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Pharmacological, Phytochemical and Medicinal use of *Cayratia Trifolia*: A Vulnerable Plant Species

Anita[1] | S. P. Choudhary[2*]

[1]Department of Botany, Vivek PG Mahavidhalaya, Kalwar, Jaipur, Rajasthan, India.
[2]Department of Botany, Vivek PG Mahavidhalaya, Kalwar, Jaipur, Rajasthan, India.

[*]Corresponding Author: choudharyshiv14@gmail.com

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ABSTRACT

The perennial medicinal climber Cayratia trifolia (Family: Vitaceae) is well known throughout Asia and Australia for its ethnomedical uses. In the current study, secondary metabolites were qualitatively screened for in the ethanolic extracts of the plant parts. Alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, and glycosides were consistently found in all organs under investigation, according to the analysis. According to phytochemical profiling, the leaves and stems are especially abundant in stilbenes like resveratrol, piceid, viniferin, and ampelopsin, as well as flavonoids like kaempferol, quercetin, myricetin, cyanidin, and delphinidin. Roots and aerial parts were consistently found to contain tannins, steroids, and terpenoids, including epifriedelanol. In all ethanolic extracts, alkaloids and glycosides were also detected using conventional reagent assays. These results support earlier research showing a wide range of phyto-constituents in C. trifolia, which is consistent with its known pharmacological properties, including anti-inflammatory, anti-bacterial, antiprotozoal, anticancer, antidiabetic, and antidiuretic effects. Significant bioactive potential is suggested by the presence of these various secondary metabolites in ethanolic extracts. While alkaloids, saponins, and terpenoids, such as epifriedelanol, are responsible for antimicrobial, cytotoxic, and enzyme-inhibitory effects, flavonoids and tannins help to scavenge free radicals and reduce inflammation. Cayratia trifolia concludes by confirming that all tested portions contain significant amounts of the important secondary metabolites alkaloids, phenols & tannins, flavonoids, saponins, terpenoids, and glycosides. The plant's widespread traditional medicinal use is supported by the distribution of these compounds, which also supports additional bioactivity-guided research aimed at identifying, describing, and assessing these compounds for potential therapeutic uses.

Keywords: *Alkaloids, Cayratia trifolia, Flavonoids, Phytochemical, Vulnerable*.

Introduction

Cayratia trifolia Linn. also called *Vitis trifolia* Linn., is a member of the Vitaceae family and is found in Australia and Asia naturally. At elevations of up to 600 meters, the perennial vine *C. trifolia* is found in mountainous areas of India, particularly in Rajasthan, Jammu to Assam, Tripura, and West Bengal (Sesagiriravu *et al.*,1986). *C. trifolia* is a flowering plant that grows throughout Asia and Australia (Fig 1). It is also known as Ambalab and Ramchna in Hindi and Amalavetash in Sanskrit (Kushwaha *et al.*,2019) and several other vernacular names are given in table 2. The flowers are brown and greenish white.

The fruits are glossy, juicy, dark purple or black, almost spherical, and have a diameter of around 1 cm. *C. trifolia*, also known as kalit-kalit, is a vulnerable herb climber found in lower elevations of dense, open jungles in the Philippines. It is widely found in subtropical and tropical Asia, India, Africa, Australia, and the Pacific islands. Both pharmacological and folk medicine studies have confirmed its widespread medicinal value (Yunus *et al.,*2021).

Worldwide, over 35,000 plant species are utilised for human medical purposes (WHO). According to Rios and Recio (2005) and Bandaranayake (2006), crude drugs are typically the dried parts of medicinal plants, such as the roots, stems, bark, leaves, flowers, seeds, fruits, and entire plants. These parts are essential raw materials for the production of traditional medicine in many different medical systems, including Ayurveda, Siddha, Unani, Homoeopathy, and others.

Figure 1: *Cayratia trifolia* A. plant B. fruit

Table 1: Taxonomical Classification of *Cayratia trifolia*

Taxonomical hierarchy	Names
Domain	Eukaryota
Subkingdom	Viridaeplantae
Kingdom	Plantae
Phylum	Tracheophyta
Subphylum	Euphyllophytina
Infraphylum	Radiatopses
Class	Magnolipsida
Subclass	Rosidae
Suborder	Vitanae
Order	Vitales
Family	Vitaceae
Subfamily	Vitoideae
Genus	*Cayratia*
Species	*trifolia*

Source: Anita *et al.*, 2019

Table 2: Vernacular names of *Cayratia trifolia*

Language	Vernacular names
Assam	Ghepeta-lat, Chepeta lota
Bengali	Amla-lata
English	Fox-grape
Gujrati	Khat-khatumbo
Hindi	Amal-bel, Ramchana, Teen panya kand, Amar chatioo,khhata-limba, Tamnaya, Gidardrak
Karnataka	Heggoli
Malyalam	Sorivalli
Marthi	Ambat-vel
Punjabi	Armal-bel
Sanskrit	Amlavetash, Atyamlaparni, Gandira

Source: Anita *et al.*, 2019

Botanical Distribution

C. trifolia is a weak vegetarian climber with dense, narrow, and somewhat succulent wood at the base. The petioles are 2-3 cm long, and the stem and leaf are tri-leafy. The leaflets have a pointed tip, are oval-shaped, and measure 2–8 cm in length and 1.5–5 cm in width. Along the leaf axis, the brown, tiny, greenish-white flowers measure 2.5 mm and are solitary (Garden and Bennett, 1956; De Freitas, 2016). Berries are almost spherical, 1 cm in diameter, dark purple or black in colour, and luscious and delicious. The triangular seeds have a rounded apex, ventral pores, and bent, slightly protruding ribs along the edge (Tutul *et al.,*2010, Sharma *et al.,*2020).

Stem Anatomy

The young stem's anatomy displays the first phase of secondary growth. The epidermal layer is cylindrical in shape and extends continuously around the stem. The walls of the epidermal cells are thin. On the epidermal layer, dense epidermal trichomes are visible. The cortex is broader and has two distinct zones: an inner zone with three or four layers of parenchyma cells and an outer zone with roughly eight layers of collenchyma cells. The vascular cylinders of multiple wide, wedge-shaped xylem segments are located in the centre and are connected by a thin layer of sclerenchyma tissue. Thick blocks of phloem with sclerenchyma caps on the outside and one to three radial short rows of wide, circular, thin-walled vessels make up the xylem segments. The pith is compact, thin-walled, parenchymatous, and broad. Calcium oxalate druses are sparsely seen in the cortical cells (Kushwaha *et al.,* 2019).

Leaf Anatomy

The epidermis layer is visible on the leaf surface. Veins, vein islets, and vein termination were among the leaf surface contents that were identified. The epidermis layer, cuticle layer, and vascular bandles (xylem and phloem) are visible in the transverse section of the leaf. The mesophyll differentiates into spongy parenchyma and palisade. From the upper epidermis, numerous covering trichomes proliferate. Trichomes are multicellular and uniseriate. Below and above the epidermis are strips of collenchyma (Kushwaha *et al.,* 2019).

Chemical Composition

In addition, this plant contains triterpenes, epifriedelanol, myricetin, quercetin, and kaempferol (Munchen 1953). According to reports, the entire *Cayratia trifolia* plant contains flavonoids, tannins, steroids/terpenoids, and yellow waxy oil (Gupta and Sharma 2007). Stilbenes like piceid, reveratrol, viniferin, and ampelopsin are found in leaves (Arora *et al.,* 2009). It has been reported that hydrocyanic acid and delphinidin are present in stem, leaves, and roots. According to reports, the leaves contain a number of flavonoids, including cyanidin (Grubben and Denton 2004; Throton 1997). Cyanogenic compounds were found in its seeds and fruits. Additionally, fruits contain calcium oxalate, which causes extreme oral irritation (Gupta and Sharma 2007).

Alkaloids, flavonoids (primarily kaempferol, quercetin, and myricetin), tannins, steroids/terpenoids, and yellow waxy oil are among the main components found in the entire plant (Sowmya *et al.,* 2015). According to Arora *et al.,* (2009), stilbenes like piceid, viniferin, reveratrol, and ampelopsin are stored in leaves. After the entire plant was analysed using Gas Chromatography Mass Spectrometry (GC-MS), 20 bioactive compounds were found. Cyclopentadecane, 9-(2-propen-1-yloxy)-.1, 4,8,12,16-Tetramethylheptadecan-4-olide, Oxirane, and Vitamin E were among the significant compounds that were isolated. (Perumal *et al.,* 2014). Using the Qiagen method, functional RNA comprising polysaccharides, polyphenols, and other secondary metabolites was extracted from CT plant tissue (Yazid and Sidik 2011).

Analysis of the stem and leaf ethanolic extract revealed a high concentration of primary metabolites (Jasuja 2019). By using the Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) methods, the ethanolic extract of the CT plant was found to contain linoleic acid (Perumal *et al.,* 2014). Tannins and phenol compounds were detected in the ethanolic extract of CT by HPTLC (High Performance thin layer chromatography) analysis (Sowmya *et al.,* 2015). The CT plant's tissue culture yielded resveratrol (Roat and Saraf 2017). The presence of phytoestrogens like daidzein, genistein, and formononetin in the CT plant was demonstrated by RP-HPLC (Reversed Phase High Performance Liquid Chromatography) analysis (Gour and Patni 2013).

n-tetradecanyl n-octadec-9, 12dienoate, n-tridecanyl n-octadec-9, 12-dienoate, n-hexadecanyl n-octadec-9, 12-dienoate, n-tetradecanyl n-octadec-9-enoate, and n-hexadecanyl n-octadec-9-enoate were detected by column chromatography of the CT stem (Singh and Sharma 2012). FTIR analysis of the CT plant's ethanolic extract revealed the presence of amine, acid, alkane, ketone acyclic, carbonyl, aromatic, ester, and alkene; HPTLC analysis confirmed the presence of alkaloids, flavonoids, glycosides, saponin, and steroids; and GC-MS analysis confirmed the presence of hexadecanoic acid-ethylester, phytol, tetratetracontane, stigmasterol, nonacosane, and octadecane-1-bromo (Sowmya *et al.,* 2016).

Ethnomedicinal Uses

The entire plant is used as an astringent (Ayyanar and Ignacimuthu, 2009; Gaur and Sharma, 2010), laxative, and to treat tumours, neuralgia, splenopathy, and leucorrhea (Gupta and Sharma, 2007). A hot root and leaf juice is fermented as a diaphoretic (Kumar *et al.* ,2011). The plant's stems and leaves are used to make aphrodisiacs. The root is used as a paste to prevent snakebite, an astringent to lessen stomach disorders, and an anaemic condition (Khare, 2008). While tuber root helps heal broken bones quickly and root bark eases muscle pain, tuber extracts containing *C. trifolia* seed extracts are administered orally to diabetic patients to monitor blood sugar levels (Kumar *et al.,*2012).

While young leaves are consumed as a vegetable in Malukas, the decoction of fresh leaves is used to treat scurvy in the Philippines, dandruff and head itching in Java, and high fever and inflammation in Peninsular Malaysia and Thailand. The root is used as a remedy for snake wounds. Bioactive components found in fruits and plants may one day be developed into anti-cancer drugs (Safarzadeh *et al.,*2014; Yin *et al.,*2013). A variety of illnesses have been treated with parts of the *C. trifolia* plant, such as the fruit, stem, and leaf (Kumar *et al.,*2012; Gupta *et al.,*2012).

Pharmacological Uses

- **Antidiabetic Activity**

In Albino rats with diabetes induced by alloxan, the ethanolic extract of *Cayratia trifolia* roots demonstrated an antidiabetic effect (Dhandapani *et al.,* 2002). To determine the toxicity in rats, an acute toxicity study of the ethanolic extract of CT roots was conducted in accordance with OECD guideline No. 420 (Veeraraghavan 2000). To find out how the ethanolic extract of CT root affected the lipid profile, body weight, glucose, plasma, and insulin level, a thorough investigation was conducted (NASSIRI-ASL *et al.,* 1986).

In one study, rats with diabetes had hypercholesterolaemia and hypertriglyceridemia, while rats given extract had higher HDL and lower triglycerides and cholesterol (Rao *et al.,* 1999). Rats with diabetes showed elevated plasma levels of urea and creatinine, which were lowered cholesterol level and triglyceride and increased HDL (Rao *et al.,* 1999). Elevation of plasma levels of urea and creatinine was seen in diabetic rats which were decreased in extract treated rats. Accordingly, it was determined that the CT root extract had the ability to lower the experimental rats' blood glucose levels and other diabetes-related problems (Batra 2013). In mice with diabetes induced by streptozocin, the ethanolic extract of CT stem also demonstrated antidiabetic activity (Yusuf *et al.,* 2018).

- **Antioxidant Activity**

Tanwer *et al.,* (2010) conducted a qualitative and quantitative analysis of the primary metabolites found in the leaves and stems of *Cayratia trifolia*. By measuring the FRAP (Ferric reducing antioxidant power), catalase activity, LPO (Lipid peroxidase), and peroxidase activity of the CT leaves and stem, the antioxidant qualities were assessed (Zou *et al.,* 2016). Following satisfactory results from FRAP, catalse activity, LPO, and peroxidase activity, it was determined that the ethanolic extract of *Cayratia trifolia* stem and leaves contains a high amount of primary metabolites and exhibits free radical scavenging activities (Jasuja 2019).

DPPH activity was used to assess the antioxidant capacity of CT stem, and the amount of antioxidant that resulted in a 50% reduction in DPPH activity was calculated by comparing it to ascorbic acid (Molyneux 2004). The IC50 value of the extract was found to be more than that of ascorbic acid which proved the presence of strong antioxidant properties (Yusuf *et al.,* 2018).

- **Hepatoprotective Activity**

The entire CT plant's ethanolic extract exhibited hepatoprotective properties. To assess the CT plant's hepatoprotective ability against nitrobenzene-induced hepatotoxicity in rats, a thorough investigation was conducted (Beauchamp *et al.,* 1982). The serum of the diseased and treated groups was used to measure the activities of liver biomarkers like ALT, AST, and ALP. ALT, AST, and ALP levels were higher in the nitrobenzene-induced group and lower in the extract-treated group (Aristatile *et al.,*2009). In contrast to the diseased group, the antioxidant enzymes SOD, Catalase, and GPx exhibited increased activity (Venukumar and Latha 2002). The extract-treated group also showed a decrease in lipid peroxidation. Thus, it was concluded that *C. trifolia* leaves extract has potent hepatoprotective action on NB induced hepattotoxicity in rats.

- **Gastric-antiulcer Activity**

In a pylorus-ligated and ethanol-induced ulcer model in wistar rats, methanolic extract of CT leaves demonstrated gastric-antiulcer activity (Paiva *et al.,*1998). The diseased and extract-treated groups were compared using a detailed analysis of the ulcer index, ulcer protection percentage, and impact on gastric juice pH and effect (Dashputre and Naikwade 20011). In rats with pylorus ligation and ethanol-induced ulcers, the extract-treated group reduced the levels of gastric juice pH, ulcer index, ulcer protection percentage, and gastric juice secretion. Therefore, it was determined that CT leaves have antiulcer activity because of their cytoprotective and antisecertory properties (Gupta *et al.,* 2012).

- **Anticancer Activity**

The CT plant's ethanolic extract has anticancer properties. Female Sprague Dawley rats with DMBA-induced cancer were used in a thorough investigation (Srivastava *et al.,*1997). VEGF-A and MMP-9 were compared between the cancer and treated groups. Matrixmetalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) expression were lower in the extract-treated group than in the cancer-induced group. Accordingly, it was determined that *Cayratia trifolia* has anticancer properties (Dewi 2017).

- **Antimalareal Activity**

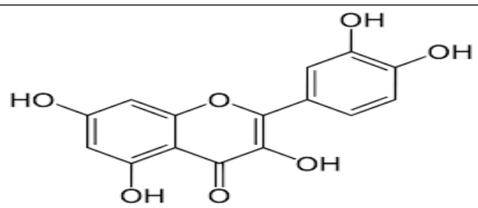
According to Rueda *et al.,* (1990), water extract from CT leaves has larvaecidal activity against Culex quinquefasciatus. The study was conducted in detail, and the mortality rate of Culex quinquefasciatus larvae was recorded in relation to the type of leaves (young or mature), the concentration of leaf extract, and the time of year. While no mortality was observed in the non-target water population, the water extract of CT leaf demonstrated increased mortality of test mosquito species within 24 hours with increased LD50 and LD90 (Deshpande *et al.,* 2014). Chromatographic analysis of CT leaf was used to assess the presence of secondary phytochemicals such as steroids, triterpene glycosides, essential oil, phenolics, and diterpenes. Thus, it was determined that CT leaf water extract possesses strong antimalarial properties (Chakraborty *et al.,* 2013).

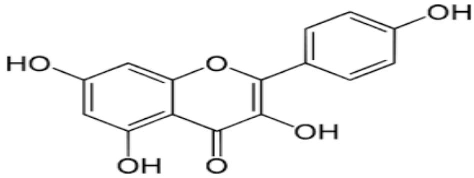
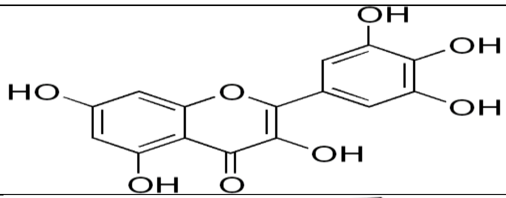
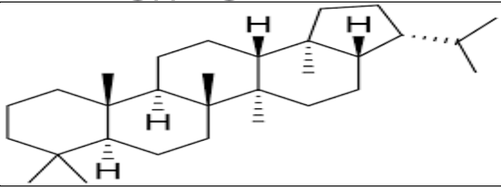
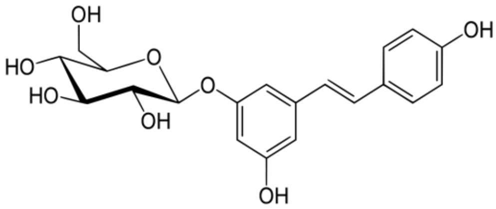
- **Phytochemistry**

Stilbenes like piceid, viniferin, reveratrol, and ampelopsin are stored in the leaves of *Cayratia trifolia*, according to Arora *et al.,* (2009). After the entire plant was analysed using Gas Chromatography Mass Spectrometry (GC-MS), 20 bioactive compounds were found. Cyclopentadecane, 9-Borabicyclononane, 9-(2-propen-1-yloxy)-.1, 4,8,12,16-Tetramethylheptadecan-4-olide, Oxirane, and Vitamin E were among the significant compounds that were isolated.

According to Yunus *et al.,* (2021), the leaf ethanol fraction has the highest yield (24.52%). Phytochemical screening revealed the presence of alkaloid compounds, flavonoids, polyphenols, and steroids in the extract and ethanol fraction of the leaves and stems. Polyphenol and steroid compounds are found in the leaves and stems of chloroform and N-hexane fraction. With an IC50 value of 58.4 ppm and the highest total phenol content of 56.2 µg/mL, the ethanol fraction of *Cayratia trifolia* rods exhibits antioxidant activity. With an LC50 value of 92.4 ppm, the toxicity test revealed that the ethanol and chloroform fraction had the highest toxicity activity. Table 3 lists some of *Caryatia trifoia's* key phytoconstituents.

Table 3: Name of some phytochemicals of *Cayratia trifolia* and their structure

Name of Componds	Structure
Quercetin	

Kaempferol	
Myricetin	
Triterpine	
Piceid	

Conclusion

The study's objective was to identify and explore *Cayratia trifolia's* pharmacological, phytochemical, and therapeutic properties. The plant's antidiabetic, antiulcer, antipyretic, hepatoprotective, antioxidant, anticancer, and antimalareal properties were discovered through preclinical research. The phytoconstituents found in CT's roots, stem, bark, leaves, flowers, and seeds may be responsible for these activities. CT offers enormous benefits that could serve as the foundation for medication supplements. As an alternative therapy, it can also be used to treat various illnesses.

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