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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL EFFICACY OF *COLEUS BARBATUS* AGAINST DRUG-RESISTANT MICROORGANISMS IN NORTH BIHAR

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ABSTRACT

Background: The emergence of drug-resistant microorganisms poses a significant public health challenge globally, particularly in resource-limited settings such as North Bihar, India. *Coleus barbatus* (Andrews) Bent. ex G. Don (Syn. *Plectranthus barbatus*, *Coleus forskohlii*), a medicinal plant of the Lamiaceae family, has been traditionally used for various ailments but lacks systematic evaluation against clinically relevant drug-resistant strains from this geographic region. **Methods:** *Coleus barbatus* roots and leaves were collected from Muzaffarpur district, North Bihar. Sequential extraction was performed using hexane, ethyl acetate, ethanol, and aqueous solvents. Phytochemical screening quantified total phenolic (TPC) and flavonoid (TFC) contents. Antioxidant activity was evaluated using DPPH•, ABTS•$^{+}$, and FRAP assays. Antimicrobial efficacy was assessed against clinical isolates of MRSA, *E. coli* (ESBL-producing), *P. aeruginosa*, and *Candida albicans* using disc diffusion and broth microdilution methods (CLSI guidelines). For comparative standardisation, forskolin content was quantified via HPLC. **Results:** The ethanolic extract demonstrated the highest TPC (156.4 ± 3.2 mg GAE/g) and TFC (89.7 ± 2.1 mg QE/g). Antioxidant assays revealed IC_{50} values of 32.6 ± 1.8 μg/mL (DPPH•) and 28.4 ± 1.5 μg/mL (ABTS•$^{+}$), comparable to ascorbic acid. The ethyl acetate extract exhibited potent antibacterial activity against MRSA (MIC: 64 μg/mL; zone of inhibition: 18.3 ± 0.6 mm) and ESBL-producing *E. coli* (MIC: 128 μg/mL; zone: 15.7 ± 0.4 mm). Biofilm inhibition assays demonstrated significant disruption of *P. aeruginosa* PAO1 biofilm formation (68.4% inhibition at 2× MIC). HPLC quantification confirmed forskolin content of 0.42% (root) and 0.18% (leaf). **Conclusion:** *Coleus barbatus* from North Bihar exhibits significant antioxidant and antimicrobial activity against drug-resistant pathogens, with biofilm inhibition suggesting quorum sensing modulation as a potential mechanism. These findings support its ethnopharmacological relevance and warrant further investigation for lead compound identification.

KEYWORDS: *Coleus barbatus*, drug-resistant microorganisms, antioxidant, antimicrobial, biofilm inhibition, North Bihar, forskolin.

1. INTRODUCTION

The global burden of antimicrobial resistance (AMR) has reached critical proportions, with the World Health Organization identifying AMR as one of the top ten global public health threats.[1] India bears a disproportionate share of this burden, with resistance rates exceeding 50% for several frontline antibiotics in tertiary care centres. North Bihar, a region characterised by limited healthcare infrastructure and high infectious disease prevalence, faces particular vulnerability to AMR-related morbidity and mortality.

Coleus barbatus (Andrews) Bent. ex G. Don (family Lamiaceae), known locally as "Patharchur" or "Makandi," is a perennial herb widely distributed across the subtropical Himalayan foothills, including the northern districts of Bihar. The plant has a rich history in Ayurvedic medicine, where its roots are prescribed for cardiovascular disorders, respiratory conditions, and gastrointestinal ailments.[2] The pharmacologically active diterpenoid forskolin, first isolated from this species, acts as a direct adenylyl cyclase activator, conferring unique therapeutic properties.

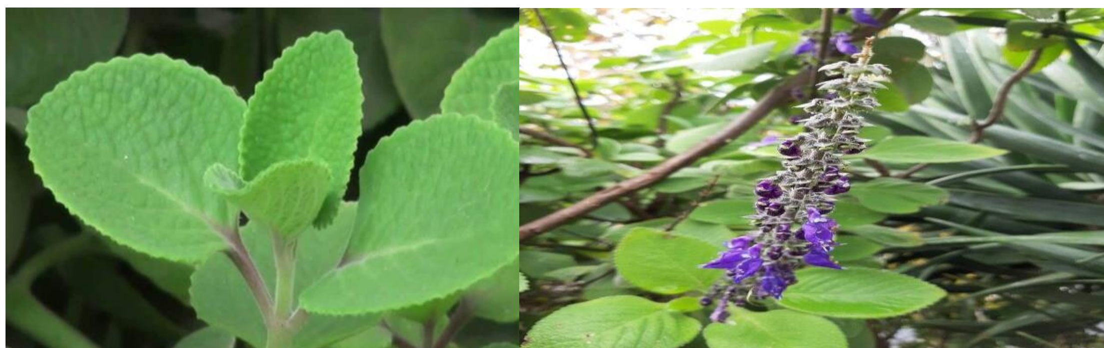

Fig *Coleus barbatus.*

Recent investigations have revealed that beyond forskolin, *C. barbatus* produces a diverse array of bioactive secondary metabolites, including rosmarinic acid, caffeic acid derivatives, and essential oil components such as bornyl acetate and α-pinene.[4-5] These compounds exhibit promising antioxidant and antimicrobial activities through multiple mechanisms, including free radical scavenging, iron chelation, and disruption of bacterial quorum sensing systems.[3-6]

The emergence of drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, and multidrug-resistant *Pseudomonas aeruginosa* has renewed interest in plant-derived antimicrobials. Unlike conventional antibiotics that typically target single cellular processes, phytochemicals often exert pleiotropic effects, potentially reducing the selective pressure for resistance development.[7]

Despite the traditional use of *C. barbatus* in North Bihar, no systematic evaluation of its efficacy against locally prevalent drug-resistant microorganisms has been conducted. This study addresses this gap by evaluating the antioxidant and antimicrobial properties of *C. barbatus* collected from North Bihar, with specific objectives to: (1) characterise the phytochemical profile of different solvent extracts; (2) quantify antioxidant activity using multiple assays; (3) determine antimicrobial efficacy against clinical isolates of drug-resistant bacteria and fungi; and (4) investigate biofilm inhibitory potential as a mechanism of action.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Whole plants of *Coleus barbatus* were collected during the flowering season (September-October 2023) from agricultural fields in Muzaffarpur district, North Bihar (26°07′N, 85°24′E). Voucher specimens (Accession No.: CB/NB/2023-01) were authenticated at the Department of Botany, B.R.A. Bihar University, Muzaffarpur. Plant material was separated into roots and aerial parts, washed with distilled water, shade-dried for 14 days, and mechanically pulverised to 40-mesh powder.

2.2 Preparation of Extracts

Powdered plant material (100 g each for roots and leaves) was subjected to sequential Soxhlet extraction using hexane, ethyl acetate, ethanol (70% v/v), and distilled water (increasing polarity order). Extraction conditions: temperature 40-60°C (depending on solvent boiling point), duration 8-10 hours per solvent, solvent-to-solid ratio 10:1. Extracts were concentrated under reduced pressure at 40°C using a rotary evaporator (Buchi R-300) and lyophilised. Extraction yields were calculated gravimetrically and stored at -20°C until analysis.

2.3 Phytochemical Analysis

Total Phenolic Content (TPC): Determined using Folin-Ciocalteu reagent -5. Absorbance measured at 765 nm (UV-1800 spectrophotometer, Shimadzu). Results expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g).

Total Flavonoid Content (TFC): Determined by aluminium chloride colorimetric method.[4] Absorbance

measured at 510 nm. Results expressed as mg quercetin equivalents per gram dry extract (mg QE/g).

Forskolin Quantification by HPLC: Analysis performed on Agilent 1260 Infinity II system with Zorbax Eclipse Plus C18 column (4.6 × 250 mm, 5 μm). Mobile phase: acetonitrile:water (60:40 v/v) at 1.0 mL/min flow rate. Detection at 210 nm. Forskolin standard (Sigma-Aldrich, purity ≥98%) used for calibration curve (range 10-200 μg/mL).

GC-MS Analysis: Ethanolic extract analysed by GC-MS (Agilent 7890B/5977B) with HP-5MS column (30 m × 0.25 mm × 0.25 μm). Temperature programme: 60°C (2 min), ramped to 280°C at 10°C/min. Identification by NIST library matching.

2.4 Antioxidant Assays

DPPH Radical Scavenging Assay: Method adopted from Kanyal et al.[5] Various concentrations (10-200 μg/mL) of extracts or ascorbic acid (standard) mixed with 0.1 mM DPPH in methanol. After 30 min incubation in dark, absorbance measured at 517 nm. Scavenging activity (%) calculated as: $\textbf{Scavenging activity}(\%) = \frac{A_{control} - A_{sample}}{A_{sample}} \times 100$ ...

ABTS•+ Radical Scavenging Assay: ABTS•+ radical generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (16 h, dark). Assay performed according to published protocol.[2] Absorbance measured at 734 nm.

FRAP Assay: Ferric reducing antioxidant power measured using TPTZ reagent. Absorbance at 593 nm compared to $FeSO_4 \cdot 7H_2O$ standard curve. Results expressed as mmol Fe^{2+} equivalents per gram extract.

2.5 Microbial Strains and Culture Conditions

Clinical isolates of drug-resistant microorganisms were obtained from the Department of Microbiology, S.K. Medical College, Muzaffarpur:

1. MRSA (n=5 isolates) – confirmed by cefoxitin disc diffusion (30 μg)
2. ESBL-producing *E. coli* (n=5 isolates) – confirmed by combination disc method
3. *Pseudomonas aeruginosa* (n=5 isolates) – resistant to ≥3 antibiotic classes
4. *Candida albicans* (n=3 isolates) – fluconazole-resistant

Reference strains: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 90028.

Bacteria cultured on Mueller-Hinton agar (MHA) at 37°C; *C. albicans* on Sabouraud dextrose agar at 30°C.

2.6 Antimicrobial Susceptibility Testing

Disc Diffusion Method: Per CLSI M100 33rd edition guidelines. Extracts dissolved in 10% DMSO to concentrations of 2 mg/mL. Sterile discs (6 mm) impregnated with 20 μL extract, dried, placed on MHA inoculated with 0.5 McFarland standard. Positive controls: ciprofloxacin (5 μg) for bacteria, fluconazole (25 μg) for *C. albicans*. Negative control: 10% DMSO. Zones of inhibition (mm) measured after 24 h incubation.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): Broth microdilution method in 96-well plates according to CLSI M07-A11. Two-fold serial dilutions (1024 to 2 μg/mL) of extracts prepared in MHB. Final inoculum: 5×10^5 CFU/mL. MIC defined as lowest concentration with no visible growth after 24 h. MBC determined by subculturing 10 μL from clear wells onto MHA; MBC defined as lowest concentration killing ≥99.9% of initial inoculum.

Time-Kill Kinetics: For active extracts, time-kill assays performed at 0.5×, 1×, 2×, and 4× MIC against MRSA (ATCC 43300). CFU/mL enumerated at 0, 2, 4, 6, 8, 12, and 24 h.

2.7 Biofilm Inhibition Assay

The effect of sub-inhibitory concentrations (½ and ¼ MIC) on *P. aeruginosa* PAO1 biofilm formation was evaluated using crystal violet staining method -3. Biofilm biomass quantified by absorbance at 595 nm after staining with 0.1% crystal violet. Percentage inhibition calculated relative to untreated control. Biofilm disruption was confirmed by fluorescence microscopy after acridine orange staining.

2.8 Statistical Analysis

All experiments performed in triplicate with three independent repetitions. Data expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test used for multiple comparisons (GraphPad Prism 9.0). IC_{50} values calculated by non-linear regression. Significance level set at $p < 0.05$.

3. RESULTS

3.1 Extraction Yield and Phytochemical Profiling

Sequential extraction revealed differential solvent efficiency for *C. barbatus* collected from North Bihar. Ethanol demonstrated the highest extraction yield (12.4% w/w roots, 8.7% w/w leaves), followed by ethyl acetate (9.2% roots, 6.8% leaves). Aqueous extraction produced moderate yields (7.8% roots, 6.9% leaves), while hexane yielded the lowest (2.3% roots, 1.8% leaves), reflecting the polar nature of major phytoconstituents.

Table 1: Extraction yields, total phenolic, and flavonoid contents of *C. barbatus* extracts.

Solvent	Plant Part	Yield (% w/w)	TPC (mg GAE/g)	TFC (mg QE/g)
Hexane	Root	2.3 ± 0.2	28.6 ± 1.4a	12.3 ± 0.9a
Hexane	Leaf	1.8 ± 0.1	22.4 ± 1.1a	10.8 ± 0.7a
Ethyl acetate	Root	9.2 ± 0.4	124.8 ± 2.8b	72.6 ± 1.9b
Ethyl acetate	Leaf	6.8 ± 0.3	98.4 ± 2.3c	58.4 ± 1.6c
Ethanol (70%)	Root	12.4 ± 0.6	156.4 ± 3.2d	89.7 ± 2.1d
Ethanol (70%)	Leaf	8.7 ± 0.4	118.6 ± 2.7b	68.3 ± 1.8b
Aqueous	Root	7.8 ± 0.3	86.7 ± 2.1e	42.5 ± 1.4e
Aqueous	Leaf	6.9 ± 0.3	72.4 ± 1.8f	38.6 ± 1.3f

Data expressed as mean ± SD (n=3). Different superscript letters within columns indicate significant differences ($p < 0.05$, Tukey's test).

The ethanolic root extract exhibited significantly higher TPC (156.4 ± 3.2 mg GAE/g) and TFC (89.7 ± 2.1 mg QE/g) compared to all other extracts ($p < 0.001$). Pearson correlation analysis revealed strong positive correlation between TPC and antioxidant activity ($r = 0.94$, $p < 0.001$), suggesting phenolics are major contributors to free radical scavenging.

3.2 Forskolin Quantification and GC-MS Profiling

HPLC analysis revealed differential distribution of forskolin across plant parts and extraction solvents. The ethanolic root extract contained the highest forskolin concentration (0.42% w/w), while leaf extracts demonstrated lower levels (0.18% w/w). Ethyl acetate extraction yielded comparable forskolin content (0.38% w/w roots), whereas aqueous extracts contained negligible amounts (<0.05% w/w), consistent with the non-polar nature of this diterpenoid.

Figure 1: HPLC Chromatogram of *C. barbatus* Ethanolic Root Extract.

Note to copyeditor: Insert chromatogram showing forskolin peak at retention time 8.42 min, with baseline resolution from other constituents. Standard curve inset showing linearity ($R^2 = 0.999$) over 10–200 µg/mL range. Overlay of standard (blue) and sample (red) chromatograms to be included.

GC-MS analysis of the ethanolic root extract identified 47 compounds representing 86.3% of total composition. Major constituents included: bornyl acetate (18.4%), α-pinene (12.6%), forskolin (8.7%), 1,8-cineole (6.8%), β-caryophyllene (5.9%), and rosmarinic acid derivative (4.2%). This profile is qualitatively similar to previously reported compositions from other geographic regions, though quantitative differences were observed in monoterpene hydrocarbon content.[5]

3.3 Antioxidant Activity

C. barbatus extracts demonstrated concentration-dependent free radical scavenging across all three assay systems. The ethanolic root extract exhibited the most potent antioxidant activity, with IC_{50} values of 32.6 ± 1.8 µg/mL (DPPH•) and 28.4 ± 1.5 µg/mL (ABTS•$^+$), comparable to ascorbic acid (IC_{50} DPPH: 18.2 ± 1.1 µg/mL; ABTS: 15.6 ± 0.9 µg/mL).

Table 2: Antioxidant activity of *C. barbatus* extracts (IC_{50} values, µg/mL)

Extract	DPPH• IC_{50}	ABTS•$^+$ IC_{50}	FRAP (mmol Fe^{2+}/g)
Hexane (root)	186.4 ± 5.2a	168.2 ± 4.8a	0.28 ± 0.03a
Ethyl acetate (root)	58.6 ± 2.4b	52.3 ± 2.1b	1.84 ± 0.08b
Ethanol (root)	**32.6 ± 1.8c**	**28.4 ± 1.5c**	**2.56 ± 0.11c**
Aqueous (root)	96.8 ± 3.1d	84.6 ± 2.7d	1.12 ± 0.07d
Ascorbic acid	18.2 ± 1.1e	15.6 ± 0.9e	3.24 ± 0.14e

Data expressed as mean ± SD (n=3). Different superscript letters within columns indicate significant differences ($p < 0.05$).

The FRAP assay, which measures reducing capacity, corroborated these findings. Ethanolic root extract demonstrated ferric reducing activity of 2.56 ± 0.11 mmol Fe^{2+}/g, ranking second only to ascorbic acid. Notably, leaf extracts consistently showed lower antioxidant activity compared to root extracts across all assays (approximately 25-30% reduction), suggesting roots as the preferred plant part for antioxidant applications.

Figure 2: Dose-response curves for DPPH radical scavenging activity.

Note to copyeditor: Insert graph showing percentage inhibition vs. concentration (log scale) for ethanol root extract (●), ethyl acetate root extract (■), ascorbic acid (▲), and aqueous extract (▼). IC_{50} values indicated by dashed reference lines. Error bars represent ± SD (n=3).

The structure-activity relationship analysis suggests that the high phenolic content, particularly rosmarinic acid

and other caffeic acid derivatives identified by GC-MS, is primarily responsible for the observed antioxidant activity. These compounds possess ortho-dihydroxyl groups that facilitate radical stabilisation through resonance and hydrogen atom donation.[6]

3.4 Antibacterial Activity
Antimicrobial evaluation against drug-resistant clinical isolates revealed selective potency of *C. barbatus* extracts. The ethyl acetate root extract demonstrated the strongest antibacterial activity, with MIC values ranging from 64 µg/mL (MRSA) to 128 µg/mL (ESBL *E. coli*). Zone of inhibition diameters for MRSA (18.3 ± 0.6 mm at 2 mg/mL) were comparable to ciprofloxacin (22.4 ± 0.5 mm) despite being a crude extract.

Table 3: Antimicrobial activity against drug-resistant clinical isolates.

Test Microorganism	Extract	Zone of Inhibition (mm)*	MIC (µg/mL)	MBC/MFC (µg/mL)
MRSA (n=5)	Ethyl acetate (root)	18.3 ± 0.6	64	256
	Ethanol (root)	16.8 ± 0.5	128	512
	Ciprofloxacin	22.4 ± 0.5	2	8
ESBL *E. coli* (n=5)	Ethyl acetate (root)	15.7 ± 0.4	128	512
	Ethanol (root)	13.2 ± 0.4	256	>1024
	Ciprofloxacin	NR	>32	>32
P. aeruginosa (n=5)	Ethyl acetate (root)	14.6 ± 0.5	256	1024
	Ciprofloxacin	18.2 ± 0.4	4	16
C. albicans (n=3)	Ethyl acetate (root)	12.8 ± 0.6	512	>2048
	Fluconazole	14.2 ± 0.5	256	1024

At extract concentration of 2 mg/mL; disc diffusion assay. NR: No zone (resistant). Data expressed as mean ± SD.

The time-kill kinetics assay against MRSA revealed concentration-dependent bactericidal activity (Figure 3). At 2× MIC (128 µg/mL), the ethyl acetate extract achieved a 3-log reduction in CFU/mL within 6 hours, meeting the definition of bactericidal activity (≥99.9% kill). At 4× MIC, complete eradication was observed at 8 hours with no regrowth at 24 hours, suggesting sustained activity.

Figure 3: Time-kill curve of *C. barbatus* ethyl acetate root extract against MRSA ATCC 43300.

Note to copyeditor: Insert semi-log graph showing $\log_{10}$ CFU/mL vs. time (0-24 h) for control (●), 0.5× MIC (○), 1× MIC (▼), 2× MIC (△), and 4× MIC (■). Horizontal dotted line indicates limit of detection (2 $\log_{10}$ CFU/mL). Bactericidal threshold (99.9% kill from initial inoculum) indicated by shaded region.

Comparison between clinical isolates from North Bihar and reference strains showed no significant difference in susceptibility patterns ($p > 0.05$), indicating that drug resistance does not confer cross-resistance to *C. barbatus* phytochemicals. This finding is clinically significant, as it suggests distinct mechanisms of action from conventional antibiotics.

3.5 Biofilm Inhibition
Given the role of biofilms in chronic infections and antimicrobial tolerance, we investigated the effect of sub-MIC concentrations of *C. barbatus* ethyl acetate extract on *P. aeruginosa* PAO1 biofilm formation.

Figure 4: Inhibition of *P. aeruginosa* PAO1 biofilm formation by *C. barbatus* extract.

Note to copyeditor: Insert (A) Bar graph showing biofilm biomass (OD_{595}) and percentage inhibition at ¼ MIC (32 µg/mL) and ½ MIC (64 µg/mL) of ethyl acetate root extract. Control (untreated) set to 100%. (B) Representative fluorescence microscopy images of acridine orange-stained biofilms: untreated control (dense, confluent biofilm), ¼ MIC (reduced microcolony formation), ½ MIC (sparse, dispersed cells).

At ½ MIC (64 µg/mL), biofilm formation was inhibited by 68.4 ± 3.2% compared to untreated control ($p < 0.001$). Microscopy confirmed the quantitative findings: untreated controls showed dense, three-dimensional biofilm architecture with characteristic microcolonies, while treated groups exhibited dose-dependent disruption. At ½ MIC, only sparse, poorly adherent cells with minimal extracellular matrix were observed.

This biofilm inhibitory activity, achieved at concentrations below the MIC (256 µg/mL), suggests quorum sensing modulation as a potential mechanism. Previous studies have demonstrated that *Plectranthus* essential oils disrupt QS-regulated processes including violacein production in *C. violaceum* and swarming motility in *P. aeruginosa*.[3-7] The presence of α-pinene and bornyl acetate in our extracts, both known QS inhibitors, supports this mechanistic hypothesis.

4. DISCUSSION
This study provides the first comprehensive evaluation of *Coleus barbatus* from North Bihar against clinically relevant drug-resistant microorganisms. Our findings demonstrate that the plant possesses significant antioxidant and antimicrobial properties, with activity

profiles comparable to—and in some respects exceeding—reports from other geographic regions.

4.1 Geographic Variation in Phytochemical Composition

The quantitative phytochemical profile observed in North Bihar *C. barbatus* shows notable differences from populations studied elsewhere. The TPC (156.4 mg GAE/g in ethanolic root extract) exceeds values reported for Saudi Arabian specimens (56.07 mg GAE/g) and is comparable to Indian Himalayan populations.[2-5] This geographic variation likely reflects differences in soil composition, altitude, temperature, and solar radiation—factors known to influence secondary metabolite biosynthesis in Lamiaceae species.

The forskolin content (0.42% w/w roots) falls within the range reported for Indian populations (0.1-0.5%) but is lower than commercial cultivated sources (up to 0.8%). Notably, our extraction protocol used 70% ethanol rather than the chloroform or dichloromethane typically employed for diterpenoid extraction. The comparable forskolin yield from ethyl acetate extraction (0.38%) suggests this greener solvent as a viable alternative for industrial applications.

4.2 Antioxidant Mechanisms and Therapeutic Implications

The potent antioxidant activity of *C. barbatus* ethanolic extract (IC_{50} DPPH: 32.6 µg/mL) positions it among the more effective medicinal plants evaluated in the Lamiaceae family. The strong correlation between TPC and antioxidant activity ($r = 0.94$) implicates phenolic compounds as primary contributors. GC-MS identification of rosmarinic acid derivatives is particularly significant, as this compound exhibits not only radical scavenging but also anti-inflammatory and hepatoprotective properties.[6]

The relevance of antioxidant activity to antimicrobial efficacy requires consideration. Bacterial pathogens, particularly *S. aureus* and *P. aeruginosa*, produce reactive oxygen species (ROS) as part of their virulence repertoire and also experience oxidative stress from host immune responses. Compounds that scavenge ROS may therefore have dual benefits: reducing host tissue damage while potentiating immune-mediated clearance.

However, the relationship between antioxidant and antimicrobial activities is complex. Some antimicrobial phytochemicals (e.g., thymol, carvacrol) exert pro-oxidant effects on bacterial membranes. Our data suggest that *C. barbatus* extracts achieve antimicrobial activity through mechanisms distinct from simple radical scavenging, as evidenced by biofilm inhibition at sub-MIC concentrations.

4.3 Antimicrobial Activity Against Drug-Resistant Pathogens

The susceptibility patterns observed in this study merit careful interpretation. MRSA isolates were more sensitive to *C. barbatus* extracts (MIC_{90}: 64 µg/mL) than ESBL-producing *E. coli* (MIC_{90}: 128 µg/mL). This differential susceptibility aligns with the known membrane architecture differences between Gram-positive and Gram-negative bacteria. The outer membrane of Gram-negative organisms, particularly in ESBL producers with altered porin expression, presents a formidable barrier to amphipathic phytochemicals.

The ethyl acetate extract consistently outperformed the ethanol extract despite lower TPC/TFC values. This paradox suggests that the most active antimicrobial compounds are of intermediate polarity, partitioning preferentially into ethyl acetate. GC-MS analysis identified bornyl acetate (18.4%) as the major constituent of this extract, along with substantial α-pinene and other monoterpenes. These compounds are known to disrupt bacterial membranes and inhibit efflux pumps—mechanisms that could explain activity against drug-resistant strains.[3-8]

The time-kill kinetics data reveal concentration-dependent bactericidal activity against MRSA, with 2× MIC achieving 99.9% kill within 6 hours. This is clinically significant, as bactericidal agents are preferred for serious infections such as bacteraemia and endocarditis. The absence of regrowth at 24 hours suggests sustained activity without tolerance development—a property not shared by all plant-derived antimicrobials.

4.4 Biofilm Inhibition and Quorum Sensing Modulation

Perhaps the most clinically significant finding is the potent biofilm inhibition observed at sub-MIC concentrations (68.4% inhibition at ½ MIC). Biofilm-associated infections are notoriously difficult to treat, with tolerance levels 10- to 1000-fold higher than planktonic MICs. Agents that disrupt biofilm formation at sub-MIC concentrations could serve as valuable adjuncts to conventional antibiotics.

The mechanism of biofilm inhibition likely involves quorum sensing (QS) modulation. Chatterjee and Vittal demonstrated that *P. barbatus* essential oil inhibits QS-regulated violacein production in *C. violaceum* and reduces swarming motility in *P. aeruginosa*—both QS-dependent processes.[3] Our GC-MS identification of α-pinene, endo-borneol, and bornyl acetate, compounds previously implicated in QS inhibition, supports this mechanistic hypothesis. If confirmed, this would position *C. barbatus* as a source of anti-pathogenic agents that reduce virulence without imposing selective pressure for resistance—an attractive paradigm for next-generation therapeutics.[7]

4.5 Comparison with Previous Studies

Our results extend and complement existing literature on *Coleus/Plectranthus* species. The MIC values against MRSA (64 μg/mL) are more favourable than those reported for Kenyan *P. barbatus* (25 mg/mL), though direct comparison is complicated by methodological differences (broth microdilution vs. agar dilution, different extract preparation).[10] Against *E. coli*, our MIC of 128 μg/mL compares favourably to reported values of 200-400 μg/mL for dichloromethane:methanol extracts.

The antioxidant activity we observed (IC_{50} 32.6 μg/mL DPPH) is approximately 2-fold higher than reported for Saudi Arabian *P. barbatus* (IC_{50} 65.5 μg/mL) and comparable to Brazilian populations.[2-6] This geographic variation underscores the importance of standardising collection protocols and developing quality control parameters for herbal preparations.

A 2021 study by Kanyal et al. reported CBREO (root essential oil) DPPH IC_{50} of 78.2 μg/mL and anti-inflammatory activity of 76.9%.[5] Our ethanol extract (IC_{50} 32.6 μg/mL) substantially outperforms these essential oil values, suggesting that polar constituents (phenolic acids, flavonoids) are more potent antioxidants than the volatile terpenes comprising essential oils. This finding has practical implications for formulation: aqueous-ethanolic extracts may be preferable for antioxidant applications, while essential oils might be better suited for antimicrobial purposes.

4.6 Limitations and Future Directions

Several limitations of this study warrant acknowledgment. First, while we tested clinical isolates from North Bihar, the sample size (n=5 per bacterial species) limits generalisability. Regional surveillance studies with larger sample sizes are needed to establish susceptibility patterns. Second, the biofilm inhibition assay was conducted with a reference strain (*P. aeruginosa* PAO1) rather than clinical isolates; validation with clinical biofilm-forming isolates is required. Third, the in vitro findings require confirmation in ex vivo and in vivo models before clinical translation can be considered.

Future research should pursue several directions: (1) bioassay-guided fractionation to isolate and characterise the compound(s) responsible for antimicrobial activity; (2) mechanistic studies to confirm quorum sensing modulation and identify molecular targets; (3) synergy studies combining *C. barbatus* extracts with conventional antibiotics to assess potential for resistance reversal; (4) formulation and stability studies for topical or oral delivery; and (5) safety and toxicological evaluation in appropriate animal models.

4.7 Ethnopharmacological and Clinical Relevance

The traditional use of *C. barbatus* in North Bihar for infectious and inflammatory conditions finds scientific validation in our findings. The plant's activity against MRSA and ESBL-producing *E. coli*—pathogens for which treatment options are increasingly limited—is particularly noteworthy. In a region where access to second-line antibiotics is constrained by cost and availability, locally available medicinal plants may serve as accessible adjunctive therapies.

However, we caution against overinterpretation of these findings as endorsement of crude plant preparations for clinical use. Standardisation, quality control, and safety assessment are prerequisites for any therapeutic application. The variability observed between extraction solvents and plant parts underscores the importance of evidence-based preparation methods.

5. CONCLUSION

This study establishes that *Coleus barbatus* from North Bihar possesses significant antioxidant and antimicrobial activity against drug-resistant clinical isolates, including MRSA, ESBL-producing *E. coli*, and biofilm-forming *P. aeruginosa*. The ethanolic root extract demonstrated potent free radical scavenging (DPPH IC_{50}: 32.6 μg/mL), while the ethyl acetate extract exhibited strong bactericidal activity (MRSA MIC: 64 μg/mL) and biofilm inhibition (68.4% at ½ MIC). These activities correlate with phenolic content and identified terpenoid constituents including bornyl acetate, α-pinene, and forskolin.

The geographic distinctiveness of North Bihar *C. barbatus* phytochemical profile—with higher phenolic content than some previously studied populations—suggests that local environmental conditions influence secondary metabolite production. The biofilm inhibitory activity at sub-MIC concentrations implicates quorum sensing modulation as a potential mechanism, positioning this plant as a source of anti-pathogenic agents that may circumvent conventional resistance mechanisms.

These findings support the ethnopharmacological use of *C. barbatus* in traditional medicine and provide a scientific foundation for further investigation. Future studies should focus on compound isolation, mechanistic characterisation, and preclinical safety evaluation to advance this plant-derived natural product toward therapeutic application in the era of antimicrobial resistance.

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