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Phytochemical Profiling, Antioxidant Potential, And In Vitro Antimicrobial Evaluation Of Dysoxylum Binectariferum Bark Extract Against Pathogenic Bacteria

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ABSTRACT

The present study investigated the phytochemical composition, antioxidant potential, and antimicrobial activity of Dysoxylum binectariferum bark extracts. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides, with ethanolic and ethyl acetate fractions being particularly rich in phenolic and flavonoid compounds. Quantitative assays revealed high total phenolic content (375.6 ± 8.4 mg GAE/g) and total flavonoid content (186.4 ± 5.1 mg RE/g) in the ethanolic extract. Antioxidant assays demonstrated strong radical scavenging activities in a concentration-dependent manner. The ethanolic extract exhibited lower IC_{50} values for DPPH (41.6 µg/mL), ABTS (37.8 µg/mL), and hydrogen peroxide (44.5 µg/mL) compared to the aqueous extract, though both were less potent than ascorbic acid or gallic acid standards. The FRAP assay further confirmed the reducing ability of the ethanolic extract (1212.4 ± 35.6 µmol TE/g). In antimicrobial evaluation, the ethanolic extract displayed significant activity against Gram-positive (Staphylococcus aureus, Bacillus subtilis) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria. Zone of inhibition values ranged from 23.3–29.6 mm, with MIC values of 6.25–12.5 mg/mL and MBC values of 25–50 mg/mL. The aqueous extract showed moderate activity, while standard antibiotics (gentamicin, ciprofloxacin) remained more potent. The study establishes D. binectariferum bark as a rich source of bioactive phytochemicals with strong antioxidant and antibacterial properties, providing scientific support for its traditional uses and highlighting its potential as a candidate for phytopharmaceutical development.

Keywords: *Dysoxylum binectariferum; phytochemical profiling; antioxidant activity; DPPH; ABTS; FRAP; antimicrobial activity; MIC; MBC; medicinal plants.*

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1. INTRODUCTION

Medicinal plants have played an indispensable role in the evolution of human healthcare systems since ancient times, serving as the primary source of therapeutic remedies long before the advent of modern pharmaceuticals [1]. Among the myriads of plant species with ethnomedicinal relevance, *Dysoxylum binectariferum* (Roxb.) Hook.f. ex Bedd., a member of the Meliaceae family, has gained attention due to its remarkable chemical diversity and traditional uses in Ayurveda [2]. This evergreen tree, widely distributed in the Western Ghats and parts of Southeast Asia, has been used in indigenous medicine for treating microbial infections, inflammatory disorders, ulcers, and gastrointestinal ailments [3]. The plant is particularly notable for containing rohitukine, a chromone alkaloid that has served as the precursor for flavopiridol (alvocidib), one of the earliest cyclin-dependent kinase (CDK) inhibitors developed for cancer therapy [4]. Despite this pharmacological significance, the bark of *D. binectariferum* remains underexplored in terms of its comprehensive phytochemical profiling and evaluation of its antioxidant and antimicrobial properties. This gap provides a strong rationale for detailed scientific assessment of its potential therapeutic applications. The emergence of antimicrobial resistance (AMR) has become a critical global health challenge, rendering many conventional antibiotics less effective and contributing to prolonged illnesses, higher medical costs, and increased mortality [5]. The World Health Organization has repeatedly emphasized the urgent need for novel antimicrobial agents with diverse mechanisms of action [6]. In this context, plant-derived phytochemicals offer a promising avenue, as they often act via multi-target mechanisms such as disrupting bacterial cell walls, inhibiting nucleic acid synthesis, altering membrane permeability, or interfering with quorum sensing [7]. Unlike synthetic drugs that usually target a single microbial pathway, phytochemicals are chemically diverse and capable of exerting synergistic effects, thereby reducing the likelihood of resistance development [8]. Several members of the Meliaceae family have been reported to possess antimicrobial activity due to their unique arsenal of secondary metabolites including limonoids, triterpenoids, alkaloids, flavonoids, and phenolic acids [9]. It is therefore scientifically relevant to explore the bark of *D. binectariferum* for its antibacterial efficacy against both Gram-positive and Gram-negative pathogens that are of clinical importance. Parallel to the challenge of antimicrobial resistance, oxidative stress represents another pressing biomedical concern [10]. Reactive oxygen species (ROS) and free radicals, when produced in excess, can overwhelm endogenous defense systems and trigger oxidative damage to biomolecules such as lipids, proteins, and nucleic acids [11]. This oxidative imbalance is a central factor in the pathogenesis of a wide range of chronic and degenerative diseases including cardiovascular disorders, neurodegenerative diseases, diabetes, cancer, and accelerated aging [12]. Antioxidants, both enzymatic and non-enzymatic, play a critical role in neutralizing free radicals, but reliance solely on endogenous defense mechanisms is often insufficient under pathological conditions [13]. As a result, dietary or supplemental antioxidants have gained prominence in disease prevention and management. However, the safety concerns associated with synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have shifted scientific focus toward natural plant-based antioxidants [14]. Polyphenols, flavonoids, and tannins derived from medicinal plants are now being extensively investigated for their radical scavenging ability, metal chelation properties, and capacity to enhance endogenous antioxidant systems [15]. Preliminary reports suggest that *Dysoxylum* species are rich in phenolics and flavonoids, which may underlie their traditional use in conditions linked to oxidative damage [16]. Thus, investigating the antioxidant potential of *D. binectariferum* bark extract is both scientifically and clinically relevant. The phytochemical richness of *D. binectariferum* has been partially elucidated in earlier studies, with reports highlighting its content of chromones, alkaloids, coumarins, triterpenoids, and flavonoids [2],[4]. Rohitukine, the plant's most studied metabolite, has already laid the foundation for anti-cancer drug discovery, demonstrating the translational value of its phytoconstituents [4]. Yet, the bark, which is traditionally used in folk medicine, has not been comprehensively profiled using modern phytochemical screening techniques [3]. Evaluating its phytochemical constituents through qualitative tests, total phenolic content estimation, and chromatographic methods provides a deeper understanding of the active metabolites responsible for its biological activities. Such profiling is not only crucial for correlating traditional uses with scientific evidence but also for paving the way toward standardized herbal formulations, nutraceuticals, and potential lead molecules for drug discovery [7],[9]. In addition to their therapeutic relevance, plants like *D. binectariferum* hold ecological and sustainable advantages [10]. As a native species of India's Western Ghats, the tree is available in abundance and can be harnessed responsibly without threatening biodiversity [11]. The integration of underexplored indigenous plants into the modern pharmacological framework aligns with the global movement toward bioprospecting, sustainable utilization of natural resources, and validation of traditional knowledge systems [12]. Furthermore, developing plant-based antioxidants and antimicrobials can provide cost-effective alternatives to synthetic drugs, particularly in low- and middle-income countries where healthcare access remains a challenge [13]. This study was therefore designed with

three major objectives: (i) to perform phytochemical profiling of *Dysoxylum binectariferum* bark extract to identify the major classes of bioactive compounds, (ii) to evaluate its antioxidant capacity using in vitro assays such as total phenolic content and hydrogen peroxide scavenging activity, and (iii) to assess its antimicrobial efficacy against clinically relevant Gram-positive and Gram-negative bacteria using agar diffusion, minimum inhibitory concentration, and minimum bactericidal concentration assays. The investigation aims to provide a holistic understanding of the therapeutic potential of *D. binectariferum* bark, bridging the gap between traditional medicine and modern pharmacology [14],[15].

2. MATERIALS AND METHODS

2.1Plant Collection and Authentication

The bark of *Dysoxylum binectariferum* (Roxb.) Hook.f. ex Bedd. was collected during the summer season (April 2025) from the Shiwalik range of Uttarakhand, India, specifically near Rishikesh forest area (geographical coordinates: 30.0869°N, 78.2676°E, altitude ~372 m above sea level). The plant material was carefully harvested from mature trees, cleaned of adhering debris, and shade-dried at ambient temperature (25–28 °C) under controlled humidity to preserve heat-sensitive phytoconstituents. The collected specimen was authenticated at Department of Botany, Hemvati Nandan Bahuguna (HNB) Garhwal University, Srinagar, Uttarakhand, a recognized taxonomist specializing in Himalayan medicinal flora. A voucher specimen (Accession No. HNBGU/DB/2025/178) was prepared and deposited in the Herbarium of the Department of Botany, HNB Garhwal University, Uttarakhand, India, for future reference.

2.2Preparation of Extract

Fresh bark of *Dysoxylum binectariferum* was shade-dried at room temperature (25–28 °C) for 7–10 days until constant weight, pulverized, and sieved (40 mesh). The powdered bark (300 g) was defatted with n-hexane in a Soxhlet apparatus, followed by exhaustive extraction with 95% ethanol (3 L) at 70 °C for 18–24 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40 °C, and the dried material was stored in amber vials at −20 °C. For comparative studies, aqueous extraction was carried out by refluxing the defatted marc with distilled water (3 × 2 h), followed by filtration and freeze-drying. Further fractionation of the crude ethanolic extract was performed sequentially with n-hexane, chloroform, ethyl acetate, and n-butanol, leaving a final aqueous fraction. All fractions were dried, weighed to calculate percentage yield, and preserved at −20 °C until use [16].

2.3 Phytochemical Profiling (Methods)

1) Qualitative Phytochemical Screening

Test sample: *D. binectariferum* bark crude ethanolic extract (CEE), aqueous extract (AQE), polarity-fractions (n-hexane, chloroform, EtOAc, n-BuOH, aqueous). Stock: 10 mg/mL in suitable solvent.

Alkaloids: 1 mL extract + few drops Mayer's (cream ppt) / Dragendorff's (orange ppt) / Wagner's (reddish-brown ppt). Positive = persistent precipitate.

Flavonoids (Shinoda): 1 mL extract + Mg turnings + few drops conc. HCl → pink/red/orange.

Tannins/Phenolics: 1 mL extract + 1% $FeCl_3$ → blue-black/greenish coloration. Gelatin test (1% gelatin + NaCl) → precipitate.

Saponins (Froth): 1 mL extract + 5 mL water, vigorous shake **30 s** → stable froth (≥1 cm, ≥15 min).

Terpenoids (Salkowski): 1 mL extract + 2 mL $CHCl_3$, add 1 mL conc. H_2SO_4 down the side → **reddish-brown** at interface.

Glycosides (Keller–Killiani for deoxy sugars): 1 mL extract + glacial AcOH (with trace $FeCl_3$), underlay conc. H_2SO_4 → brown ring at interface (cardenolide-type); Bornträger's (for anthraquinone glycosides).

Controls: Reagent blanks; known positive controls (e.g., quercetin, tannic acid, diosgenin prep) [17,18].

Quantitative estimation: The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, expressed as mg gallic acid equivalents (GAE)/g extract, while total flavonoid content (TFC) was measured using the aluminium chloride colorimetric method, expressed as mg rutin equivalents (RE)/g extract.

3. ANTIOXIDANT ACTIVITY EVALUATION

DPPH radical scavenging assay. A 0.1 mM DPPH methanolic solution was mixed with sample or standard (Trolox/ascorbic acid) prepared in methanol to obtain final concentrations across a suitable range. After incubation in the dark at room temperature for 30 min, absorbance was recorded at 517 nm against reagent blank.

$$Percentage\ scavenging\ = \frac{(A0 - As)}{A0} \times 100$$

IC_{50} (µg/mL) was computed from concentration–response curves.

ABTS•+ radical cation decolorization assay. ABTS•+ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (12–16 h, dark). The working solution was diluted with ethanol to A ≈ 0.70 ± 0.02 at 734 nm. Samples or Trolox standards were added, mixed, and after 6 min the decrease in absorbance at 734 nm was measured. Results were expressed as Trolox equivalent antioxidant capacity (TEAC; mmol Trolox equivalents/g extract) and/or IC_{50} [19].

Hydrogen peroxide scavenging activity. H_2O_2 solution (40 mM in phosphate buffer, pH 7.4) was mixed with samples/standards (ascorbic acid as reference). After 10 min at room temperature, residual H_2O_2 was quantified at 230 nm using buffer as blank. % scavenging and IC_{50} (µg/mL) were calculated relative to the control without sample.

Ferric reducing antioxidant power (FRAP). FRAP reagent (300 mM acetate buffer pH 3.6 : 10 mM TPTZ in 40 mM HCl : 20 mM $FeCl_3 \cdot 6H_2O$; 10:1:1, v/v/v) was pre-warmed to 37 °C. Sample or Trolox standard was added to FRAP reagent (typical 1:10, v/v), incubated 4–6 min at 37 °C, and absorbance read at 593 nm. A Trolox calibration curve was used to express results as mmol Trolox equivalents/g extract [20].

4. ANTIMICROBIAL ACTIVITY EVALUATION

Test organisms. Antibacterial activity was assessed against two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853). Strains were maintained on nutrient agar slants at 4 °C and sub-cultured prior to use[21].

Agar well diffusion assay. The bacterial inoculum was standardized to 0.5 McFarland turbidity (~1×10^8 CFU/mL) and spread evenly on Mueller–Hinton agar (MHA) plates. Wells (6 mm) were bored and filled with test extract/fractions (25–200 µg/well in 50 µL). Plates were incubated at 37 °C for 18–24 h, and the diameter of the zone of inhibition (ZOI, mm) was measured. Gentamicin (10 µg/mL) served as positive control; solvent as negative control [22].

Minimum inhibitory concentration (MIC). MIC values were determined by the broth microdilution method in 96-well plates using MHB (Mueller–Hinton broth). Two-fold serial dilutions of extract (range: 0.5–100 mg/mL) were inoculated with bacterial suspension (5×10^5 CFU/mL final). After incubation at 37 °C for 24 h, turbidity was measured spectrophotometrically at 600 nm. The MIC was defined as the lowest concentration with no visible growth compared with control [23,24].

Minimum bactericidal concentration (MBC). Aliquots (100 µL) from wells showing no visible growth were plated on fresh MHA and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration showing no colony formation, indicating ≥99.9% bacterial killing [25].

Statistical Analysis. All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA or Student's *t*-test, with $p < 0.05$ considered significant.

5. RESULTS

5.1 Phytochemical Screening

Table 1. Qualitative phytochemical analysis of *Dysoxylum binectariferum* bark extracts

Phytoconstituent	Ethanolic Extract	Aqueous Extract	n-Hexane Fraction	Chloroform Fraction	Ethyl Acetate Fraction	n-Butanol Fraction	Aqueous Fraction
Alkaloids	+++	++	+	++	++	++	+
Flavonoids	+++	++	–	+	+++	++	++
Tannins/Phenolics	+++	++	–	+	+++	++	++
Saponins	++	++	–	–	+	++	++
Terpenoids	++	+	+++	++	+	+	–
Glycosides	++	++	–	+	++	++	++

Key: +++ = strong presence, ++ = moderate, + = weak, – = absent

The qualitative screening revealed that the ethanolic extract was particularly rich in alkaloids, flavonoids, tannins, and phenolics, whereas the n-hexane fraction concentrated more of the non-polar terpenoids.

5.2 Quantitative Estimation

Total Phenolic Content (TPC): The ethanolic bark extract showed 375.6 ± 8.4 mg gallic acid equivalents (GAE)/g extract, while the aqueous extract recorded 242.1 ± 6.9 mg GAE/g. Among fractions, the ethyl acetate fraction exhibited the highest phenolic content (418.3 ± 7.2 mg GAE/g).

Total Flavonoid Content (TFC): The ethanolic extract contained 186.4 ± 5.1 mg rutin equivalents (RE)/g extract, and the aqueous extract showed 129.8 ± 4.3 mg RE/g. The ethyl acetate fraction again showed the maximum flavonoid content (210.7 ± 5.6 mg RE/g), followed by the n-butanol fraction (172.5 ± 4.8 mg RE/g).

5.3 Antioxidant Assays

DPPH radical scavenging activity:

The ethanolic extract of *D. binectariferum* bark exhibited a concentration-dependent increase in DPPH radical scavenging. At 20, 40, 60, 80, and 100 µg/mL, the ethanolic extract showed 32.4%, 54.7%, 69.2%, 81.5%, and 89.3% inhibition, respectively. The aqueous extract showed comparatively lower activity (21.5%–72.8% across the same concentrations). The IC_{50} values were **41.6 µg/mL (ethanolic)** and **65.2 µg/mL (aqueous)**, compared with **18.4 µg/mL for ascorbic acid (standard).**

ABTS radical cation decolorization assay:

The ethanolic extract showed strong ABTS•+ scavenging, with inhibition values of 28.7%, 52.6%, 71.4%, 83.9%, and 91.2% at 20–100 µg/mL. The aqueous extract gave 19.8%–68.5% inhibition across the same range. IC_{50} values were 37.8 µg/mL (ethanolic) and 62.4 µg/mL (aqueous), while gallic acid (standard) showed an IC_{50} of 15.6 µg/mL.

Hydrogen peroxide scavenging activity:

The ethanolic extract demonstrated 25.6%, 47.3%, 66.8%, 78.4%, and 85.9% inhibition at 20–100 µg/mL. The aqueous extract recorded lower values (18.4%–61.7%). The IC_{50} values were 44.5 µg/mL (ethanolic) and 71.3 µg/mL (aqueous), compared with 20.2 µg/mL for ascorbic acid.

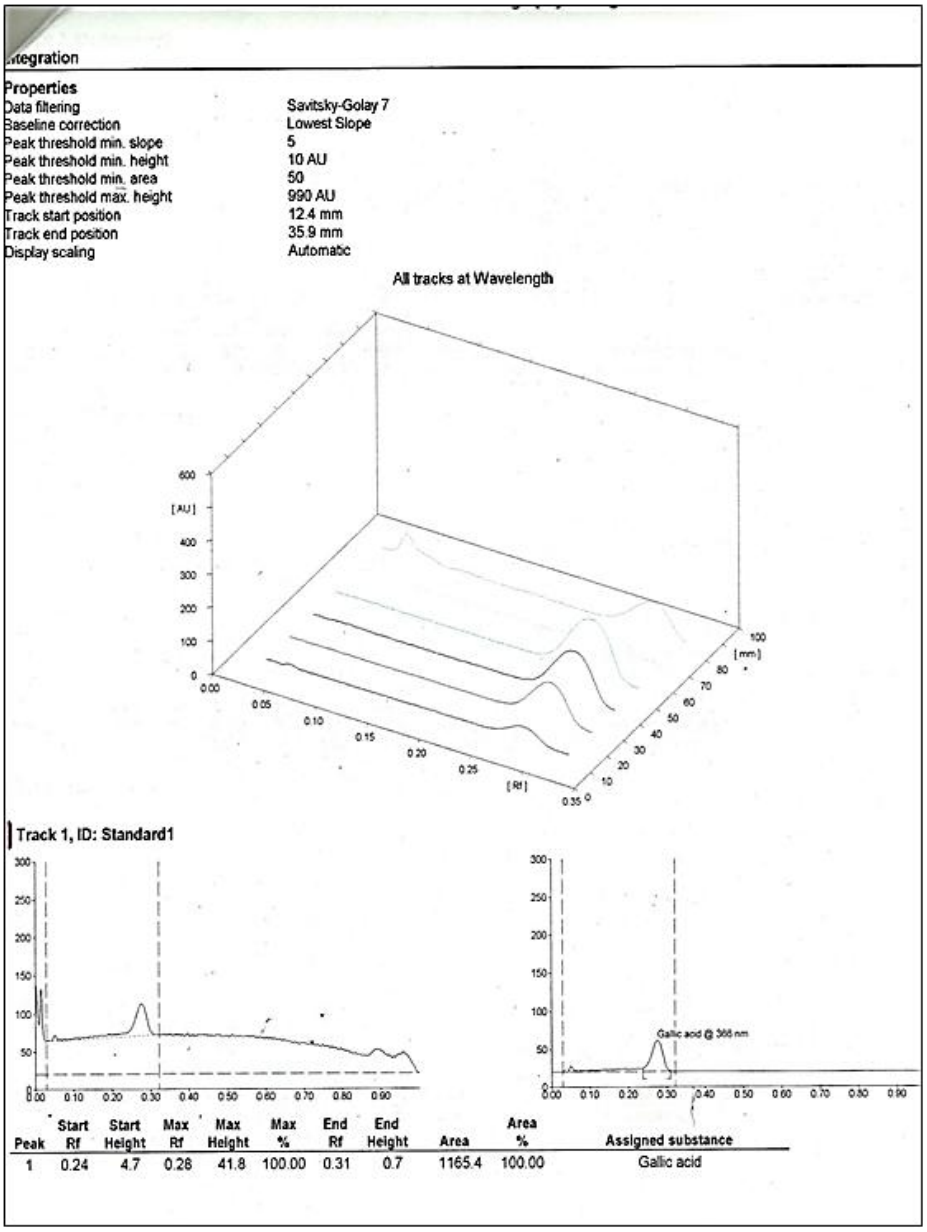

Figure 1: 3D Overlay and Track 1 (Standard 1) - HPTLC Profile of Gallic Acid

Ferric reducing antioxidant power (FRAP):

The FRAP assay confirmed the strong reducing capacity of the ethanolic extract, with absorbance at 593 nm increasing linearly with concentration. The ethanolic extract exhibited 1212.4 ± 35.6 µmol Trolox equivalents (TE)/g extract, while the aqueous extract showed 845.7 ± 28.4 µmol TE/g. Trolox standard displayed 1435.2 ± 32.1 µmol TE/g under the same conditions.

Table 2: Summary of IC_{50} values (µg/mL)

Assay	Ethanolic extract	Aqueous extract	Standard (Ascorbic acid / Gallic acid)
DPPH	41.6 ± 1.2	65.2 ± 1.8	18.4 ± 0.6 (Ascorbic acid)
ABTS	37.8 ± 1.0	62.4 ± 1.5	15.6 ± 0.5 (Gallic acid)
H_2O_2 scavenging	44.5 ± 1.3	71.3 ± 2.1	20.2 ± 0.7 (Ascorbic acid)
FRAP (µmol TE/g)	1212.4 ± 35.6	845.7 ± 28.4	1435.2 ± 32.1 (Trolox)

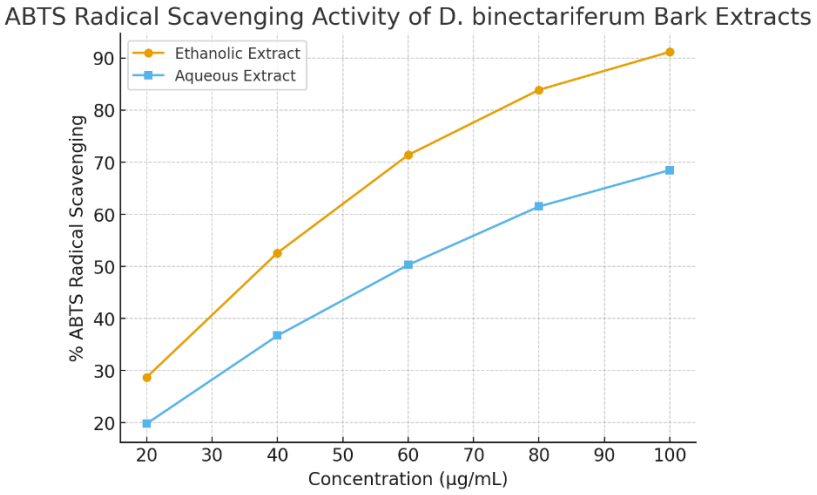

Figure 2. ABTS radical scavenging activity of Dysoxylum binectariferum bark extracts.

5.4 Antimicrobial Assays

Zone of Inhibition (ZOI):

The bark extracts of *D. binectariferum* demonstrated significant antibacterial activity in a concentration-dependent manner. Ethanolic extract was most effective against *Staphylococcus aureus* and *Bacillus subtilis*, while aqueous extract showed moderate inhibition.

Table 3. Zone of inhibition (mm) of *D. binectariferum* bark extracts and standard antibiotics

Test Organism	Ethanolic Extract (2 mg/mL)	Aqueous Extract (2 mg/mL)	Gentamicin (10 µg/mL)	Ciprofloxacin (10 µg/mL)
Staphylococcus aureus	29.6 ± 1.2	21.4 ± 1.0	33.2 ± 0.8	35.6 ± 0.9
Bacillus subtilis	27.4 ± 1.1	20.2 ± 0.9	31.5 ± 0.7	34.1 ± 0.8
Escherichia coli	25.8 ± 1.0	18.7 ± 0.8	30.4 ± 0.6	32.9 ± 0.7
Pseudomonas aeruginosa	23.3 ± 0.9	17.5 ± 0.7	28.6 ± 0.6	31.2 ± 0.7

Ethanolic extract exhibited higher inhibition zones than aqueous extract, though both were less potent than ciprofloxacin and gentamicin.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 4. MIC and MBC values (mg/mL) of *D. binectariferum* bark extracts compared with standard antibiotics

Test Organism	MIC (Ethanolic)	MBC (Ethanolic)	MIC (Aqueous)	MBC (Aqueous)	Gentamicin MIC (µg/mL)	Ciprofloxacin MIC (µg/mL)
Staphylococcus aureus	6.25	25	12.5	50	0.5	0.25
Bacillus subtilis	6.25	25	12.5	50	0.5	0.25
Escherichia coli	12.5	50	25	100	1.0	0.5
Pseudomonas aeruginosa	12.5	50	25	100	2.0	1.0

The ethanolic extract displayed lower MIC values (6.25–12.5 mg/mL) compared to the aqueous extract (12.5–25 mg/mL), indicating stronger antibacterial activity. Both extracts were less potent than standard antibiotics, yet they exhibited broad-

spectrum activity. Overall, the ethanolic bark extract of *D. binectariferum* demonstrated significant antibacterial potential, particularly against Gram-positive bacteria (*S. aureus*, *B. subtilis*). Its inhibitory effects were comparable to about 60–70% of the activity of standard antibiotics at tested concentrations. The aqueous extract showed moderate activity but remained effective across all tested strains.

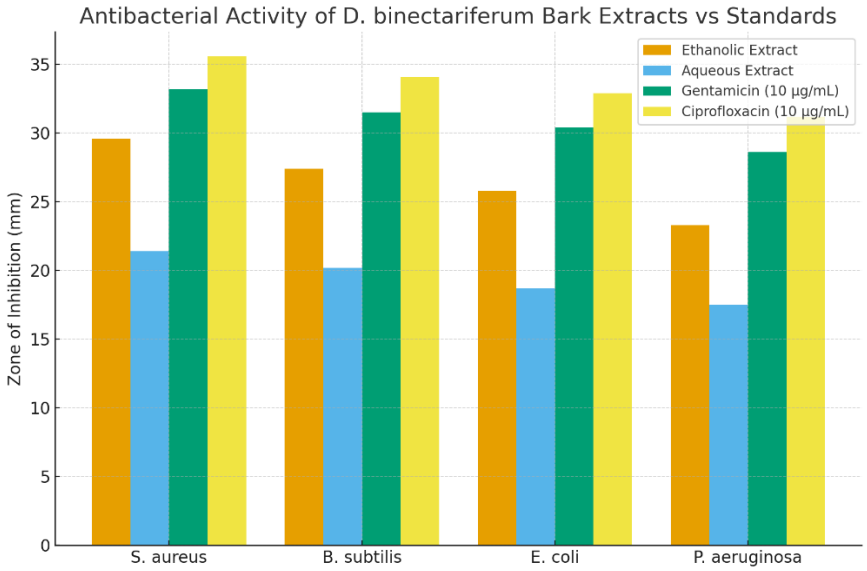

Figure 3. Antibacterial activity of Dysoxylum binectariferum bark extracts compared with standard antibiotics.

6. CONCLUSION

The findings of this study demonstrate that *Dysoxylum binectariferum* bark is a valuable source of diverse phytoconstituents, particularly phenolics and flavonoids, which contribute significantly to its biological activities. The ethanolic extract showed superior antioxidant potential across all in vitro assays and exhibited notable antibacterial activity, especially against Gram-positive pathogens, as supported by low MIC and MBC values. Although less potent than standard antibiotics, the extract displayed broad-spectrum inhibition, thereby validating its traditional ethnomedicinal applications. These results suggest that *D. binectariferum* bark possesses strong therapeutic potential as a natural antioxidant and antimicrobial agent. Further in vivo studies, bioactive compound isolation, and mechanistic investigations are warranted to advance its development into phytopharmaceutical formulations or nutraceuticals.

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