IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

Evaluation of Antioxidant and Antibacterial Activity of Cnidoscolus Aconitifolius

Phadtare Vijay Babaso[1], Mane-Patil Poonam A.[2], Sutar Mohmmedzaid Isak[3], Kalangade Ayan Irshad[3]
B Pharmacy Final Year Student Eklavya College of Pharmacy, Tasgaon, India[1,3]
Assistant Professor Eklavya College of Pharmacy, Tasgaon, India

Abstract: *The Cnidoscolus aconitifolius is commonly known as Tree spinach and it belongs to the family euphorbiaceae. Chaya possess 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 anti-oxidant and anti-bacterial activity of leaves of Cnidoscolus aconitifolius. Chaya (Cnidoscolus aconitifolius) is a domesticated leafy green vegetable of the Kerala region of India. Though relatively unknown outside of this area, evidence suggests that chaya was of significant importance to ancient peoples of the kerala and perhaps elsewhere within this region. Here we review what little research has been done on this impressive plant.*

Due to its ease of cultivation, potential productivity, and above all its substantial nutritional value, we propose chaya as a potential crop. the leaves of Chaya plant were collected from nearby region dried, powdered and extracted with ethanol. These crude extracts were tested for antibacterial activity by Well Diffusion Method the extracts found to be active were subjected to (MIC) the extracts were prepared according to the MIC and antibacterial susceptibility test was carried out using Agar well diffusion method. Extracts showed antibacterial activity against the tested strain of S, aureus. The present study showed the effectiveness of the crude plant extract against the tested bacterial strains and indicates the potential use of the extract as antimicrobial agent for the control of infectious diseases. 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) is a popular, quick, easy, and affordable approach for the measurement of antioxidant properties that includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS)...

Keywords: Cnidoscolus aconitifolius, DPPH assay, antibacterial activity, phytochemicals, oxidative stress, Staphylococcus aureus.

I. INTRODUCTION

Chaya (*Cnidoscolus chayamansa*) is a *Euphorbiaceae* shrub growing in Central America and the hot tropics of southern Mexico. [1] However, the plant shows great adaptability to milder climates and can be found growing in northern latitudes, under dryer environments and in different soils. The leafy vegetable was consumed by Mayan Indians and is traditionally incorporated in salads and regional dishes[2]. This plant has been associated with several health benefits such as maintaining healthy blood sugar levels, acting as an anti-inflammatory, anti-microbial, and antioxidant effect etc. 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 tree spinach, a member *of Euphorbiacae* family Chaya and its related species are a group of *arborescent* shrubs, of the section c*alyptrogen* 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] .

DOI: 10.48175/IJARSCT-25357

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

Tree spinach is exceptionally high in fibre (31.165%), calcium (50 mg/g), iron (10 mg/g), potassium (20 mg/g) and vitamin C (892.025 mg/100 g). Although chaya is primarily collected as a food plant, it has been used therapeutically for a number of ailments such as diabetes arteriosclerosis, gall- stones and high cholesterol. It is also believed that chaya cleans the circulatory system, stimulates lactation, improves eyesight, strengthens nails, improves digestion, and is a diuretic and laxative agent. Chaya is also an important component of the regular diet of indigenous communities because of its nutritional value; it contains dietary fiber, protein, minerals, vitamins A and C, flavonoids, and polyphenols. Kaempferol and Quercetin are the most abundant phenolic compounds identified in Chaya[3].

Fig.no.[1]

Fig no.[2]

Fig:*cnidoscolus aconitifolius* 1) plant 2)leaves

Plant profile :

Cnidoscolus varieties are of broadly, wildly 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. 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[4].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, favourable 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 is done by cutting because it roots slowly. At early stages, the growth is low, but soon leaves are being harvested, growth is rapid[7]

Scientific classification

Common name- Chaya, Tree Spinach, Spinach Tree
Scientific name- Cnidoscolus aconitifolius
Family- Euphorbiaceae
Kingdom- Plantae
Order- Malpighiales
Genus- Cnidoscolus
Species- C. aconitifolius

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

Chemical constituent

fatty acid -1.445%	palmitic acid,steric acid,oleic acid
vitamin derivatives	ascorbic acid,thiamin,niacin
phenolic acid-33.0mg gae/100g	chlorogenic acid,protocatechunic acid,vanelic acid.
amino acid-7.68%	alanine,arginine,glutamic,histadine
Alkaloids	choline,catharanthine,nicotinic,palmatine
Saponins	arabinopyranosyl-3,23,30-trihydroxyolean[8]

II. DIFFRERENT METHODS USED IN PLANT EXTRACTION

I] Extraction:

Extraction techniques of Medicinal plants Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. 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. [5]

i] Methods of extraction of medicinal plants :

A]Conventional extraction technique :

Maceration
percolation
Digestion
Decoction

B] Modern extraction technique :

Counter-current Extraction
Ultra sound Extract
Supercritical Fluid
Aqueous Extraction
Ethanol Extraction
Fractionational Extraction
Soxhlet Extraction [6]

i) Hydrogen atom transfer (HAT) :

The HAT-based assays measure the capability of an antioxidant to quench free radicals (generally, peroxyl radicals considered to be biologically more relevant) by H-atom donation.

ii) Single electron transfer (SET) assay:

The antioxidant action is simulated with a suitable redox-potential namely, the antioxidants react with a fluorescent or coloured probe (oxidising agent) instead of peroxyl radicals. Spectro-photometric SET-based assays measure the capacity of and antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change (either an increase or decrease of absorbance of the probe at a given wavelength) is correlated to the concentration of antioxidants in the sample.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

1) Hydrogen atom transfer methods (HAT) methods :

Oxygen radical absorbance capacity (ORAC) [9]
Lipid peroxidation inhibition capacity (LPIC)
Total radical trapping antioxidant parameter (TRAP)
Inhibited oxygen uptake (IOC)
Crocin bleaching nitric oxide radical inhibition activity
Hydroxyl radical scavenging activity by p-NDA (p-butrisidun ethyl aniline)
Scavenging of H2O2 radicals
ABTS radical scavenging
Scavenging of super oxide radical formation by alkaline (SASA)

2] Electron transfer methods (ET) :

Trolox equivalent antioxidant capacity (TEAC) decolourization
Ferric reducing antioxidant power (FRAP)
DPPH free radical scavenging
Copper
Total phenols by Folin-Ciocalteu
N,N-dimethyl-p-phenylenediamine (DMPD).
2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is the most commonly used antioxidant assay for plant extract.

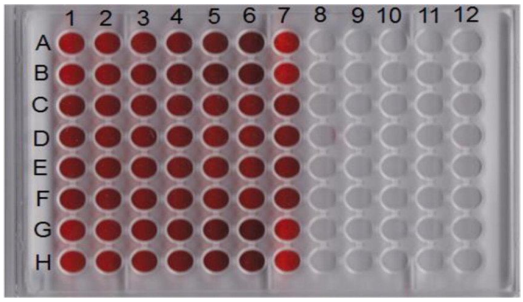
E] Principle of DPPH activity :

1,1-Diphenyl 2- Picryl Hydroxyl is a stable (in powder form) free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show 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 indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability[10].

F] Advantages of DPPH method :

The application of this test allows the comprehension of the various chemical phenomena and has obvious advantages, like low cost, ease of performing experiments, Reproducibility, applicability at room temperature, as well as automation possibilities.

96 micro well plate method is A micro-plate, also known as a micro-plate micro well plate or multi well is a flat plate with multiple "wells “used as small test tubes. The micro-plate has become a standard tool in analytical research and clinical diagnostic testing laboratories. A micro plate typically has 6, 12, 24, 48, 96, 384 or 1536 sample wells arranged in a 2:3 rectangular matrix. 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 micro well plates are read on ELISA plate reader.



Fig[3]:96micro well plate reader

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

III] Anti-bacterial activity :

Alcoholic extraction of *Cnidoscolus aconitifolius* showed growth inhibition for Staphylococcus aurous;.The *Cnidoscolus aconitifolius* was also found to be active against multidrug-resistant strains of S.aureus that are also resistant to common beta lactam antibiotics. Chaya was also found to be effective as anti diabetic, as well as anti inflammatory [11].

A] Staphylococcus aureus :

Staphylococcus aureus (S. aureus) is a bacterium that can cause a variety of illnesses through suppurative or non-supportive(toxin mediated) means. Staphylococcus aurous is a common cause of skin and skin structure infections as well as osteoarticular infections in the human population. Scale skin syndrome- In 1878, Ritter described exfoliative dermatitis in 297 infants less than one month old. The disease he described is now called Ritter. The sudden appearance of local erythema around the mouth (redness and inflammation), which takes over the entire body over two days, is a characteristic of this disease.

Staphylococcal food poisoning- Staphylococcal food poisoning is one of the most common food borne illnesses as a result of the presence of toxin in food and not due to infection. In other words, instead of being an infection, it is a kind of poisoning; therefore, instead of direct effect of the organism on the individual, the illness is due to the bacterial toxin in the food. Bacteraemia and endocarditic staphylococcus aurousis the common agent for bacteraemia. Although bacteraemia produced by a large body of other organisms originates from a recognizable infection site, such As: lung, urethra, or gastrointestinal tract infections. osteomyelitis and infectious arthritis Staphylococcus aureus the cause of most cases of primary osteomyelitis[12].

This disease is predominantly occurring in boys Under the age of 12, and is often followed by the diffusion of a Primary haemorrhage (ulcer or furuncle). Escherichia coli (E. coli) diarrheal Infections ETEC strains have been associated with self-limited gastrointestinal illness characterized by abdominal cramping and watery stools lasting 1 to 5 days. Urinary Tract Infections- strains are responsible for approximately 80% of Community acquired and 30% of nosocomial-acquired UTIs. Infections in children are often due to blockages in the urinary tract, resulting in pools of stagnant urine. Septicaemia and Meningitis in Neonates , both term and preterm, are susceptible to septicaemia and meningitis. Presentation in the first week after birth (early onset) and particularly in the first 2 days after birth reflects vertical transmission, whereas late- onset infection suggests no socioeconomically or community acquisition

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 coloured bottles.

2) Phytochemical screening:

Test for Alkaloid's:

Test	Process	Observation	Positive/negative
Dragendroffs test	2ml of methanolic extract +2ml dragendroffs reagent	Reddish brown precipitated formed.	Alkaloids are present
Haggers Test	2ml methanolic extract+1mlof haggers reagent	Yellow precipitated is formed	lkaloids are formed.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

Test for Glycosides:

Test	Process	Observation	Positive/negative
Legal Test	2ml extract dissolve in pyridine +nitroprusside solution+10%NAOH solution	Pink colour precipitated formed	Glycosides present

Test For Phenolic Compound:

Test	Process	Observation	Positive/negative
Ferric-Chloride TEST	2ml extract dissolve in-distilled water + nitroprusside solution+5%drops of ferric chloride solution.	Blue green precipitated formed	Phenolic compound present [13,14]

3) Determination Of anti-oxidant activity :

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).

100µL of test compounds water were taken in the micro titer plate.

100 µL of 0.1% methanolic DPPH was added over the samples carbon dots at different concentration (10, 20, 50µg/ml) and incubated for 30 minutes in dark condition.

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.

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

4) Determination of antimicrobial activity :

The inoculums of the microorganism were prepared from the bacterial cultures.

15 ml of nutrient agar (Highmedia) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.

100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly.

Sample was placed on the well and plates were incubated at 37 degree celcius

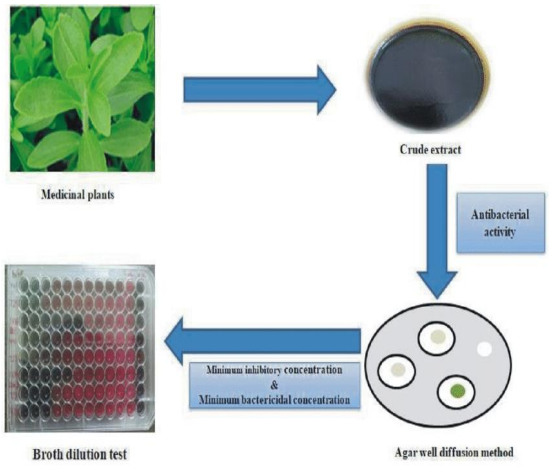


Fig [4] : antibacterial Activity of Chaya Extract Against *S.aureus*

DOI: 10.48175/IJARSCT-25357

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 6, April 2025
Impact Factor: 7.67

III. CONCLUSION

The *Cnidoscolus aconitifolius* have rich source of phytoconstituents. Phenolic compounds are essential for the antioxidant and antibacterial activity[14]. In the chaya leaf, there is a general trend toward the presence of different phenolic groups, such as coumarin, flavonoids, phenols, tannins, anthraquinones, and flobotanins in aqueous and alcoholic extracts. The chaya plant has potential for production as food and as a medicinal plant, but the task of comparing the results obtained from the different research articles is complicated by the different processes used by each of the researchers to report the phenolic compounds and the antioxidant capacity of this plant. Apart from the analysis of different extraction methods, solvents and forms of preparation, as well as the diversity of extracted compounds, further research is also important and necessary through in vitro and in vivo studies of each type of extract in order to evaluate their biological effects on health, for example, in reducing glucose levels, or as a possible chemo-preventive or chemo-protector agent against colon cancer. [15]

REFERENCES

[1]. Cnidoscolus chayamansa Mc Vaugh, an important antioxidant, anti-inflammatory, and cardioprotective plant used in Mexico. Journal of Ethnopharmacology, 151(2), 937–943.

[2]. Panghal, A., Shaji, A. O., Nain, K., Garg, M. K., & Chhikara, N. (2021). Cnidoscolus aconitifolius: Nutritional, phytochemical composition and health benefits–A review. Bioactive Compounds in Health and Disease, 4(11), 260–286.

[3]. Ebel, R., Méndez Aguilar, M. de J., Castillo Cocom, J. A., & Kissmann, S. (2019). Genetic diversity in nutritious leafy green vegetable—chaya (Cnidoscolus aconitifolius).

[4]. Rasul, M. G. (2018). Conventional extraction methods used in medicinal plants, their advantages and disadvantages. International Journal of Basic Science and Applied Computing, 2(6), 10–14.

[5]. Seabra, R. M., Andrade, P. B., Valentão, P., Fernandes, E., Carvalho, F., & Bastos, M. L. (2006). Antioxidant compounds extracted from several plant materials. In Biomaterials from Aquatic and Terrestrial Organisms (pp. 115–174). Science Publishers

[6]. Alemán, L. R., Ramirez-Mares, M. V., Flores-Gallegos, A. del R., Guzmán-De-Peña, D., & Rocha-Guzmán, N. E. (n.d.). Antioxidant and anti-inflammatory potential of Cnidoscolus aconitifolius leaf extracts.

[7]. Benjamin, A. E., Edem, A. E., & Enos, O. J. (n.d.). Review on Cnidoscolus aconitifolius: A green vegetable with medicinal value.

[8]. Vinci G, Nair SA, Sacchi R. Role of antioxidants in food preservation: Potential application and health benefits. Nova Biotechnol Chim. 2017;16:7-21.

[9]. Ou, B., & Huang, D. (2003). Oxygen radical absorbance capacity (ORAC) assay. Journal of Agricultural and Food Chemistry, 51(11), 3273–3279.

[10]. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25–30.

[11]. Abere, T. A., Okoye, E. L., & Abosi, A. O. (2012). Antibacterial activity of crude leaf extracts of Cnidoscolus aconitifolius on Staphylococcus aureus. Journal of Medicinal Plants Research, 6(44), 5787–5793.

[12]. Benharroch, R. I., Csukas, G., Carmeli, Y., & Kreft, B. (1993). Staphylococcus aureus exfoliative toxins: Diagnostic and therapeutic considerations. Clinical Microbiology Reviews, 6(3), 422–433.

[13]. Khatun, M. A. N., & Pillay, N. G. J. (2020). Traditional medicine and traditional healers in South Africa: A scoping review. South African Journal of Botany, 132, 304–320..

[14]. Kuri-García, A., & Guzmán, S. H. (2017). Phenolic profile and antioxidant capacity of Cnidoscolus chayamansa and Cnidoscolus aconitifolius: A review. Journal of Medicinal Plants Research, 11(45), 713–727

[15]. Al-Snai, A. E. (2019). Iraqi medicinal plants with antibacterial effect—A review. IOSR Journal of Pharmacy, 9(8), 22–103.