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Eco-Friendly Antibacterial Potential of *Catharanthus roseus* Leaf Extracts: A Green Approach to Natural Drug Discovery

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Abstract: Virulent diseases continue to cause millions of global deaths annually, exacerbated by the growing issue of microbial resistance, which casts uncertainty on the future use of antimicrobial drugs. *Catharanthus roseus* (Periwinkle or Sadabahar), a medicinal plant belonging to the *Apocynaceae* family, is abundantly found in tropical regions and is rich in phytochemicals. The leaves of *C. roseus* are commonly used in traditional medicine for the treatment of various critical illnesses. This research aims to explore the phytochemical composition and antimicrobial activity of both aqueous and methanolic extracts of *C. roseus* leaves. The study evaluated the presence of key phytochemicals such as flavonoids, glycosides, phenols, saponins, tannins, terpenoids, and steroids, alongside testing the antibacterial potential of the extracts. The extracts were tested against four common bacterial strains using the agar well diffusion method, with Ciprofloxacin serving as the standard control. The results revealed that the aqueous extract exhibited the highest antibacterial activity, with a zone of inhibition of 55 ± 0.6 mm against *Staphylococcus aureus* (*S. aureus*), followed by 45 ± 0.3 mm against *Salmonella enterica*. The methanolic extract also showed significant antibacterial activity, with a zone of inhibition of 40 ± 0.2 mm against *S. aureus*. Conversely, the methanol extract showed the smallest inhibition zones against *Bacillus subtilis* (24.0 mm) and *Escherichia coli* (*E. coli*) (25.0 mm). Phytochemical analysis of methanolic extract revealed the presence of glycosides, flavonoids, phenols, tannins, steroids, saponins, and terpenoids. These findings confirm the promising antimicrobial efficacy of both the methanolic and aqueous extracts of *C. roseus*, particularly against *E. coli*, *S. aureus*, *S. enterica*, and *B. subtilis*. The study supports the therapeutic potential of *C. roseus* as a valuable medicinal resource and eco-friendly alternative for combating microbial infections and producing value-added products, including treatments for complex diseases such as cancer.

Keywords: *Catharathus roseus*; antibacterial; agar well diffusion assay; methanolic extract; microorganisms; aqueous extract

1. Introduction

Many wild and weedy plant species present in our surroundings remain largely undocumented, despite possessing significant medicinal potential capable of treating a wide spectrum of health disorders [1,2]. In recent years, the overuse of chemically synthesized antibiotics has led to the emergence of drug-resistant pathogens, thereby weakening the efficacy of conventional therapeutic approaches and adversely impacting the human immune system. This alarming trend has intensified the global pursuit of alternative, natural remedies capable of countering such conditions. As a result, there has been a renewed interest in exploring plant-based compounds for

drug development, particularly those with the potential to combat antimicrobial resistance. Nature offers an abundant reservoir of medicinal plants that are pivotal in promoting human health and survival [3]. In the contemporary era, the reliance on herbal medicine has gained renewed relevance, especially as pharmaceutical industries continue to isolate and synthesize bioactive constituents from medicinal plants to develop novel therapeutic agents.

Catharanthus roseus is a well-recognized herbal medicinal plant with a long-standing history of therapeutic use [4]. Native to Madagascar, it is now widely distributed across South Africa, Australia, the United States, Southern Europe, and is commonly cultivated as an ornamental plant throughout India. Various parts of *C. roseus* have been traditionally employed in folklore medicine to treat numerous infectious diseases. Notably, the leaves of *C. roseus* are rich in pharmacologically important vinca alkaloids, including vinblastine and vincristine—two potent anticancer agents that have been extensively utilized in chemotherapy [5]. Beyond these, *C. roseus* leaves contain over 70 bioactive compounds, such as ajmalicine, reserpine, and serpentine, which contribute to the plant's broad therapeutic potential. Alkaloids, as the principal class of active constituents in *C. roseus*, are widely used to treat several serious health conditions including diabetes, hypertension, dysmenorrhea, asthma, constipation, and cancer [6].

The present study evaluated the antibacterial activity and phytochemical properties of *Catharanthus roseus*, an important medicinal plant known for its diverse therapeutic potential.

Commonly known by several synonyms, including *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea*, *C. roseus* is believed to have originated in India and is found growing wild throughout the Indian subcontinent and southern Asia [7]. Cultivated varieties are often distinguished by flower color, with the pink variety referred to as *Rosea* and the white variety as *Alba* [8]. Traditionally, the leaves of *C. roseus* have been widely used in herbal medicine for the treatment of a range of ailments including menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, cancer, menstrual disorders, skin diseases, and bleeding diarrhea. Additionally, the plant is noted for its sedative and antiviral properties [9].

Catharanthus roseus is known to synthesize a variety of bioactive compounds exhibiting antimicrobial, antioxidant, anti-diarrheal, hypolipidemic, and wound-healing activities [10]. The present study focuses on the phytochemical profiling and evaluation of the antimicrobial potential of both methanolic and aqueous extracts of *C. roseus*. The objective is to explore natural sources for the development of novel antimicrobial agents to address the growing global demand for effective alternatives to synthetic antibiotics. This work builds upon our previous investigations into the phytochemical and antimicrobial properties of other medicinal plant species, including *Taxus wallichiana Zucc.* [11], as well as *Prosopis cineraria* (ghaf) and various mangrove species [12].

2. Material and Methods

2.1. Collection and Preparation of Plant Material

Fully mature *Catharanthus roseus* plants leaves (Figure 1) were collected in January 2025 from the premises of the Environment Laboratory, Ras Al Khaimah Municipality, United Arab Emirates. The collected plant leaves were initially washed thoroughly under running tap water to remove dust and surface contaminants, followed by rinsing with sterilized distilled water to ensure aseptic conditions. Excess moisture was removed using sterile filter paper. The leaves were carefully separated, air-dried at 45 °C for 6 h in a controlled drying oven, and subsequently ground into fine powder using a laboratory-grade electric mixer [11]. The powdered material was stored in airtight containers at room temperature until further use for extraction and analysis.

(**a**) (**b**)

Figure 1. (**a**) Plant with flower, (**b**) Leaves, of *Catharathus roseus.*

2.2. Preparation of Plant Extracts

For extraction, 10 g of powdered *Catharanthus roseus* leaves were soaked in 100 mL of each solvent—aqueous (distilled water) and methanol—separately. The aqueous extraction was performed by soaking the plant material overnight in a rotary shaker to ensure uniform mixing and optimal solubilization of bioactive compounds. The supernatant was subsequently collected and filtered for further analysis.

The methanolic extract was prepared using the continuous hot extraction method, following the Soxhlet apparatus technique described by [13]. The extract was concentrated by evaporating the solvent under reduced pressure and stored at 4 °C in sterile containers for subsequent phytochemical and antimicrobial analysis.

2.3. Microbial Strains and Culture Conditions

In the present study, the antibacterial activity of *Catharanthus roseus* extracts was evaluated against four standard bacterial strains: *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), and *S. aureus* (ATCC 6538). All strains were obtained from the American Type Culture Collection (ATCC) and provided by LTA srl, Italy. Pure cultures of each bacterial strain were maintained on nutrient agar slants at 4 °C for preservation. For the experimental assays, subcultures were prepared and used in triplicates to ensure the reproducibility and reliability of results.

2.4. Methodology for the Detection of Antibacterial Activity

2.4.1. Inocula Preparation

Bacterial isolates were cultured in 5 mL of sterile Nutrient Broth and incubated at 37 °C for 18 h to obtain actively growing cultures. These overnight cultures were used as inocula for subsequent antimicrobial assays.

2.4.2. Agar Well Diffusion Assay

The antibacterial activity of *Catharanthus roseus* leaf extracts (methanolic and aqueous) was assessed using the agar well diffusion method, as described by [11]. A volume of 30 µL of each extract was introduced into wells (6 mm in diameter) punched into Mueller-Hinton agar plates inoculated with standardized bacterial suspensions. The plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured in millimeters to evaluate antimicrobial efficacy. All assays were performed in triplicate to ensure accuracy and reproducibility, and the mean values were recorded. The entire experiment was conducted under strict aseptic conditions to prevent any contamination.

2.5. Phytochemical Analysis

Qualitative phytochemical screening was conducted on the plant extracts to identify the presence of bioactive compounds using standard procedures.

To detect flavonoids, the extract was mixed with ammonia solution in a 1:5 ratio, followed by the addition of concentrated sulfuric acid. The appearance of a yellow color that disappears on standing indicated the presence of flavonoids.

For glycoside detection, the extract was treated with glacial acetic acid, a few drops of ferric chloride, and concentrated sulfuric acid. A brown ring formation at the interface confirmed the presence of glycosides.

Phenolic compounds were tested by adding a few drops of 0.5% neutral ferric chloride solution to the extract. A dark green coloration indicated a positive result.

Steroids were identified by dissolving the extract in chloroform and carefully layering it with concentrated sulfuric acid. A red upper layer and a yellow lower layer exhibiting green fluorescence were indicative of steroids.

To test for saponins, the extract was combined with distilled water, boiled in a water bath, and vigorously shaken. Persistent froth formation confirmed the presence of saponins.

The presence of tannins was determined by boiling the extract with distilled water, cooling it, and adding 0.1% ferric chloride solution. The development of a brownish-green or blue-black color indicated the presence of tannins.

Terpenoids were tested by mixing the extract with chloroform and concentrated sulfuric acid. The appearance of a reddish-brown layer at the interface confirmed their presence.

3. Results and Discussion

The present study aimed to evaluate the phytochemical composition and antimicrobial activity of methanolic and aqueous extracts of *Catharanthus roseus* leaves. Both extracts were prepared at 10 g per 100 mL of solvent.

The antibacterial efficacy of these extracts was tested against four bacterial strains: *Bacillus subtilis*, *E. coli*, *Salmonella enterica*, and *S. aureus*. The results of the phytochemical screening and the agar well diffusion assay is discussed below, highlighting the plant's potential as a source of bioactive antimicrobial agents.

3.1. Antibacterial Activity of Catharanthus roseus Leaf Extracts

The antibacterial potential of *Catharanthus roseus* leaf extracts were evaluated using the agar well diffusion method. Among the tested samples, the aqueous extract exhibited the highest antibacterial activity, producing a zone of inhibition measuring 55 ± 0.6 mm against *S. aureus*. The methanolic extract also showed notable activity, with a maximum inhibition zone of 40 ± 0.2 mm against *S. aureus* (Table 1).

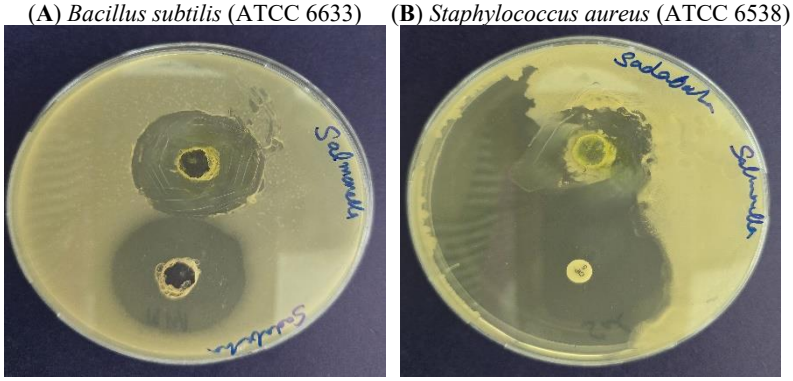
The minimum inhibitory effect was recorded for the methanolic extract against *Bacillus subtilis* (Figure 2A), with a zone of 24.0 mm, followed by 25.0 mm against *E. coli* (Figure 2C). These results suggest a differential sensitivity of bacterial strains to the type of solvent used in the extraction process.

Table 1. Zone of inhibition (mm) of methanolic and aqueous extracts of *Catharanthus roseus* leaves against test bacterial strains.

S No.	Microorganisms	Zone of Inhibition (mm) Methanol Extract (ME)	Zone of Inhibition (mm) Aqueous Extract (Aq E)
1	*Bacillus subtilis* (ATCC 6633)	24 ± 0.0	30 ± 0.5
2	*E. coli* (ATCC 8739)	25 ± 0.5	39 ± 0.8
3	*Salmonella enterica* (ATCC 14028)	33 ± 0.2	45 ± 0.3
4	*Staphylococcus aureus* (ATCC 6538)	40 ± 0.2	55 ± 0.6

Note: Values represent the mean ± standard deviation of triplicate experiments.

(**A**) *Bacillus subtilis* (ATCC 6633) (**B**) *Staphylococcus aureus* (ATCC 6538) (**C**) *E. coli* (ATCC 8739)



(**D**) *Salmonella enterica* (ATCC 14028) (**E**) Ciprofloxacin standard

Figure 2. Illustrates the zones of inhibition for both methanolic and aqueous extracts of *C. roseus* leaves against the tested bacterial strains, providing a visual comparison of antibacterial efficacy. (**A**) Zone of inhibition produced by aqueous and methanolic extracts against *Bacillus subtilis.* (**B**) showing zone of inhibition difference between aqueous and methanolic extract against *S. aureus* (**C**) Zone of inhibition produced by aqueous and methanolic extracts against *E. coli* (**D**) showing zone of inhibition difference between aqueous and methanolic extract against *Salmonella enterica* (**E**) Last picture is ciprofloxacin standard against *Salmonella enterica.*

The findings agree with previous studies, [14] reported that leaf extracts of *C. roseus* demonstrated substantial activity against *Salmonella* and *S. aureus*, with comparatively lower inhibition against *E. coli*. Similarly, [15] observed pronounced antimicrobial activity of *C. roseus* leaf extracts against *Salmonella typhimurium* and *S. aureus*, supporting the outcomes of the present study. Furthermore, [16] assessed the antibacterial efficacy of

ethanolic extracts of *C. roseus* against *E. coli* and five additional bacterial strains, reporting the highest inhibition against *Klebsiella pneumoniae*, followed by *E. coli*, with ethanol being more effective than methanol. These findings parallel our results, indicating the promising antibacterial properties of *C. roseus* leaf extracts, particularly in aqueous form.

[17] also reported similar outcomes, where aqueous extracts of *C. roseus* demonstrated strong antibacterial effects, especially against *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, and *Bacillus cereus*, corroborating the antibacterial potential observed in the current study.

3.2. Phytochemical Analysis of Catharanthus roseus Leaves

In this study, methanolic extracts of *Catharanthus roseus* leaves were analyzed for the presence of key phytochemicals, including steroids, saponins, tannins, terpenoids, flavonoids, glycosides, and phenolic compounds. The results indicated the presence of all tested secondary metabolites in the methanolic leaf extract, as detailed in Table 2 and illustrated in Figure 3. These bioactive constituents are known to contribute to the plant's therapeutic potential, particularly its antimicrobial and antioxidant properties.

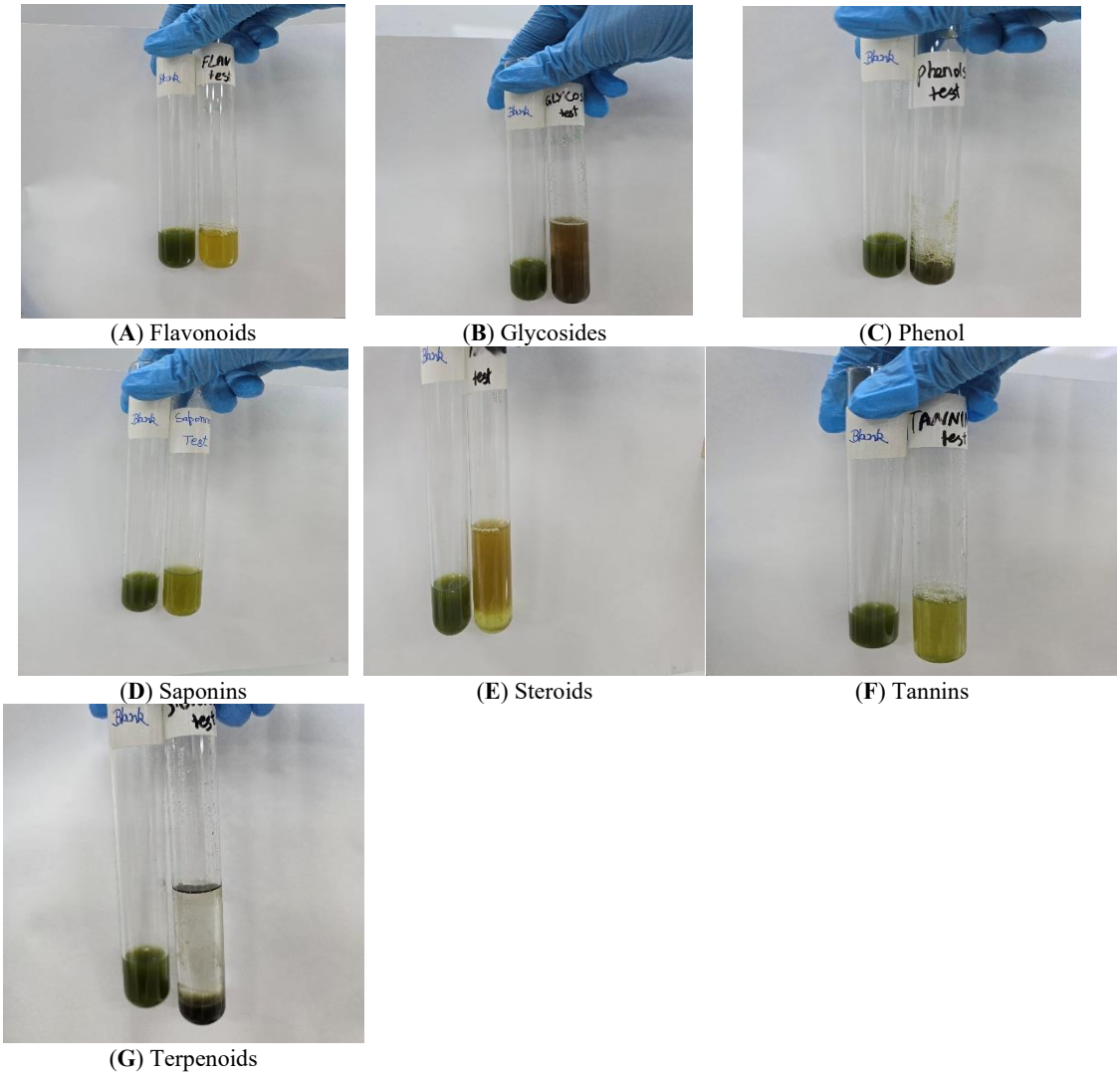


Figure 3. Showing the confirmation of Phytochemicals present in crude extract of *Catharathus roseus* leaves (**A**)Flavonoids (**B**) Glycosides (**C**) Phenol (**D**) Saponins (**E**) Steroids (**F**) Tannins (**G**) Terpenoids.

Table 2. Phytochemicals in methanolic crude extracts of *Catharathus roseus* leaves.

S No.	Phytochemicals	*Catharathus roseus* Leaves Methanolic Extract
1	Flavonoids	+
2	Glycosides	+
3	Phenol	+
4	Saponins	+
5	Tannins	+
6	Terpenoids	+
7	Steroids	+

The findings are consistent with those reported by [18], who also documented the presence of significant phytoconstituents such as saponins, terpenoids, alkaloids, and tannins in the methanolic extracts of *C. roseus* leaves. The presence of such a wide array of phytochemicals reinforces the pharmacological relevance of *C. roseus* and supports its traditional use in herbal medicine.

These results confirm the presence of multiple phytochemical groups known for their therapeutic relevance, particularly antimicrobial, antioxidant, and anti-inflammatory activities. The higher efficacy of the methanolic extract may be attributed to its superior solvent capacity for polar and non-polar bioactive compounds.

The present study highlights the promising antibacterial activity of *Catharanthus roseus* leaf extracts, particularly the aqueous extract, against both Gram-positive and Gram-negative bacterial strains. The observed antibacterial effects are attributed to the rich phytochemical profile of the plant, including flavonoids, glycosides, phenolics, saponins, tannins, terpenoids, and steroids, which possess antimicrobial potential through various mechanisms.

Flavonoids and phenolic compounds are widely reported to exert antibacterial activity by disrupting bacterial cell membranes, chelating metal ions essential for microbial metabolism, and interfering with nucleic acid synthesis. Saponins can increase cell membrane permeability, leading to leakage of essential intracellular components. Tannins can bind with proteins and enzymes, inactivating microbial adhesins and enzymes necessary for pathogenicity. Terpenoids and steroids are believed to interfere with the lipid layer of microbial cell membranes, affecting membrane integrity and leading to cell lysis.

Interestingly, the aqueous extract exhibited a significantly larger zone of inhibition than the methanolic extract across all tested bacteria, particularly against *S. aureus* (55 ± 0.6 mm). This suggests that water may be a more efficient solvent in extracting certain polar bioactive compounds from *C. roseus* leaves. While methanol is commonly used for phytochemical extraction due to its polarity, the effectiveness of aqueous extraction in this study may reflect the nature of specific compounds more soluble or active in water, such as glycosides and tannins.

The tested bacterial strains are medically significant. *S. aureus* is a major cause of skin infections, pneumonia, and bloodstream infections, often associated with antibiotic resistance, including methicillin-resistant *S. aureus* (MRSA). *E. coli* and *Salmonella enterica* are leading causes of foodborne illnesses and urinary tract infections, while *Bacillus subtilis*, although generally non-pathogenic, serves as a model for Gram-positive bacteria and can cause opportunistic infections. The increasing emergence of drug-resistant strains of these pathogens has necessitated the search for novel antimicrobial agents, particularly from natural sources.

From a therapeutic standpoint, the results support the potential application of *C. roseus* extracts in developing alternative or complementary antimicrobial treatments. In conclusion, the present study confirms the antimicrobial potential of *Catharanthus roseus* leaf extracts, with aqueous extracts demonstrating superior efficacy. These findings provide a scientific basis for the traditional use of *C. roseus* in herbal medicine and warrant further investigation for potential pharmaceutical development, especially in the context of growing antimicrobial resistance.

4. Conclusions

In the present study, phytochemical and antimicrobial analyses were conducted on aqueous and methanolic leaf extracts of *Catharanthus roseus* (commonly known as Sadabahar). Between the two solvent systems, the aqueous extract exhibited superior antibacterial activity across all tested microbial strains, while the methanolic extract proved more effective in phytochemical screening, revealing the presence of key secondary metabolites such as glycosides, flavonoids, phenols, tannins, steroids, saponins, and terpenoids.

These findings underscore the promising antibacterial potential of *C. roseus* leaf extracts and validate its traditional medicinal use. Moreover, the demonstrated broad-spectrum efficacy positions *C. roseus* as a viable candidate for the development of plant-based antimicrobial agents. This research highlights the utility of *C. roseus*

in green synthesis approaches and offers an eco-friendly, sustainable, and cost-effective alternative for combating emerging and re-emerging infectious diseases. The study contributes to the expanding field of medicinal plant research, supporting the therapeutic potential of *C. roseus* for future biomedical and pharmaceutical applications.

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Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The relevant data and materials are available in the present study.

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Conflicts of Interest

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

Abbreviations

SD, standard deviation; ATCC, American Type Culture Collection; E, Extract; h, hours; C, ciprofloxacin; *C. roseus*, *Catharanthus roseus*.

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