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EVALUATION OF ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL SCREENING OF CATHARANTHUS ROSEUS

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ABSTRACT

Plants have long been the focus of the medical sector due to their numerous biological and medicinal properties.Natural alternatives must be investigated because antibiotic resistance has grown to be a global concern. The antibacterial potential and phytochemical components of *Catharanthus roseus* in four solvent extracts chloroform (C), ethyl acetate (EA), methanol (M), and aqueous prepared from the leaf material of this medicinal plant, which is rich in bioactive alkaloids, are examined in this work.Alkaloids, flavonoids, saponins, tannins, and terpenoids were detected by phytochemical screening; methanol and ethyl acetate extracts showed the greatest phytochemical variety across all seven constituent classes. The extract with the highest alkaloid concentration was chloroform . Using the agar well diffusion method and streptomycin (40 µg/ml) as the positive control, antibacterial activity was assessed against four clinically significant bacterial strains: *Klebsiella pneumoniae* (MTCC#8911), *Bacillus subtilis* (MTCC#736), *Escherichia coli* (MTCC#45), and *Staphylococcus aureus* (MTCC#10787). The results showed that the chloroform extract had the strongest antibacterial activity, resulting in the biggest zones of inhibition against *B. subtilis* (25 mm) and *E. coli* (26 mm). Methanol and aqueous extracts showed selective and somewhat modest inhibition, but ethyl acetate extract consistently showed broad-spectrum action across all four test species. *B. subtilis* and *E. coli* were not

affected by the aqueous extract. These results imply that *Catharanthus roseus* contains bioactive substances with noteworthy antibacterial qualities, especially in non-polar to semi-polar solvent fractions.

KEYWORDS: Antimicrobial activity, *Catharanthus roseus*,zone of inhibition, solvent extracts, alkaloids, agar well diffusion, bioactive compounds.

1. INTRODUCTION

Antibiotic resistance among bacterial pathogens has emerged as one of the most significant global health threats (WHO, 2023). To address this, medicinal plants are being extensively investigated as natural reservoirs of antimicrobial agents (Smith, Thomas, & Williams, 2023). The Apocynaceae family includes *Catharanthus roseus*, also referred to as Madagascar periwinkle, which has shown strong antibacterial action against a range of harmful pathogens. *Catharanthus roseus* is a perennial herb traditionally used to treat diabetes, malaria, and microbial infections (Pandey *et al*., 2016) They show potential as natural antibiotic sources due to their capacity to fight a variety of bacterial infections.

The plant is globally recognized for producing antineoplastic alkaloids vincristine and vinblastine but is also known for secondary metabolites exhibiting antibacterial and antioxidant effects (Gupta *et al.*, 2023). Previous studies by Khan *et al.* (2016) and Sharma & Agrawal (2016) demonstrated strong antibacterial activity of methanolic and ethanolic extracts of *Catharanthus roseus* leaves, suggesting its potential as a natural antibacterial source. Recent investigations (Bhardwaj, 2025; Jamal & Ahmad, 2024; Rekha & Mamatha, 2023) have identified compounds such as vindoline, quercetin, and chlorogenic acid responsible for microbial inhibition. This research evaluates antibacterial potential and phytochemical profile of C. roseus using various solvent extracts to establish a correlation between chemical constituents and biological activity.Alkaloids and tannins were abundant in methanol extract, while chloroform and ethyl acetate extracts were rich in flavonoids. Aqueous extract contained fewer compounds. This chemical variation corresponds with antibacterial efficiency, suggesting that alkaloids and flavonoids contribute significantly to antibacterial action (Rani & Raj, 2024).

The antibacterial activity of Catharanthus roseus can be attributed to the synergistic effect of multiple phytochemicals. Chloroform extract showed maximum inhibition, which aligns with findings of Jamal & Ahmad (2024), who noted nonpolar extracts possess higher antibacterial

efficacy. The study also supports Rekha & Mamatha (2023), who reported that Gram-positive bacteria are generally more susceptible due to simpler cell walls. Compounds such as vindoline, serpentine, and ajmalicine may act by disrupting bacterial membranes or inhibiting nucleic acid synthesis (Singh et al., 2018). The significant correlation between phytochemical richness and antibacterial potency emphasizes the pharmacological value of plant-derived compounds. These results reaffirm Catharanthus roseus as a potential bioresource for developing natural antibiotics, especially in the context of growing resistance (Brown & Johnson, 2023).

2. MATERIALS AND METHODS

Fresh leaves of *Catharanthus roseus* were collected from Dumna Nature Reserve Jabalpur, Madhya Pradesh, India, carefully stored in a collection bag and dried at room temperature and grinded to powder using mortar and pestle in the laboratory.

Solvent Extraction:1gm of powdered plant leaf samples were dissolved in 10ml of different solvents chloroform, ethyl acetate, methanol, and water. The extract was then filtered with the help of Whatmann No.1 filter paper. The filtrate was used for antibacterial activity and phytochemical screening using various bioassays.

Test Organisms used for antibacterial activity Assay: Four Bacterial species were used in screening for antibacterial activities which were procured from Central Research Laboratory, Mata Gujri Mahila Mahaidyalaya (Autonomous) Jabalpur,M.P.

Antibacterial Activity via Agar Well Assay

The Antibacterial activity of the filtrates was tested against *Bacillus subtilis, Staphylococcus aureus, and Escherichia coli and Klebsiella pneumoniae* via agar well diffusion.Antibacterial Activity via Agar Well Assay In this Assay Nutrient Agar Media (Peptone:5 gm; Beef extract: 3 gm; NaCl: 5 gm; Agar: 20 gm; Distilled Water: 1000 ml) plates. Plates were prepared and 25 µl of bacterial suspension was applied on each of these prepared plates under laminar air flow by spread plate method. Wells of 4-5 mm diameter were prepared on the plates with the help of sterilized cork borer. The filtrates were obtained after solvent extraction was filled in the wells with the help of micropipette (100 µl). These plates were then kept in bacteriological incubator at 37 °C for 24 hrs. After incubation the activity was recorded in terms of zone of inhibition in mm.

Standard qualitative assays were used for phytochemical screening: Hager's test (alkaloids), alkaline reagent test (flavonoids), ferric chloride test (tannins), and froth test (saponins).

Phytochemical Screening

Test for alkaloids: Hager's test – extracts were dissolved individually in dilute HCl and filtered. Filtrate was treated with Hager's reagent. Formation of yellow coloured precipitate indicated the presence of alkaloids.

Test for flavonoids: Alkaline Reagent test–In the test solution having extract few drops of NaOH solution was added. Formation of intense yellow colour which turns to colourless by addition of few drop of dilute acetic acid indicated the presence of flavonoids.

Test for Tannins: Ferric chloride test: To the test solution having extract few drops of ferric chloride solution was added. An intense green, purple, blue or black colour indicated the presence of tannin.

Test for Saponins: Froth test: - In the test solution having extract, 2- 3 ml of distilled water was added. The mixture was shaken vigorously. Formation of foam indicated the presence of saponins.

Test for Regins: In a dry test tube 1ml of extract was taken and one drop of concentrated Sulphuric Acid was added to it. Formation of purple colour changes into violet indicating the presence of regins.

3.RESULTS AND DISCUSSION

The results revealed distinct variations in antibacterial potential among solvent extracts. Chloroform extract showed the highest inhibition against all test organisms, while aqueous extract was least active.

Table 3.1 shows the antibacterial activity of *Catharanthus roseus* plant extracts prepared in different organic solvents Chloroform (C), Ethyl Acetate (EA), Methanol (M), and Aqueous extract tested against four pathogenic bacteria. Activity is measured as the zone of inhibition (mm) using the disc diffusion method, compared against Streptomycin (40 µg/ml) as a positive control.The *Klebsiella pneumoniae* MTCC#8911 Chloroform extract showed no activity, while Ethyl Acetate shows 18mm zone, Methanol shows 12mm, and Aqueous extract 10mm, showed moderate inhibition.The positive control Streptomycin gave 15mm,

suggesting Ethyl Acetate extract performed comparably better.*Bacillus subtilis* MTCC#736 Chloroform extract showed 25mm zone and Ethyl Acetate 18mm showed strong activity, matching the positive control 18mm.Methanol and Aqueous extracts showed no activity.Escherichia coli MTCC#45the strongest inhibition overall was seen with Chloroform 26mm, followed by Ethyl Acetate 18mm and Methanol 15mm.Aqueous extract showed no activity, yet Chloroform exceeded the positive control 16mm.*Staphylococcus aureus* MTCC#10787 moderate activity with Chloroform 20mm, Methanol 12mm, and Ethyl Acetate 10mm while aqueous extract showed only weak inhibition 5mm, and no aqueous data was recorded for methanol.

Chloroform extract was the most potent solvent fraction overall, showing the highest zones of inhibition across multiple organisms.Ethyl Acetate extract showed consistent moderate activity against all four bacteria.Methanol and Aqueous extracts were generally less effective, with no activity against *B. subtilis and E. coli.*The negative control produced no inhibition in all cases, confirming the results are due to the plant extracts.These findings suggest *Catharanthus roseus* contains bioactive compounds with broad spectrum antibacterial potential, particularly concentrated in non-polar,semi-polar fractions.

Table 3.1: Antibacterial activities of the different organic solvents by using *Catharanthus roseus.*

S.No.	Test organism	Antibacterial activity Zone of inhibition (in mm)				Positive control Streptomysin (40µg/ml)	Negative control (100 µl solvents)
		C	EA	M	Aqueous extract		
01	*Klebsiella pneumoniae* MTCC#8911	----	18mm	12mm	10mm	15mm	---
02	*Bacillus subtilis* MTCC#736	25mm	18mm	00mm	00mm	18mm	----
03	*Escherichia coli* MTCC#45	26mm	18mm	15mm	00mm	16mm	----
04	*Staphylococcus aureus* MTCC#10787	20mm	10mm	12mm	-------	5mm	----

Phytochemical screening detected alkaloids, flavonoids, tannins, phenolics, and saponins across extracts. Methanol and ethyl acetate extracts exhibited higher phytochemical diversity. Table 3.2.The phytochemical screening of Catharanthus roseus across four solvent extracts

methanol, ethyl acetate, chloroform, and aqueous revealed a diverse array of bioactive compounds, with varying intensities depending on the polarity of the solvent used.

Table 3.2. The phytochemical screening of Catharanthus roseus across four solvent extracts methanol, ethyl acetate, chloroform, and aqueous extract.

Phytochemicals	Methanol	Ethyl acetate	Chloroform	Aqueous
Alkaloids	++	+	+++	-
Flavonoids	+	+	+	-
Saponins	+	+	-	+
Tannins	++	+	-	+
Glycosides	+	+	+	+
Phenolics	++	+	+	+
Terpenoids	+	+	-	-

Alkaloids detected in three of four extracts. Chloroform yielded the strongest presence (+++), suggesting these compounds are predominantly non-polar in nature. Methanol showed moderate levels (++), while ethyl acetate showed weak presence (+). The aqueous extract showed no detectable alkaloids (−), consistent with the low water-solubility typical of most alkaloids. Flavonoids were uniformly weak (+) across methanol, ethyl acetate, and chloroform extracts, and entirely absent in the aqueous extract. This suggests flavonoids are present but not in high concentrations, and their glycosylated forms may be limited. Saponins were present in methanol (+), ethyl acetate (+), and aqueous (+) extracts but absent in chloroform (−), which aligns with their known amphiphilic nature they partition into both polar and moderately polar solvents but not purely non-polar ones.Tannins showed moderate presence in methanol (++) and weak presence in ethyl acetate (+) and aqueous (+), but were absent in chloroform (−). This is expected, as tannins are predominantly polar, water- and alcohol-soluble polyphenols. Glycosides were uniformly detected (+) across all four extracts, making them the most consistently distributed phytochemical. Their presence in both polar and non-polar fractions reflects the structural diversity of glycosides in the plant. Phenolics demonstrated moderate presence in methanol (++) and weak but consistent detection in ethyl acetate, chloroform, and aqueous extracts (+). Their broad distribution reflects the diversity of phenolic subclasses ranging from polar to semi-polar. Terpenoids were detected only in methanol (+) and ethyl acetate (+), and were absent in both chloroform and aqueous extracts. This moderate-polarity distribution may indicate the presence of oxygenated terpenoids rather than purely non-polar terpene hydrocarbons.

CONCLUSION

Catharanthus roseus exhibits notable antibacterial potential against both Gram-positive and Gram-negative bacteria.Methanol and ethyl acetate are the most effective solvents for broad spectrum phytochemical extraction from *C. roseus*, capturing all seven compound classes. Chloroform is superior specifically for alkaloid extraction, which is pharmacologically significant given that C. roseus is the source of the important anticancer alkaloids vincristine and vinblastine.The aqueous extract showed the narrowest phytochemical profile, though its detection of saponins, tannins, glycosides, and phenolics still supports some biological activity.The overall richness of phytochemicals across extracts supports the well-established medicinal value of *C. roseus* and justifies its use in traditional and pharmaceutical medicine.Further studies should isolate active compounds, evaluate cytotoxicity, and explore synergistic combinations with existing antibiotics. Thus, *Catharanthus roseus* represents a promising source for development of plant-based antimicrobial agents.

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