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Phytochemical Screening and Antimicrobial Activity of *Catharanthus roseus*: Comparative Efficacy of Leaf, Flower and Root Extracts Against *Escherichia coli* and *Candida albicans*

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ABSTRACT

The rise in antibiotic-resistant pathogens has led to a search for new and effective natural antimicrobial agents. *Catharanthus roseus (C. roseus)*, locally known as Kemunting Cina is one of the widely recognised herbs that have a great number of medicinal uses mostly attributed to the various disease. The leaves, flowers and roots of the *C. roseus* collected from Sungai Siput, Perak *C. roseus* were identified and then subjected to a cold maceration extraction method using ethanol. The resultant crude extracts were analysed for preliminary phytochemicals to identify bioactive compounds. The cup plate method assessed the antimicrobial activity with *Escherichia coli* (*E. coli)* and *Candida albicans* (*C. albicans*) as representative bacterial and fungal pathogens, respectively. The results indicated high antimicrobial activity in the leaves, while the flowers and root extracts showed the lowest MIC values. The extraction yields were 12.3% for flowers, 16.25% for leaves, and 5.6% for roots. The antimicrobial properties of the 3 plant parts were then studied through cup plate method assay using concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml. The leaves showed the strongest antimicrobial activity, with inhibition zones of 12–14 mm against *E. coli* and 10 mm against *C. albicans*, demonstrating dose-dependent effects. These results were comparable to standard antibiotics such as Amoxicillin and Griseofulvin. The data supporting the potential use of *C. roseus* in developing plant-based antimicrobial agents.

1. INTRODUCTION

Natural products have played a central role in human health throughout history, serving both as remedies and as foundations for modern drug discovery [1]. They represent a vast reservoir of structurally diverse molecules, many of which continue to inspire new therapeutic agents and prototypes. Consequently, the exploration of plant-derived compounds remains a critical priority for sustainable biodiversity conservation and the development of novel pharmaceuticals. The global demand for herbal medicines has risen considerably, with new products continuously entering the market. However, alongside their growing use, concerns regarding safety, quality, and public health risks have also gained prominence. Despite their widespread application, many herbal preparations remain poorly regulated, inadequately tested, and lacking in robust evidence to support their safety and efficacy [13,20,31].

Reports of poisoning and adverse events linked to herbal remedies have further highlighted these concerns [3]. Given these challenges, in-vitro investigations into medicinal plants are warranted to generate scientific data that could guide future multidisciplinary research and validate their therapeutic potential [12,31].

Catharanthus roseus (*C. roseus*), is a herb plant native to Madagascar but due to imperialism and trade, it is now found worldwide. For example, in Malaysia it is known as Kemunting Cina [16]. Due to its abundance and diverse pharmacological profile, *C. roseus* has been utilised as a traditional remedy for centuries. As documented in a comprehensive review by [6], the plant's therapeutic applications vary significantly by tissue

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type: leaf juice is traditionally employed to manage metabolic and systemic ailments such as diabetes, menorrhagia, and dysentery, whereas root extracts are specifically applied to treat fevers and septic wounds. These medicinal effects are fundamentally attributed to a rich repository of secondary metabolites, including phenolic compounds, flavonoids, and tannins. [6] emphasise that this complex phytochemical matrix provides the scientific basis for the plant's broad-spectrum healing properties, validating its long-standing role in ethnopharmacology.

Botanically, *C. roseus* can grow up to one meter tall, with glossy green leaves arranged in opposite pairs and delicate flowers that vary in colour from white to deep pink, often featuring a prominent red center. The corolla measures 2–5 cm across and is divided into five petal-like lobes, while the fruit comprises paired follicles approximately 2–4 cm in length [14]. Beyond its traditional medicinal applications, the plant is highly valued for its ability to produce monoterpenoid indole alkaloids (MIAs), such as vinblastine and vincristine, which serve as key chemotherapeutic agents. However, its pharmacological potential is limited by low alkaloid content, slow growth rates, and sensitivity to environmental stresses. Despite these challenges, natural products continue to be a vital source of new drug leads, particularly as the development of novel pharmaceuticals has slowed [30,13]. Their chemical complexity and biological diversity have renewed interest in plant-based drug discovery.

In addition to its anticancer properties, *C. roseus* demonstrates a broad spectrum of biological activities, including antifungal, antioxidant, antiviral and antimicrobial effects. With current efforts to heighten antibiotic resistance, this plant is literally under the microscope of many researches. The present study investigates the antimicrobial potential of *C. roseus* extracts, identifies their phytochemical constituents and evaluates their suitability as a sustainable, eco-friendly source of new antibiotics. By doing so, it aims to contribute to ongoing drug discovery efforts while addressing the urgent need for alternatives to synthetic antimicrobials

2. EXPERIMENTAL

2.1 Collection of plant material

Fresh *C. roseus* specimens were gathered in January 2023 from Taman Muhhibah, Ipoh, Perak, Malaysia. An officer from the Biodiversity Unit at the Institute of Bioscience, Universiti Putra Malaysia, verified the plant's identity and a voucher specimen (No. KM 0108/24) was recorded and stored for reference. After collection, the samples were transported to the laboratory, where the leaves, flowers and roots were carefully separated for further analysis.

The plant materials were initially rinsed with tap water and then washed with distilled water to wash off impurities. They were then air-dried under shade for 10–15 days, cut into smaller fragments and processed into a coarse powder with the use of a mechanical blender. It is stored in airtight containers for planned evaluations

Fig. 1. Clockwise a. fresh leaves, b. fresh flower, c, fresh roots, d. dried leaves, e. dried flower, f. dried roots

2.2 Extraction

Based on literature, cold maceration method was used with 95% ethanol (v/v) mixed with distilled water at a 1:4 ratio as the extraction solvent. The coarsely ground plant material was placed in a sealed container, completely immersed in the solvent and left at 22-25 °C for at least three days with occasional agitation. To maximise extraction, the process was extended to seven days [2].

Following maceration, the solution was first passed through Whatman No. 1 filter paper and then through a cotton plug for further clarification. To produce the concentrated extract, a rotary evaporator was used. It was set at 175 mmHg, 60 rpm, and 55 °C. Any remaining moisture was eliminated by gentle heating on a mantle until a completely dry extract was obtained. The final product was stored in airtight containers at 4 °C until further use [18,24,27].

2.3 Characterization of *C. roseus* Extracts via Maceration

Factors influencing the phytochemical composition of extracts obtained via maceration are numerous and interrelated. The most significant determinant is the plant part used because leaves, flowers and roots contain different classes and concentrations of phytochemicals, the resulting extracts vary in both colour and compound distribution. For instance, flower extracts appeared yellowish-green, leaf extracts were green and root extracts were light brown, suggesting distinct phytochemical signatures across plant parts (Table 1) [26].

Table 1. Characterisation of *C. roseus* plant parts

Characteristic/Parts	Flowers	Leaves	Roots
Colour	Yellowish green	Green	Light brown
Consistency	Liquid	Liquid	Liquid

Extract colour often reflects the types of active constituents present. Chlorophylls produce a green hue particularly prominent in leaves, whereas flavonoids, tannins or other phenolic compounds may impart yellowish or brown tones to flowers and roots. Thus, the observed colour variations serve as crude visual indicators of differing phytochemical profiles.

Extract consistency can also be informative, although in this investigation all extracts remained liquid. Extract consistency is influenced by solvent choice and solute solubility, which are dictated by the nature and concentration of extracted compounds. Even among liquid extracts, differences in viscosity or density may impact how effective phytochemicals can be separated, stabilised and analysed [14].

2.4 Yield extraction

Maceration is one of the common extraction methods whereby plant materials are soaked in a solvent. This aims to extract bioactive compounds. The method is popular due to its simplicity and efficiency. The extraction yields from leaves, flowers, roots dried extracts have been determined and are presented in Table 2. The formula used for calculating yield is:

$$\text{Yield} = \frac{\text{Mass of dried Extract}}{\text{Mass of powdered } C.roseus \text{ (leaves, flowers and roots)}} \times 100 \quad (1)$$

Table 2. The yields of *C. roseus* flower, leaves and roots

Yield /Parts	Flowers	Leaves	Roots
Yield of extraction in ethanol (%)	12.29	16.25	5.6

2.5 Preliminary phytochemical screening

The supernatant obtained from the ethanolic (95%) extracts of different plant parts was used for qualitative phytochemical screening. Based on literature, the following procedures were adopted to identify the secondary metabolites that are present in the extract [23].

2.6 Test for Alkaloids Determination

- Wagner's Test

Alkaloids will show its presence with the mixture of a few drops of Wagner's reagent and several milliliters of the plant extract in a test tube. A red-brown precipitates indicate positive results.

- Mayer's Test

To minimise the risk of false negatives or reagent specific bias, this method is also used to test for alkaloids, around 2 mg of the plant extract was mixed with a few drops of Mayer's reagent. A pale-yellow precipitate indicates a positive results indicating the presence of even trace amounts of alkaloids.

2.7 Test for Carbohydrates Determination

- Molisch's Test

With the addition of 2 mL of a 2.0% sodium hydroxide solution and the crude leaf extract of our sample extract and two drops of an alcoholic α-naphthol solution, a formation of a purple ring indicates a positive identification of carbohydrates.

2.8 Test for Flavonoids Determination

The presence of flavonoids is verified with the dissipation of the dark yellow colour of the solution between, 2 mL of a 2.0 % sodium hydroxide solution and the crude leaf extract when two drops of dilute acid are added to it.

2.9 Test for Glycosides Determination

- Borntranger's Test

3mL of extract are combined with dilute sulfuric acid (H_2SO_4) and brought to a boil. After filtration, the solution is allowed to cool before adding an equal volume of benzene. The mixture is shaken thoroughly, followed by the addition of an equal volume of dilute ammonia solution to the organic layer. The appearance of a pink-colored ammonia layer indicates the presence of anthraquinone glycosides [32].

2.10 Test for Terpenoids Determination

- Salkowski's Test

To screen for terpenoids, 2 mL of the plant extract was mixed with an equal volume of chloroform, after which an equal amount of concentrated sulfuric acid (H_2SO_4) was carefully added. The solution was gently agitated, leading to the development of a red coloration in the chloroform layer and a greenish-yellow fluorescence in the acid layer. These characteristic color changes confirmed the presence of terpenoids [10].

2.11 Test for Protein Determination

- Ninhydrin Test

A few drops of ninhydrin reagent were added to the extract solution, followed by gentle heating. The development of a pink to purple coloration indicated a positive reaction for proteins, peptides or free amino acids [30].

2.12 Test for Tannins Determination

- Ferric Chloride Test

To detect phenolic compounds, a few drops of 5% ferric chloride ($FeCl_3$) solution were added to 1 mL of each plant extract. The development of a blue, green, or black coloration indicated the presence of phenolic groups such as tannins or flavonoids [10,20].

2.13 Test for Saponin Determination

- Ferric Chloride Test

One mL of the extract was combined with 20 mL of distilled water in a measuring cylinder and shaken vigorously for 15 minutes. The formation of a persistent foam layer indicated the presence of saponins [10].

3. MICROBIOLOGICAL EVALUATION OF *C. ROSEUS* LEAVES, FLOWER AND ROOTS EXTRACT

3.1 Preparation of Media and Reagents

3.1.1 Preparation of bacterial smear

To prepare the smear, a small oval-shaped area was marked on a clean glass slide using a marker. A sterile inoculating loop was then used to transfer the bacterial specimen to the marked area. If the specimen was obtained from a bacterial colony on solid culture media, a drop of normal saline or distilled water was first placed on the slide before emulsifying the colony in the liquid. If a liquid culture was used, a loopful of the suspension was directly placed on the slide. The smear was then allowed to air dry completely before heat fixation. Heat fixation was performed by passing the slide, smear side up, through a Bunsen burner flame 3-4 times. Care was taken to avoid excessive heating, which could distort bacterial morphology [16].

3.1.2 Gram staining

Bacterial smears were first heat-fixed onto clean glass slides and placed on a staining rack. The smears were covered with crystal violet, the primary stain, for one minute and then gently rinsed with distilled water to remove excess dye. Gram's iodine was applied for an additional minute to act as a mordant, forming a crystal violet–iodine complex that helps retain the stain in Gram-positive bacteria. After rinsing with distilled

water, decolorization was performed by adding acetone or 95% ethanol dropwise for 5–10 seconds, followed immediately by a rinse to prevent over-decolorization.

Counterstaining was then carried out using either 0.25% (w/v) safranin or a 1:10 dilution of carbonyl fuchsin for 30–60 seconds, allowing Gram-negative bacteria to absorb the secondary stain. After a final rinse with distilled water, the slides were air-dried. Residual stain on the back of the slides was removed with tissue paper, and a drop of cedarwood oil was placed on the smear. Examination under a bright-field microscope with a 100× oil immersion objective revealed Gram-positive bacteria as purple due to crystal violet retention, while Gram-negative bacteria appeared pink from the counterstain [16].

3.2 Direct Colony Suspension

3.2.1 *Selection and Preparation of Inoculum*

Fresh bacterial cultures, not older than 30 hours, were selected to ensure optimal viability. Using a sterile inoculating loop, 3–5 well-isolated colonies were carefully transferred from the culture plate into a sterile test tube containing 0.85% saline or an appropriate broth medium, such as Mueller–Hinton or tryptic soy broth.

3.2.2 *Turbidity Standardization*

The bacterial suspension was mixed thoroughly using a vortex mixer to ensure an even distribution of cells. Its turbidity was then adjusted to match the 0.5 McFarland standard, which corresponds to an approximate bacterial density of 1.5×10^5 CFU/mL. Standardisation was achieved by visually comparing the suspension with a prepared McFarland reference standard.

3.2.3 *Dilution for Testing*

Further diluted where 50 µL of the 0.5 McFarland suspension was transferred to 10 mL of broth to obtain the appropriate final inoculum concentration.

3.2.4 *Inoculation and Testing*

To maintain bacterial viability, the prepared inoculum was used within 30 minutes of preparation).

3.3 Antimicrobial assessment

- • Microbial Strains

The study utilised specific microbial strains, *E. coli* for bacteria, and *C. albicans* for fungi. The microbial cultures were prepared in tryptone soup and potato dextrose broth, respectively, and incubated for 24 hours at 37 °C for bacteria and 5 to 7 days at room temperature for fungi

3.3.1 *Antibacterial Susceptibility Testing: Well Diffusion Assay*

The antibacterial property of the plant extracts was assessed using agar well diffusion method. The literature reported this as a common approach to assess natural and laboratory produced antimicrobial activity [20, 29]. Using this method, the measurements of the diameter of inhibition zones of control, blank and plant extracts demonstrate ability and efficacy against microbes [29].

4. RESULTS AND DISCUSSION

4.1 Phytochemical Screening

Phytochemical screening of *C. roseus* leaves, flowers and roots shows a wide variety of secondary metabolites. Alkaloids, carbohydrates, flavonoids, proteins and terpenoids were consistently detected across all plant parts (Table 3). In contrast, the distribution of saponins and glycosides was tissue-specific, being present in leaves and roots but absent in flowers. This chemical profile is consistent with earlier reports identifying *C. roseus* as a rich source of pharmacologically important metabolites with antimicrobial, anticancer and antioxidant properties [15,22].

4.1.1 Alkaloids

The detection of alkaloids in all parts of *C. roseus* is consistent with its well-established pharmacological importance. Among these, the dimeric indole alkaloids vincristine and vinblastine remain two of the most clinically significant anticancer agents derived from the plant [27]. Their presence across leaves, flowers and roots suggests that alkaloid biosynthesis occurs systemically within *C. roseus* tissues [15]. Beyond anticancer activity, alkaloids are also known to exert antimicrobial effects through mechanisms such as DNA intercalation and enzyme inhibition [23], which may account for the inhibitory activity observed in this study against *E. coli* and *C. albicans*.

4.1.2 Flavonoids and Terpenoids

Flavonoids were uniformly present in all plant parts, which agrees with earlier studies highlighting their broad distribution and antioxidant potential in *C. roseus* [19]. These compounds act as free-radical scavengers and exhibit anti-inflammatory and antimicrobial properties [21]. Similarly, terpenoids detected in all tissues are known for antimicrobial and anticancer activities [13] and their presence likely contributes synergistically with alkaloids to the antimicrobial effects seen in the extracts

4.1.3 Carbohydrates and Proteins

The consistent presence of carbohydrates and proteins across plant parts indicates both structural and metabolic functions but also suggests potential roles in plant microbe interactions. Carbohydrates may act as precursors for glycosylated secondary metabolites, while proteins include enzymes that mediate biosynthetic pathways for alkaloids and phenolics [27].

4.1.4 Glycosides and Saponins

The selective distribution of glycosides and saponins highlights tissue-specific specialization. Glycosides were found in leaves and roots but absent in flowers. Their therapeutic relevance, especially as cardiac glycosides, has been widely documented [32]. Saponins, absent in flowers but present in leaves and roots, are notable for their immunomodulatory and antifungal effects [13]. Their absence in flowers may partly explain the relatively lower antifungal activity of flower extracts compared to leaves in this study.

The phytochemical profile observed here broadly agrees with previous reports on *C. roseus* [22]. However, variation in the distribution of specific compounds such as the absence of saponins and glycosides in flowers likely reflects both intrinsic tissue-specific metabolism and extrinsic factors such as soil, climate and growth conditions [5]. Similar variability across

plant parts and environments has been noted in other medicinal plants, underscoring the need for standardised phytochemical screening approaches [3,12].

The rich secondary metabolite profile of *C. roseus* offers a strong biochemical rationale for both its traditional and contemporary pharmacological applications. The consistent presence of alkaloids, flavonoids and terpenoids reflects the plant's broad therapeutic potential, whereas the selective distribution of saponins and glycosides points to tissue-specific bioactivities. Collectively, these results not only support the plant's historical use in ethnomedicine but also highlight its promise as a reservoir of novel antimicrobial and anticancer compounds, warranting further pharmacological and biotechnological investigation.

Table 3. Phytochemical screening of three different parts of *C. roseus*

Chemical compound	Results		
	Leaves	***Flowers***	***Roots***
Alkaloids	+	+	+
Carbohydrates	+	+	+
Flavonoids	+	+	+
Glycosides	+	-	+
Protein	+	+	+
Saponin	+	-	+
Terpenoids	+	-	+

Note: (+): presence, (-): absence

4.2 Antimicrobial evaluation

The antibacterial and antifungal activities of the leaf, flower and root extracts against E. coli and C. albicans are presented in Tables 4, 5 and 6, respectively.

Table 4. Antimicrobial activity of the *C. roseus* leaves

Pathogen	**Bacteria**	**Fungal**	**p-value**
Part of plant Extract (200mg/ml)	*E. coli*	*C. albicans*	
Leaves (mm)	12.9	6	
Positive Control: Amoxicillin (200 mg/ml)	24	-	p < 0.001
Positive Control: Griseofulvin (200 mg/ml)	-	14	p < 0.001
Negative Control (0.1 % DMSO)	Na	Na	

* Values are expressed as mean ± SD (n=3). Na: No activity
Mean (± SD), Zone of Inhibition (mm)

Table 5. Antimicrobial activity of the *C. roseus* flower

Pathogen	**Bacteria**	**Fungal**	**p-value**
Part of plant Extract (200mg/ml)	*E. coli*	*C. albicans*	
Leaves (mm)	9.0	4.8	
Positive Control: Amoxicillin (200 mg/ml)	23	-	p < 0.001
Positive Control: Griseofulvin (200 mg/ml)	-	13	p < 0.001
Negative Control (0.1 % DMSO)	Na	Na	

* Values are expressed as mean ± SD (n=3). Na: No activity
Mean (± SD), Zone of Inhibition (mm)

Table 6. Antimicrobial activity of the *C. roseus* roots

Pathogen	**Bacteria**	**Fungal**	**p-value**
Part of plant Extract (200mg/ml)	*E. coli*	*C. albicans*	
Leaves (mm)	6.1	6.8	
Positive Control: Amoxicillin (200 mg/ml)	22	-	p < 0.001
Positive Control: Griseofulvin (200 mg/ml)	-	12	p < 0.001
Negative Control (0.1 % DMSO)	Na	Na	

* Values are expressed as mean ± SD (n=3). Na: No activity
Mean (± SD), Zone of Inhibition (mm

4.2.1 Antimicrobial activity against E. coli

The ethanolic extracts of *C. roseus* demonstrated inhibitory effects against *E. coli*, with the level of activity differing among plant parts. The leaf extract exhibited the most pronounced antibacterial action, yielding a mean inhibition zone of 12.9 mm, although this was notably smaller than that produced by the reference antibiotic, Amoxicillin (24 mm; $p < 0.001$). The flower extract showed moderate inhibition, forming a 9.0 mm zone, while the root extract displayed the least activity, with an inhibition zone measuring 6.1 mm. We have contextualised the 12.9 mm inhibition zone by referencing CLSI M100 (2024) clinical breakpoints, noting that while the activity is statistically significant ($p<0.05$), it remains below the ≥18 mm threshold for clinical susceptibility. This is further supported by University of Utah's study on extract standardisation, which emphasise that crude extracts require further fraction isolation to achieve standalone therapeutic efficacy [4].

It should be noted that the 200 mg/ml concentration used for plant extracts represents a crude mixture of numerous compounds and is therefore not directly comparable to the active ingredient concentration in Amoxicillin (200 mg/ml), which contains a single purified antibacterial agent. This distinction limits direct potency comparisons and highlights the need for fractionation and isolation of active constituents in future studies.

These results suggest that leaves contain a higher concentration of antibacterial phytochemicals, possibly alkaloids, flavonoids and terpenoids, which are consistently reported as major contributors to antimicrobial effects in *C. roseus* [17, 25]. Both Wagner's and Mayer's tests confirmed the presence of alkaloids in all three plant parts, with red-brown precipitates (Wagner's) and pale-yellow precipitates (Mayer's)

consistently observed across leaf, flower and root extracts, indicating that alkaloid biosynthesis occurs systemically in *C. roseus*. Flowers and roots, though less effective, still demonstrated measurable activity, indicating that bioactive metabolites are present across tissues but in varying concentrations.

The 10–15 days air-drying period, though conducted under shade to minimise direct photodegradation, may have resulted in the partial loss of heat or moisture-sensitive bioactive compounds. Research on drying kinetics demonstrates that prolonged shade drying specifically over extended durations similar to those in this study can lead to a significant reduction in essential oil content and a shift in volatile terpenoid profiles due to continued enzymatic activity and oxidative stress during the slow moisture-loss phase [11]. This is further contextualised in a 2025 study that emphasise while traditional air-drying remains a common practice, it often fails to meet the rigorous standards of modern bioprospecting, as it allows for the gradual degradation of sensitive secondary metabolites that would otherwise be preserved through rapid stabilization techniques [9].

As the authors of this study, we acknowledge that while shade-drying provides a culturally and economically accessible baseline for evaluating *C. roseus*, it likely presents a conservative representation of the plant's true pharmacological potential. The sensitive indole alkaloids detected in this species, such as vindoline, are highly susceptible to post-harvest environmental fluctuations [7]. Therefore, we argue that while our results validate the antimicrobial presence in traditionally processed samples, the transition to novel drying applications, such as lyophilization, is a necessary next step to maximise the extraction of the most volatile and potent therapeutic fractions. Future research should prioritise a direct comparison between these drying kinetics to quantify the "metabolic gap" between traditional processing and high-tech preservation methods [23].

4.2.2 Antimicrobial activity against C. albicans

The antifungal evaluation showed a more balanced activity among plant parts. The leaf extract inhibited *C. albicans* with a zone of 6 mm, whereas the flower extract showed weaker activity (4.8 mm). Interestingly, the root extract demonstrated the highest antifungal activity among the three, producing a 6.8 mm inhibition zone, although this remained significantly lower than Griseofulvin (12 mm; $p < 0.001$).

This observation is discussed in a 2019 study where the root system is buried in the rhizosphere which is a microbial rich spot in the soil that has a significant amount of higher density and much diverse population of fungus compared to above-ground plant tissues [8]. The higher amount of saponins in the roots is highly likely due to the nature of the roots being able to survive far longer past the above ground tissues. Since they store nutrients more and longer during this period, they are subjected to constant invasion from microbes. This in turn trigger higher amount of saponins as a defence mechanism [13]. This observation is consistent with the findings of a 2015 study, which discussed the phyllosphere as a dynamic and stressful interface [4]. The generalised activity of the leaf extract likely reflects an adaptation to this environment, which is exposed to a wide variety of aerial pathogens and fluctuating environmental stressors, necessitating a broad-spectrum rather than tissue-specific defence strategy.

4.2.3 Antimicrobial evaluation in relation to phytochemical screening

The antimicrobial effects observed in the ethanolic extracts of *C. roseus* can be closely linked to the phytochemical distribution across plant parts. The leaves, which demonstrated the strongest antibacterial activity against *E. coli* (12.9 mm), were shown in the phytochemical screening to contain all the tested secondary metabolites, including alkaloids, flavonoids, terpenoids, glycosides, saponins and proteins. Alkaloids and flavonoids in particular are widely reported for their antibacterial activity through mechanisms such as inhibition of nucleic acid synthesis, disruption of bacterial membranes and interference with enzymatic activity [25]. The abundance of alkaloids and flavonoids detected in the leaves may therefore account for their superior antibacterial efficacy as these compounds have been observed to demonstrate antibacterial activity in the literature.

Flowers, which displayed moderate inhibition against *E. coli* (9.0 mm) and weak antifungal activity (4.8 mm against *C. albicans),* were found to lack glycosides and saponins. These missing phytochemical classes could explain their reduced antimicrobial potential compared to leaves and roots. Roots, in contrast, showed limited antibacterial activity (6.1 mm) but the highest antifungal effect (6.8 mm). The phytochemical profile of the roots revealed the presence of glycosides and saponins, the latter of which are recognised for their ability to interact with sterols in fungal cell membranes, causing permeability changes and cell death. This likely explains why roots, despite weaker antibacterial activity, displayed relatively stronger antifungal potential.

Thus, the antimicrobial results mirror the phytochemical composition: leaves emerge as the most broadly active due to their rich metabolite profile, roots are selectively antifungal because of saponins and glycosides, while flowers exhibit the weakest bioactivity owing to the absence of key antimicrobial constituents. This phytochemical-antimicrobial correlation reinforces the pharmacological significance of *C. roseus* and highlights the importance of tissue-specific chemical variation in determining therapeutic efficacy [28].

5. CONCLUSIONS

This study revealed that *C. roseus* possesses a rich and diverse array of phytochemicals, each contributing differently to its antimicrobial potential across various plant parts. Phytochemical screening confirmed the widespread occurrence of alkaloids, flavonoids, terpenoids, proteins and carbohydrates, while glycosides and saponins were notably absent in the flowers. These compositional differences were clearly reflected in the antimicrobial assays. Among the tested extracts, the leaves displayed the strongest antibacterial activity against *E. coli*, indicating a higher concentration or bioavailability of active constituents in leaf tissues. In contrast, the roots exhibited the most pronounced antifungal effect against *C. albicans*, suggesting a distinct chemical defense mechanism. The flower extract, meanwhile, showed only moderate inhibition, likely due to its comparatively lower levels of bioactive metabolites.

Although none of the plant extracts matched the potency of standard antibiotics or antifungal agents, their statistically significant inhibition zones ($p < 0.001$) demonstrate meaningful antimicrobial efficacy. Collectively, these findings validate the

traditional medicinal use of *C. roseus* and highlight its potential as a sustainable source of plant-derived antimicrobial compounds and an important avenue in addressing the growing challenge of antibiotic resistance. Further work should focus on isolating and characterizing the active molecules, elucidating their mechanisms of action, and exploring potential synergistic interactions with conventional therapies.

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