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Phytochemical and Pharmacological Evaluation of Catharanthus pusillus for Antioxidant and Antimicrobial Activity

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Abstract

The plant Catharanthus pusillus (commonly known as tiny periwinkle) is an underutilized species belonging to the Apocynaceae family. This study investigates the phytochemical composition, antioxidant potential, and antimicrobial efficacy of Catharanthus pusillus extracts, aiming to evaluate its suitability as a source of bioactive compounds. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and phenolic compounds. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, and antimicrobial activity was evaluated against a panel of bacterial and fungal pathogens. The findings highlight the plant's potential as a natural antioxidant and antimicrobial agent.

Keywords: Catharanthus pusillus, tiny periwinkle, Terpenoids, DPPH and ABTS, Antimicrobial, Antioxidants

Introduction

Medicinal plants have been an integral part of traditional medicine systems for centuries, serving as a source of therapeutic agents and inspiration for modern drug development. In recent years, the demand for natural products has increased significantly, driven by growing concerns over the side effects of synthetic drugs and the emergence of antibiotic-resistant pathogens. Plants offer a vast repository of bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, and phenolics, which exhibit diverse pharmacological properties. The systematic exploration of these compounds not only enriches our understanding of plant-based therapeutics but also paves the way for the development of novel drugs and nutraceuticals.

One such plant with untapped potential is Catharanthus pusillus, a lesser-known member of the Apocynaceae family. This family is well-regarded for its therapeutic properties, with its most prominent representative, Catharanthus roseus (commonly known as Madagascar periwinkle), being a source of vinca alkaloids used in cancer therapy. While C. roseus has been extensively studied, C. pusillus has received comparatively little attention. Commonly referred to as tiny periwinkle, C. pusillus is a herbaceous plant widely distributed in tropical and subtropical regions, particularly in India and Southeast Asia. Traditional medicine practitioners have long utilized C. pusillus to treat various ailments, including fever, skin disorders, and inflammation, hinting at its pharmacological potential.[1-4]

The therapeutic properties of plants are largely attributed to their secondary metabolites, which are often produced as a response to

environmental stressors, such as UV radiation, microbial attack, and herbivory. These compounds play a critical role in the plant's defense mechanisms and are now recognized as valuable resources for human health. Among the most studied bioactivities of plant-derived compounds are their antioxidant and antimicrobial properties. Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, has been implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, diabetes, neurodegenerative conditions, and cancer. Antioxidants are compounds that can scavenge free radicals and reduce oxidative stress, thereby mitigating disease progression. Natural antioxidants, particularly those derived from plants, have gained prominence due to their efficacy and low toxicity. Phenolic compounds, for example, are known for their ability to donate hydrogen atoms or electrons to free radicals, rendering them harmless.[5-6]

Antimicrobial resistance (AMR) poses another significant challenge to global public health. The overuse and misuse of antibiotics have led to the emergence of multidrug-resistant pathogens, rendering many conventional antibiotics ineffective. This has necessitated the search for alternative antimicrobial agents. Plants, with their rich repository of secondary metabolites, offer a promising solution. Compounds such as alkaloids, flavonoids, and tannins exhibit antimicrobial activity through diverse mechanisms, including disrupting microbial membranes, inhibiting enzyme function, and interfering with nucleic acid synthesis. Despite its promising therapeutic potential, C. pusillus remains underexplored compared to its more famous relative, C. roseus. The few existing studies on C. pusillus have reported the presence of alkaloids, flavonoids, and other phenolic compounds, but comprehensive investigations into its phytochemical composition and biological activities are lacking. This represents a significant gap in knowledge, as the unique phytochemical profile of C. pusillus could yield novel bioactive compounds with significant therapeutic applications. The present study aims to address this gap by conducting a detailed phytochemical and pharmacological evaluation of Catharanthus pusillus. [7-9] Specifically, the study focuses on the antioxidant and antimicrobial activities of the plant's extracts. By employing a combination of qualitative and quantitative phytochemical analyses, the study seeks to identify the major classes of bioactive compounds in C. pusillus. Additionally, in vitro assays, such as the DPPH and ABTS radical scavenging assays for antioxidant activity and the agar well diffusion method for antimicrobial activity, are utilized to assess the plant's pharmacological potential. The rationale for focusing on antioxidant and antimicrobial activities is multifaceted. Firstly, oxidative stress and microbial infections are two of the most common underlying factors in the pathogenesis of human diseases. The ability to combat these conditions with natural compounds could significantly reduce the burden on healthcare systems. Secondly, antioxidant and antimicrobial activities are often interrelated, as oxidative stress can weaken the immune system, making the body more susceptible to infections. Thus, plants like C. pusillus that exhibit both activities hold immense therapeutic potential. [10-12]

This study also contributes to the growing body of literature on the pharmacological properties of lesser-known medicinal plants. By highlighting the bioactivities of C. pusillus, it not only underscores the plant's potential for drug development but also emphasizes the need for the conservation of medicinal plant biodiversity. The findings of this study could serve as a foundation for future research, including the isolation and characterization of individual bioactive compounds and the exploration of their mechanisms of action. Unlike synthetic drugs, which often have significant side effects and environmental impact, plant-based compounds are generally considered safer and more sustainable. This makes them particularly

attractive for applications in the food, pharmaceutical, and cosmetic industries.[13]

Catharanthus pusillus represents an underutilized resource in the realm of natural product research. Its rich phytochemical profile and potential therapeutic properties warrant a detailed investigation, particularly in the context of antioxidant and antimicrobial activities. By filling the existing gaps in knowledge, this study aims to unlock the pharmacological potential of C. pusillus, contributing to the broader goal of harnessing plant biodiversity for human health and well-being.[14-16]

Materials and Methods

Plant Collection and Extract Preparation

Leaves of Catharanthus pusillus were grind through mechanical grinding and powdered by electrical blender. Ten grams of this powder was soaked in 100 ml of methanol for 48 Hrs. The contents were then filter through whattman filter paper no.1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -20°C until use. The extract was then dissolved in different solvent for testing. Extract was stored at 4°C until further use. [17-20]

Methodology

a) **Physical Characteristics**- Extract was investigated for its solubility in water, methanol, acetone, chloroform, ethylacetate, DMSO, petroleum ether.

Phytochemical investigation- Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents [17]

In vitro evaluation of antibacterial activity

Antibacterial Susceptibility Assay

Extract obtained was evaluated for antibacterial activities by disc diffusion assay (DDA). The extract was filtered sterilize before it is use in the experiment. Petri dish containing 20 ml of Nutrient Agar (NA) was inoculate with approximately 100 µl of seed culture and allow to solidify. Sterile disc was load with 100 µl of extract and incubated at 37°C overnight along with positive and negative controls. [17]

Agar disk diffusion assay

Cultures used for antimicrobial activity: The microorganisms used were as follows, Staphylococcus aureus NCIM 5021, Pseudomonas aeruginosa NCIM 2036, Bacillus subtilis NCIM 2010 and Salmonella typhimurium NCIM 2501. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Catharanthus pusillus* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes.[18]

Agar well diffusion assay

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Result:

Solubility determination

Table 1: Solubility determination of extract

S. No.	Solvent	Solubility of methanolic extract
1.	Water	Soluble
2.	Acetone	Insoluble
3.	Chloroform	Partial soluble
4.	Methanol	Soluble
5.	Petroleum ether	Partial soluble
6.	Ethylacetate	Partial soluble
7.	DMSO	Soluble

Phytochemical testing

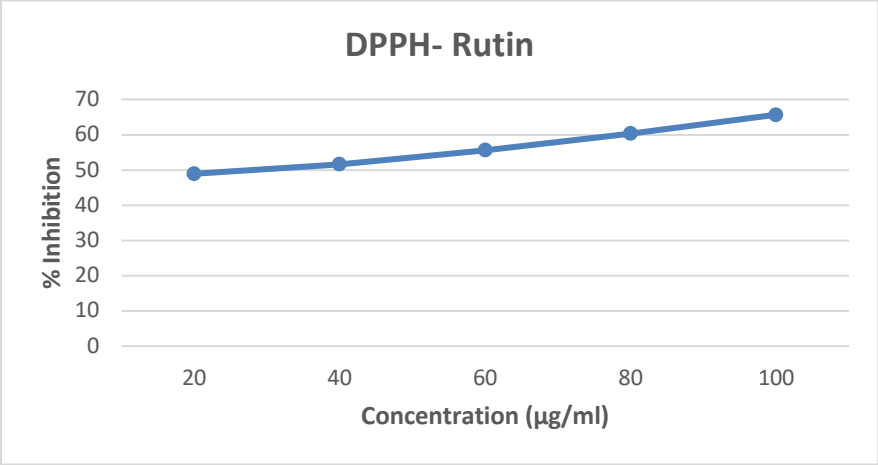
Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test
1.	**Alkaloids**	
1.1	Mayer's reagent test	Absent
1.2	Wagner's reagent test	Absent
1.3	Hager's reagent test	Absent
2.	**Carbohydrates**	
2.1	Molish's test	Present
2.2	Fehling's test	Absent
2.3	Benedict's test	Absent
2.4	Barfoed's test	Absent
3	**Proteins and Amino Acids**	
3.1	Biuret test	Absent
4.	**Flavonoids**	
4.1	Alkaline reagent test	Present
4.2	Lead Acetate test	Present
5.	**Glycoside**	
5.1	Borntrager test	Absent
5.2	Legal's test	Absent
5.3	Killer-Killiani test	Absent
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present
6.2	Lead Acetate test	Present
6.3	Gelatin test	Absent
7.	**Saponin**	
7.1	Foam test	Absent
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Present
8.2	Libbermann-Burchard's test	Present

Antioxidant assay (DPPH assay)
DPPH readings

Table 3: DPPH readings for control group

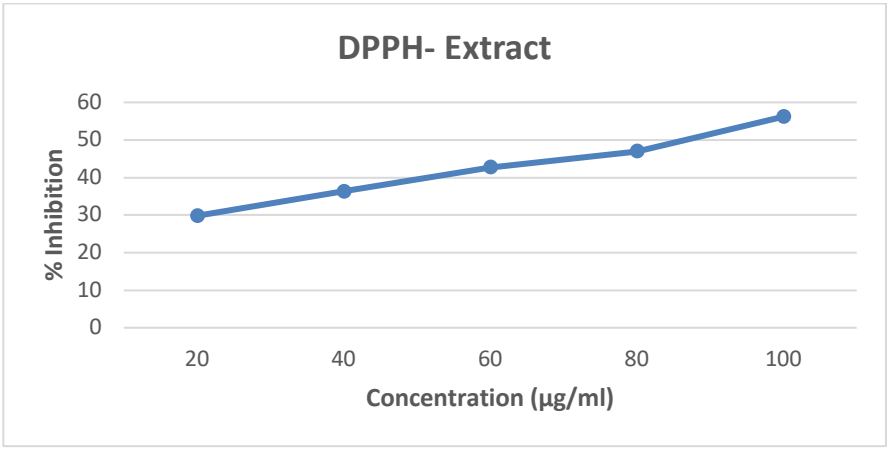
S. No.	Concentration (mcg/ml)	% inhibition
1.	20mcg/ml	48.9011
2.	40mcg/ml	51.64835
3.	60mcg/ml	55.63187
4.	80mcg/ml	60.3022
5.	100mcg/ml	65.65934



Graph 1: DPPH Control group

Table 4: DPPH readings for test group

S. No.	Concentration (mcg/ml)	% inhibition
1.	20mcg/ml	29.80769
2.	40mcg/ml	36.26374
3.	60mcg/ml	42.71978
4.	80mcg/ml	46.97802
5.	100mcg/ml	56.18132



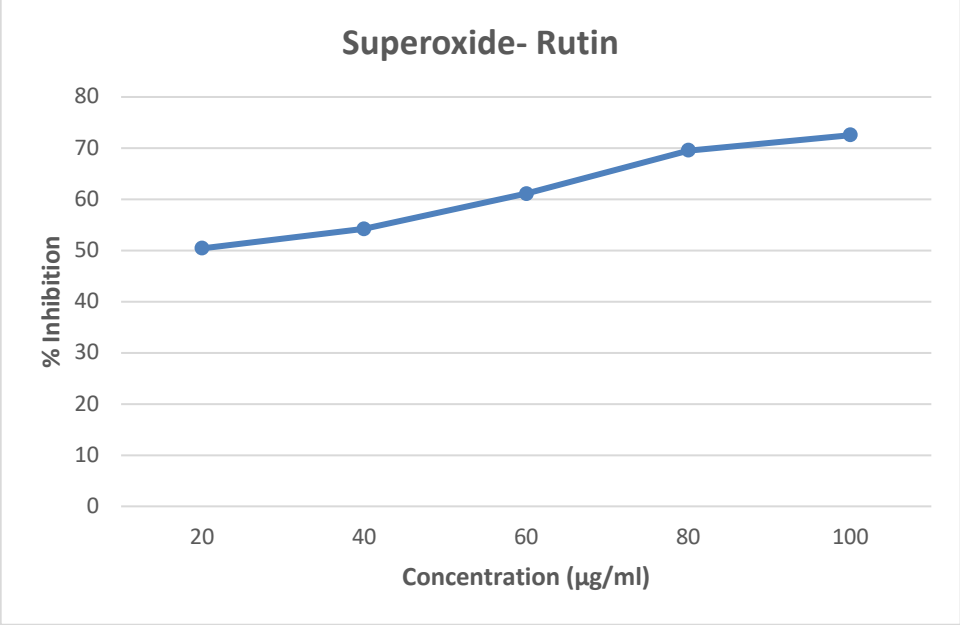
Graph 2: DPPH extract group

The control group have IC50= 22.9. Which is its 50% inhibition concentration and extract treated group have IC50= 81.3, which is close to the control group so, we can say that the extract consist antioxidant property.

Superoxide assay

Table 5: Superoxide assay readings for control group

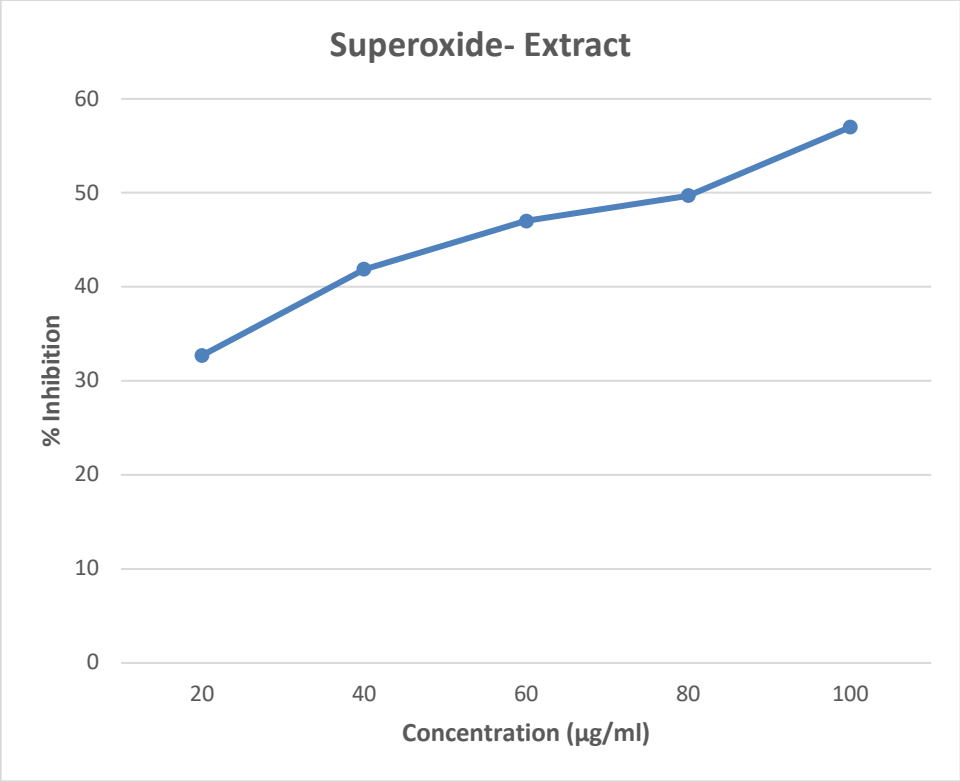
S. No.	Concentration (mcg/ml)	% inhibition
1.	20mcg/ml	50.41152
2.	40mcg/ml	54.21811
3.	60mcg/ml	61.11111
4.	80mcg/ml	69.54733
5.	100mcg/ml	72.53086



Graph 3: Graph for superoxide assay control group

Table 6 Superoxide assay readings for extract group

S. No.	Concentration (mcg/ml)	% inhibition
1.	20mcg/ml	32.71605
2.	40mcg/ml	41.87243
3.	60mcg/ml	47.01646
4.	80mcg/ml	49.69136
5.	100mcg/ml	56.99588



Graph 4: Graph for superoxide assay extract group

The control group have IC50= 11.18. Which is its 50% inhibition concentration and extract treated group have IC50= 68.3, which is close to the control group so, we can say that the extract consist antioxidant property and on the basis of these two studies it is confirmed that the extract have an antioxidant property.

Antimicrobial Activity (In vitro evaluation of antibacterial activity)

Antibacterial Susceptibility Assay

Table 7: Anti-microbial activity of *C. pusillus* methanol extract

S.No.	Organism	Extract
1.	Escherichia coli	++
2.	Pseudomonas aeruginosa	+
3.	Serratia marcescens	+++
4.	Salmonella typii	+++
5.	Staphylococcus aureus	-
6.	Streptococcus pyrogens	+
7.	Bacillus cereus	++
8.	Bacillus subtilis	+

Phytochemical extract was found to be inhibitory. Gram-negative bacteria were found more susceptible as compared to Gram-positive species. However, the efficacies of plant extract was less than the standard.

Agar disk diffusion assay

The antimicrobial activity of the extract of *Catharanthus pusillus* were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* NCIM 5021, Bacillus subtilis NCIM 2010) and two Gram-negative.

Table 8: Antibacterial activity of hydroalcoholic extract of leaves of *Catharanthus pusillus* against bacterial test organism.

S.No.	Microorganism	Zone of inhibition in mm Concentration in (µg/ml)				
		5	25	50	100	125
1.	*Staphylococcus aureus*	-	13	15	16	18
2.	*Bacillus subtilis*	-	11	12	13	15
3.	*Salmonella typhimurium*	-	14	16	18	20
4.	*Pseudomonas aeruginosa*	-	10	12	13	16

Values are mean ±SD of three parallel measurements, - = No zone of inhibition

Table 9: Antibacterial activity of standard drugs against bacterial test organism

S.No.	Drug	Microorganism	Zone of inhibition in mm Concentration in (µg/ml)				
			5	25	50	100	125
1.	*Ampicillin*	*Staphylococcus aureus*	13	14	16	20	24
		Bacillus subtilis	12	15	18	22	25
		Salmonella typhimurium	14	15	18	20	26
		Pseudomonas aeruginosa	10	13	15	18	22
2.	*Chloramphenicol*	*Staphylococcus aureus*	11	13	16	18	23
		Bacillus subtilis	14	15	18	22	25
		Salmonella typhimurium	12	14	16	19	24
		Pseudomonas aeruginosa	15	17	20	23	26

Values are mean ±SD of three parallel measurements.

The results show that the extracts of *Catharanthus pusillus* were found to be effective against all the microbes tested.

Agar well diffusion assay

The antimicrobial activity of the extract of *Catharanthus pusillus* were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* NCIM 5021, Bacillus subtilis NCIM 2010) and two Gram-negative (Salmonella typhimurium NCIM 2501, *Pseudomonas aeruginosa* NCIM 2036).

Table 10: Antibacterial activity of hydroalcoholic extract of leaves of *Catharanthus pusillus* against bacterial test organism.

S.No.	Microorganism	Zone of inhibition in mm Concentration in (µg/ml)				
		5	25	50	100	125
1.	*Staphylococcus aureus*	-	9	11	14	18
2.	*Bacillus subtilis*	-	11	14	16	19
3.	*Salmonella typhimurium*	-	10	13	14	18
4.	*Pseudomonas aeruginosa*	-	10	14	17	19

Values are mean ±SD of three parallel measurements, - = No zone of inhibition

Table 11: Antibacterial activity of standard drugs against bacterial test organism

S.No.	Drug	Microorganism	Zone of inhibition in mm				
			Concentration in (µg/ml)				
			5	25	50	100	125
1.	*Ampicillin*	*Staphylococcus aureus*	11	14	15	18	20
		Bacillus subtilis	12	14	17	19	22
		Salmonella typhimurium	11	13	16	17	20
		Pseudomonas aeruginosa	10	13	16	19	22
2.	*Chloramphenicol*	*Staphylococcus aureus*	12	15	17	20	21
		Bacillus subtilis	13	14	18	20	23
		Salmonella typhimurium	11	14	16	19	22
		Pseudomonas aeruginosa	11	13	16	17	21

Values are mean ±SD of three parallel measurements.

Conclusion:

The present research was aimed at screening medicinal plants and extracts for their antimicrobial activity and thereby identifying potential plant extracts for further development as safe, effective, affordable, alternative therapeutic agents, most likely new antimicrobials. The objectives have been met to an appreciable extent, though further research and efforts are warranted to realize the absolute goal. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and phenolic compounds have been shown to posses antimicrobial activities against a number of microorganisms.

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