 Equipment and Reagents

Standard analytical grade biochemical reagents, chemicals and materials were used in this study. They include phosphate buffer, deionized water, ascorbic acid, bromocresol green (BCG) solution, chloroform, atropine, sodium nitrite, aluminum chloride, sodium hydroxide, Folin-Ciocalteu's reagent (FCR), catechol solution, carbonate buffer, adrenalin, sulphuric acid, acetone, potassium hydroxide, salicylic acid, pyrogallol [0.05 M in 0.1M phosphate buffer (pH 6.5)], H_2O_2 (1% in 0.1M phosphate buffer, pH 6.5). The equipment include UV-2450 Spectrophotometer (Schimadzu, Japan), Laboratory Oven or incubator (Genlab, E8A64403), water bath (UT4301E), weighing balance (DS Hortitrade), measuring cylinder, crucibles, pair of thongs, beakers, burette, pipette, Whatman paper, funnel, test tubes, and ceramic mortar.

2.2 Collection and Preparation of the Sample Extract

Fresh sample of *Cnidoscolus aconitifolius* was harvested from a vegetable garden at Choba, Rivers State, and was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. 10 g of the leaves sample was weighed and 100 mL of phosphate buffer was added before crushing in a ceramic mortar, after which it was left to macerate for 24 h in a shaker at room temperature. The sample was afterwards filtered with grade 1 Whatman paper (Whatman International Ltd, Maidstone. England) and the extract was analyzed for phytochemicals (flavonoids, alkaloids and phenols), nitrate, moisture content, ash content, and crude fibre; and antioxidant capacity [2,2-diphenyl-1-picryhydrazyl (DPPH) scavenging assay, superoxide dismutase (SOD) and peroxidase activity (PD)].

2.3 Methods of Analyses

The alkaloid, flavonoid and phenolic content was quantitatively assayed using the method described by [12]. The concentrations of alkaloid, flavonoid and phenolic were estimated using atropine, catechin and catechol as reference standards respectively. Nitrate content was assayed by salicylic acid nitration method as described by [13], and was estimated with a standard curve made with potassium nitrate. The nutritional composition was examined using the method described by the Association of Analytical Chemists [14]. Peroxidase activity was estimated using the method described by [15]. Superoxide dismutase activity was assayed based on the rapid auto-oxidation of adrenaline in the presence of superoxide anions as described by [16], while the DPPH scavenging activity was determined using the method proposed by [17].

2.4 Data Analysis

The primary data was analysed using descriptive statistics and results presented as Mean ± standard deviation for each of the tests. Meanwhile, the 50 percent inhibitory concentrations of samples and standards were obtained by plotting the percentage inhibitions against the standards and generating the R-square and regression equations from the plots for the necessary calculations.

3. RESULTS AND DISCUSSION

3.1 Results

The current study investigated the biophysicochemical and some in-vitro antioxidant parameters of *Cnidoscolus aconitifolus* leaves harvested from a vegetable farm in Choba, Rivers State, Nigeria. The assayed biological active compounds include alkaloid, flavonoid, nitrate, and phenol. Also analyzed were ash, crude fibre and moisture content. The result presented in Table 1 revealed that *C. aconitifolus* is rich in nitrate (461.29 ± 5.83 mg/100g), phenol (351.53±2.33 mg/100g), flavonoid (132.83±9.25 mg/100g), and alkaloid (105.91±1.52 mg/100g). The concentration of crude fibre, ash content, and moisture content in *C. aconitifolus* sample were 13.35 ± 0.27 %, 6.61 ± 0.27 %, and 79.12 ± 0.16 % respectively.

The result of the in-vitro antioxidant capacity assayed by estimating the activities of some antioxidant enzymes (superoxide dismutase and peroxidase), and the free radical scavenging ability of the sample against 2, 2-diphenyl-1-picryhydrayl (DPPH) revealed that the sample recorded SOD activity of 0.076 ± 0.004 Units/g fresh weight, and peroxidase activity of 0.442 ± 0.027 (activity/min/u/g) (Table 2).

The free radical scavenging ability of the extract against DPPH as presented in Table 3 indicated that the sample recorded an inhibitory concentration (IC_{50}) value of 12.95, which is higher than 0.13 recorded by the standard (ascorbic acid).

Table 1. Biophysicochemical properties of *Cnidoscolus aconitifolius*

Parameter	Concentration
Flavonoids (mg/100g)	132.83 ± 9.25
Alkaloids (mg/100g)	105.91 ± 1.52
Phenols (mg/100g)	351.53 ± 2.33
Nitrate (mg/100g)	461.29 ± 5.83
Crude fibre (%)	13.35 ± 0.27
Ash (%)	6.61 ± 0.27
Moisture (%)	79.12 ± 0.16

Values represent Mean ± Standard deviation; number of test, n = 3.

Table 2. Antioxidant composition of *Cnidoscolus aconitifolius*

Antioxidant	Concentration
Superoxide Dismutase (Units/g fresh weight)	0.076 ± 0.004
Peroxidase (Activity/min/u/g)	0.442 ± 0.027

Values represent Mean ± Standard deviation; number of test, n = 3.

Table 3. DPPH Assay of *Cnidoscolus aconitifolius* compared with Ascorbic acid

Sample	Inhibitory concentration (IC_{50})	R^2	Equation
Ascorbic acid	0.13397	0.8979	y = 23.151 ln(x) + 96.536
C. aconitifolius	12.95	0.97	y = 19.663ln(x) - 0.3602

Values represent Mean; number of test, n = 3.

3.2 Discussion

Vegetables has been known to contribute significantly in the general wellbeing of man through its nutritional and therapeutic potential. There is hardly any balanced diet that is void of vegetable. This signifies the essential role of vegetables in supplying important nutrients. According to [9], intake of diet enriched in vegetables and fruits has a significant effect on the prophylaxis and treatment of cardiovascular diseases (CVDs). The current study investigated the biophysicochemical and some in-vitro antioxidant parameters of *Cnidoscolus aconitifolus* leaves harvested from a vegetable farm in Choba, Rivers State, Nigeria. The assayed biological active compounds include alkaloid, flavonoid, nitrate, and phenol. Also investigated were ash, crude fibre and moisture content. The result presented in Table 1 revealed that *C. aconitifolus* is rich in nitrate, phenol, flavonoid, and alkaloid. The result also revealed significant concentrations of ash content and crude fibre, with a very high moisture content. These compounds are known to for their well-established biological properties and activities. For instance, the antioxidant and anti-inflammatory capacity of nitrate was reported by [18]. [9] reported that most biologically active phytochemicals including nitrate present in green leafy and root vegetables may confer cardioprotective benefits via various mechanisms. According to [18], nitrate intake has some beneficial effects on the cardiovascular system such as triglyceride reduction, blood pressure regulation, and stroke and atherosclerosis prevention. Nitrate has also been reported by [19], to act as an anti-nutrient which have direct and indirect effects ranging from mild reactions to death. Remarkably, [20], posited that the ecological risks associated with high nitrate intake signifies that high nitrate content in vegetable is beneficial considering its high natural antioxidants compounds such as polyphenols, betalain pigments, and vitamins that inhibits nitrosamines synthesis. According to [21], flavonoids consist of natural substances with variable phenolic structures and are dominant in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wines. Considering its anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function, they have become an essential constituent in various industries such as cosmetics, nutraceutical, pharmaceutical, and medicinal firms. According to [22], phenolic compounds are bioactive constituents of plant that possess good health promoting activities. The phenolic compounds possess anti-inflammatory, anti-clotting, antioxidant, and act as immune enhancers and hormone modulators [23]. [24], reported the antioxidant properties of phenols. Alkaloid on its part has been reported by [25], to act as CNS stimulant, topical anesthetic in ophthalmology, powerful pain relievers, antipyretic action, among other uses. The high concentration of phenols, flavonoids, alkaloid, and nitrate recorded in this study is a clear indication that *C. aconitifolus* possess various medicinal effect especially antioxidant potential. Also, the appreciable amount of crude fibre present in the sample is an indication that *C. aconitifolus* has the ability to enhance reduction of cholesterol level, maintain body weight, stabilize glucose and minimize the risk of other health complications such as heart disease, hemorrhoids and cancer as well as help to keep the digestive system healthy by adding bulk to faeces and preventing constipation. The aforementioned activities of crude fibre has been reported by [26]. The ash content recorded in this study is an indication that the *C. aconitifolus* sample contains appreciable amount of inorganic mineral. [27], posited that the ash content of food samples can affect different characteristics of food including physiochemical and nutritional properties. The result also revealed high moisture content in the sample indicating short shelf-life and poor resistance to bacterial activities which can lead to easy perishability of the sample. Also, high moisture content is an indication of significant amount of micronutrients such as carotene, vitamins, and minerals in leafy vegetables, which makes them an essential component of a nutritious diet [28]. The result of the in-vitro antioxidant capacity assayed by estimating the activities of some antioxidant enzymes (superoxide dismutase and peroxidase), and the free radical scavenging ability of the sample against 2, 2-diphenyl-1-picryhydrayl (DPPH) revealed that the sample recorded low SOD activity, and significant peroxidase activity (Table 2). This outcome is in line with the study of [29] that vegetables are rich in antioxidant enzymes such as SOD, peroxidase, catalase (CAT), and some other antioxidant compounds that ameliorate the oxidative damage caused by accumulation of reactive oxygen species (ROS). [30], reported that SOD activity regulates the concentration of H_2O_2 and O_2 and serves as the initial line of defense against toxicity caused by different stresses in plants. The SOD activity recorded in this study is an indication that *C. aconitifolus* has a potential to mitigate H_2O_2 and O_2 activities. The study also recorded a high peroxidase activity indicating a rapid degradation of hydrogen peroxide (H_2O_2) by peroxidase through oxidative reaction. The free radical scavenging ability of the extract against DPPH as presented in Table 3 indicated that the sample recorded a higher inhibitory concentration (IC_{50}) value than the standard (ascorbic acid). The higher IC_{50} value recorded by *C. aconitifolus* is an indication that the plant sample has a lesser potent DPPH radical scavenging activity than ascorbic acid. According to [31], an IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. It is inversely related to the antioxidant activity of crude extracts. Thus, the lower the IC_{50} value, the higher the antioxidant activity. DPPH radical scavenging activity remains one of the most vital analysis applied in assaying the antioxidant activity of plant extract [32]. The antioxidant capacity shown by the sample can be attributed to the presence of flavonoids and other phenolic compounds. The results obtained in this study almost correlate with some other outcome of the same assay on *C. aconitifolus* harvested in other areas, as geographical location and soil content can impact the primary data.

4. CONCLUSION

In conclusion, findings from this study revealed the phytochemical, nutritional value, and antioxidant capacity of *C. aconitifolus* leaves harvested from Choba, Rivers State, Nigeria. The sample has high concentration of nitrate, phenol, flavonoids and alkaloid as well as a very high moisture content and a good amount of crude fibre. The sample also showed a relatively high peroxidase and DPPH radical scavenging activities, though not to be compared with that of ascorbic acid. This indicates the sample's capacity to combat oxidative stress and ensure protection against free radicals. It is imperative to state that *C. aconitifolus* leaves harvested from the study location should be considered a good source of antioxidant, and further studies on its *in-vivo* antioxidant capacity should be investigated to ascertain its potential as a therapeutic remedy for free radical induced disorders. The nutritional and health benefits of *C. aconitifolus* leaves from the study area will go a long way in addressing certain diet-related ailments. Thus, the populace of the research area should be sensitized on the need to grow more of this vegetable and adopt it as a valuable dietary component.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/122751