Evaluation of Antioxidant Activity of *Cnidoscolus aconitifolius* (Chaya Plant) Extract.

Om Santosh Varude[1*], Rohan Haridas Babar[2], Nikita Hanmant Kolse[3], Sacchidanand Dhanaji Lengare[4], Vinayak Hanumant Dhage[5], Nandini Vasudev Kalyankar[6], Dr. Pravin P. Honamne[7].

[1,2,3,4,5,6] Students Adarsh College of Pharmacy, Vita.

[7] Assistant Professor Adarsh College of Pharmacy, Vita.

Corresponding author Email: omvarude205@gmail.com

ABSTRACT: *Cnidoscolus aconitifolius* is commonly known as Tree Spinach and it belongs to the family *Euphorbiaceae. Chaya* possesses excellent medicinal properties for the treatment of different ailments. The different parts of the *Cnidoscolus aconitifolius* plant including leaves, seeds, latex, and fruit exhibited to have medicinal value. This research focuses on the antioxidant and anti-bacterial activity of leaves of *Cnidoscolus aconitifolius.* Here, we examine the study that has been conducted on this remarkable plant. We suggest chaya as a promising crop due to its simplicity of growing, potential yield, and most importantly its significant nutritional value. The leaves of the *Chaya* plant were collected from nearby regions dried, powdered, and extracted with ethanol. We studied the identification of antioxidants using (DPPH) 2, 2-Diphenyl-1-picrylhydrazylradical scavenging activity in *Cnidoscolus aconitifolius*, as *Cnidoscolus aconitifolius* is an important herbal plant. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method is a popular, fast, simple, and inexpensive method for measuring the antioxidant activity of compounds. It uses free radicals to evaluate whether substances have the capacity to act as hydrogen suppliers or free-radical scavengers (FRS).

Keywords: *Chaya, Cnidoscolus aconitifolius,* Antioxidant, DPPH.

Introduction: Chaya (Cnidoscolus chayamansa) is a plant from the family Euphorbiaceae and grows in Central America and in southern Mexico.[1] The plant shows great affinity to milder climates and can be found growing in northern latitudes, under dryer environments, and in different soils.[2] The leafy vegetable was consumed by Mayan Indians and is traditionally incorporated in salads and regional dishes.[3] This plant has been associated with several health benefits such as maintaining healthy blood sugar levels, acting as an anti-inflammatory, antiemetic, anti-microbial, and antioxidant effect, etc.[4] Chaya leaf powder or leaf extract has gained huge importance and thus increased the commercial value of the plant. The Chaya plant is commonly known as tree spinach, a member of the Euphorbiacae family. Chaya and its species are a group of arborescent shrubs, of the section calyptosolen of the genusCnidoscolus. 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.[5] 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).[6] Although chaya is primarily collected as a food plant, it has been used therapeutically for a number of ailments such as diabetes, [7] arteriosclerosis, gall- stones, and high cholesterol. Chaya is believed to be diuretic and laxative, clean the circulatory system, induce breastfeeding, strengthen vision, improve nails, and boost digestion.8 Due to its nutritional content, which includes dietary fiber, protein, minerals, vitamins A and C, flavonoids, and polyphenols, chaya is also an important element of indigenous populations' regular diets. Kaempferol and Quercetin are the most abundant phenolic compounds identified in Chaya.[9]

Plant profile: Cnidoscolus varieties are wildly distributed from southern America along the gulf coast, through Colombia. Chaya has been spread to Maya families throughout Mexican country and the southwest United States of America.[10] It has now spread to the Caribbean, Mexico, and the US. Throughout the nearby range, Chaya was typically only harvested for ornamental or as living fences earlier. Though recently food and medicinal usage of plant lant has increased due to its inherent nutritional and health-promoting attributes. Chaya is cold-sensitive and typically grows much faster and better at 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, the Chaya plant does not need much care and it grows well on less 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. In the early stages, the growth is low, but soon after leaves are 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. Chaya plant can growup to a 16-19 feet shrub. But it’s recommended to maintain in 2m height. Although it’s kept very small, it can actively produce a great amount of leaf material. The harvest period ranges from two tothree months. Leaves are giant and succulent, up to 32 cm long and 30 cm wide, on petioles up to 28 cm long.[11] Leaves exhibit stinging hairs, in the petiole and bottom margin of the lamina. Mature leaves are mosthree-lobedobed with rough margins and no stinging hairs, young leaves lack the distinct lobes the mature leaves have. Mature plant flowers produce pollen but are mostly non-viable. Mature fruit is rare, without viable seeds with thick succulent stems.

Figure 1. Cnidoscolus aconitifolius leaves

Some of the widely used varieties can be categorized in two groups

1) Deeply-Lobed Chaya**: -** Tends to bloom quickly and when still short (3-4′). Small white blooms attract an abundance of zebra-longwing butterflies**.** Leaves are somewhat coarser in texture. Leaves tend to be deeper green. Possibly because of a larger, tree-sized plant over time. Seems less tolerant of frost than the maple-leaf varieties. Easy to grow – few pest issues. A giant tree form appears possible.

2) Maple-Leafed Chaya: - More tender leaves. Less likely to flower until high, occasionally won't flower at all. Higher yield per leaf. Not as desirable as an in sectary plant. Easy to grow – few pest issues. Maximum size unknown.

Scientific classification:
Common name- Chaya, Tree Spinach, Spinach Tree

Scientific name- *Cnidoscolus aconitifolius*

Family- *Euphorbiaceae*

Kingdom – *Plantae*

Order- *Malpighiales*

Genus- *Cnidoscolus*

Species- *C. aconitifolius*

Traditional uses: Chayamansa is an excellent remedy for a variety of health problems, hence it is strongly advised that we include it in our diet in order to obtain all the nutrients we need in a balanced ratio as well as a treatment for a variety of health-related concerns. The major benefits are Enhanced blood circulation, increase digestion, increase eyesight, Prevents varicose vein, controls cholesterol, helps in losing weight, reduce cough and helping in the healthy growth of bone and teeth, helps in the functioning of lungs, prevents anemia, Enhance the functioning of the brain and memory power, Reduces bone disease, Stimulate the functioning pancreas and controls the diabetes, helps in treatment of kidney stone, Prevents acne.

Pharmacological profile

Activity from leaf extract.

- Anti-inflammatory
- Anti-alopecic
- Hemolytic
- Antitumor
- Antibacterial

- Antifungal
- Lubricant
- Antioxidant
- Hyper cholestrolemic
- Nematicide
- Flavor
- Anti-androgenic
- Anti-diabetic
- Antimutagenic activity

Chemical constituents:

Table No. 1- Chemical constituents

FATTY ACIDS- 1.145%	Palmitic acid, Stearic acid, Oleic acid.
VITAMIN DERIVATIVES	Ascorbic acid, thiamin, Niacin.
PHENOLIC ACID-33.0 mg GAE/ 100g	Chlorogenic acid, Protocatechuic acid, Vanillic acid, Caftaric acid
AMINO ACIDS -7.68%	Alanine,Arginine,Glutamic,Histidine
ALKALOIDS	Choline, Catharanthine, Nicotinic, Palmatine, Sitsirikine, Vindoline, Vinleurosine
SAPONINS	3-O-β-D-glucopyranosyl-(1--3)-α-L-arabinopyranosyl-3,23,30-trihydroxyolean-12-en-28-oic acid 3-O-hexose-pentose-pentose phytolaccagenic acid 28-O-hexose
FLAVONOIDS	Rutin, Kaempferol, Quercetin-3-O-glycoside, Procyanidin B1
ALCOHOL	1,2,3-Propanetriol

Extraction

Extraction techniques of Herbal plants Extraction, as this is used pharmaceutically, involves the separation of medicinally active plant or animal tissues from the inert components by using selective solvents in standard extraction procedures. It includes classes of medications known as infusions, decoction, fluid excerpts, tinctures, pilular (circumfluous) excerpts and pulverized excerpts.

Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent used in making the extract.

Methods of Extraction of Medicinal Plants

A. Conventional extraction technique

- Maceration
- Infusion
- Digestion
- Decoction
- Percolation
- Hot Continuous Extraction (Soxhlet apparatus method)
- Aqueous Alcoholic Extraction by Fermentation

B. Modern extraction technique

- Counter-current Extraction
- Ultrasound Extraction (Sonication)
- Supercritical Fluid Extraction.

Experimental work:

Extraction

Techniques for extracting herbs by utilizing selected solvents according to accepted extraction practices, extraction as it is applied in the pharmaceutical industry includes the separation of medicinally useful plant or animal tissues from the inert components. Infusions, decoctions, fluid excerpts, tinctures, pilular (circumfluous) excerpts, and pulverized excerpts are among the groups of drugs that are included.

Extracts have been defined as preparations of unprocessed medications that contain all ingredients

soluble in the solvent used to create the extract..

Methods of Extraction of Medicinal Plants

B. Conventional extraction technique

- Maceration
- Infusion
- Digestion
- Decoction
- Percolation
- Hot Continuous Extraction (Soxhlet apparatus method)
- Aqueous Alcoholic Extraction by Fermentation

C. Modern extraction technique

- Counter-current Extraction
- Ultrasound Extraction (Sonication)
- Supercritical Fluid Extraction.

Hot Continuous Extraction (Soxhlet apparatus method)

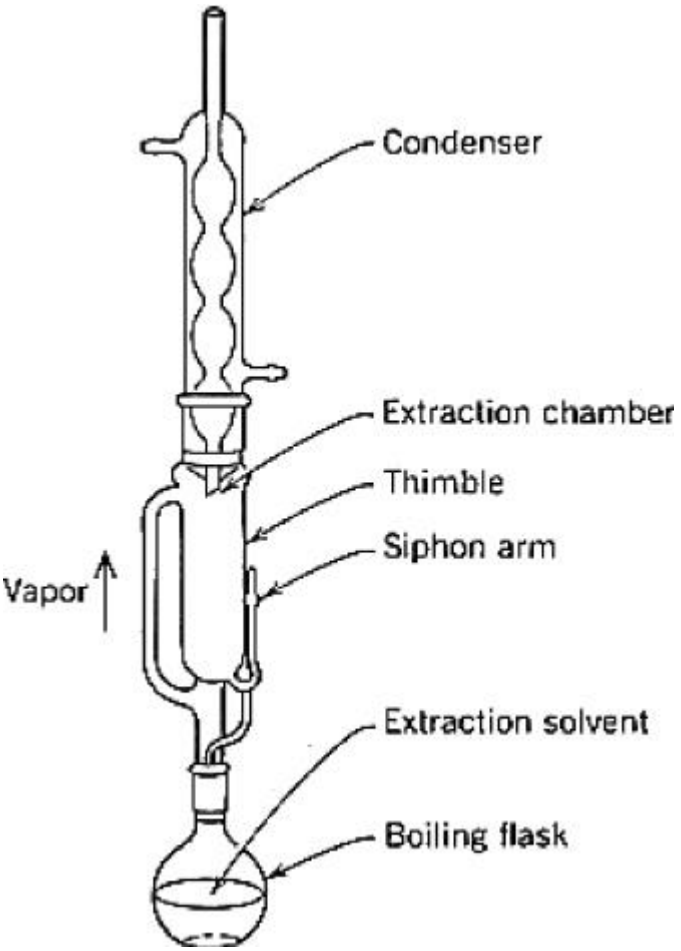

Figure 2. Soxhlet apparatus.

As illustrated in the diagram, chamber E of the Soxhlet apparatus is where the finely crushed crude medication is deposited in a porous bag or "thimble" composed of sturdy filter paper. The heating of the extracting solvent in flask A causes its vapors to condense in the condenser. The crude medication is extracted by contact as the condensed extractant drips into the thimble containing it. The liquid in chamber E siphons into fluid chamber A when the amount of liquid in chamber E reaches the top of siphon tube C. This operation is carried out continuously until an evaporated drop of solvent from the siphon tube leaves no residue. Compared to the approaches previously discussed, this method has the advantages.

Antioxidant

The antioxidant is a substance that protects cells from the damage caused by unstable free radicals

which are made in the process of oxidation during normal metabolism. It also plays an important major role in ensuring that our foodstuffs keep their taste and color and remain edible over a longer period. Their use is particularly important for avoiding the oxidation of fats and fats-containing products. Oxidation is a chemical reaction that transfers electrons or hydrogen from substances to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions, when the chain reactions occur in a cell, they can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidative reactions. Antioxidants are substances that prevent oxidation, a chemical process that can result in the production of free radicals (typically seen as autoxidation).

Various type antioxidants

A variety of antioxidants, including natural antioxidants, synthetic antioxidants, dietary antioxidants, and endogenous antioxidants, are now present in food and play a significant role in food preservation.

- Dietary antioxidants for example, ascorbates, tocopherols, and carotenoids,
- Vitamin C, vitamin E, and beta carotene, Beta carotene and other carotenoids and oxy carotenoids,lycopene and lutein are among the most widely studied dietary antioxidants.
- Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation.
- Natural antioxidant: Many fruits and vegetables contain natural antioxidants.
- Endogenous antioxidants are in addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage.
- Exogenous: Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc.

Sources of antioxidants

- Vitamin C, Vitamin E, a-carotene, Licopein, Selenium, Polyphenol, Glutathione, Peroxidase, Cystine are the main sources of antioxidants.
- Antioxidants like polyphenols, vitamin C, and vitamin E can be found in large quantities in fruit juices, beverages, and hot liquids.
- The recommendations grounded on epidemiological studies are similar, that fruits, vegetables and lower reused staple foods ensure the stylish protection against the development of conditions caused by oxidative stress, similar as cancer, coronary heart complaint, rotundity, type 2 diabetes,

hypertension and cataract.

Functions of antioxidant

The Food and Drug Administration (FDA) defines antioxidants only as dietary supplements to be taken in addition to normal food consumption in an effort to prevent these diseases. It has been proven that eating fruits and vegetables often lowers the chance of developing chronic illnesses. Studies demonstrate that an antioxidant-rich diet has a very positive health impact in the long run of life. Four possible mechanisms have been suggested John in 1989 by which antioxidants function to reduce the rate of oxidation of fats and oils. These are - hydrogen donations by antioxidants, electron donation by antioxidants, the addition of lipid to the antioxidants and the formation of a complex between lipid and antioxidants.[12]

Mode of action of antioxidants

Antioxidants can drop the oxidative damage directly via responding with free revolutionaries or laterally by inhibiting the exertion or expression of free radical-generating enzymes or enhancing the exertion or expression of intracellular antioxidant enzymes.

Based on their mode of action, antioxidants can be divided into two main groups, namely:

1. Hydrogen Atom transfer (HAT)

   These tests measure an antioxidant's ability to scavenge free radicals (peroxy radicals are generally considered to be more biologically active).

2. Single electron transfer (SET) assays

The antioxidant action is simulated with a suitable redox potential namely; the antioxidants react with a fluorescent or colored probe (oxidizing agent) instead of peroxyl radicals. SET spectrophotometric tests measure the ability of an antioxidant to reduce an oxidant that changes color when it is reduced. The quantity of color change (either an increase or reduction in the probe's absorbance at a particular wavelength) is proportional to the amount of antioxidants present in the sample.

Hydrogen Atom Transfer methods (HAT) methods-

1) Oxygen radical absorbance capacity (ORAC)
2) Lipid peroxidation inhibition capacity (LPIC)
3) Total radical trapping antioxidant parameter (TRAP)
4) Inhibited oxygen uptake (IOC)

5) Crocin bleaching nitric oxide radical inhibition activity
6) Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline)
7) Scavenging of H2O2 radicals
8) ABTS radical scavenging
9) Scavenging of super oxide radical formation by alkaline (SASA)

II) Electron Transfer methods (ET)

1) Trolox equivalent antioxidant capacity (TEAC) decolorization
2) Ferric-reducing antioxidant power (FRAP)
3) DPPH free radical scavenging
4) Copper(II) reduction capacity
5) Total phenols by Folin-Ciocalteu
6) N, N-dimethyl-p-phenylenediamine (DMPD).[13]

The most common antioxidant for plant extract is the determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Principle of DPPH activity

1, 1-Diphenyl-2-picrylhydrazyl is a stable free radical (in powder form) that is red in color and turns yellow when harvested. The DPPH test uses this sign to indicate free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, DPPH-H + (A) (DPPH) + (H-A) Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration reveals the antioxidant compounds' or extracts' scavenging capacity in terms of hydrogen-donating capacity.

Advantages of DPPH Method

Using this assay provides insight into various chemical phenomena and offers clear advantages such as low cost, ease of experimentation, reproducibility, applicability at room temperature, and opportunities for automation.

96 microwell plate method:- A microplate, also known as a microtiter plate microwell plate or multi-well is a flat plate with multiple "wells" used as small test tubes. The microplate has become a standard tool in laboratories for analytical research and clinical diagnostic testing. The microplate typically has 6,

12, 24, 48, 96, 384, or 1536 sample wells arranged in a 2:3 rectangular matrix.[14] 96-well plates are designed for High Throughput Screening (HTS), for biochemical activities like antioxidant testing, sample storage, cell culture, and DNA extraction involving a large sample size. These microwell platesare read on an ELISA plate reader.

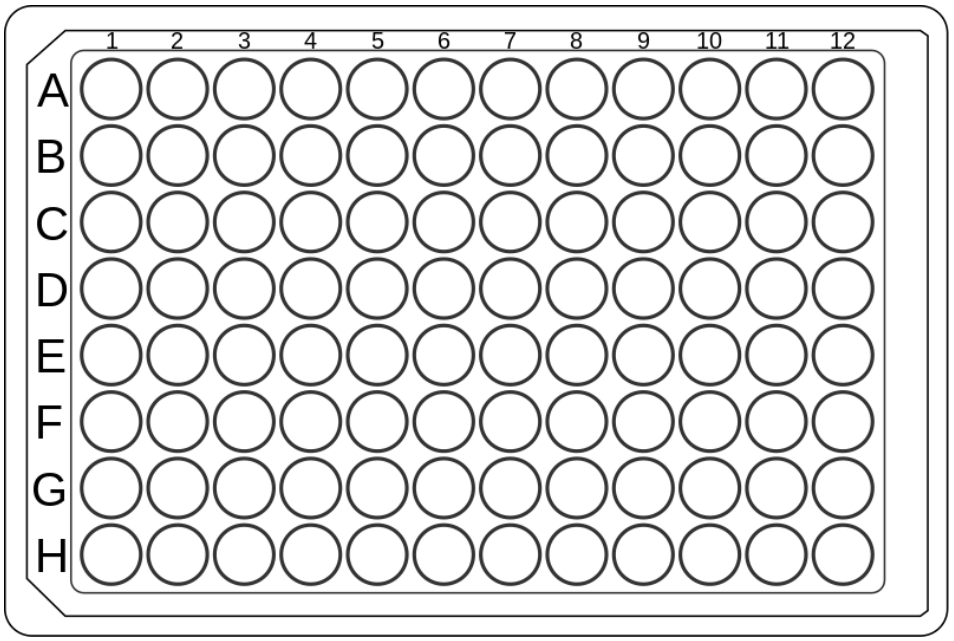

Figure 3. 96 micro well plate titer.

ELISA plate reader Enzyme immunoassay (ELISA) is a method of capturing target antigens (or antibodies) in samples using a specific antibody (or antigen) and detecting/quantifying the target using an enzymatic reaction with its substrate**.** Here, we use ELISA plate reader for determination of antioxidant purpose. The principle of working is light absorption of the extract formed after substrate addition of DPPH is measured and converted to numeric values depending on the how much antioxidant activity is takes place.[15]

EXPERIMENTAL WORK:

1) Preparation of crude extract

i) The collected leaves were shade dried under normal environmental condition. Then, the powder is ground into uniform powder using mixer.

ii) Powder of Chaya species were extracted with ethanol by using Soxhlet extraction apparatus.

Then extract were filtered and collected in well closed amber colored bottles

2) Phytochemical screening

Table no. 2- Test for Alkaloids

Test	Process	Observation	Positive/Negative
Dragendroff's test	2ml methanolic extract + 2ml Dragendroff's reagent	Reddish brown precipitated formed.	**Alkaloids present**
Hager's test	2ml methanolic extract + 1ml of Hager's solution	Yellow precipitated formed.	**Alkaloids present**

Table no. 3- Test for glycosides

Test	Process	Observation	Positive/Negative
Legal's test	2ml extract dissolve in pyridine + Sodium nitroprusside solution + 10% NaOH solution	Pink color precipitated formed.	**Glycosides present**

Table no. 4- Test for Phenolic compounds

Test	Process	Observation	Positive/Negative
Ferric chloride test	2ml extract dissolve in distilled water + 5% drops of ferric chloride solution	Blue green precipitated formed.	**Phenolic compounds present**

3) Determination of Antioxidant activity:

i. Antioxidant activity in the sample compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (George et al., 1996).

ii. 100µL of test compounds water was taken in the microtiter plate.

iii. 100µL of 0.1% methanolic DPPH was added over the sample's carbon dots at different concentrations (10, 20, 50µg/ml) and incubated for 30 minutes in dark condition.[16]

iv. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm.[17]

v. Radical scavenging activity was calculated by the following equation.

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) /(Absorbance of control)] x 100

RESULTS

1) Phytochemical screening

Table no.5 Alkaloids test

Test	**Result**
Dragendroff'stest	**Alkaloids present**
Hager's test	**Alkaloids present**

Table no.6 Test for glycosides

Test	**Result**
Legal's test	**Glycosides present**

Table no.7 Test for Phenolic compounds

Test	Result
Ferric chloride test	**Phenolic Compound present**

2) Antioxidant activity

Table no. 5- Effect of samples by using Antioxidant activity by DPPH (96 well method)

Sample code	Concentration	Absorbance	Mean	% inhibition
Control	**-**	**2.101**		
		1.912	**1.942**	
		1.813		
Standard	**1000 µg**	**0.150**		
Ascorbic acid		**0.106**	**0.125**	**93.56**
		0.120		
Sample A	**1000 µg**	**0.888**		
		0.850	**0.827**	**57.41**
		0.745		

(4) **(5)**

Figure 4, 5. Effects of different Chaya Extract - against DPPH

DISCUSSION

Antioxidant activity

Cnidoscolus aconitifolius have rich source of phytoconstituents. Phenolic compounds are essential for antioxidant and antibacterial activity. Cnidoscolus aconitifolius show very good antioxidant activitywith 57.41%. DPPH radical scavenging activity respectively where standard ascorbic acid shows 93.56% DPPH radical scavenging activity.

REFERENCE

1) Kolterman DA, Breckon GJ, Kowal RR Chemotaxonomic studies in Cnidoscolus (Euphorbiaceae). II. Flavonoids of C. aconitifolius, C. souzae, and C. spinosus. Systematic Botany **1984** 9(1): 22–32.

2) Martin FW, Rubert´e R (1978) Vegetables for the Hot, Humid Tropics. Part 3. Chaya, Cnidoscolus chayamansa. USDA, New Orleans, **1978**.

3) Martin FW, Telek L, Rubert ´e R Some tropical leaves as feasible sources of dietary protein.J Agric University of Puerto Rico **1977** 61: 32–40.

4) https://ffhdj.com/index.php/BioactiveCompounds/article/download/865/1524

5) Berkelaar, D., Chaya. Technical Note, Echo, Florida, **2006**.

6) Pillai, K.K., Chidambaranathan, N., Halith, M.M., Jayaprakash, S. and Narayanan, N. Anti-hyperglycemic effect of alcoholic extracts of Cnidoscolus chayamansa in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. International Journal of Research in Pharmacy and Chemistry, **2012**, 2(1), 164-178.

7) Kuti JO, Torres ES Potential Nutritional and Health Benefits of Tree Spinach (Cnidoscoluschayamansa). Progress in New Crops, American Society of Horticulture Science Press, **1996**.

8) D´ıaz Bolio J La Chaya Planta Maravillosa—Alimenticia y Medicinal. Cr´onicaEtnobot´anica. Gobierno de Quintana Roo, M´exico, **1974**.

9) Perez-Gonzalez MZ, Macias-Rubalcava ML, Hernandez-Ortega S, Siordia-Reyes AG, Jimenez- Arellanes MA. Additional compounds and the therapeutic potential of Cnidoscolus chayamansa (McVaugh) against hepatotoxicity induced by antitubercular drugs. *Biomed Pharmacother*. (2019) 117:109140.10.1016/j.biopha.**2019**.109140

a. PubMed] [CrossRef] [Google Scholar].

10) Ross-Ibarra, J. and Molina-Cruz, A., The ethnobotany of chaya (Cnidoscolusaconitifolius ssp. aconitifolius Breckon): a nutritious maya vegetable. Economic Botany, **2002** 56(4), 350. https://doi.org/10.1663/0013- 0001(2002)056[0350: TEOCCA] 2.0.CO; 2.

11) Awoyinka, O.A., Balogun, I.O., Ogunnowo, A.A. Phytochemical screening and in vitro bioactivity of Cnidoscolus aconitifolius (Euphorbiaceae). J. Med. Plant. **2007** Res. 1, 63-65. https://doi.org/10.4103/0974-8490.138290.

12) Res. Environ. Life Sci. review article on "Antioxidants and its functions in human body - A Review" by Anuj Yadav, 2016, Page no. 1328-1331.

13) Alex. J. Fd. Sci. & Technol. article on Methods for Determining the Antioxidant Activity: A Review by H.A. Moharram, **2014** Vol. 11, No. 1, Page no. 31-42.

14) Kavitha Vijayaraghavan, S.Mohamed Ali, studies on Phytochemical screening and antioxidant activity of chromolaena odorata and annona squamosa, international journal of innovativeresearch in science Engineering and technology Vol 2 issue 12 **2013**, 7315-7321.

15) Dr Prieto'S DPPH microplate Protocol, (**2012**), 1-3.

16) Kavitha Vijayaraghavan, S.Mohamed Ali, studies on phytochemical screening and Antioxidant activity of chromolaena odorata and annona squamosa, international journal of innovativeresearch in science Engineering and technology Vol 2 issue 12, **2013**, 7315-7321.

17) Datatraya Raut synthesis, molecular docking and biological evaluation of 2- (2- hydrazinyl) thiazoles as potential Antioxidant, Anti-inflammatory and significant anticancer agent, Recent advances in inflammation and allergy drug discovery, **2022**, 16, 96-106.