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Comprehensive phytochemical screening and assessment of antibacterial and antifungal activities of *Holarrhena antidysenterica* bark extract

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ABSTRACT

Background: *Holarrhena antidysenterica* is a medicinal plant widely used in traditional medicine for treating gastrointestinal disorders. Plant-derived antimicrobial agents have gained increasing attention as potential alternatives to synthetic drugs owing to the rising antimicrobial resistance. **Objective:** This study aimed to evaluate the phytochemical profile and antibacterial and antifungal activities of the ethanolic extract of *H. antidysenterica*. **Materials and Methods:** Bark powder was macerated in 80% ethanol, and the resulting extract underwent qualitative phytochemical evaluation. Analysis revealed major groups such as alkaloids, phenolics, tannins, steroids, glycosides, and coumarins, whereas saponins, terpenoids, carbohydrates, proteins, and anthraquinones were not detected. Antibacterial efficacy was evaluated using the agar well diffusion assay against *E. coli*, and *S. flexneri* (MTCC strains) at extract concentrations between 50 and 200 µg/mL. **Results:** A clear dose-dependent inhibition was recorded, with the largest inhibition zone observed for *E. coli* (28 mm), followed by *S. flexneri* (24 mm) at 200 µg/mL, approaching the efficacy of standard azithromycin. The extract, however, did not display any measurable antifungal activity against *C. albicans* or *A. niger*. The antibacterial activity is due to the combined action of alkaloids, phenols, and tannins. **Conclusion:** These results support the traditional medicinal use of *H. antidysenterica* and indicate its potential as a natural antibacterial source for the development of plant-based antimicrobial agents.

Keywords: *Holarrhena antidysenterica*, Phytochemical profiling, Antibacterial evaluation, Natural antimicrobials, *Shigella flexneri*, Ethanol extract.

INTRODUCTION

Antimicrobial resistance (AMR) has become a critical global challenge in modern medicine one of the most pressing challenges in modern healthcare and public health. The World Health Organization (WHO) has declared AMR a major threat to human health, food security, and economic development. It is estimated that by 2050, drug-resistant infections could cause up to 10 million deaths annually if no urgent interventions are made [1]. Excessive and improper antibiotic use has accelerated the development of resistant bacterial strains [2].

This alarming trend has created an urgent need for alternative strategies to combat pathogenic microorganisms, particularly Gram-negative bacteria, which are known for their strong resistance and ability to acquire resistance genes [3].

Medicinal plants have long formed foundation of traditional healing systems worldwide and are increasingly recognized as reservoirs of structurally diverse bioactive compounds with antimicrobial properties [4,5]. In contrast to most synthetic antibiotics, that act on single microbial targets, plant-derived phytochemicals are chemically heterogeneous and exert their effects through multiple mechanisms, thereby reducing the likelihood of resistance development. Phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, have been extensively studied for their antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties. Tannins exert antibacterial activity by binding to proteins and bacterial cell walls, whereas flavonoids inhibit nucleic acid synthesis and disrupt cell membranes. Phenolic compounds are known to cause protein precipitation and enzyme inhibition in microbial cells [6,7].

Recent studies in ethnopharmacology highlight how traditional medicinal knowledge provides valuable leads for modern drug discovery [8,9]. India, in particular, with its rich biodiversity and centuries-old medicinal systems such as Ayurveda, Siddha, and Unani, serves as an important source of bioactive medicinal plants. Several herbal formulations mentioned in Ayurvedic texts continue to be used in rural healthcare, particularly for treating infections and gastrointestinal ailments. Among these, *Holarrhena antidysenterica* Wall. ex A. DC. (syn. *Holarrhena pubescens*), belonging to the family Apocynaceae, has a long-standing reputation in Ayurvedic and folk medicine. Commonly known as Kutaja, Kurchi, or Tellicherry bark, this plant has been traditionally used to treat a range of conditions, including diarrhea, dysentery, fever, malaria, piles, asthma, and intestinal worms [10-12].

The bark of *H. antidysenterica* is especially valued in traditional medicine for treating diarrhea and microbial infections [13,14]. Chemical studies show it holds key compounds like conessine, holarrhenine, holarrhinine, and kurchicine alongside flavonoids, phenolics, and tannins [15,16]. Notably, one compound, conessine, draws attention because of proven effects on amoebae and bacteria [17]. Though evidence supports its use for gut-related illnesses and some gram-positive strains, thorough lab testing of bark extracts on significant gram-negative species still lacks depth [18].

Moreover, previous studies often lack comprehensive phytochemical profiling alongside antimicrobial testing, making it difficult to correlate the observed biological activity with specific phytoconstituents [19]. This gap is particularly significant because Gram-negative pathogens, such as *E. coli* and *S. flexneri*, are among the leading causes of gastrointestinal diseases worldwide, contributing to high morbidity and mortality, especially in developing countries [20].

Fungal pathogens also remain a major health concern, particularly *Candida albicans* and *A. niger*. *C. albicans* is a common cause of opportunistic fungal infections in immunocompromised patients, whereas *A. niger* is associated with respiratory infections and aspergillosis [21]. The growing problem of antifungal resistance has highlighted the need for novel plant-derived antifungal agents [22]. However, the antifungal potential of *H. antidysenterica* bark remains poorly explored, with most studies emphasizing its antibacterial and antidiarrheal properties [23].

Given this context, the present study aimed to bridge the existing knowledge gap by performing a detailed phytochemical screening of the ethanolic bark extract of *H. antidysenterica* and evaluating its antibacterial and antifungal activities against selected clinically relevant pathogens. Antibacterial activity was assessed against *E. coli*, *S. flexneri*, while antifungal activity was tested against *C. albicans* and *A. niger*. Standard antibiotics azithromycin and clotrimazole served as reference controls for bacterial and fungal assays, respectively [24,25].

By correlating the phytochemical composition with the observed antimicrobial activity, this study provides experimental support for the ethnopharmacological use of *H. antidysenterica* bark in traditional medicine [26,27]. The absence of antifungal activity suggests selectivity toward bacterial targets, highlighting the specificity of its bioactive compounds. These findings not only strengthen the scientific basis for traditional knowledge but also emphasize the plant's potential as a natural source of antibacterial agents [28-31]. Future work should focus on isolating and characterizing the active constituents and exploring possible synergistic effects with existing antibiotics to combat multidrug-resistant pathogens.

MATERIAL AND METHODS

Chemicals and reagents

All reagents and chemicals used were of analytical grade and obtained from certified laboratory suppliers. Ethanol (80%), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), acetic anhydride, chloroform ($CHCl_3$), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), lead acetate, Mayer's reagent, Dragendorff's reagent, and Liebermann-Burchard reagent were procured from HiMedia Laboratories Pvt.Ltd., Mumbai, India. Nutrient agar, Mueller–Hinton agar, and potato dextrose agar media were also obtained from the same supplier. Azithromycin and clotrimazole, obtained from HiMedia Laboratories Pvt.Ltd., Mumbai, India, were used as positive controls for antibacterial and antifungal assays, respectively.

Collection of Plant Material and Preparation of Extract

The bark of *H. antidysenterica* was purchased from a certified Ayurvedic store in Kalaburagi, Karnataka, India to ensure authenticity and quality. The dried bark was pulverized into fine powder with the help of a mechanical grinder. Approximately 10 g of the powdered bark was macerated in 80% ethanol for 72 h with occasional shaking to facilitate the extraction of phytoconstituents following standard procedures described by Harborne (1973) [32], Trease and Evans (1989) [33], and Sofowora (1993) [34]. After maceration, the extract was first filtered through Whatman No. 1 filter paper (Grade 1 90 mm diameter ~11 µm pore size) followed by sterile filtration using 0.22 µm Axiva Syringe filter to remove plant particles and debris. The filtrate was evaporated at room temperature to produce a semisolid extract, which was transferred into airtight container and refrigerated at 4 °C until further use in phytochemical screening and antimicrobial testing.

Qualitative phytochemical screening

The ethanolic extract was subjected to qualitative phytochemical analysis. The ethanolic bark extract of *H. antidysenterica* was carried out following the standard procedures described by Harborne [32], Trease and Evans [33], and Sofowora [34]. Qualitative analyses were performed using colorimetric and precipitation-based assays to identify major classes of phytoconstituents. Standard qualitative tests such as Mayer's and Dragendorff's for alkaloids, alkaline reagent and lead acetate tests for flavonoids, Ferric chloride test for phenols and tannins, Liebermann-Burchard test for steroids and glycosides, and Salkowski's test for terpenoids were employed. The appearance of characteristic color changes, precipitates, or reactions indicated the presence of the respective phytochemical groups. The observations are summarized in Table 1.

Microorganisms Used

Cultures of *E. coli* (MTCC 443), and *S. flexneri* (MTCC 1457) were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

All bacterial isolates were maintained on nutrient agar slants and maintained at 4 °C until use. Before each assay, a loopful of each strain was inoculated in Mueller–Hinton Broth (MHB) and incubated at 37 °C for 6 h to obtain actively growing cultures standardized to the 0.5 McFarland turbidity standard.

Antibacterial Activity

All solutions and culture media were prepared freshly and handled under aseptic conditions throughout the experiments.

The antibacterial efficacy of the ethanolic bark extract was assessed using the agar well diffusion method against clinical bacterial isolates of *E. coli*, and *S. flexneri* [35]. Wells (6 mm diameter) were prepared aseptically in Mueller–Hinton agar plates, and the test samples were introduced into each well. Different concentrations of the extract (50, 100, 150, and 200 µg/well) were introduced into the wells, while azithromycin (30 µg/mL) served as the positive control and sterile

distilled water as the negative control. Plates were incubated at 37 °C for 24 h, and the diameter of the inhibition zones (in mm) was measured. The assay was conducted in triplicate, and the average inhibition zone diameters were recorded following the protocol by Holder and Boyce [36] and Magaldi *et al*. [37].

Antifungal Activity

The antifungal potential of the extract was determined using the agar well diffusion technique against *C. albicans* and *A. niger* cultured on Potato dextrose agar. Clotrimazole (30 µg/mL) was employed as a reference (positive) control, while sterile distilled water served as the negative control. Inoculated plates were incubated at 28 °C for 48-72 hours, and the zones of inhibition were subsequently measured. All experiments were conducted in triplicate to ensure accuracy, following the standard procedures described by Magaldi *et al*. [38] and Rex *et al* [39].

RESULTS

Phytochemical Analysis

Qualitative phytochemical evaluation of the ethanolic bark extract of *H. antidysenterica* indicated the presence of alkaloids, flavonoids, phenolic compounds, tannins, steroids, glycosides, and coumarins (Table 1). In contrast, constituents such as saponins, terpenoids, anthraquinones, carbohydrates, and proteins were not detected.

Table 1 shows test perform for phytochemical screening of ethanolic bark extract showing the presence of major bioactive constituents. Alkaloids, flavonoids, phenols, tannins, steroids, glycosides, and coumarins were present, while saponins, terpenoids, anthraquinones, carbohydrates, and proteins were absent (Table 2).

Antibacterial Activity

The ethanolic bark extract of *H. antidysenterica* exhibited a clear concentration-dependent antibacterial activity against all the tested MTCC strains (Figure 1). The maximum zone of inhibition was recorded against *E. coli* (28 mm), and *S. flexneri* (24 mm) at the concentration of 200 µg/mL. No inhibition was observed in the control wells containing the solvent alone. All assays were performed in triplicate, and the mean zone diameters (in millimeters) were recorded for statistical reliability.

Table 3 shows zone of inhibition (mm) of ethanolic bark extract against clinical isolates of *E. coli* and *S. flexneri*. Azithromycin was used as a positive control and sterile water as a negative control.

Antifungal Activity

No antifungal activity was observed against *C. albicans* or *A. niger* at any of the tested concentrations (Figure 2). The ethanolic bark extract of *H. antidysenterica* did not exhibit detectable inhibitory effects on fungal growth, indicating its specificity towards bacterial pathogens.

Table 1: Test performed for preliminary phytochemical analysis of the bark extract of *H. antidysenterica*

Phytochemical	Test Performed	Observation
Alkaloids	Mayer's / Dragendorff's Test	Development of a yellowish or reddish-brown precipitate confirms the presence of alkaloids.
Flavonoids	Alkaline reagent / Lead acetate Test	Appearance of a yellow coloration that disappears upon acid addition indicated flavonoids.
Phenols	Ferric chloride Test	Formation of bluish-black coloration shows the presence of phenolic compounds.
Tannins	Ferric chloride Test	Blue-green coloration signifies the presence of tannins.
Steroids	Liebermann–Burchard Test	Development pf a greenish-blue coloration denotes the presence of steroids.
Glycosides	Keller–Killiani Test	Reddish-brown ring at the junction indicates cardiac glycosides.
Coumarins	Alkaline reagent Test	Yellow fluorescence under UV light confirms coumarins.
Saponins	Froth Test	Absence of persistent forth indicates that saponins are not present.
Terpenoids	Salkowski's Test	No violet coloration observed, suggesting terpenoids are absent.
Anthraquinones	Borntrager's Test	Lack of pink coloration indicates anthraquinones are not detected.
Carbohydrates	Benedict's Test	No brick-red precipitate observed, indicating absence of carbohydrates.
Proteins	Biuret Test	Absence of violet coloration confirms lack of proteins

Table 2. Preliminary phytochemical analysis of the bark extract of *Holarrhena antidysenterica*

S. No.	Phytochemical Constituent	Result
1	Alkaloids	+
2	Anthraquinones	–
3	Carbohydrates	–
4	Coumarins	+
5	Flavonoids	_
6	Glycosides	+
7	Phenols	+
8	Proteins	–
9	Saponins	–
10	Steroids	_
11	Tannins	+
12	Terpenoids	–

+: Presence; –: absence

Table 3: Antibacterial activity of ethanolic bark extract of *H. antidysenterica*

Organism	0 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	Control (Azithromycin 30 µg/mL)
Escherichia coli	-	9	11	13	16	28
Shigella flexneri	-	8	8	12	14	25

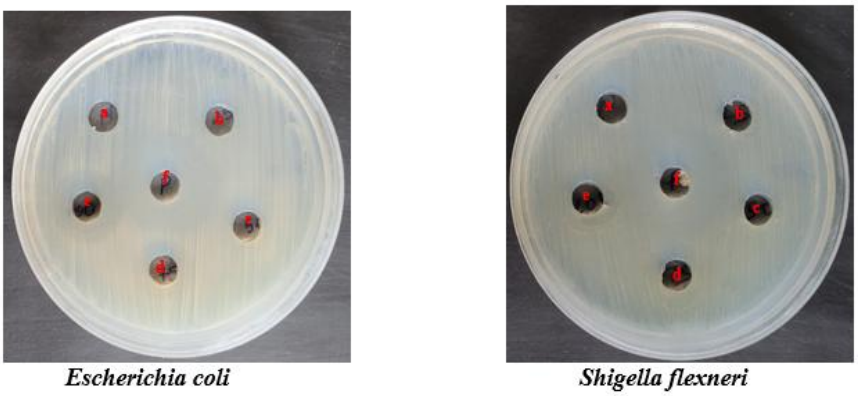

Figure 1. Antibacterial activity of *H. antidysenterica* ethanolic bark extract

Agar well diffusion assay showing inhibition zones produced by ethanolic bark extract against bacterial isolates at varying concentrations. a: 0 µg/well, b: 50 µg/well, c: 100 µg/well, d: 150 µg/well, e: 200 µg/well, f: Azithromycin (30 µg/mL)

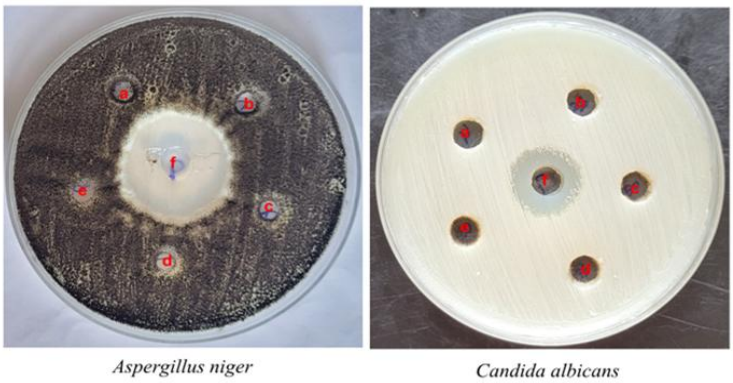

Figure 2: Antifungal activity of *H. antidysenterica* ethanolic bark extract

Representative plates showing antifungal activity of ethanolic bark extract against *C. albicans* and *A. niger* compared to clotrimazole. a: 0 µg/well, b: 50 µg/well, c: 100 µg/well, d: 150 µg/well, e: 200 µg/well, f: Azithromycin (30 µg/mL)

DISCUSSION

The present study demonstrates that the 80% ethanolic bark extract of *H. antidysenterica* exhibits significant antibacterial activity against clinically relevant bacterial strains, whereas no antifungal activity was detected under the tested conditions. The pronounced antibacterial effect may be attributed to the presence of key secondary metabolites, including alkaloids, phenols, and tannins, identified in the extract [40-42]. These compounds are known to exert antimicrobial effects through multiple mechanisms, such as disrupting microbial cell membranes, inhibiting nucleic acid synthesis, and inactivating essential enzymes, ultimately leading to bacterial cell death.

Previous studies have confirmed that alkaloids, particularly conessine, holarrhenine, and kurchicine, isolated from *H. antidysenterica*, exhibit potent antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [43-46]. Bhutani *et al*. and Dua *et al*. reported that conessine significantly inhibited bacterial growth by disrupting membrane permeability and interfering with protein transport [44,47]. Similarly, Siddiqui *et al*. and Cheenpracha *et al*. demonstrated that steroidal alkaloids derived from *H. antidysenterica* disrupt bacterial DNA replication and energy production leading to growth inhibition [48,49]. These findings are consistent with the present study, where strong antibacterial activity was observed against both Gram-positive and Gram-negative bacteria, further supporting the broad-spectrum antimicrobial potential of the bark extract.

The concentration-dependent inhibition observed in this study aligns with the findings of Kavitha *et al*. and Kaundal & Sagar, who reported enhanced antibacterial efficacy of ethanolic bark and leaf extracts of *H. antidysenterica* with increasing concentrations [50,51]. This pattern suggests that higher extract concentrations facilitate improved diffusion of active phytochemicals across bacterial cell walls, resulting in stronger inhibitory effects.

In contrast, the absence of antifungal activity against *C. albicans* and *A. niger* may be attributed to the structural complexity of fungal cell walls, which are composed of chitin and β-glucans, that restrict phytochemical penetration [52]. Similar observations were made by Lilhare and Kawale and Kuntal *et al*. who reported weak or negligible antifungal effects of *H. antidysenterica* extracts against common fungal strains [53,54]. Moreover, certain bioactive compounds may be present in lower concentrations or may require solvent systems with higher polarity for effective extraction and antifungal efficacy [55].

Overall, the findings of this study reaffirm the ethnopharmacological claims that *H. antidysenterica* bark is traditionally used to manage bacterial dysentery and gastrointestinal infections [56,57]. The observed antibacterial activity is likely attributed to steroidal alkaloids and phenolic compounds, which act synergistically to inhibit pathogenic bacteria [58-60]. Comparable ethnomedicinal validation has been documented in India, Sri Lanka, and West Africa, where *H. antidysenterica* and *H. floribunda* are used to treat diarrhea, malaria, and intestinal disorders [61-63].

Therefore, this study provides experimental evidence supporting the traditional therapeutic use of *H. antidysenterica* as an antimicrobial agent and underscores its potential for the development of plant-derived antibacterial formulations. Future studies should focus on the chromatographic purification, structural elucidation, and mechanism-based analysis of conessine and related alkaloids to further explore their pharmacological potential [64-66].

The antibacterial potential of *H. antidysenterica* can be attributed to its rich content of phenols, tannins, alkaloids, and glycosides, which are known to interfere with microbial cell wall synthesis and enzymatic activity, ultimately contributing to bacterial growth inhibition.

CONCLUSION

The 80% ethanolic bark extract of *H. antidysenterica* demonstrated significant antibacterial potential against MTCC strains of *E. coli* and *S. flexneri*, while showing no detectable antifungal activity. Phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins, and glycosides, which are likely responsible for the observed antibacterial effects. The absence of flavonoids in the extract may be attributed to solvent polarity or complexation during extraction. These findings provide strong experimental validation for the ethnomedicinal use of *H. antidysenterica* bark in managing of bacterial infections, particularly those associated with dysentery and gastrointestinal disturbances.

Future investigations should focus on bioassay-guided isolation of active constituents, determination of minimum inhibitory and bactericidal concentrations, and advanced structural characterization through chromatographic and spectroscopic analyses to further elucidate the plant's therapeutic potential.

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Conflict of interest

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