

REVIEW ARTICLE

Exploring the Therapeutic Potentials of *Diplocyclos palmatus*: Insights from *In Vitro*, *In Vivo*, and *In Silico* Approach –an Overview

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ABSTRACT

Research on medicinal plants has grown significantly due to their enduring reputation, economic benefits, and sustainability. This review focuses on the climbing plant *Diplocyclos palmatus*, commonly known as striped cucumber, which belongs to the Cucurbitaceae family. The plant has numerous health benefits, including antioxidant, antibacterial, anti-infective, anti-diabetic, antipyretic, anticancer, antifertility, antithrombotic, antivenom, antidote, and anti-inflammatory properties. It continuously re-examines the morphology, bioactive compounds, and the activity of secondary metabolites using *in vitro*, *in vivo*, and *in silico* approaches for *Diplocyclos palmatus*. Finally, the reassessment outlines the various analytical techniques, including TLC, FTIR, SEM, GC-MS, HPLC, and LC-MS, while revisiting the traditional applications of *Diplocyclos palmatus*.

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INTRODUCTION

Since the start of human civilization, plants have served as sources of food, energy, and medicine due to their safety and non-toxicity, leading to a recent focus on plant based antioxidant compounds. An around 80% of the population in developing countries still relies on traditional medicine, particularly herbal remedies. The plant *Diplocyclos palmatus* was chosen to quench the demand for a new herbal Medicine (Bannerman Buton & Wen-chieh, 1983).

Diplocyclos palmatus belongs to the cucurbitaceae family and is commonly referred to as brynoy or lined cucumber. It is indigenous to both rainforests and dry rainforests and is a twining vine with stems that can grow up to 6 meters long. The plant is native to Australia and is often called marble vine (Gupta & Wagh, 2014).

The Shivalingi plant is a perennial climbing species characterized by a bare stem that thickens and develops pale speckles along the edges, becoming more mature over time. Leaves are broadly ovate, measuring between 3.5 and 14 cm, and are palmately lobed. The petiole ranges between 1.5 and 9 cm in length. Flowers are small, pale or yellowish, and appear in clusters of two to eight without stalks, including five female blooms in the same axil. The sepals in the inflorescence measure approximately 3 to 4 mm in length, while those in the female blooms are 1.5 to 2.5 mm long, and the sepals are smaller than the corolla (Gupta & Wagh, 2014).

Generally, blossom chap is larger than feminine. It has an oblong shape, measuring 1.5 to 2.5 cm when mature, and is scarlet with prominent light stripes. It is native to India and the Himalayas, at altitudes ranging

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from 200 to 1500 meters. Usually, the blossom period of *D. palmatus* falls in the autumn season (Gupta & Wagh, 2014). The general composition of *Diplocyclos palmatus* is given in Table 1

Common Names:

Gujarati	:	Shivalingi
Kannada	:	Lingatonda balli
Hindi	:	Shivalingi
Malayalam	:	Aiviralikova
Nepal	:	Shivalingi
Tamil	:	Aivirali
Telugu	:	Lingatonda

Taxonomic Hierarchy:

The taxonomic hierarchy of *Diplocyclos palmatus* (L) C. Jeffrey is as follows:

Kingdom	:	Plantae
Division	:	Angiospermae
Class	:	Dicotyledonae
Order	:	Cucurbitales
Family	:	Cucurbitaceae
Genus	:	<i>Diplocyclos</i>
Species	:	<i>Diplocyclos palmatus</i> (L) C. Jeffrey

Table 1: General composition of *Diplocyclos palmatus*

General composition of <i>D. palmatus</i>	Percentage
Moisture	16.39
Protein	40
Oil	12
Total ash	8.41
Alcohol soluble extractive	10.43
Water-soluble extractive	12.42
Other substances	< 2

(Venkateshwarlu et al., 2011 & Dave et al., 2006)

General constitutions of fatty acids:

Acid number of *D. palmatus* is predicted at 2.9%. Iodine value reflects the extent of unsaturation in a fat or oil the *D. palmatus* showed an iodine number of 171.5. The peroxide value refers to the quantity of

peroxide oxygen per 1 kg of fat or oil, which is recorded at 0.3 mEq/kg for *D. palmatus*. The saponification value indicates the number of milligrams of potassium hydroxide required to saponify 1g of fat under certain conditions. The saponification value of *D. palmatus* is 208.3 (Venkateshwarlu et al., 2011 & Dave et al., 2006).

Morphological evaluation of *D. palmatus*:

Stem, leaves, and root:

D. palmatus plant is a slender vine, with a maximum stem diameter of 2cm. The leaves are deeply palmately lobed, with five prominent, projecting lobes. The size of leaves is approximately 6-13×6-12cm, and the petioles measure about 2-4cm long. Normally, the leaves are crushed to release an unpleasant smell. Also, the upper side of the leaf blade is covered with scattered scab-like hairs. Two extended rings were observed, denoting the leaf’s unfavorable attributes (Gupta & Wagh, 2014). *D. palmatus* has a tap root system with secondary roots and numerous root hairs. Seldom do fibrous roots arise from the nodes of vines (Thakur & Puri, 2025). Roots have a beneficial effect on asthma (Kranti et al., 2022). Leaf of *Diplocyclos palmatus* (L.) Jeffrey is depicted in Figure 1.



Figure 1: Leaf of *Diplocyclos palmatus* (L.) Jeffrey

Flowers:

Generally, one female flower and three male flowers are found in each leaf axil. The female flowers can grow to 15mm in length, while the male flowers reach about 20mm. Both types of flower petals are 8-10mm long and feature a

dense fur covering on their inner surfaces. Each flower contains two anthers, which may be unilocular or bilocular. The anthers and fibers vary in length from 2-4mm, with a shaggy texture near the base. The hypanthium measures 3-4mm long, and the thick, shaggy staminodes extend towards three stigmas. Leaf flaps are roughly 2mm long with swollen bases and scattered outgrowths (Gupta & Wagh, 2014). Flower of *Diplocyclos palmatus* (L.) Jeffrey is represented in Figure 2.



Figure 2: Flower of *Diplocyclos palmatus* (L.) Jeffrey

Fruits:

Fruits of *Diplocyclos palmatus* are ovoid to ellipsoid, measuring around 20-30×15-32mm, and feature random longitudinal markings on their surface. *D. palmatus* fruit has an alkaline nature, with a pH of around 8.04 (Venkateshwarlu *et al.*, 2011). In terms of traditional usage, *D. palmatus* fruit has several medicinal properties and applications. It's recognized for its bitter, aperient, and tonic characteristics and is commonly used to relieve bilious ailments, viz., stomach ache, and diarrhoea. Typically, the fruit is used to treat external abscesses



Figure 3: Fruit and seeds of *Diplocyclos palmatus* (L.) Jeffrey

(Gupta & Wagh, 2014). In addition, the intake of these fruits has been linked to health complications and mortality in children (Gupta & Wagh, 2014). Likewise, fruits are an effective treatment for chronic colitis (Thakur & Puri, 2025).

Seedlings:

Normally, each plant holds nearly 6 to 10 seeds, with each seed roughly 6 to 8 mm long, irregularly shaped, and resembling teardrops. Cotyledons extend about 4 to 5 mm. The radicle is very small, measuring around 0.8 mm, making it considerably shorter than the cotyledons (Gupta & Wagh, 2014). An elliptical cotyledon measures about 20-26 X 10-15 mm, and the petioles are almost 2 mm long. Both the petioles and the stalks above the cotyledons are adorned with small, curved trichomes (Gupta & Wagh, 2014).

The first pair of exact leaves is deeply serrated and tri-lobed. At the end of the tenth leaf, crushed and emit an unpleasant odor. The margins of the deeply lobed leaves feature 3-5 main lobes that taper into long, narrow points at their tips. The hairs on the stalk are curved and take the place of thorns. The upper side of the leaf margin is hispid, covered with short hairs (Gupta & Wagh, 2014). Ethanolic and methanolic extracts of *D. palmatus* seeds exhibit anti-arthritis and anti-diabetic properties (Kadam & Bodhankar, 2013; Tripathi *et al.*, 2012; Jaynarayan *et al.*, 2012). *D. palmatus* seeds contain 12% essential oil and various proteins, i.e., bryonin, punicic acid (a type of trans fatty acid), goniotalamin, non-ionic glucomannan, and lipids (Singh & Malviya, 2006). Fruit and seeds of *Diplocyclos palmatus* (L.) Jeffrey is shown in Figure 3.

Qualitative and Quantitative analysis of *D. palmatus*:

Alkaloids, flavonoids, triterpenoids, saponins, resins, glycosides, phenolic compounds, and steroids were positive in *D. palmatus* (Gokulakrishnan *et al.*, 2019). The methanolic extract of *D. palmatus* leaf and fruit had a higher phenolic content (7.51±0.08 and 9.29±0.01 mg TAE/g extract). In contrast, the aqueous extracts of leaves and fruits showed moderate levels of phenolic compounds, while hexane and chloroform extracts exhibited lower phenolic content (Attar & Ghane, 2017). Phenols are a category of natural secondary metabolites that effectively scavenge free radicals in biological systems. Increased phenol levels are directly related to enhanced antioxidant activity (Roya & Fatemeh, 2013).

Tannins are high molecular weight phenolic compounds with various biological functions, including the ability to chelate metal ions and precipitate proteins, and are recognized as effective antioxidants. Reports indicate that tannins possess stronger antioxidant properties than low molecular weight phenolic compounds (Yokozawa et al., 1998). Chloroform extract of *D. palmatus* leaf (22.07 ± 0.06 mg CE/g) and fruit (6.99 ± 0.10 mg of catechin equivalent/100), the total tannin content exceeded that of carbinol, hexyl hydride, and water extract (Attar & Ghane, 2017).

Flavonoids are the most common type of antioxidants due to their strong redox potential, acting as singlet oxygen quenchers, reducing agents, hydrogen donors, and metal chelators (Cao et al., 2009). The methanolic extract of *D. palmatus* fruit has high flavonoids (15.02 ± 0.96 mg CE/g) than the aqueous extract of leaf (9.55 ± 0.65 mg CE/g) (Attar & Ghane, 2017). Terpenoids are the largest and most diverse group of compounds, functioning as growth promoters, influencing fertilization, and acting as anti-feedants (Cao et al., 2009). The methanolic extract of leaves and fruits has the highest concentration of terpenoids (Attar & Ghane, 2017).

Biological activity of *Diplocyclos palmatus*:

***In vitro* study of antioxidant activity**

Patel et al. (2020) assayed the ABTS radical on *D. palmatus* fruit. He concludes that the antioxidant activity is higher than that of ascorbic acid ($\mu\text{g/g}$).

Antimicrobial activity

Ethanol extract of *D. palmatus* exposed to antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* by the well diffusion method. Leaf extract of *D. palmatus* exhibited the highest inhibitory activity against *Candida albicans*, *S. aureus*, *Shigella*, *E. coli*, and *S. typhi*. Fruit extract showed good inhibitory effects on *S. shigella*, *Candida albicans*, *S. typhi*, and *S. aureus* (Gupta & Wagh, 2014).

Anti-cancer activity:

Fruit extract of *D. palmatus* had anti-cancer activity against breast (MCF-7) and colon (HT-29) cell lines. Generally, it's considered independent of cellular metabolic activity not obstructed by the test compounds. Significant effect of anti-cancer on the MCF-7 (GI₅₀<10, TGI 5.14, LC₅₀ 44.27 $\mu\text{g/mL}$) and HT-29 cell lines (GI₅₀<10, TGI 46.88, LC₅₀ 68.31

$\mu\text{g/mL}$), compared with the standard drug Adriamycin (Alexpandi et al., 2019; Nath et al., 2024 & Manda & Yellu, 2024). Normally, the combined effects of cucurbitacin B, gemcitabine, docetaxel, methotrexate, and cisplatin are used to treat cancer (Cai, 2015). The efficacy of cucurbitacins B and I in inhibiting the MCF-7 and HT-29 cell lines (Kim et al., 2014 & Gupta & Shrivastava, 2014). Biological activities of *Diplocyclos palmatus* are shown in Table 2.

Anti-diabetic activity:

D. palmatus plant allowed for various extraction methods, including microwave-assisted extraction, steam bath-assisted extraction, continuous shaking extraction, and ultra-assisted extraction. The microwave-assisted extraction achieved the highest alpha-amylase inhibition ($68.68 \pm 0.66\%$). Conversely, steam bath-assisted extraction showed the lowest inhibition ($33.52 \pm 1.87\%$). But moderate inhibition was observed in continuous shaking extraction ($46.55 \pm 1.50\%$) and ultra-assisted extraction ($44.43 \pm 0.11\%$), respectively (Patel et al., 2020).

Generally, α -glucosidase plays a vital role in postprandial hyperglycemia by hydrolyzing linear and branched isomaltose oligosaccharides, which enhances glucose release. *D. palmatus* extracts from ultra-assisted extraction exhibited very consistent inhibitory activity ($56.27 \pm 0.60\%$). The microwave-assisted extraction method showed lower activity ($48.34 \pm 0.72\%$). Similarly, steam bath extraction and continuous shaking extraction have significant inhibitory effects (Zhang, 2011).

Anti-thrombotic activity:

Usually, thrombosis is considered the formation of a blood clot or the presence of a blood clot within a blood vessel. Ethanol extract of *D. Palmatus* was used to treat thrombosis. Results confirmed that *D. Palmatus* extract controls thrombolytic activity due to phytochemicals found in the plant that can activate plasminogen through fibrin-dependent and fibrin-independent pathways (Gokulakrishnan et al., 2019).

Anti-pyretic activity & anti-pasmodic effect:

Kore et al., 2024 found that the alcoholic extract of *D. palmatus* (L) C. boosts the antispasmodic effect. Over 70% of the alcoholic extract derived from the aerial parts of the *D. Palmatus* plant exhibited



Table 2: Biological activities of *Diplocyclos palmatus*

S.No	Parts of the plant	Pharmacological action	References
1	Root	Asthma	Kranti, et al., 2022
2	Fruits	Chronic colitis & Anti-malaria	Thakur & Puri 2025, Dwivedi et al., 2021
		Anticancer for breast cancer (MCF-7)	Thakur & Puri 2025, Dwivedi et al., 2021
		Anticancer colon cell line (HT-29)	Thakur & Puri 2025, Dwivedi et al., 2021
		Anti diarrhoea	Gupta & Wagh, 2014
3	Seed	Anti-arthritic and anti-diabetic	Kadam & Bodhankar 2013, Tripathi et al., 2012, Jaynarayan, et al., 2012
		Anti infertility	Chauhan et al., 2018
		Anti inflammatory	Khan & Khan 2006
		Anti fungal	Gowrikumar et al., 1981
		Anti microbial	Mosaddik et al., 2001
		Analgesic	Kamble et al., 2010
		Anti hyperlipidemic	Paras et al., 2019
		Anti cytotoxicity	Nalinaksh & Anand 2024
Anti Neuro effect	Manda & Yellu 2024		
4	Leaf	In vitro Callus stimulation	Ramar & Ayyadurai, 2015
	Leaf paste	Anti venom activity	Gupta & Wagh 2014
	Leaf paste	Anti dote activity	Gupta & Wagh 2014
	Leaf, stem, fruit, & seed	Antimicrobial activity	Alexpandi et al., 2021
	Shoot tip & nodal segment	In vitro Callus stimulation	Mishra, et al., 2025
	Stem	Antioxidant activity	Nath et al., 2024
5	Dried aerial part	Anti pyretic	Gupta & Wagh 2014
6	Methanolic extract of <i>D. palmatus</i>	Anti Quorum	Alexpandi et al., 2021
		In vivo anti infection activity	Alexpandi et al., 2021

analgesic activity in mice (Ram Kishnan 2019). *D. Palmatus* fruits are used to control malaria infections (Thakur & Puri, 2025).

Anti-infertility, anti-venom, and antidote activity:

Recently, a uterine tonic was developed using *D. Palmatus* seeds for infertility treatment. *D. Palmatus* seeds play a significant role in ethnomedicine to treat infertility. This uterine tonic enhances androgenic activity, increases sperm count, raises testosterone levels, stimulates luteinizing hormone, and elevates fructose levels in the seminal vesicles, as measured in serum (Chauhan et al., 2018). The *D. Palmatus* seed powder (less than 5 grams), mixed with water or milk and consumed regularly for 21 days, aids in regulating the menstrual cycle in women (Gupta & Wagh, 2014). *D. Palmatus* seeds have anti-inflammatory, antifungal,

antimicrobial, analgesic, and anti-hyperlipidemic properties due to the presence of protein (Khan & Khan 2006, Paras et al., 2019, Kamble et al., 2010, Mosaddik et al., 2001, Gowrikumar et al., 1981). Usually, after a snakebite, fifty grams of *D. Palmatus* leaf paste mixed with betel leaves should be taken orally three times daily until recovery occurs (Gupta & Wagh, 2014).

Anti -Quorum Sensing activity:

Methanolic extract of *D. Palmatus* leaf significantly reduced the biofilm thickness, as confirmed by light microscopy and confocal laser scanning microscopy. Also, reduced the prodigiosin pigment production. In contrast, untreated *S. marcescens* showed their intricate structure and a dense layer of biofilm cells

on glass slides. Nowadays, extracellular virulence in *S. marcescens*, modulated by quorum sensing, plays a significant role in host infections. Methanolic extract of *D. Palmatus* leaf significantly reduced the extracellular polysaccharide production against *S. marcescens* and cell surface hydrophobicity. Decreased protease activity was observed due to tocopherol and phytol in the extract. Worms exposed to UV-A light, employing the 2',7'-dichlorodihydrofluorescein diacetate stain to evaluate the levels of intracellular reactive oxygen species. Considerably higher ROS level in the UV-A-treated sample than in the control. Extract treated to downregulate ROS levels and improve the lifespan of *S. marcescens* by hindering bacterial proliferation within the worms (Alexpandi *et al.*, 2019).

Normally, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-tetrazolium-5-carboxanilide forms a water soluble colored formazan when it interacts with metabolically active cells. The methanolic extract of *D. Palmatus* on *S. marcescens* treated cells exhibited a similar level of formazan as the control. In contrast, he confirmed that the anti-QS activity of the methanolic extract of *D. Palmatus* was not due to antibacterial effects (Gowrishankar *et al.*, 2016).

In vivo anti-infection assay:

Methanolic extract of *D. palmatus* increased the lifespan of *Caenorhabditis elegans* by diminishing the virulence of bacterial load within the worms. A colony forming unit assayed on the extract treated worms had fewer colonies (6×10^4 CFU/mL) than the control (34×10^4 CFU/mL). *C. elegans* survival assay assessed the efficacy of *D. palmatus* against *S. marcescens*. Worms infected with *S. marcescens* showed complete mortality within 70 hours. Whereas the extract treated at 200 to 600 $\mu\text{g}/\text{mL}$ concentrations, shown to extend the life span by up to five days. 600 $\mu\text{g}/\text{mL}$ was recommended for safety and effectiveness (Alexpandi *et al.*, 2019).

In vitro callus induction activity:

An efficient *in vitro* callus stimulation of *D. palmatus* was achieved from node, internode, and leaf explants on Murashige and Skoog medium with B5 vitamins and different concentrations and combinations of BAP (benzylaminopurine), IAA (indole-3-acetic acid), and IBA (indole-3 Butyric acid). The MS medium formulation containing BAP (1.5 mg/L) + NAA (Naphthalene acetic acid) (1 mg/L) + IBA (0.5 mg/L) led to the highest rate of callus induction (Ramar and Ayyadurai, 2015). Regenerated calluses were then transferred to half-

strength MS medium fortified with GA3 (1 mg/L) for callus elongation (Upadhyay *et al.*, 2021 & Roopa & Thomas, 2022).

In silico activity:

The 3D structure of the SmAR protein was modeled using Phyre2 software. Outcomes of docking analysis showed tocopherol (5.3), β -tocopherol (5.2), tocopherol (5.0), and phytol (3.9) have a higher binding score compared to C4HSL (3.8 K cal/mol) natural ligand (Kelley, 2015). The homologystudied on glyceraldehyde-3-phosphate dehydrogenase using *D. palmatus*; the protein sequences closely match known plant protein sequences (Rubalakshmi, 2020).

Analytical methods of *Diplocyclos palmatus*:

FTIR analysis and SEM analysis:

Fourier transform infrared spectroscopy (FTIR) is primarily used for identifying functional groups. The *D. palmatus* extract treated and untreated samples showed differences in functional groups. The four key spectral ranges, 3700-3100 cm^{-1} (hydration), 3050-2750 cm^{-1} (cell membrane fatty acid content), 1700-1500 cm^{-1} (amide linkages in proteins and peptides), and 1300-1000 cm^{-1} (combined region of proteins and fatty acids), are particularly valuable for evaluating alterations in bacterial cellular mechanisms (Santhakumari *et al.*, 2017).

The significance of trichome micromorphology in cucurbits was explored using Scanning Electron Microscopy (SEM) across 23 different species (Ali & Alhemaïd, 2011). *D. palmatus* of young and older leaves displayed minimal, poorly developed trichomes with a flattened base at a magnification of X 300.

Chromatographic analysis:

Fruits of *D. palmatus* were analyzed by Thin Layer Chromatography (TLC) using various solvents, viz., petroleum ether, chloroform, ethanol, benzene, toluene, ethyl acetate, and aqueous extract. Equal amounts of these extracts were loaded on the TLC plates. After developing the spots, the plates were viewed under ordinary light and UV light (254 nm & 365 nm), and Rf values were calculated. Results proved that the best separation was achieved using benzene as mobile phase (Rf values: 0.14, 0.31, 0.42, 0.50, 0.68, and 0.86), followed by toluene and ethyl acetate in a ratio of 93:7 (Rf values: 0.14, 0.25, 0.51, 0.74, and 0.9), Venkateshwarlu *et al.*, 2011.

The methanolic extract of *D. palmatus* was analyzed by gas chromatography-mass spectrometry (GC-MS). Nearly 17 compounds were identified; among them, palmitic acid (78.27%) and phytol (3.14%) were more significant, and octadecadienoic acid (0.23%) had the least area (Alexpandi *et al.*, 2019). The *D. palmatus* leaf and fruit were analyzed using high performance liquid chromatography to quantify rutin and quercetin. Results showed that the fruits contain 0.0805% rutin and 0.0024% quercetin, respectively. Similarly, leaf had 0.0055% rutin (mg/mL) (Rodge & Biradar, 2016).

Patel *et al.*, (2020) studied the fruit of *D. palmatus* using liquid chromatography mass spectrometry. Totally, eleven chief compounds were identified. Beta-hederin, a triterpenoid saponin had the highest mass, recorded at 752.491 at m/z 734.45 ([M+H]⁺). In contrast, Isovaleric acid, a member of the fatty acid group, confirmed the smallest molecular mass at 125.05 g/mol

Concluding remark and Future research

This review emphasizes that *D. palmatus* has potential antioxidant and anti-thrombotic actions and is especially advantageous for individuals with diabetes. It could also function as a nutraceutical supplement in the creation of various nutrient dense products. Nevertheless, a standardized mechanism is required to isolate and identify its compounds. Additionally, *in vivo* studies are critical to determine whether these plants interact with pharmaceuticals, including their mechanisms of action, drug absorption, detoxification, interactions, apoptosis, and cell signaling pathways. Further research should be conducted to verify the effectiveness of these approaches in the pharmaceutical and biotechnology industries for developing new drugs targeting antibacterial effects. These drugs are intended to impede the growth of abnormal cells. More research is necessary to assess the product's shelf life.

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The author confirms full accountability for data collection and manuscript preparation.

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