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Phytochemical Characterization and Antimicrobial Potential of *Catharanthus roseus* Methanol Extract Against Vaginal Microflora

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KEYWORDS

Catharanthus roseus, Methanol extract, Phytochemical screening, antimicrobial activity, Nutritional and mineral composition.

ABSTRACT:

Catharanthus roseus is a medicinally important plant recognized for its alkaloid-rich composition and therapeutic applications. The present study aimed to evaluate the phytochemical profile, nutritional composition, mineral content, and antimicrobial activity of the methanol extract of C. roseus leaves. The crude extract was greenish-black, pungent in odor, with a yield of 4.08 g. Proximate analysis revealed high crude fat (42.82%), carbohydrate (40.27%), and fibre (17.58%) contents, with moderate protein (4.70%), suggesting potential nutritional value. Mineral analysis demonstrated significant amounts of calcium (36.3 mg/100 g) and potassium (23.8 mg/100 g), along with sodium, magnesium, and trace elements such as iron and zinc, indicating the plant's potential as a source of essential micronutrients. Phytochemical screening confirmed the presence of alkaloids, phenols, tannins, saponins, proteins, and lipids, whereas flavonoids, terpenoids, and steroids were absent. Antimicrobial evaluation of six chromatographic fractions (M1–M6) showed that only M1 and M2 exhibited considerable antibacterial activity, while other fractions were inactive, suggesting that bioactive compounds were concentrated in specific polar fractions. The strong activity of these fractions may be attributed to alkaloids and phenolic constituents, known for their antimicrobial efficacy.

Introduction:

Medicinal plants have played a fundamental role in the treatment of diseases and the maintenance of human health since ancient times. According to the World Health Organization (WHO), nearly 80% of the global population relies on traditional herbal remedies as their primary source of healthcare, particularly in developing countries (5). The increasing incidence of antimicrobial resistance and the rising cost of synthetic drugs have revived interest in plant-based therapies, which are often considered safer, cost-effective, and enriched with bioactive compounds capable of targeting multiple disease pathways (1). Among such plants, *Catharanthus roseus* (L.) G. Don, also known as Madagascar periwinkle or Sadabahar, holds a distinguished place owing to its wide range of medicinal properties and its ability to produce pharmacologically significant alkaloids (3,7).

Catharanthus roseus, belonging to the family Apocynaceae, is a perennial herb widely distributed across tropical and subtropical regions. Traditionally, the plant has been utilized in Ayurveda, Unani, and folk medicine systems to manage a variety of ailments, including diabetes, hypertension, microbial infections, menstrual disorders, and skin diseases. The leaves, flowers, roots, and stems of *C. roseus* are known to harbor

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diverse classes of phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins, which contribute to its therapeutic potential. The plant is particularly renowned for its anticancer alkaloids—vincristine and vinblastine—that revolutionized the treatment of leukemia and Hodgkin's lymphoma, demonstrating the enormous pharmacological significance of its phytoconstituents (6).

Phytochemical studies of *C. roseus* have revealed that its leaves are a rich source of alkaloids, tannins, saponins, phenols, and proteins, with solvent polarity significantly influencing the yield and diversity of extracted metabolites. Methanol, being a polar solvent, is widely used in extraction protocols due to its ability to solubilize a broad spectrum of bioactive compounds including phenolics, alkaloids, and glycosides. Such phytoconstituents are known for their antimicrobial, antioxidant, anti-inflammatory, and cytoprotective properties, making methanol extracts of *C. roseus* a promising candidate for pharmacological evaluation. Despite these known properties, systematic investigations on the nutritional profile, mineral composition, and antibacterial activities of *C. roseus* extracts, particularly methanol-based fractions, remain limited (11).

The nutritional and elemental composition of medicinal plants also plays an essential role in their pharmacological attributes. Minerals such as calcium, magnesium, potassium, sodium, and iron not only contribute to physiological functions in humans but also serve as cofactors for enzymes involved in secondary metabolite biosynthesis (4). These secondary metabolites are often responsible for antimicrobial and antioxidant activities, highlighting the interconnectedness of elemental composition and therapeutic efficacy. Therefore, understanding the proximate composition (moisture, ash, protein, fat, fiber, and carbohydrates) along with elemental analysis is essential for correlating nutritional attributes with medicinal properties (2,13).

The increasing global concern over multidrug-resistant pathogens has necessitated the discovery of new antimicrobial agents from natural sources. Plants like *C. roseus* provide a reservoir of novel bioactive molecules that can potentially address this challenge. Preliminary screening of its crude extracts has shown antibacterial potential, but further fractionation and evaluation of solvent-specific extracts are needed to isolate active principles. In particular, methanol fractions have shown higher bioactivity compared to non-polar solvents such as hexane or petroleum ether, underscoring the importance of polarity in extracting potent phytochemicals (10).

In this context, the present study was undertaken to systematically investigate the phytochemical composition, nutritional and elemental content, and antimicrobial activity of *Catharanthus roseus* leaves with a special focus on the methanol extract. The study also aimed to identify bioactive fractions through column chromatography and evaluate their antibacterial efficacy against selected bacterial isolates (12). By correlating the proximate and elemental analyses with phytochemical screening and bioactivity, the research attempts to provide an integrative understanding of the therapeutic potential of *C. roseus* methanol extracts. Such an approach not only validates traditional claims but also provides a scientific basis for the development of plant-derived antimicrobial agents (8).

2. Materials and Methods

2.1 Study Area and Plant Collection

Fresh specimens of *Catharanthus roseus* were collected from the Raipur District, Chhattisgarh, India. The leaves were separated, shade-dried, and ground into fine powder using a sterile grinder. The powdered material was stored in airtight containers until further use (13).

2.2 Extraction Procedure

Approximately 12.5 g of powdered leaf material was subjected to sequential extraction in a Soxhlet apparatus using a thimble. Methanol solvent was employed successively in increasing polarity. The obtained extract was concentrated and stored at 4 °C for further analysis.

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2.3 Proximate Analysis

Proximate composition, including moisture, crude fiber, ash, crude fat, crude protein, and total carbohydrate content, was determined in triplicates following standard AOAC (2005) procedures (9).

2.3.1 Moisture Content

Two grams of powdered sample were weighed in triplicate and subjected to drying in a hot air oven. Initial drying was done at 80–90 °C, followed by final drying at 100–102 °C until a constant weight was obtained (16).

$$\text{Moisture Content (\%)} = W_F\text{-}W_D/W_f \times 100$$

Where:

- WfW_fWf = Weight of fresh sample
- WdW_dWd = Weight of dry sample

2.3.2 Crude Fat Content

Two grams of dried sample were placed in a thimble and extracted with 150–175 mL petroleum ether in a Soxhlet apparatus for 8 h. The solvent was evaporated, and the flask with extract was weighed.

$$\text{Crude Fat (\%)} = W_F/W_s \times 100$$

Where:

- WfW_fWf = Weight of extracted fat
- WsW_sWs = Weight of sample

2.3.3 Ash Content

Two grams of powdered sample were placed in pre-weighed crucibles and incinerated in a muffle furnace at 600 °C. After cooling in a desiccator, the crucibles were reweighed.

$$\text{Ash (\%)} = W_a/W_s \times 100$$

Where:

- Wa = Weight of ash
- Ws = Weight of sample

2.3.4 Crude Protein Content

Crude protein was determined by the micro-Kjeldahl method. One hundred milligrams of powdered sample were digested with a catalyst mixture (K_2SO_4:$CuSO_4$, 9:1) and concentrated H_2SO_4. The digest was diluted to 100 mL, and aliquots were distilled with 40% NaOH. Released ammonia was trapped in N/100 H_2SO_4 and titrated with N/100 NaOH (10).

$$\text{Crude Protein (\%)} = \%N \times 6.25$$

2.3.5 Crude Fiber Content

Two grams of sample were refluxed with 200 mL of 1.25% H_2SO_4 for 30 min, filtered, washed, and then refluxed with 200 mL of 1.25% NaOH for 30 min. The residue was washed, oven-dried (80–110 °C), weighed, and ashed at 550–660 °C in a muffle furnace.

$$\text{Crude Fiber (\%)} = W_{cf}/W_s \times 100$$

2.3.6 Total Carbohydrates

Total carbohydrate content was estimated by difference:

$$\text{Carbohydrates (\%)} = 100 - [\text{Moisture} + \text{Crude Fat} + \text{Ash} + \text{Crude Protein} + \text{Crude Fiber}]$$

2.4 Elemental Analysis

The concentrations of sodium (Na) and potassium (K) were estimated using a flame photometer. Calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe) were analyzed from aqueous digests using an Atomic Absorption Spectrophotometer (Varian AA 240FS, Australia) with flame and graphite furnace attachments. All determinations were carried out in triplicates.

2.5 Phytochemical Analysis

2.5.1 Qualitative Phytochemical Screening

The extracts were tested for the presence of proteins, amino acids, alkaloids, flavonoids, tannins, glycosides, saponins, anthraquinones, steroids, terpenoids, reducing sugars, fats, oils, and lignin using standard phytochemical assays (15).

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2.6 Antimicrobial Activity (Agar Well Diffusion Method)

Antibacterial activity was tested by the agar well diffusion method on Luria Bertani–Muller Hinton agar plates. Bacterial cultures (1×10^6 CFU/mL) were uniformly spread. Wells (8 mm diameter) were made with a sterile cork borer.

- Test wells: extracts dissolved in DMSO at concentrations of 5, 10, 15, and 20 mg.
- Positive control: standard antibiotic.
- Negative control: DMSO only.

Plates were incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters to assess antibacterial activity (14).

3. Results

3.1 Organoleptic Characteristics

The methanol extract of *Catharanthus roseus* leaves yielded **4.08 g**. The extract was **non-foamy** in texture, with a **greenish-black** coloration and a **pungent odor**.

Table 1. Organoleptic properties of different solvent extracts of Catharanthus roseus leaves (weight, texture, color, and odor).

Parameter	Observation
Weight	4.08 g
Texture	Non-foamy
Color	Greenish black
Odor	Pungent

3.2 Proximate Analysis

Proximate analysis of *Catharanthus roseus* leaves revealed the following composition:

Table 2. Elemental composition of Catharanthus roseus leaves showing essential minerals (sodium, potassium, calcium, magnesium, iron, and zinc). Values represent mean ± SD (n=3).

Parameter	Value (Mean ± SD)
Moisture (%)	15.72 ± 1.3
Dry Matter (%)	24.28 ± 0.1
Crude Fat (%)	42.82 ± 1.1
Ash (%)	8.96 ± 0.6
Crude Protein (%)	4.70 ± 0.3
Crude Fibre (%)	17.58 ± 0.6
Total Carbohydrates (%)	40.27 ± 2.5

3.3 Elemental Analysis

Essential mineral content (mean ± SD of triplicates) in *C. roseus* leaves was as follows:

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Table 3. Proximate composition of Catharanthus roseus leaves including moisture, ash, crude fat, crude protein, crude fiber, and carbohydrate content. Values are expressed as mean ± SD (n=3).

Mineral	Concentration (Mean ± SD)
Sodium	4.79 ± 0.5
Potassium	23.8 ± 5.0
Calcium	36.3 ± 3.0
Magnesium	5.17 ± 0.3
Iron	1.6 ± 0.1
Zinc	0.025 ± 0.1

3.4 Phytochemical Analysis (Methanol Extract)

Qualitative phytochemical screening of the methanol extract demonstrated the presence of:

Table 4. Phytochemical screening of Catharanthus roseus extracts in different solvents showing the presence (+) or absence (–) of secondary metabolites.

Phytochemical	Presence (+) / Absence (–)
Alkaloids	+
Terpenoids	–
Phenols	+
Tannins	+
Sugar	–
Saponins	+
Flavonoids	–
Steroids	–
Proteins	+
Fats & Oils	+

3.5 Antimicrobial Activity (Column Fractions of Methanol Extract)

Column chromatography of the methanol extract yielded **six fractions** (M1–M6). Out of these, **two fractions (M1 and M2)** demonstrated significant antibacterial activity against the tested bacterial isolates. These bioactive

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fractions were selected for further evaluation of their **antioxidant activity** and determination of their **Minimum Inhibitory Concentration (MIC)**.

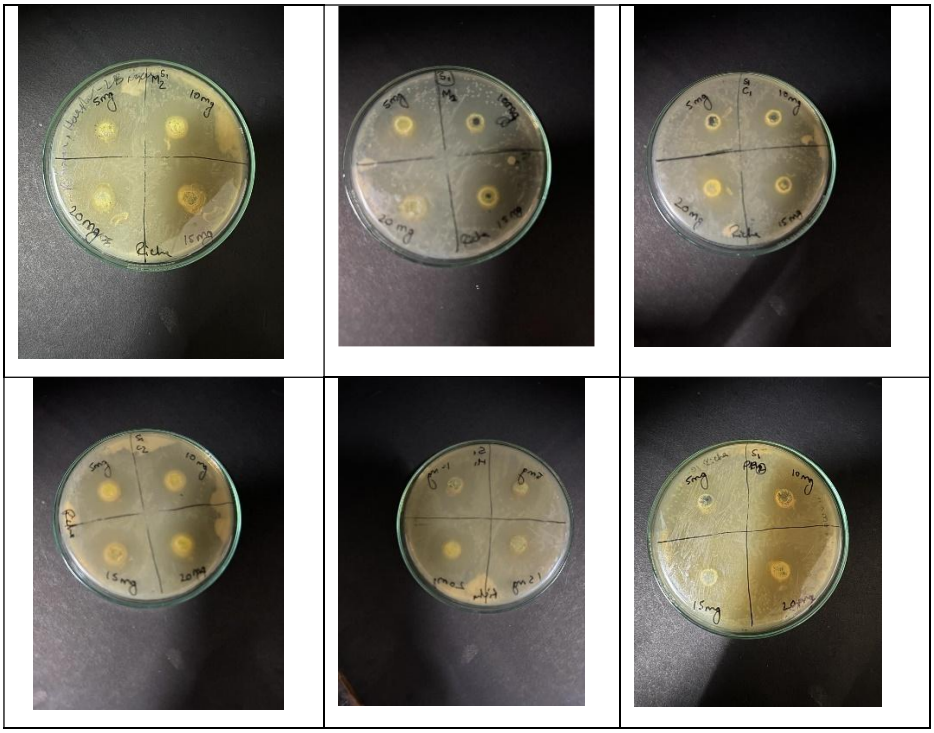

Figure 1. Showing antimicrobial activity of Methanolic extract of C.roseus against Gram positive and Gram negative strains.

Table 5. Antimicrobial activity of column chromatography fractions of methanol extract of Catharanthus roseus leaves against bacterial isolates.

Methanol Fractions	Antibacterial Activity
M1	Significant
M2	Significant
M3	Not active
M4	Not active
M5	Not active
M6	Not active

4. Discussion:

The present investigation explored the phytochemical composition, nutritional profile, mineral content, and antimicrobial potential of the methanol extract of *Catharanthus roseus* leaves. The findings provide valuable insight into the bioactive

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potential of this medicinal plant, traditionally known for its therapeutic importance. The methanol extract was greenish-black, non-foamy, and had a pungent odor, indicating the presence of volatile bioactive compounds, possibly alkaloids and phenolic derivatives. Such physical characteristics are consistent with earlier reports where *Catharanthus* extracts exhibited strong odor due to the abundance of indole alkaloids and phenolic compounds, which are known contributors to bitterness and medicinal aroma. The moderate yield (4.08 g) further suggests that methanol was an efficient solvent in extracting polar and semi-polar metabolites compared to non-polar solvents. The proximate analysis revealed significant levels of crude fat (42.82%), carbohydrates (40.27%), and fibre (17.58%), along with moderate protein content (4.70%). The high lipid content is unusual for leafy tissues but may reflect the presence of essential oils, fatty acids, and waxy compounds in the cuticular layers of *C. roseus*. Fibre and carbohydrate richness indicate its potential nutritional and functional food applications, especially as dietary fibre plays a role in gastrointestinal health and in modulating glycemic response. The relatively low protein percentage is expected since *Catharanthus* is primarily medicinal rather than a protein-rich edible plant. Mineral analysis demonstrated a predominance of calcium (36.3 mg/100 g) and potassium (23.8 mg/100 g), followed by appreciable amounts of sodium and magnesium. Calcium is essential for bone and metabolic functions, while potassium contributes to electrolyte balance and cardiovascular health. Trace minerals such as iron and zinc, though present in low concentrations, are critical cofactors for enzymatic and antioxidant functions. The presence of these essential minerals enhances the nutraceutical potential of the leaves, supporting their use in herbal formulations aimed at mineral supplementation. Phytochemical screening confirmed the presence of alkaloids, phenols, tannins, saponins, proteins, and lipids, while flavonoids, terpenoids, and steroids were absent. Alkaloids are the most well-known metabolites of *C. roseus*, especially indole alkaloids like vincristine and vinblastine, which are established anticancer agents. Phenols and tannins contribute antioxidant, antimicrobial, and anti-inflammatory properties, while saponins may enhance membrane permeability and exhibit antifungal/antibacterial effects. The absence of flavonoids in the methanol extract contrasts with reports in aqueous or ethanol extracts, suggesting solvent specificity in extracting particular classes of phytochemicals. Overall, the detected compounds provide a biochemical basis for the traditional therapeutic claims of the plant. Among the six methanolic fractions (M1–M6) obtained through column chromatography, only M1 and M2 showed significant antibacterial activity, while the remaining fractions were inactive. This indicates that the antimicrobial constituents were concentrated in the early polar fractions, possibly due to alkaloids, phenols, or tannin-rich fractions. Previous studies have demonstrated that alkaloid-rich fractions of *C. roseus* possess broad-spectrum antibacterial properties, particularly against Gram-positive organisms. The inactivity of other fractions could be attributed to the separation of compounds lacking antimicrobial activity or to sub-inhibitory concentrations of active metabolites. The strong activity of M1 and M2 suggests that purification and characterization of these fractions may yield bioactive compounds with pharmaceutical relevance.

The results align with earlier findings where methanolic extracts of *C. roseus* were reported to contain indole alkaloids and phenolics with antimicrobial and antioxidant properties. However, the current study contributes by correlating nutritional, mineral, and phytochemical parameters with fraction-specific antimicrobial potential. The high crude fat, coupled with the presence of phenols and alkaloids, may synergistically enhance bioactivity. Moreover, the demonstration of fraction-dependent antimicrobial activity highlights the importance of chromatographic separation in identifying bioactive leads from crude extracts. The study reinforces the ethnomedicinal value of *Catharanthus roseus* and highlights the methanol extract as a promising source of nutraceutical and pharmaceutical compounds. The bioactive methanol fractions (M1 and M2) hold potential for development into antibacterial agents after further characterization (e.g., HPLC, FTIR, NMR studies). The presence of essential minerals also supports its

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role as a functional plant with dual nutritional and medicinal applications. Future research should extend to antioxidant assays, cytotoxicity evaluation, and purification of active metabolites to validate the therapeutic potential of these extracts.

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