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Ramdas Bhat
Associate Professor, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Bhavyashree K
UG student, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Charan DS
UG student, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Deeksha
UG student, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Deepa K
UG student, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Deepak
UG student, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Corresponding Author:
Ramdas Bhat
Associate Professor, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka, India

Evaluation of antibacterial activity of *Coleus aromaticus* stem extract in ointment formulations

Ramdas Bhat, Bhavyashree K, Charan DS, Deeksha, Deepa K and Deepak

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Abstract

Background: The global surge in antimicrobial resistance (AMR) necessitates novel plant-based antimicrobials. *Coleus aromaticus* (Indian borage), traditionally used for dermatological and respiratory ailments, is underexplored for its stem-derived antibacterial properties.

Objective: This study evaluates the antibacterial efficacy of ethanolic *Coleus aromaticus* stem extract in topical ointment formulations against *Escherichia coli* and *Staphylococcus aureus*.

Methods: Ethanolic stem extract was obtained via maceration and subjected to phytochemical screening. Ointments (5% and 10% w/w) were formulated using an emulsifying base and assessed for physicochemical properties (appearance, pH, spreadability, homogeneity, washability). Antibacterial activity was determined using the agar well diffusion method, with norfloxacin as the positive control.

Results: Phytochemical analysis revealed alkaloids, phenolics, saponins, and steroids. The 10% ointment showed superior inhibition zones (18.6±0.5 mm for *E. coli*; 19.4±0.6 mm for *S. aureus*), with statistically significant inhibition compared to the base control (one-way ANOVA followed by Dunnett's multiple comparison test, $p < 0.001$). Ointments were stable, with a skin-compatible pH of 4.9 and excellent spreadability.

Conclusion: *Coleus aromaticus* stem extract demonstrates promising antibacterial activity, supporting its potential as a natural topical antimicrobial. Further studies should focus on compound isolation and *in vivo* validation.

Keywords: *Coleus aromaticus*, antibacterial activity, herbal ointment, *Escherichia coli*, *Staphylococcus aureus*, phytochemicals

1. Introduction

Antimicrobial resistance (AMR) is recognized as one of the most urgent global health challenges of the 21st century. According to the World Health Organization, resistant bacterial infections are responsible for nearly 1.3 million deaths annually, with projections indicating that this figure may rise substantially in the coming decades if effective alternatives are not developed [1]. Resistance has emerged due to the misuse and overuse of antibiotics, inadequate infection control measures, and limited discovery of new antimicrobials. As a result, infections that were once easily treatable, such as pneumonia, urinary tract infections, and skin infections, are now associated with prolonged hospitalization, higher healthcare costs, and increased mortality. The crisis underscores the necessity of exploring novel and sustainable approaches to infection management [2].

Medicinal plants are considered one of the most promising resources for new antimicrobial agents, as they provide a wide variety of bioactive phytochemicals with therapeutic potential. Unlike synthetic antibiotics that act through a single mechanism, phytoconstituents often function through multiple pathways, including disruption of bacterial cell walls and membranes, inhibition of efflux pumps, and suppression of protein and nucleic acid synthesis [3]. These multi-targeted modes of action reduce the risk of resistance development and enhance antimicrobial efficacy. Additionally, herbal remedies are cost-effective, widely available, and generally associated with fewer side effects compared to conventional drugs, making them highly relevant in both developed and developing countries [4].

Among medicinal plants, *Coleus aromaticus* (syn. *Plectranthus amboinicus*), a member of the Lamiaceae family, holds significant ethnomedicinal importance. Commonly known as

Indian borage or country borage, this perennial aromatic herb is traditionally employed in South Asian systems of medicine for the treatment of respiratory ailments such as cough, asthma, and bronchitis, as well as for skin infections, fever, and inflammatory conditions [5]. Pharmacological studies on the leaves of *C. aromaticus* have demonstrated antioxidant, antimicrobial, anti-inflammatory, and antiepileptic activities, which are largely attributed to its essential oil constituents such as thymol, carvacrol, and eugenol, along with flavonoids and phenolic compounds. These findings validate its traditional applications and highlight its pharmacological versatility [6].

Despite extensive studies on the leaves and essential oils of *C. aromaticus*, its stem remains relatively overlooked in scientific investigations. Interestingly, in folk medicine, the stem has been incorporated in topical remedies for skin ailments, suggesting that it may contain bioactive compounds of therapeutic value [7]. Given the urgent need for new antimicrobials and the limited data on *C. aromaticus* stem, a systematic investigation of its antibacterial potential is warranted.

Therefore, the present study was conducted to evaluate the antibacterial activity of ethanolic stem extract of *C. aromaticus* formulated into topical ointments and tested against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. This work aims to validate the traditional claims and assess the plant's potential as a natural topical antimicrobial.

2. Review of literature

Escherichia coli and *Staphylococcus aureus* are among the most common bacterial pathogens, responsible for a wide range of hospital- and community-acquired infections such as wound infections, urinary tract infections, and sepsis [8, 9]. The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *E. coli* has created major therapeutic challenges, driving the urgent search for alternative antimicrobial strategies [10].

Plant-derived antimicrobials have been widely investigated for their ability to combat resistant bacteria. Extracts from plants like clove (*Syzygium aromaticum*), rosemary (*Rosmarinus officinalis*), and pomegranate (*Punica granatum*) have demonstrated significant antibacterial effects, largely attributed to their phenolic and terpenoid compounds [11, 12]. These natural agents act through mechanisms such as membrane disruption, enzyme inhibition, and interference with bacterial protein synthesis.

Within this context, *Coleus aromaticus* has gained attention for its strong antimicrobial and antioxidant activities. Its leaves and essential oils, rich in thymol, carvacrol, and flavonoids, have shown activity against foodborne and resistant pathogens [13, 14], with studies suggesting mechanisms like membrane damage and protein synthesis inhibition [15, 16]. However, despite its ethnomedicinal use in topical remedies, the stem remains underexplored [7]. The present study was therefore undertaken to evaluate the antibacterial potential of *C. aromaticus* stem extract in ointment formulations.

3. Materials and Methods

3.1 Plant Material

Stems of *Coleus aromaticus* were collected from Punjalkatte, Karnataka, India, in July 2024. The plant was authenticated by a botanist at Srinivas College of Pharmacy, Mangalore. Stems were washed, shade-dried for 10 days, and pulverized into a coarse powder using a mechanical grinder.

3.2 Extraction

A 300 g sample of stem powder was macerated in 600 mL of 95% ethanol (1:2 ratio) for 72 hours with intermittent stirring. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator, yielding a dark green semi-solid extract (2% w/w). The extract was stored in an airtight container at 4°C until further use [17].

3.3 Phytochemical Screening

The ethanolic extract was screened for alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda test), phenolics (ferric chloride test), saponins (foam test), steroids (Salkowski test), and glycosides (Keller-Kiliani test) using standard pharmacognostic protocols [17].

3.4 Ointment Formulation

Ointments were prepared using an emulsifying base (30 g emulsifying wax, 20 g liquid paraffin, 50 g white soft paraffin). The ethanolic extract was incorporated at 5% (0.5 g/10 g base) and 10% (1 g/10 g base) w/w concentrations. The mixture was melted at 60 °C, homogenized, and cooled to form a uniform ointment [18].

3.5 Ointment Evaluation [18]

Ointments were evaluated for:

- **Appearance**: Visual inspection for color and texture.
- **Consistency**: Assessed by touch for smoothness.
- **Spreadability**: Measured using the glass slide method (weight required to separate slides).
- **pH**: Determined using a digital pH meter.
- **Washability**: Time to remove ointment under running water.
- **Homogeneity**: Microscopic observation for uniformity.

3.6 Antibacterial Assay [19]

Antibacterial activity was assessed using the agar well diffusion method. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were cultured on nutrient agar plates. Wells (6 mm diameter) were filled with 50 µL of 5% or 10% ointment, norfloxacin (10 µg/mL, positive control), or ointment base (negative control). Plates were incubated at 37°C for 24 hours. Inhibition zones were measured in millimeters (mean± SEM, n=3).

3.7 Statistical analysis:

Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test to compare treatment groups with the base control. Values were expressed as mean± SEM, and differences were considered statistically significant at $p < 0.05$.

4. Results

4.1 Extract Yield

The ethanolic extraction yielded a dark green semi-solid extract with a 2% w/w yield, indicating moderate extractable phytochemical content.

4.2 Phytochemical Screening: Phytochemical analysis confirmed the presence of alkaloids, phenolics, saponins, and steroids, with flavonoids and glycosides absent (Table 1).

Table 1: Phytochemical Screening of *Coleus aromaticus* Stem Extract

Phytochemical	Result
Alkaloids	+
Phenolics	+
Saponins	+
Steroids	+
Flavonoids	–
Glycosides	–

4.3 Ointment Quality

Both 5% and 10% ointments were smooth, homogeneous, and stable over 30 days at room temperature. The pH was 4.9, suitable for topical application. Spreadability ranged from 10–12 g/cm², and washability was achieved in approximately 13 seconds, indicating ease of application and removal.

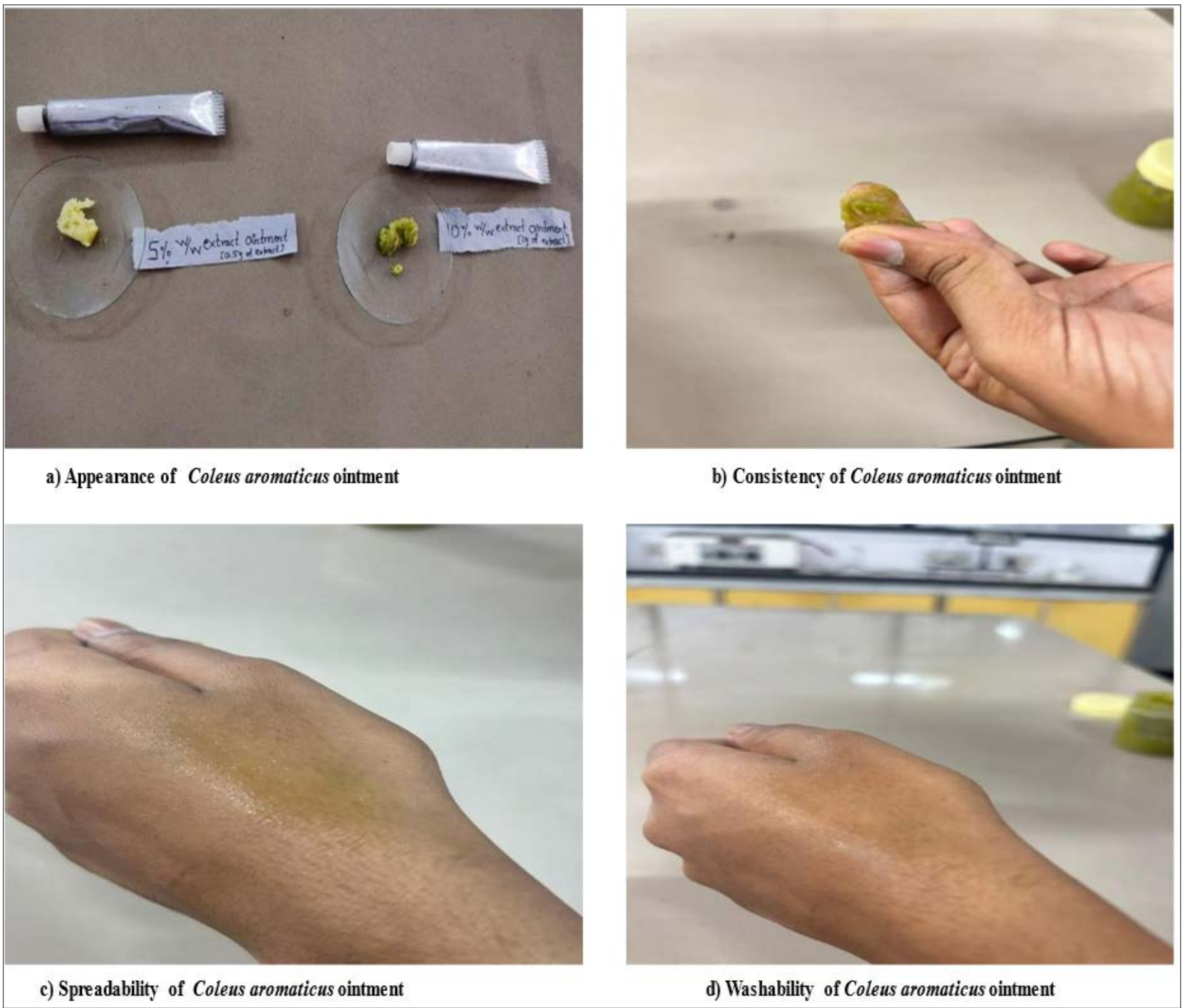

Fig 1: Physicochemical evaluation of 5% and 10% *Coleus aromaticus* stem ointments.

4.4 Antibacterial Activity

The 5% and 10% ointments showed dose-dependent antibacterial activity (Table 2). The 10% ointment exhibited larger inhibition zones (18.6±0.5 mm for *E. coli*; 19.4±0.6 mm for *S. aureus*) compared to the 5% ointment (12.8±0.4 mm for *E. coli*; 13.2±0.4 mm for *S. aureus*). Norfloxacin showed superior activity (>24 mm for both bacteria). The ointment base showed no activity. Statistical analysis confirmed significance ($p < 0.01$ for 5%, $p < 0.001$ for 10% and norfloxacin).

Table 2: Antibacterial Activity of *Coleus aromaticus* Stem Ointments

Test Sample	*E. coli* (mm)	*S. aureus* (mm)
5% Ointment	12.8±0.4**	13.2±0.4**
10% Ointment	18.6±0.5***	19.4±0.6***
Norfloxacin	24.3±0.6***	25.1±0.5***
Ointment Base	Nil	Nil

Values represented as mean± SEM (n=3). ***p* < 0.01, ****p* < 0.001 compared to ointment base (one-way ANOVA followed by Dunnett's multiple comparison test).

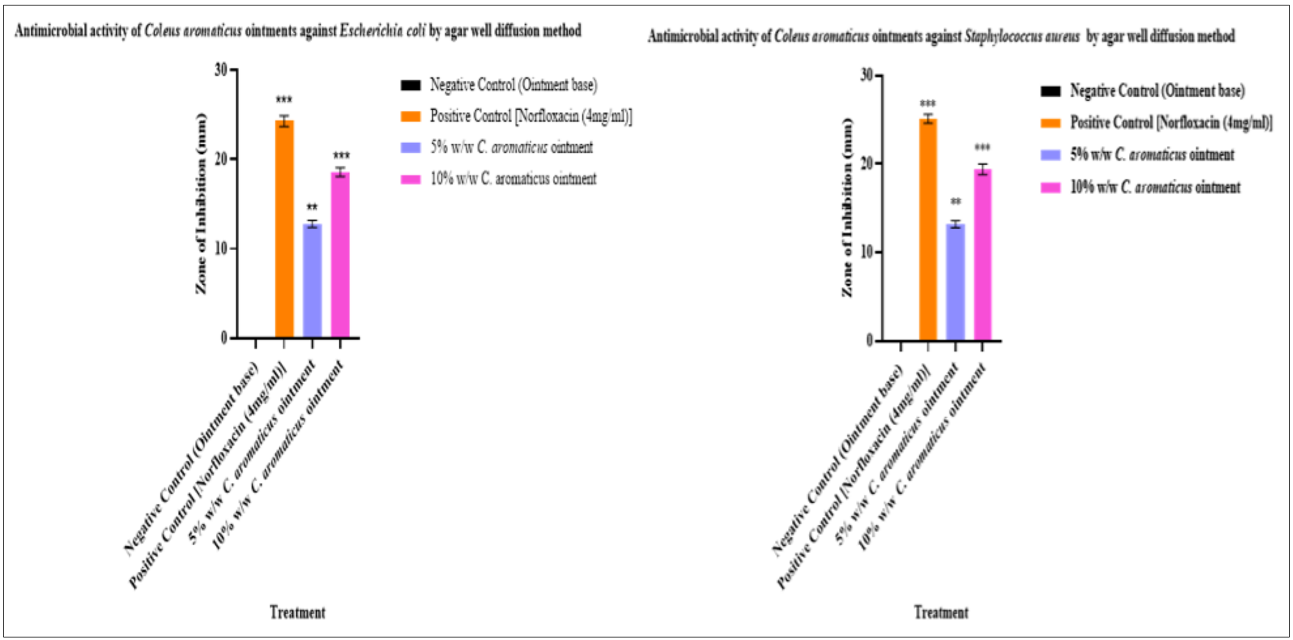

Fig 2: Antibacterial activity of 5% and 10% *Coleus aromaticus* stem ointments against *E. coli* and *S. aureus* by agar well diffusion method.

Fig 3: Zones of Inhibition for 5% and 10% *Coleus aromaticus* Ointments Against *E. coli* and *S. aureus*

5. Discussion

The global AMR crisis underscores the need for alternative antimicrobials [1]. The ethanolic *Coleus aromaticus* stem extract, rich in alkaloids, phenolics, saponins, and steroids, demonstrated significant antibacterial activity, likely due to phenolic compounds like thymol and carvacrol, which disrupt bacterial cell membranes and inhibit protein synthesis [15, 20]. The dose-dependent effect (10% > 5%) aligns with studies showing concentration-dependent bioactivity in plant extracts [21]. Norfloxacin's superior performance reflects its synthetic optimization, but its efficacy is increasingly limited by resistance, particularly in *S. aureus* [22].

The ointment's physicochemical properties, including a skin-compatible pH of 4.9 and excellent spreadability, make it suitable for topical use [23]. The absence of flavonoids, typically found in *Coleus aromaticus* leaves, suggests a distinct stem phytochemical profile, warranting further investigation [6]. These findings support the ethnomedicinal use of *Coleus aromaticus* stems for skin infections, particularly in resource-limited settings [7].

Limitations include the *in vitro* design and use of a single solvent (ethanol), which may not extract all bioactive compounds [24]. Future research should explore other solvents (e.g., methanol, aqueous), isolate active compounds using HPLC or GC-MS, and evaluate *in vivo* efficacy in wound healing models [25]. Synergistic effects with antibiotics could enhance clinical applicability [26].

6. Conclusion

The ethanolic extract of *Coleus aromaticus* stem, formulated as 5% and 10% ointments, exhibited significant dose-dependent antibacterial activity against *E. coli* and *S. aureus*. Its phytochemical richness and favorable physicochemical properties highlight its potential as a natural topical antimicrobial. Further studies are needed to isolate bioactive compounds, validate *in vivo* efficacy, and explore clinical applications to combat AMR.

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Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this research work.

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