



## ***Cnidoscolus aconitifolius*: Nutritional, phytochemical composition and health benefits – A review**

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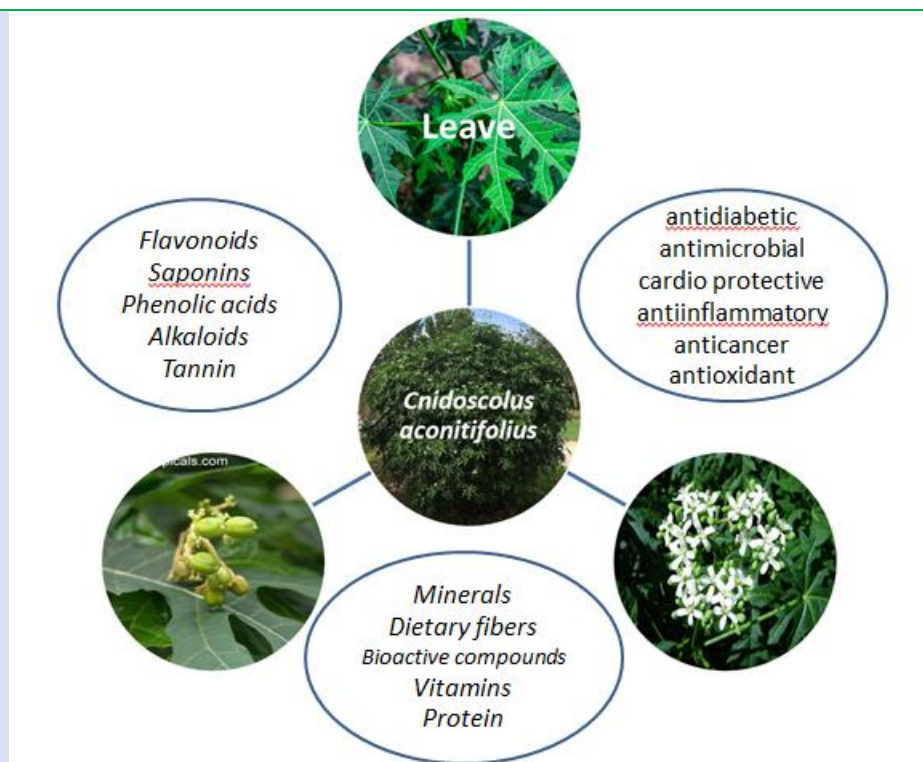
**Submission Date:** November 10<sup>th</sup>, 2021; **Acceptance Date:** November 28<sup>th</sup>, 2021; **Publication Date:** November 30<sup>th</sup>, 2021

**Please cite this article as:** Panghal A., Shaji A.O., Sajitharan D., Nain K., Garg M.K., Chhikara C. *Cnidoscolus aconitifolius*: Nutritional, phytochemical composition and health benefits – A review. *Bioactive Compounds in Health and Disease* 2021; 4(11): 260-286. DOI: <https://www.doi.org/10.31989/bchd.v4i11.865>

### **ABSTRACT**

*Cnidoscolus aconitifolius* belonging to Euphorbiaceae family is widely used as a folk medicine among South American countries such as Mexico and Brazil. *C. aconitifolius* can be considered as a potential leafy green vegetable, largely due to the presence of various secondary metabolites. Different bioactive compounds such as phenolic acids, Alkaloids, saponins, flavonoids etc. along with terpenoids have unique structure that are exclusive for this genus. *C. aconitifolius* has numerous nutraceutical benefits proven with pharmacological studies such as anti-diabetic, hepatoprotective, anti-microbial etc. The recent upsurge of consumer interest for health promoting products has opened up new vistas for *C. aconitifolius* application in food product research and development field. Limited knowledge about this underutilized plant is presenting an opportunity to explore the plant as novel ingredient with vast functional attributes.

**Keywords:** *Cnidoscolus aconitifolius*, phytochemicals, nutraceutical potential, biological activity, diabetes



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## INTRODUCTION

The concern for improvement in quality of life has increased in the modern world as rates of disease have also steadily increased. Fruits and vegetables have been systematically linked with reduction of risk of chronic diseases such as cardiovascular diseases and neurodegenerative diseases. The demand for health promoting foods has increased among consumers. There is a need for healthy and nutrient rich foods that can improve overall health of the society, which has further increased consumers demand for plant-based foods [1]. Leafy green vegetables are a rich source of nutrients, phytochemicals and other health promoting components. *Cnidoscolus aconitifolius* (Chaya plant, tree spinach) can be a prime example of a green leafy vegetable that has strong health promoting connotation, an association that is supported by scientific literature. This plant has been associated with several health benefits such as maintaining healthy blood sugar levels, acting as an anti-inflammatory, anti-

anemic, anti-microbial, and antioxidant effect etc. [Table 1, Table 2]. Chaya leaf, leaf powder or leaf extract has gained huge importance and thus enhanced the commercial value of the plant. Chaya plant is commonly known as a tree spinach, a member of Euphorbiaceae family. Chaya and its related species are a group of arborescent shrubs, of the section calyptosolen of the genus *Cnidoscolus*. It is a fast-growing attractive shrub, usually 3 to 5 m tall with attractive, large, dark green leaves. It can grow in a wide range of climates, but at higher temperatures it grows particularly easy and quicker. The leaves have a lower moisture content than most green leafy plants like spinach [2]. Many native plants with high potential health benefits are commonly underutilized and ignored. *C. aconitifolius* is commonly underutilized due to the poor knowledge regarding its nutritional and health benefits [3]. Tree spinach is exceptionally high in fiber (31.165%), calcium (50 mg/g), iron (10 mg/g), potassium (20 mg/g) and vitamin C (892.025 mg/100 g) [4]. It provides a wide range of

health benefits such as anti-diabetic activity, cardio protective activity, antimicrobials, anti-inflammatory, hematopoietic property and anticancer properties. This comprises the main dietary sources of indigenous people of Yucatan peninsula of Mexico and ketch people [5-6]. While nutritional potential of the chaya plant has been suggested, little scientific experimentation has been done on the nutritional value of chaya leaves. However, there are studies based on therapeutic benefits of chaya. Considering the argument made above, the present work is aimed to compile the available evidence that link the tree spinach and chaya based products with several health promoting properties.

**Plant Description: Origin, Distribution and Soil**

**Conditions:** *Cnidoscolus* varieties are of broadly, widely distributed from southern Texas along the gulf coast, through Yucatan and Chipas, and through Colombia. Chaya has been spread to Maya families in urban and south urban areas throughout Mexico and southwest United States [6]. It has now spread to Cuba, Florida, Mexico, and the US. Throughout the native range, Chaya was typically only cultivated for ornamental or as living fence earlier. Though recently food and medicinal usage of plant have increased due to its inherent nutritional and health promoting attributes. Chaya is cold sensitive and typically grows better in the beginning of the summer months. Chaya flourishes in a wide range of conditions, including extensive sun or rain and humid or

dry climates. Remarkably, Chaya plant needs very little care and it grows well on low fertile soil as well. Although it can grow in extreme climates, favorable climates result in good yield and ample leaf production. Chaya plant is easy to propagate and is highly resistant to pests and disease. If it is propagated, it is done by cutting because it roots slowly. At early stages, the growth is low, but soon after leaves are being harvested, growth is rapid.

**Morphological Properties:** The plant is evergreen in color, drought resistant shrubs up to 6 m tall with alternate or palmate lobate leaves, milky sap, and small, white flowers on dichotomously branched cymules (Figure1). Chaya plant can grow up to a 5-to-6-meter shrub (Figure 1). But it's recommended to maintain in 2m height. Although it's kept very small, it can actively produce great amount of leaf material. The harvest period ranges from two to three months. Leaves are giant and succulent, up to 32 cm long and 30 cm wide, on petioles up to 28 cm long [7]. Leaves exhibit stinging hairs, in petiole and bottom margin of lamina. Mature leaves are mostly three lobed with rough margins and no stinging hairs, young leaves lack the distinct lobes the mature leaves have. Mature plant flowers produce pollen but mostly non-viable. Mature fruit is rare, without viable seed with thick succulent stems.



(a)



(b)



(c)

**Figure 1.** *Cnidoscolus aconitifolius*: plant (a); leaves (b); flowers (c).

**Nutritional and Chemical Composition of Tree Spinach:**

The leaf protein concentrates of *C. aconitifolius* contain high amounts of carbohydrate content (41.895%), 7.68% of protein, 31.165% of crude fiber, 4.85% ash content, 1.145% crude fat and a moisture content of 13.53% which can essentially provide a gross energy of 14.6 MJ/kg [8-9] (Figure 1). It also contains most of the essential amino acids and vitamins like carotene, vitamin A, vitamin C, niacin, riboflavin, and thiamin. Twenty phyto-chemotypes with different therapeutic activities along with different secondary metabolites such as flavonoids, saponins and Alkaloids are also revealed from *C. aconitifolius* [10]. Processing generally improved the composition of the tree spinach, and its low moisture content makes it resistant to spoilage microorganisms [11]. Carbohydrate content, crude fiber and its moisture content reveal its nutritional quality. This is also rich in fibers which in turn can stimulate weakening hunger, stimulating peristaltic movements and lower serum cholesterol level. Likewise, dietary fats have a role in food absorption and flavor retention [12]. Chaya leaves when consumed in adequate amounts can meet the RDA values of carbohydrates for children (40%), adult (40%), pregnant women (30%), and lactating women's (25%). The high fiber- low fat content of tree spinach indicates the clinical relevance in treating digestive associated diseases.

Proteins are essential nutrients needed for the building up of body tissue and regulatory substance such as enzymes and hormone. The quality of protein relays on its amino acid composition, its bioavailability and the essential amino acids that are needed according to RDA of each organism. A total of 44.80% essential amino acids and 55.19% non-essential amino acids are

present in the leaf protein concentrate of tree spinach [8]. *C. aconitifolius* leaf contains alanine (4.60 g), arginine (5.17 g), glutamic acid (13.50 g), methionine (1.35 g), histidine (2.60 g), isoleucine (4.25 g), lysine (5.05 g), threonine (3.70 g), tyrosine (3.33 g), aspartic acid (8.50 g), serine (3.36 g), and valine (4.54 g) in 100g of protein [8] (Figure 1).

Micronutrients are essential elements needed in very small quantities, including minerals as well as vitamins. Vitamins are the group of substance that is needed for normal cell function, growth, and development. Chaya leaves contain vitamin A (59.644 mg/100g), vitamin C (892.02 mg/100g), one of the most important categories of vitamins are the B complex vitamins (vitamin B<sub>1</sub> 9.425 mg/100g, vitamin B<sub>2</sub> 5.84 mg/100g, vitamin B<sub>9</sub> 9.425 mg/100g), which are essential for growth, development, and maintenance of other bodily functions [13]. Carotene, riboflavin, and thiamin are also present in *C. aconitifolius*. [2]. (Table 2). Only 25g of Chaya leaves can meet the daily requirement of vitamin C and can enhance the absorption of non-heme iron [14]. The consumption of Chaya leaves helps to maintain the rigidity of cell membranes and slows down the aging process due to the antioxidant properties possessed by the vitamins [2]. Chaya leaf has high amounts of vitamin C, which serves as a potent antioxidant. This facilitates the transport and uptake of the non heme iron at the mucosa, the reduction of folic acid intermediates, and synthesis of cortisol. Vitamin C deficiency may cause fragility to blood capillaries, gum decay, and scurvy.

Chaya plant also has an appreciable amount of vitamin A, which is essential for normal vision, gene expression, growth, and immune function by the

maintenance of epithelial cell functions. Vitamin E is also an essential vitamin and a powerful antioxidant which can prevent the damage from free radicals. This has also role in formation and normal function of red blood cells and muscles [15-16]. In short, tree spinach is an affordable and nutritious source of vitamins.

The minerals contained in Chaya leaf are essential for maintenance of human health and wellness. It helps in osmoregulation, bone health, hemoglobin formation, prevention of prostate cancer, reduction of stroke etc. The leaf protein concentrate contains sodium of 41.47 mg/100g, potassium of 33.88 mg/100g, calcium of 29.16 mg/100g, phosphorus of 24.68 mg/100g, copper of 7.1 mg/ 100g, zinc of 15.64 mg/100g, iron of 14.83 mg /100g, manganese of 0.18 mg /100g. Mineral composition of the leaf was affected by the methods of processing (cooking, sun drying) when compared to leaf protein concentrates [3].

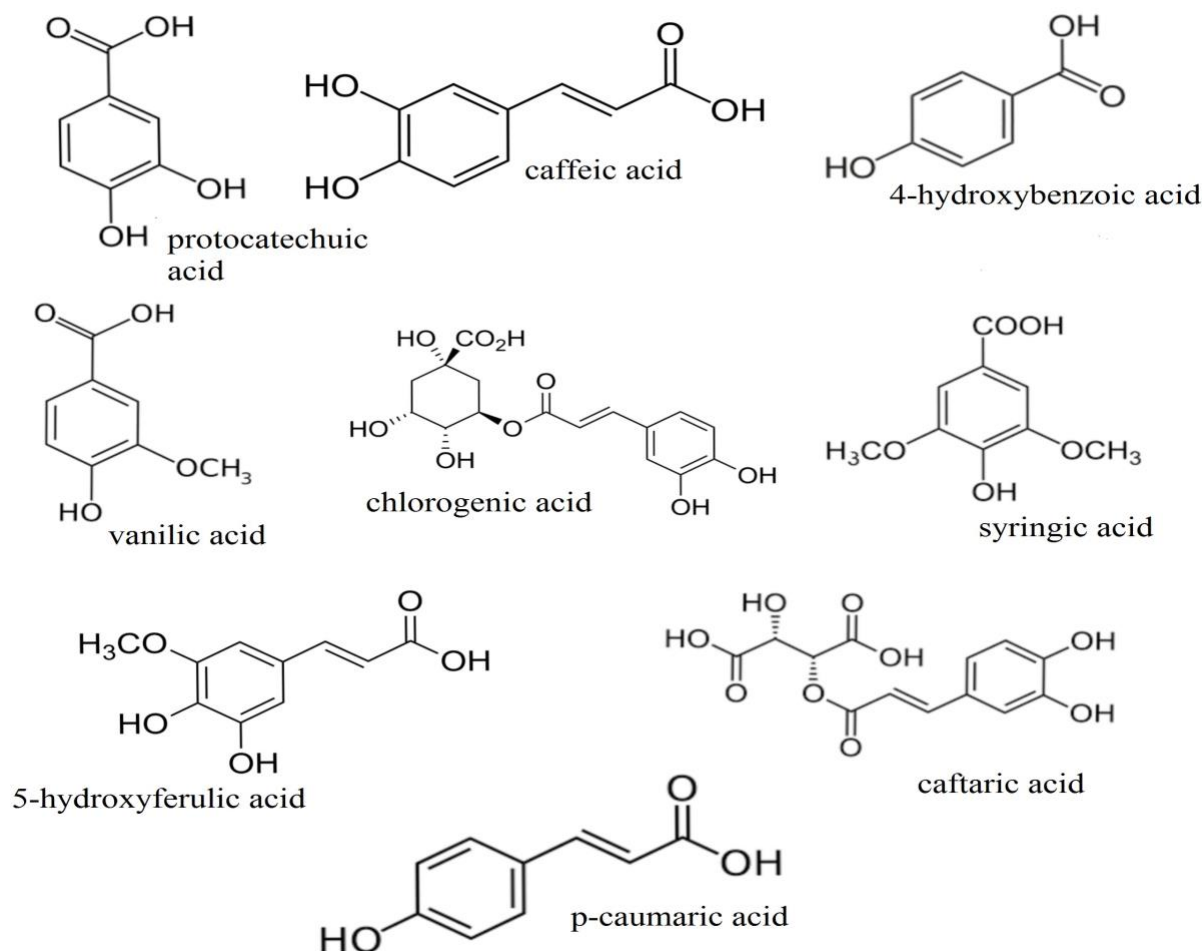
Sodium and potassium are the activators of energy potential across nerve membrane and can be used in management of disease associated with the central nervous system and neurodegenerative disease. They also have role in maintaining acid-base balance along with nerve transmissions. Calcium is a rich source of mineral that involved in bone formation. Along with fibrinogen, collagen and fibrin, calcium is an important component in fibrin net formation. Calcium ions along with clotting factor fibrin can help in maintenance of normal nervous function and gut peristalsis [17]. Phosphorus and copper present in tree spinach aid in brain functioning. Chaya leaves can be used as traditional means for increasing blood content of body

[17]. Zinc is an important component for more than ten important enzymatic functions of the body. Deficiency in zinc may result in various health concerns including inability to heal wounds, store insulin, as well as defend against various skin infections. Magnesium is also an essential component in many intermediary metabolism and enzymatic actions. It is an important cell function modulator along with vital role in control of diabetes [18].

**Bioactive Compounds:** Bioactive compounds are the secondary metabolites present in plant and accounts for numerous health benefits on prolonged consumption in specified amount [1]. Tree spinach contains many bioactive compounds such as phenolic acids, Alkaloids, saponins, flavonoids etc. Terpenoids with unique structures were also identified from *C aconitifolius*, which is exclusive factor of this genus [19].

**Phenolic acids:** These are substances containing phenolic ring and an organic carboxylic acid function. The total phenolic content in tree spinach is 33.02 mg/100g and can be exploited in pharmaceutical for treatment of many disease conditions [Table 1] [11]. Major phenolic acids that are isolated from the tree spinach are chlorogenic acid, vanillic acid, protocatechuic acid, caftaric acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, p-caumaric acid, synapic acid and ferulic acid (Figure 2). These phenolic compounds offer antioxidant properties, can prevent cancer and cardiovascular diseases, and possess anti-mutagenic property [Table 1] [20].

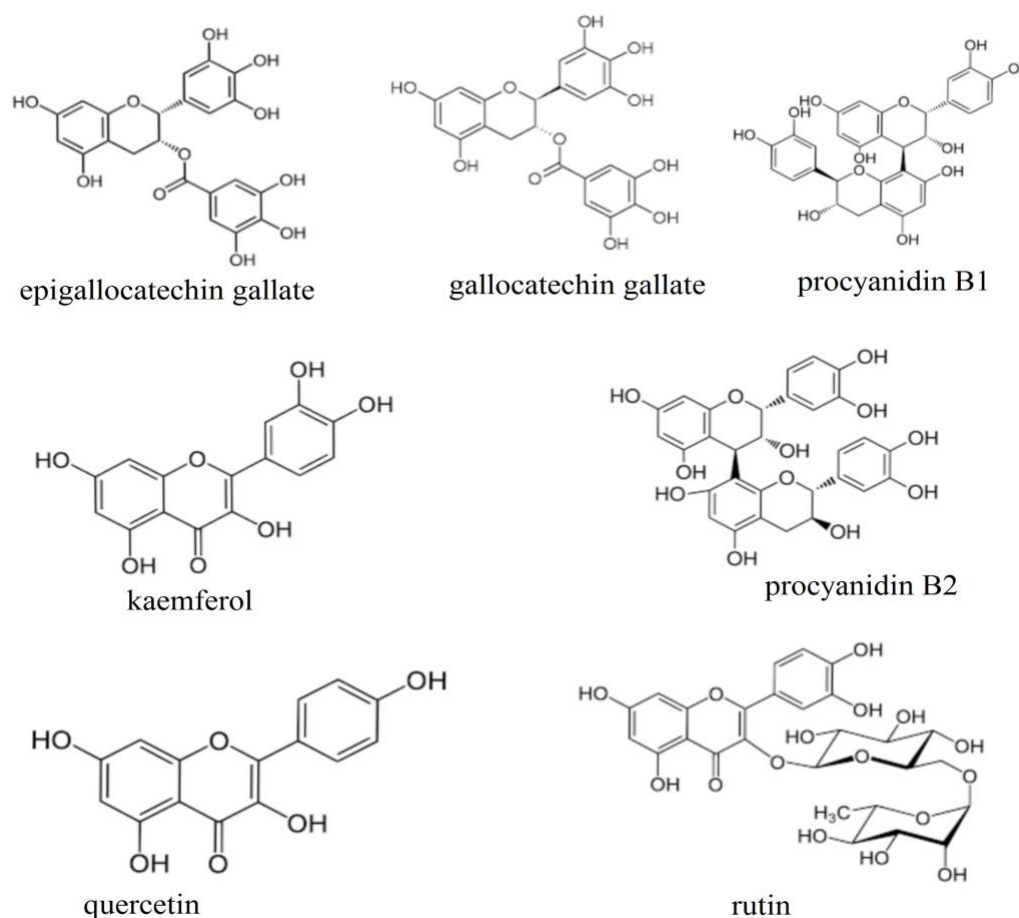




**Figure 2.** Phenolic acids present in *Cnidoscolus aconitifolius*

**Flavonoids:** Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. They are the largest group of phytonutrients with more than 6000 types. Chaya contains abundant amounts of flavonoids (332.89 µg catechin equivalents (CE) per gram of sample fresh weight). Yuan et al [21] isolated 15 flavonoids from tree spinach, primarily from the aerial parts. Flavonoids isolated from tree spinach involve procyanidin B1, procyanidin B2, catechin, rutin, gallic acid, epigallocatechin gallate, epicatechin-3-O-gallate, quercetin-3-O-galactoside, quercetin-3-O-glycoside,

quercetin-3-O-rhamnoside, trans-reverseritol, quercetin and Kaempferol (Figure 3) [Table 3]. These can account for antioxidant properties, play a role in modulation of several enzymatic pathways, reduce the risk of chronic diseases like cancer and cardiovascular disease, and neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis [Table 1]. Flavonoids can neutralize the free radicals and protect against development of heart disease. They can also inhibit tumor invasion and potentially kill growing cancer cells.



**Figure 3.** Flavonoids present in *Cnidocolus aconitifolius*

**Saponins:** These are the class of steroid and terpenoids glycosides and can impart some health benefits in combination with other bioactive compounds. Chaya contains 6.3.5 mg/100g and is an anti-nutritional factor associated with tree spinach. Some of the saponins found to be present in tree spinach are:

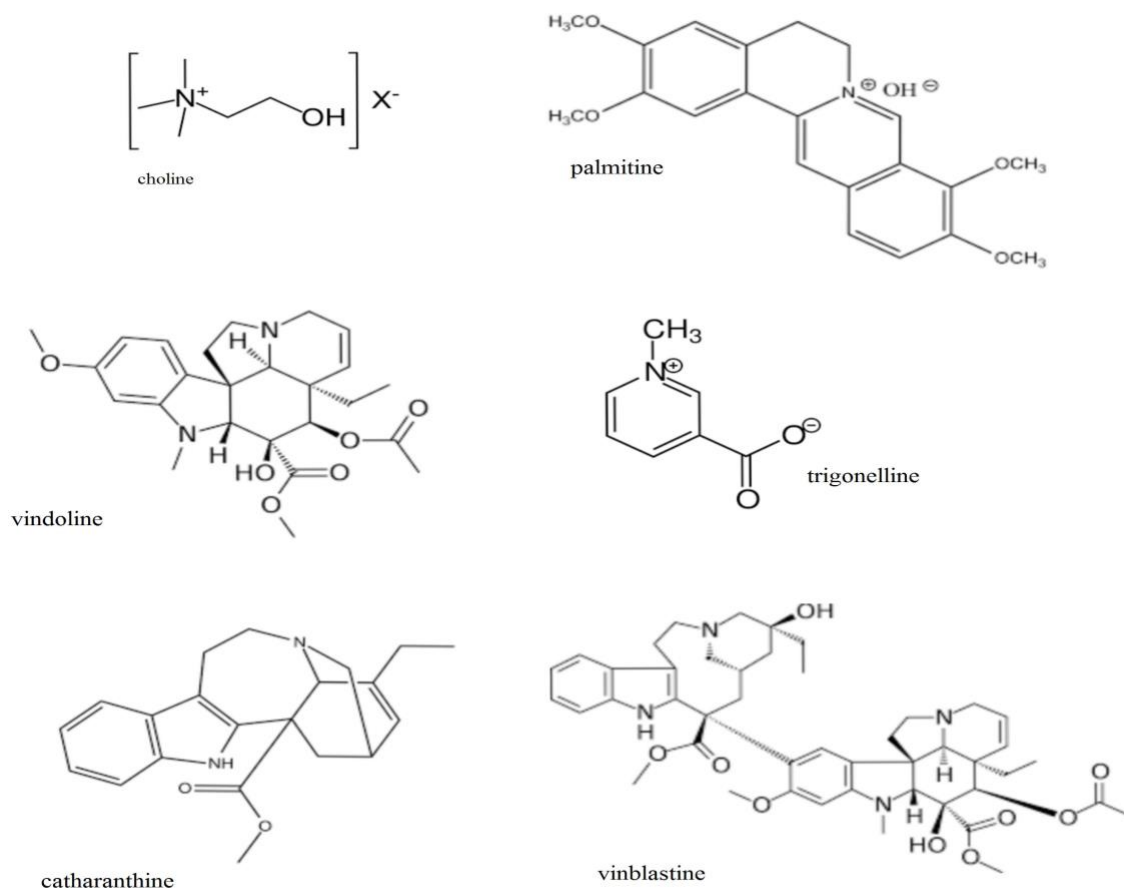
3-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl-3,23,30-trihydroxyolean-12-en-28-oic acid, 3-O-β-D-glucopyranosyl-(1-3)-β-D-galactopyranosyl phytolaccagenic acid, 28-O-β-D-glucopyranosylester, 3-O-hexose-pentose-pentose phytolaccagenic acid-28-O-hexose, 3-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl phytolaccagenic acid 28-O-β-D-glucopyranosylester, 3-O-β-D-xylopyranosyl-(1-3)-β-D-glucuronopyranosyl hederagenin 28-O-β-D-

glucopyranosyl ester, 3-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl serjanic acid 28-O-β-D-glucopyranosyl ester, 3-O-β-D-glucuronopyranosyl oleanolic acid 28-O-β-D-glucopyranosyl ester.

Saponins can provide benefits like boosting the immune system in various ways by offering a chemo preventive strategy in lowering the risk of cancer, lowering blood lipids, lowering blood glucose response, hyperglycemia, hypercholesterolemia, and hypertension [Table 1] [22-23]. Saponins also possess a natural tendency to ward off microbes, which make them effective therapy for fungal and yeast infections [24]. They exhibit antioxidant activity by reducing the level of lipid hydroperoxidase.

**Alkaloids:** These are plant secondary metabolites, a class of nitrogenous organic compounds, that are biologically active. There is total of 1.96 mg/100g of alkaloids content in tree spinach. Choline, trigonelline, palmitine, sirsirine, dihydrosirsirine, vinblastine, vindoline, catharanthine, vinleurosine (Figure 4) are various alkaloids that have been isolated from Chaya [22, 25]. These saponins play a role in hepatoprotective

and anti-inflammatory effects, and in the prevention of various degenerative diseases, and possess free radical scavenging property [Table 3]. This is one of the largest groups of phytochemicals in plants with amazing effects on humans and has been used for treatment of intestinal infections associated with AIDS and hypertension [22].



**Figure 4.** Alkaloids present in *Cnidocolus aconitifolius*

**Tannins:** These are organic substances found in plants and the content can vary with the species, variety, maturity, and season. In tree spinach, it's found that tannins can react with some proteins to provide some tanning effect which is important for the treatment of inflamed or ulcerated tissue [26]. The healing of wound and anti-inflammatory activities is directly linked with tannin content [27].

**Phenols:** These are a class of bioactive compounds consisting of hydroxyl group bonded directly to aromatic hydrocarbon group which accounts for both anti-microbial and antioxidant properties [28]. Total phenolic compounds in tree spinach were found to be 5.66 mg GAE (gallic acid equivalents)/g of fresh weight of sample [29]. Natural polyphenols can remove free radicals, chelating metal catalysts, activate antioxidant enzymes,



reduce  $\alpha$ -tocopherol radicals, and inhibit oxidase [Table 1]. Phenolic content of raw Chaya leaves is 241.1 mg/100 g and that of cooked were 285.1 mg/100 g and the percentage gain of phenolic content was 18.3%. This implies phenolic compounds are trapped in fibers of green leafy vegetables and are more available in cooked compared to raw forms. The percent gain in the total phenol content during cooking may be due to the breakdown of tough cell walls and release of phenolic compounds trapped in fiber of green leafy vegetable for easier absorption in the small intestine [30].

**Functional Properties of Tree Spinach:** The incidence of chronic diseases such as cancer, diabetes and cardiovascular diseases are increasing worldwide. Human diet and this physiopathology are interlinked and hence there is a huge want for functional foods as a cost-effective mean for prevention and treatment of many diseases. Chaya plant is also used as a traditional medicine for the treatment of diabetes, rheumatism, gastrointestinal disorders, and inflammation [14].

**Antioxidant Effect:** Antioxidants are substances that inhibit oxidation, mainly used to counter act deterioration of stored food products. Various studies have been conducted over the same to identify the antioxidant potential of the Chaya plant. The presence of bioactive compounds such as phenol and flavonoids can contribute to the antioxidant property. Chronic diseases and food spoilage are associated with pro-oxidants. The antioxidant components found in Chaya can be effective in disease prevention, food preservation and drug formulations. Kuti and Konuru [31] observed that raw leaves possess more antioxidant potential as compared to cooked sample when evaluated with ORAC (oxygen radical absorbance capacity) assay. The ORAC assay measures a fluorescent signal from a probe that is reduced in the presence of ROS, allowing the fluorescent signal to persist and the

results are compared with a standard that is a vitamin E analogue called Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acids). Modification in ORAC assay includes the use of fluorescein as a fluorescent probe, for the separation of hydrophilic and lipophilic antioxidants to obtain the total antioxidant capacity.

Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) can be tumorigenic [32]. The synthetic counterparts of these antioxidants can be replaced by potential antioxidant compounds that isolated from these kinds of medicinally important plants. The in-vitro antioxidant activities were related against 2,2-Diphenylpicryl-1-hydrazyl radicle (DPPH) and vitamin C. This antioxidant potential was due to the phytochemicals present in tree spinach. This antioxidant property in turn facilitates to prevent the oxidative damage occurring in the body.

**Anti-Diabetic Property:** Diabetes is a metabolic disorder in which the body does not produce or properly use insulin which in turn causes disturbance in carbohydrate, protein and lipid metabolism resulting in number of diseases like retinopathy, blindness, and neuropathies. The hypoglycemic ability and toxicity by the addition of hydroponic Chaya leaves were studied by [33]. Wister albino rats were used for demonstrating the hypoglycemic ability while toxicity in *Artemia salina* larvae and the results implied that the ingestion of Chaya leaves induced hypoglycemic effect in rats rather than glibenclamide, which is a medication used for type II diabetes. This ameliorative effect was mainly due to the hypoglycemic agents such as catechin and rutin present in Chaya leaves. Infusion of leaves of Chaya can also be considered as non-toxic as well as safe for consumption as a potential hypoglycemic agent [33-34]. The mechanism involved in controlling the high glucose

level was the presence of major secondary metabolites that can effectively act on  $\alpha$ -glycosidase enzyme [9] [Table 2].

The anti-diabetic potential of Chaya plant was also studied in streptozotocin- induced diabetic rats [Table 2, Table 3]. The compounds responsible for the anti-diabetic property were flavonoids and phenolic acids present in the leaf extracts. This study showed that the hyperglycemic effect produced was not related with or with starch digestive enzymes but with the decrease in glucose absorption. But the long-term intake on these extracts suggests possible alteration in renal function by the increase in urine micro albumin concentration [25].

The ethyl acetate fraction of *C. aconitifolius* can act as an anti- diabetic agent possibly by the inhibition of the carbohydrate metabolism. This can extend the time needed to absorb the glucose and thereby decreasing the level of ingested glucose [35]. The glucose absorption can also be delayed by the inhibition to two major carbohydrate metabolism enzymes such as  $\alpha$  amylase and  $\alpha$ - glycosidase, which are present in the mouth and small intestine [35-36]. The inhibitory effect of ethyl acetate fraction on both enzymes showed a good concentration dependent activity. This inhibitory effect on carbohydrate metabolizing enzyme was possibly due to the presence of phenolic compounds in *C. aconitifolius*.

In another research approach, extract of *C. aconitifolius* imparted anti-hyperglycemic effect possibly by the presence of flavonoid and saponins that can stimulate insulin from the pancreatic  $\beta$  cell [37]. The amount given to the experimental model was 5, 10, 50, 100, 150, 200 mg/kg and the best recommended dosage was 100mg/kg [Table 3] of body weight.

**Protective Effect against Alzheimer:** The increased activity of cholinesterase in the brain of patients having Alzheimer's reduces the accessibility of acetylcholine,

and thereby imparting a cholinergic shortage and memory loss. Acetyl choline is an important neurotransmitter in cholinergic neurons. Cholinesterase can regulate the neurotransmission through the hydrolysis of acetylcholine. The compounds present in *C. aconitifolius* especially phenolic compounds such as caumaric acid, amentoflavone, and kaemferol can act on cholinesterase substrate and blocks the enzyme active site there by alternating the cholinergic dysfunction and less inclination towards Alzheimer's [38].

The inhibition of monoamine oxidase is a vital biomarker for the treatment of Alzheimer disease. The degradation of amines by the reactive oxygen species can be controlled by increasing the neurotransmitters like dopamine. This can be triggered by the decrease in monoamine oxidase level [39]. This inhibition is due to the presence of phenolic compounds in the plant fraction. One of the main reasons for this inhibitory activity is due to structural similarity that is shared by the synthetic monoamine inhibitors towards the phenolic compounds present in ethyl acetate extract of *C. aconitifolius* [40].

#### **Protective Effect on Neurodegenerative Diseases:**

Neurodegenerative disease is marked by the irregular increase in E-NTDase (ecto-5'-nucleotidase) along with neural-inflammation and neuro-immunoreaction [41]. E-NTDase can hydrolyze AMP to adenosine and thereby regulate the purinergic transmission and nucleotide mediated cell signaling. The variation in the E-NTDase activity may cause an increase in adenosine level in the synaptic cleft. The increase in levels of adenosine can disturb the release of neurotransmitters like acetylcholine, dopamine, glutamate, and noradrenaline [42]. Adenosine can protect the brain against neural dysfunction. By increasing the accessibility of adenosine to the synaptic cleft the ethyl acetate fraction of Chaya leaves can inhibit the activity of E-NTDase in a

concentration dependent manner. These inhibitory effects are mainly due to the phenolic compounds that present in the ethyl acetate extract of *C. aconitifolius* [43].

Neurodegenerative diseases are partially due to dopamine deficiencies [44]. L-tyrosine is first converted to L-DOPA (L-3,4-dihydroxyphenylalanine) by Tyrosinase. L-3,4-dihydroxyphenylalanine is then converted to dopamine by DOPA decarboxylase inside the nervous system. The capability of ethyl acetate extract of chaya leaves to inhibit the activity of tyrosinase is linked to the antioxidant properties of phenolic compounds present in plants, which tend to compete with substrates by binding to the active site of enzymes. Thus, the ethyl acetate fraction of *C. aconitifolius* can be used for developing drugs that can be used for treatment of neurodegenerative diseases, as tyrosinase is a critical enzyme for the production of dopamine [43].

The ethyl acetate extract of Chaya leaves increased the  $\text{Na}^+/\text{K}^+$  ATPase activity in brain in a concentration dependent manner.  $\text{Na}^+/\text{K}^+$  play a significant role in maintaining the membrane potential across cell as well as the neuronal excitability [45]. The ethyl acetate fraction with increased  $\text{Na}^+/\text{K}^+$  content may help in preventing the neuronal dysfunction [43].

**Protective Effect on Hepatic Damage:** The inability of liver to perform its normal physiological function as part of normal physiology or any kind of exposure to harmful chemicals which in turn result in liver failure can be termed as hepatic damage. The protective effect of Chaya leaf extracts against the hepatic damage that induced by the chronic ethanol administration in rats was studied by [46] and study concluded that the ethanol treated rats had high degree of lipid peroxidation. Antioxidant resistant indices such as reduced glutathione and catalases were lowered due to the ethanol activity. At the same time, there was

increase in serum cholesterol level, triglycerides, serum alanine, aspartate aminotransferase and low-density lipoproteins in the ethanol treated rats. Rats administered with leaf extracts showcased significant decrease in the liver marker enzymes. A stabilized lipid profile was observable, and it also restored the antioxidant status of ethanol treated rats and thereby indicating the protection against hepatic damage as well as the possible antioxidant effect of Chaya which offered protection against ethanol induced toxicity [46, 34].

Adaramoye and Aluko [46] studied the protective effect on hepatic damage induced by the chronic ethanol administration in-vivo model. The dosage administrated to the experimental model was 100 and 200 mg/kg of animal body weight. The flavonoids present in the ethanol extract of *C. aconitifolius* can protect the damage caused to glomerular filtration rate by the action of reactive oxygen species. It can also elevate superoxide dismutase (SOD), and catalase activity and thereby protecting the hepatic damage [47]. SOD is an important enzyme that can convert super oxide to hydrogen peroxide and oxygen which in turn fights free radicals. The aqueous extract of Chaya also demonstrated protective effect on carbon tetrachloride induced hepatotoxicity. The phenols, saponins and the cardiac glycosides that present in the extract can prevent the oxidative damage by the carbon tetra chloride by converting them into trichloromethanide in hepatocytes. This can cause cell death by the accumulation of lipid peroxidase, and it also can protect from the inflammatory process [47].

Oyagbemi and Odeitola [48] studied the hepatoprotective effect of ethyl alcohol extract of Chaya leaves and found that hepatic damage is prevented by the inhibition of serum alkaline phosphatase and bilirubin levels by improving the secretory mechanisms of hepatic cells. The amount of ethyl alcohol extract

given was 100, 500 and 1000 mg/ kg and the best protective effects were shown by the experimental model supplied with 1000 mg/ kg.

**Antimicrobial Activity:** The antimicrobial substances inhibit the growth of microorganism by hindering the essential pathways, physiological metabolic and reproduction activities. The usage of natural antimicrobial agents will reduce the chemical load of food also. The antibacterial activity of *C. aconitifolius* essential oil against three bacterial strains such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* was studied using agar disc diffusion technique. The oil exhibited significant antimicrobial activity against *S. typhi* and *E. coli*, but ineffective against *P. aeruginosa*. This proves that essential oil of Chaya leaf has an inhibitory activity against *E. coli* and more particularly against *S. typhi*. This provides a scientific basis for the use of essential oil against typhoid fever and *E. coli* associated with gastroenteritis. The purified active compounds in Chaya can enhance the antimicrobial activity [49] (Table 1).

Antibacterial activity of Chaya was studied in Microplate Alamar Blue Assay in the mycobacterium tuberculosis strain, which is sensitive to streptomycin, isoniazid, rifampicin, Ethambutol, pyrazinamide and against four strains of mycobacterium tuberculosis [50]. Antibacterial activity was again evaluated by many other methods using the aqueous extract of Chaya leaf, but antimicrobial assay turned negative every time [51]. Aqueous extract of the leaf didn't show any kind of inhibitory mechanism against *S. typhi* and *Staphylococcus aureus* [7]. In later studies, it was found that the hot ethanolic extract of Chaya leaves can contribute to the antimicrobial activity possibly due to the cytotoxic effect associated with the plant alkaloids content. More work is needed to identify the actual mechanism and the compound responsible for this

biological activity. This can be step forward in the drug development of alternative natural antimicrobial agents.

The possible antifungal property of Chaya has also been analyzed, and it shown to have inhibit the mycelial growth of test fungi (*Aspergillus niger* and *Aspergillus tamari*). Concentration of the extract directly depended on the inhibition zone [52]. A proper study on the antimicrobial potentials of Chaya plant will be a breakthrough in the development of antibiotics with wide range of activity against many allergies and diseases. Thus, the identification of active component responsible for different antimicrobial property may result in innovation of new antimicrobial drugs against pathogenic infection.

Adeniran et al. [11] conducted a study on antimicrobial property of crude ethanol extract of all plant parts such as root, leaves and stem against different bacterial strains (*K. oxytoca*, *Proteus spp.*, *B. subtilis*, *E. coli*, *P. aeruginosa*). All extracts exhibited appreciable activity against *B. subtilis* and *E. coli*. But stem provided antibacterial as well as antibiotics inhibitory activities against almost every bacterial strain used. Acaricidal effect is having a lethal effect on mites. Acaricidal activity of ethanolic extract of *C. aconitifolius* was studied by Numa et al. [53]. 10 to 2000 µg of Chaya extract was supplied to the experimental in-vivo model. The possible lethal effect was possibly because of Hamaudol and Xanthones present in ethanolic extract of *C. aconitifolius*. The best recommended dosage was 2000 µg/ml.

**Hematopoietic Property:** Chaya leaves can contribute to the production of blood cells and have hematopoietic properties. This can increase the lifespan of erythrocytes, by reducing the osmotic fragility in protein energy malnutrition [54]. Diseases that can affect blood, especially such as malaria and sickle cell anemia are a burden on health in this developing world. Though

malaria and sickle cell anemia are entirely different diseases, they also have some common clinical presentations. In both anemia and impaired immune system, there will be excessive generation of free radicals. Vitamin C, vitamin A and zinc have the capacity to control the impaired growth and immune function which can contribute to cure malaria also. There are studies that show vitamin A can provide host resistant to malaria [55]. Chaya leaves are also enriched with high amounts of vitamins and metal ions also [56], which can be used to treat malaria also. There are many on the treatment of anemia using the micronutrients. Fe, Zn and Cu are abundant in Chaya leaves, and this can be utilized in management of sickle cell anemia. Sickling, generation of ROS and decrease in rate of physical development are the major metabolic changes associated with the sickle cell anemia. This metabolic imbalance is associated with stress on erythrocyte membrane resulting to hemolysis and to large extent loss of hemoglobin and essential micronutrients. Minerals (Fe, Zn, Cu and folate) present in tree spinach can be used to ameliorate such metabolic changes [57]. Iron and copper also have a biological importance. Iron helps in the transport of oxygen as well as metalloenzymes involved in oxidative phosphorylation and copper is essential in proper functioning of these metalloenzymes such as ceruloplasmin involved in iron metabolism. The anti-calcium action of zinc can also contribute to anti-sickling effect [58].

**Effect on Erectile Dysfunction:** The inhibition of Phosphodiesterase-5 (PDE-5) is key component for penile erectile process. PDE-5 inhibitors such as sildenafil, tadalafil and verdanafil can stimulate and increase the levels of cyclic guanosine monophosphate (cGMP), as well as enhance no bioavailability for the easing of penile tissue for the penile erection. This PDE-5 can catalyze the breakdown of cGMP and also reduce

the levels of nitric oxide in the endothelial cells and thereby decreasing the signaling [43].

The impairment in penile erection can also be caused by the increased arginase activity, which is associated with diabetic penile corpus cavernosum. Erectile dysfunction can sometimes be found in patients with decreased arginase activity also. This can occur due to the vaso-relaxation of corpus cavernosum smooth muscle. The inhibition of arginase activity can be beneficial method in the treatment of patients with erectile dysfunction. The ethyl acetate extract of Chaya leaves inhibited in arginase activity due to the presence of phenolic compounds present in the fraction and can be considered as a useful treatment to the patients with erectile dysfunction [59-60].

The main reason for the erectile dysfunction is associated with the increased blood pressure or hypertension. This erectile dysfunction is combined with angiotensin II. Increased blood pressure can be managed by decreasing the secretion of angiotensin II by the angiotensin converting enzyme (ACE) inhibitor. This hypertensive induced erectile dysfunction can also be controlled by managing blood pressure through the use of phytochemicals present in Chaya leaves [60].

**Antifertility Activity:** The effect of Chaya leaf extract on the reproductive hormone of female rats were studied and the administration of extract result in significant increase in serum prolactin concentration and reduction in the serum concentration of estradiol, progesterone, follicle stimulating and luteinizing hormone. Not only that, but it also created an adverse effect on maturation and formation of follicle. Therefore, leaf extract may impair fertility and conception in female rats and can be a potential replacement for the chemical contraceptive agents [61-62].

**Anticancer Properties:** Cancer is the serious concern of developing and developed countries due to hectic lifestyle leading to oxidative stress [63]. The anticancer activity of Chaya was studied using the ethanolic extract at various concentrations against the colon carcinoma cell lines using MTT assay method. The extract showed moderate cytotoxicity over cancerous and normal cell lines. The ethanolic extract was dose dependent and non-selective irrespective of cell origin [22].

**Chaya as Animal Feed:** *Cnidoscolus aconitifolius* can be used for animal fed formulations also. The effect of garlic, ginger and Chaya at graded levels on the growth performance, hematological indices and serum biochemistry of pullet chicks were studied [64]. The mixture of chaya leaf along with the ginger and garlic was termed as 'phytomix'. The pullet chicks were supplemented with phytomix at different level. Final weight along with daily weight gain was improved by phytomix supplementation compared to normal soybean based dieted chicks. phytomix was enriched with highest quantity of alkaloids. These compounds played a significant effect on increasing RBC and WBC of the pullet chicks also. The dietary phytomix supplementation up to 10g/kg will generate a positive effect on growth performance and health status of pullet chicks. Supplementation had positive effect on livability of pullet chicks [65]. The palatability and digestion due to stimulation of enzyme secretion, which can increase nutrient role for development of digestive system, muscle tissue accretion, and improve the growth performance. Ginger and garlic can aid the chicks in providing protective mechanism against oxidative damage and decreasing the susceptibility towards infections [66].

**Traditional Usage:** The use of Chaya leaf as a part of food in Meso America goes back without doubt to pre-Columbian period [67]. The major parts used for consumption is the leaves, sometimes apical shoots also. The mature leaves were used as wraps. These are consumed mainly after boiling them in water with salt. Cooking time depends approximately 10 to 20 minutes. Broths were also consumed along with greens.

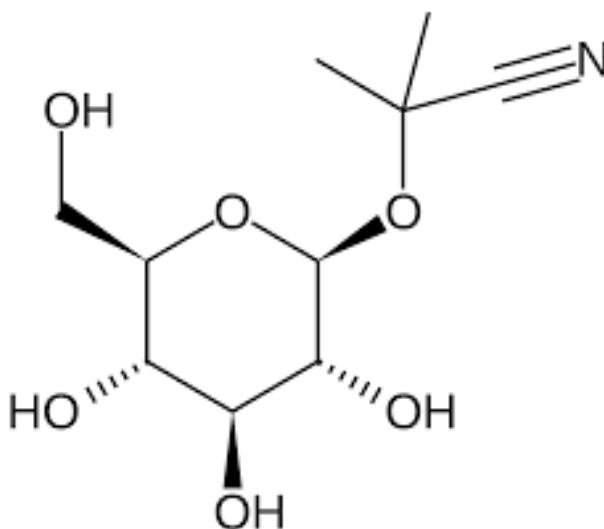
There are many varieties of recipes available with Chaya, but the main one being used along with other vegetables or meat in soup and stews [68]. This is mainly used as a side dish. Chaya tea can be prepared by adding the dried leaves to boiling water along with ginger, honey, and some lemon juice. This is then refrigerated and served. There are several other preparations also by which this healthy diet can be part of our eating habits.

**Toxological Aspects of Chaya Plant:** Genus *Cnidoscolus* is well known for their toxological characteristics. This can be toxic to animal as well. High doses in animals can lead to death also. A well grown plant is characterized with the presence of trichomes that can cause an irritating allergic reaction to skin. Raw tree spinach holds high levels of cyanogenic glycosides. These cyanogenic glycosides on hydrolysis liberate hydrogen cyanide (HCN) and cyanide molecules, a potent toxin and anti-nutritional factor. This also suggests that it is a cyanogenic species. The total cyanogenic content in the leaf extract of tree spinach was 2.51 mg HCN equivalents/ 100 g fresh weight [69]. The total cyanogen content in fresh leaves of edible tree spinach is relatively lower than the recommended safe level of consumption of food containing cyanogen which is 10 mg HCN equivalent/g fresh weight.



Researchers found that the toxicity is because of the presence of hydrocyanic acid and can be decreased by drying processes. The leaves and root bark extracts have low toxicity than other plant parts. The total cyanogen content in fresh leaf tissue is lower when compared to frozen tree spinach [70]. The increased cyanogenic content in freeze product may be due to the freeze induced damage. Freezing can impact on the changes in lipid membrane, intercellular concentration of carbohydrate and even the protein synthesis in plant cell [69, 71]. High temperature usually lowers the cyanogenic content of the leaves of tree spinach [70].

The predominant cyanogenic glycoside that found in *Cnidioscolus* is Linamarin (Figure 5). Various methods can be employed for linamarin determination, but as per the studies of Kuti and Konuru [69], quantification of linamarin by HPTLC method requires fewer amounts of chemicals and recovery degree is great. Linamarin consist of a  $\beta$ -linkage that can only broke under high pressure or high temperature by the use of mineral acids and enzymes which can cleave the  $\beta$  linkage [69, 73]. Linamarin can be hydrolyzed by removing the glucose portion by  $\beta$ -glycosidase enzyme [73].



**Figure 5.** Linamarin- a predominant cyanogenic glycoside in *Cnidioscolus aconitifolius*

Akachukwu et al. [74] carried out toxicological study from the aqueous extracts of leaves of *C. aconitifolius* in Wistar rats. These rats were treated with different proportions of therapeutic leaf extracts with their diets for 28 days. After this period also there were no hematological or biochemical changes were observed. The animals treated with the extracts were not affected the toxic effect according to this study suggesting that

this plant is not that toxic and can be safely consumed after drying or heat treatment. HCN is a systemic poison, and its toxicity is due to the inhibition of cytochrome oxidase, which prevents cellular utilization of oxygen, inhibition of terminal step of electron transport in cells of brain results in loss of consciousness, respiratory arrest, and ultimately death.

**Table 1.** Biological activities of different constituents of *Cnidoscolus aconitifolius*

Name of Constituents		Type	Amount (%)	Biological Activities	Reference
Fatty acids- 1.145%					
n-octadeconoic acid		Steric acid	8.36	Flavorant	[75]
9-octadeconoic acid		Oleic acid	43.13	Anti-inflammatory, anti-alopecic, hemolytic, antitumor, antibacterial, antifungal , lubricant	[47, 75-76]
n-Hexadecanoic acid		Palmitic acid	12.82	Antioxidant, hypercholestrolemic, lubricant, nematocide, flavor, anti-androgenic	[47, 75, 77]
Hexadecanoicacid methyl ester	Palmitic acid	1.51	Antioxidant, Hypercholestroemic, lubricant		[47, 75, 77]
9,12-octadeconoic acid		Linoleic acid	43.13	Hypercholestroemic, hepatoprotective, anti-coronary, anti- arthritic	[75]
Palmitic (C:16)		Fatty acid	2.1	Profound effects on human disorders and diseases, prevention against cardiovascular diseases and cancer	[47, 75, 77]
Stearic acid (C:18)			1.2		
Oleic (C 18;1)			1.8		
Linoleic acid(C 18:2)			3.3		
VITAMIN DERIVATIVES					
Ascorbic acid 2,6-dihexadecanoate		Vitamin derivative	12.53 mg/100g	Antioxidant, anti-inflammatory, Antibacterial	[52]
Ascorbic acid		Vitamin derivative	892.025 mg/100g	Antioxidant, absorption of iron, Formation of collagen, wound healing properties.	[2]
thiamin		Vitamin B1	1.36 mg/100g	Anti-diabetic, anti- inflammatory, essential for the flow of electrolytes	[2, 13]
Niacin		Vitamin B3	-	Lowers cholesterol, ease arthritis, boost brain function	[13]
Ascorbic acid -2,6-dihexadecanoate		Vitamin C dipalmitate derivative	12.53 mg/100g	Antioxidant, anti-inflammatory	[15]
PHENOLIC ACID-33.0 mg GAE/ 100g					
Chlorogenic acid		Phenolic acid	3.8 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[11, 20, 25]
Protocatechuic acid		Phenolic acid	12.6 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[11, 20]
p-Coumaric acid		Phenolic acid	33.0 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[11, 20]
Vanillic acid		Phenolic acid	9.6 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
Caftaric acid		Phenolic acid	14.0 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
4-Hidroxi benzoic acid		Phenolic acid	17.7 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[11, 20]

Name of Constituents	Type	Amount (%)	Biological Activities	Reference
<b>Coutaric acid</b>	Phenolic acid	20.9 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
<b>Syringic acid</b>	Phenolic acid	25.5 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
<b>Synapic acid</b>	Phenolic acid	32.6 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
<b>Caffeic acid</b>	Phenolic acid	23.5 mg GAE/ 100g	Antioxidant property, antimutagenic activity, scavenging activity on free radicles, prevention in cancer, cardiovascular diseases	[20, 25]
<b>FLAVONOIDS -2.955%</b>				
<b>Procyanidin B1</b>	Flavonoid	13.4%	Role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders, Antioxidant.	[25, 78]
<b>Gallocatechin gallate</b>	Flavonoid	38.4%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78]
<b>Epigallocatechin gallate</b>	Flavonoid	38.4%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78]
<b>Epicatechin-3-O-gallate</b>	Flavonoid	41.8%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[25, 78]
<b>Catechin</b>	Flavonoid	17.5%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[25, 78]
<b>Procyanidin B2</b>	Flavonoid	21.2%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[25, 78]
<b>Rutin</b>	Flavonoid	27.5%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[25, 78]
<b>Quercetin-3-O-gallate</b>	Flavonoid	41.6%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[25, 78]

Name of Constituents	Type	Amount (%)	Biological Activities	Reference
<b>Quercetin-3-O-galactoside</b>	Flavonoid	49.6%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78-79]
<b>Quercetin-3-O-glycoside</b>	Flavonoid	50.2%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78-79]
<b>Quercetin-3-O-rhamnoside</b>	Flavonoid	53.9%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78-80]
<b>Trans- reversatrol</b>	Flavonoid	59.6%	Role in modulation of several enzymatic pathways, disease prevention, Antioxidant, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78-80]
<b>Kaempferol</b>	Flavonoid	70.3%	Antioxidant, role in modulation of several enzymatic pathways, disease prevention, reduced chronic diseases including cardiovascular diseases, cancer, and neurodegenerative disorders	[78-80]
<b>SAPONINS -6.305 mg/100g</b>				
<b>3-O-β-D-glucopyranosyl-(1--3)-α-L-arabinopyranosyl-3,23,30-trihydroxyolean-12-en-28-oic acid</b>	Saponins	20.9 mg/100g	Protective effect on CCl <sub>4</sub> induced hepatotoxicity and hematotoxicity, Antihyperglycaemic effect.	[22, 25]
<b>3-O-β-D-xylopyranosyl-(1-3)-β-D-glucuronopyranosyl hederagenin 28-O-β-D-glucopyranosyl ester</b>	Saponins	21.6 mg/100g	Protective effect on CCl <sub>4</sub> induced hepatotoxicity and hematotoxicity, Antihyperglycaemic effect	[22, 25]
<b>3-O-β-D-glucopyranosyl-(1!3)-β-D-galactopyranosyl phytolaccagenic acid 28-O-β-D-glucopyranosyl ester</b>	Saponins	22.5 mg/100g	Protective effect on CCl <sub>4</sub> induced hepatotoxicity and hematotoxicity, Antihyperglycaemic effect	[80]
<b>3-O-hexose-pentose-pentose phytolaccagenic acid 28-O-hexose</b>	Saponins	25.8 mg/100g	Protective effect on CCl <sub>4</sub> induced hepatotoxicity and hematotoxicity, Antihyperglycaemic effect	[25]
<b>3-O-b-D-glucopyranosyl-(1!3)-a-larabinopyranosyl phytolaccagenic acid 28-O-b-D-glucopyranosyl ester</b>	Saponins	24.7 mg/100g	Protective effect on CCl <sub>4</sub> induced hepatotoxicity and hematotoxicity, Antihyperglycaemic effect	[25]
<b>ALKALOIDS-1 .96 mg/100g</b>				
<b>Choline</b>	Alkaloids	6.7 mg/100g	Prevention in various degenerative diseases, free radical scavenging property, pharmaceutical importance.	[25]
<b>Catharanthine</b>	Alkaloids	42.3 mg/100g	Prevention in degenerative diseases, free radical scavenging property, pharmaceutical importance.	[22]

Name of Constituents	Type	Amount (%)	Biological Activities	Reference
Trigonelline	Alkaloids	6.7 mg/100g	Prevention in various degenerative diseases, free radical scavenging property, pharmaceutical importance.	[80]
Vinleurosine	Alkaloids	50.6 mg/100g	Free radical scavenging property, pharmaceutical importance	[81]
Nicotinic acid	Alkaloids	13.4 mg/100g	Prevention in various degenerative diseases, free radical scavenging property, pharmaceutical importance.	[25, 81]
Palmitine	Alkaloids	15.6 mg/100g	Free radical scavenging property, Prevention in various degenerative diseases	[25, 81]
Sitsirikine	Alkaloids	18.5 mg/100g	Free radical scavenging property, pharmaceutical importance	[22, 25, 81]
Vindoline	Alkaloids	37.7 mg/100g	Free radical scavenging property, Prevention in various degenerative diseases	[22, 25, 81]
Vinleurosine	Alkaloids	50.6 mg/100g	Free radical scavenging property, Prevention in various degenerative diseases	[22, 25, 81]
Dihydrositsirikine	Alkaloids	19.9 mg/100g	Pharmaceutical importance	[22, 25, 81]
<b>CYCLIC AMINE</b>				
$\gamma$ -amino butyric lactam 6,10,14-trimethyl-2-Pentadecanone	Pyrrolidone ketone	0.79	Anticonvulsant, solvent and excipient in veterinary pharmaceutical products. Arthropod repellent	[75]
<b>ALCOHOL</b>				
1,2,3-Propanetriol	Glycerol	0.64	For pharmaceutical preparations, sweetener	[75]
<b>ACID ESTERS</b>				
Hexadecanoic methyl ester	Palmitic acid ester	0.82	Antioxidant, hypercholesterolemic, flavor, hemolytic, alpha 5 reductase inhibitor, nematocide, lubricant	[82]
1,2,3-propanetriyl ester octadecanoic acid	Trielaidin	3.86	For proxisomal metabolism	
<b>AMINO ACIDS -7.68%</b>				
Alanine	Amino acids	3.5%	Potential against protein deficiency disease like kwashiorkor, energy production	[8]
Arginine		2.3%		
Glutamic acid		1.9%		
Glutamine		2.7%		
Histidine		2.3%		
Isoleucine		1.4%		
Lysine		2.0%		
Threonine		3.2%		
Valine		4.6%		

**Table 2.** Functional properties of compounds extracted/compounds isolated from *Cnidoscolus aconitifolius*

Functional Property	Compound Responsible	Experimental model/extract/fraction	Experimental Method	Amount given	Amount Recommended	Mechanism	Reference
<b>Antihyperglycaemic effect</b>	Flavonoid Saponins	CHCl <sub>3</sub> fraction	In vivo	5,10,50, 100,150 and 200 mg/kg	100mg/kg	Possibly by stimulating insulin from pancreatic $\beta$ cell	[37]
<b>Anti-anemic effect</b>	Iron Vitamin	Dietary supplementation	In vivo	10 and 20 %	10%	Higher reticulosis leads to increasing mean corpuscular volume results in reduced osmotic fragility of erythrocytes and helps in protein energy metabolism	[47, 54]
<b>Hepatoprotective effect</b>		EtOH extraction	In vivo	100,500 and 1000 mg/kg	1000 mg/kg	Inhibition of serum ALP and Bilirubin levels there by improving secretory mechanism of hepatic cells	[47-48]
<b>Antibacterial activity</b>	Alkaloids Phlobatannins Flavonoid	EtOH extraction	In vitro	20 mg/ml		Showed some sensitivity towards <i>S.typhi</i>	[7, 47]
<b>Antimicrobial Activity</b>	Alkaloids Phlobatannins Flavonoid	EtOH extraction	In vitro	20 mg/ml		No sensitivity against bacterial strains	[47, 52]
<b>Acaricidal activity</b>	Hamaudol Xanthoness	EtOH extraction	In vitro	10 to 2000 $\mu$ g/ml	2000 $\mu$ g/ml	Lethal effect on mites.	[47, 53]
<b>Anti-nociceptive and Anti-inflammatory effect</b>	Terpenoids Flavonoid	EtOH extraction	In vivo	100 and 200 mg/kg	200 mg/kg	By improving secretory mechanism	[37, 47]
<b>Protective effect on CCl<sub>4</sub> induced hepatotoxicity and hematotoxicity</b>	Phenols Saponins Cardiac Glycosides	H <sub>2</sub> O Extract	In vivo	100,250 ,500 and 750 mg/kg		Prevents oxidative damage by metabolism of CCl <sub>4</sub> to CCl <sub>3</sub> in hepatocytes which ultimately results in cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process	[47, 83]
<b>Antioxidant activity</b>	Kaemferol 3-O glycosides Quercetin 3-O glycosides	H <sub>2</sub> O Extract	In vitro	Not Defined	Not Defined	Combinations with other flavonoids are producing synergistic effects	[47]
<b>Protective effect on hepatic damage induced by chronic ethanol administration model</b>	Flavonoids	H <sub>2</sub> O Extract	In vivo	100 and 200 mg/kg	200 mg/kg	ROS in impairment of the glomerular filtration rate is blocked and by elevating superoxide di maltase (SOD) and Catalase activity	[46-47]
<b>Antioxidant activity</b>	Kaemferol 3-O glycosides Quercetin 3-O Glycosides	Organic extract	In vitro	ND	ND	Combinations with other flavonoids are producing synergistic effects	[47]



**Table 3.** Biological activities of different extracts from *Cnidoscopus aconitifolius*

Extract used	Experimental model	Biological activity or Findings	Reference
<b>Aqueous extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis of GC-MS	Remarkable inhibition of bacterial growth, when compared to chloramphenicol. The major inhibition percentage for pathogen was 61.3% of chloramphenicol. Alkaloids and flavonoids were also found using GC-MS contributing to functional properties of extract.	[10, 84]
<b>Aqueous extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and analysis of GC-MS	Presence of cardiac glycosides, flavonoids, phenols, anthraquinones and triterpenoids was found in phytochemical analysis. 42 compounds were identified using GC-MS and the major ones includes borneol, caryophyllene oxide, 1H- Cycloprop and azulene, 4 ( 1-5-dimethyl-4-enyl)cyclohex-2-enone%), farnesol, longipinane and benzene.	[84-85]
<b>Aqueous extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and Wistar albino rats	Tannins, alkaloids, saponins, flavonoids, cyanogenic glycosides and phytates were identified in phytochemical analysis. Along with the presence of vitamin A, vitamin B1, vitamin B3, vitamin B6, vitamin B12, vitamin C and vitamin E. Extract exhibited reversal effect on selected oxidative and hematological markers.	[84, 86]
<b>Methanolic extract of</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and Wistar albino rats	The active fraction which showed the highest hyperglycemic effect was identified in albino rats was further analysed by GC-MS and identified dodecanoic acid ester-1,2,3-propanetrile, cyclotetradecane, arachidic acid, octadecanoic acid, 4-propionate, benzene acetic acid, phenyl malonic acid and 3-oxo-4-phenyl butyronitrile.	[84]
<b>Ethanol extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and <i>T. urticae</i> adult female	The extract was able to increase the mortality and decrease the fertility in a dose dependent manner. Major metabolites identified were sesquiterpene type flavonoid compounds, xanthone type chromone as minor compounds with potential effect on acaricidas.	[48, 84]
<b>Aqueous extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and male albino rats	Reduction of alkaline phosphatase, alanine aminotransferase, total proteins, total bilirubin, triglycerides, low-density proteins, urea nitrogen blood, total cholesterol and creatinine had a significant decrease and an increase in HDL and complete restoration of plasma electrolytes.	[74, 84]
<b>Methanolic extract</b> <i>Cnidoscopus aconitifolius</i>	Preliminary phytochemical analysis and in-silico	The extract supplementation significantly reduced the activity of liver marker enzymes that elevated by the use of ethanol, stabilized the lipid profile and restored the status of treated antioxidants. The activity of extract was compared with kolaviron in rats that treated with ethanol and it imparted protective effect which was proved with histopathological examination of the liver slides.	[84]

Extract used	Experimental model	Biological activity or Findings	Reference
<b>Extract of <i>Cnidoscolus aconitifolius</i></b>	Wistar albino rats	The extract use deceased the biochemical indexes for normal parameters induced by chronic administration of ethanol, the serum and renal malondialdehyde levels increased besides levels of catalase, superoxide dismutase and antioxidant state, there was a reduction of glutathione. These findings contribute to renal protective effect.	[48, 84]
<b>Methanolic extract of <i>Cnidoscolus aconitifolius</i></b>	Wistar albino rats	Significant reduction in glycemia was shown by the group supplied with 100 mg/kg, along with gradual increase in treatment group compared to diabetic control, which had weight loss. Plasma cholesterol levels also decreased in those treated with 1000 mg/kg of extract.	[46, 84]
<b>Methanolic extract of <i>Cnidoscolus aconitifolius</i></b>	Wistar albino rats	Hematological parameters were restored by the pretreatment, with an associated decrease in activities of hepatic injury enzyme maker( serum transaminase (ALT and AST) and phosphatase (ALP) by haloalkane	[46, 84]
<b>Ethanolic extract of <i>Cnidoscolus aconitifolius</i></b>	Preliminary phytochemical analysis and anti-microbial activity and antioxidant activity	Presence of secondary metabolites such as tannins, steroids, alkaloids, cardiac glycosides, terpenoids and saponins. All parts of plant exhibited Anti-microbial activity against <i>Escherichia coli</i> and <i>Bacillus subtilis</i> . Stem exhibited broadest spectrum of activity against different strains of bacteria used.	[48, 84]
<b>Extract of <i>Cnidoscolus aconitifolius</i></b>	Male wistar albino rats newly weaned	The recovery diet result in an increase in blood count of platelets, white cells, corpuscular mean, platelets, haemoglobin and decrease in osmotic fragility.	[83-84]
<b>Ethanolic activity and water extract</b>	Preliminary phytochemical analysis and anti-microbial activity	Phytochemical screening of both extracts was positive to phenols, saponins and cardiac glycosides. Phlobatannin was identified in aqueous extract and alkaloids in ethanolic extract. <i>Salmonella typhi</i> was sensitive to ethanolic extract. The fresh aqueous extract and chloramphenicol showed 2.0±0.5, 3.0±0.1 and 11.5±0.1 mm bioactivity against <i>S. aureu</i> .	[7, 48, 84]
<b>Methanolic extract of <i>Cnidoscolus aconitifolius</i></b>	Male swiss mice	In normal animals with moderate diabetes, Ca decreased the diabetes by 25.6%, for severely diabetic patients the glucose level was decreased by 43.7%. Indicates Ca has an insulogenic property that possibly stimulates latent $\beta$ cells to secrete insulin. The histopathological study showed that the islets of Langerhans were preserved in period examined and the liver and kidney had moderate pathology.	[84, 87]

## CONCLUSION

*Cnidoscolus aconitifolius* is an unexplored important genus of the Euphorbiaceae family as it has significant nutritional properties and health benefits. It is

commonly used as folk medicine. The active components of this edible horticulture crop can be utilized for many nutraceutical and pharmacological studies. The research based on tree spinach, which is

richer in nutritional values than spinach, is quite limited. If utilized properly, tree spinach can be a good nutritive and healthy food source to help manage the symptoms of many diseases. Chaya contains lots of secondary metabolites such as alkaloids, flavonoids, saponins, etc. If properly harnessed, this plant can be utilized as a healing aid beyond just the local use and create a worldwide market for its products, either in the production of nutraceutical or pharmaceutical. Overall, the results indicate that, while the evidence that support a useful role of *Cnidocolus aconitifolius* consumption in treating many diseases, further human based studies is still needed. Limited knowledge about this underutilized plant is representing an opportunity to investigate new secondary metabolites along with possible functional property that could provide.

**List of Abbreviations:** CE: catechin equivalents, GAE: gallic acid equivalent, ORAC: oxygen radical absorbance capacity, ROS: Reactive oxygen species, BHT: butylated hydroxytoluene, BHA: butylated hydroxyanisole, DPPH: 2,2-Diphenylpicryl-1-hydrazyl radicle, E-NTDase: ecto-5'-nucleotidase, AMP: Adenosine Mono Phosphate, L-

DOPA: L-3,4-dihydroxyphenylalanine, ATPase: adenosine triphosphatase, SOD: superoxide dismutase, Fe: Iron, Zn: Zinc, Cu: Copper, cGMP: guanosine monophosphate, PDE-5: Phosphodiesterase-5, ACE: angiotensin converting enzyme, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, RBC: Red blood cell, WBC: white blood cell, HCN: hydrogen cyanide, HPTLC: high pressure thin layer chromatography, RDA: Recommended Dietary Allowance, g: gram, mg: milligram, m: meter, cm: centimeter.

**Author's contribution:** AP and NC conceived the idea of review. AOS, KN and MKG participated in drafting the manuscript. AP, NC and MKG reviewed the manuscript, and all authors were engaged in drafting and editing the review. All authors have read and approved the final manuscript.

**Conflict of Interest:** All authors confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

**Acknowledgment and Funding:** The authors declare no acknowledgments / funding.

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