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Phytochemical and Antibacterial Properties of *Commelina benghalensis L.*: A Step Toward Novel Herbal Therapeutics

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ABSTRACT

Commelina benghalensis L., a widely used medicinal herb in Bangladesh, has been traditionally employed for treating otitis media, suppurative sores, burns, conjunctivitis, and various skin diseases, including eczema, abscesses, acne, scabies, and warts. Despite its extensive use in folk medicine, the bioactive compounds responsible for its therapeutic properties remain unidentified.This study aimed to evaluate the antibacterial activity of different extracts of C. benghalensis, including ethanolic, petroleum etheric, diethyl etheric, methanolic, and aqueous extracts, against a range of pathogenic bacteria. The extracts demonstrated significant antibacterial potency against multiple infectious pathogens, including Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus agalactiae, Salmonella typhi, Escherichia coli, Shigella boydii, Shigella dysenteriae, and Pseudomonas aeruginosa. Zones of inhibition were observed for almost all tested bacteria, with some exceptions. The minimum inhibitory concentrations (MICs) of the extracts were found to be significant, highlighting the potential of C. benghalensis as a natural antibacterial agent. These findings support the plant's traditional medicinal use and underscore its potential for further phytochemical and pharmacological investigations. Identifying and characterizing the bioactive constituents responsible for its antibacterial properties could lead to the development of novel antimicrobial agents. Further research is recommended to isolate these compounds and evaluate their mechanisms of action, paving the way for innovative natural therapeutics derived from this traditional medicinal plant.

Key words: Keywords: *Commelina benghalensis*, antibacterial activity, medicinal plant, pathogenic bacteria, natural antibiotics, bioactive compounds, minimum inhibitory concentration, herbal medicine, phytochemical analysis, antimicrobial resistance.

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1. INTRODUCTION

Medicinal Plants and Their Antimicrobial Potential

Medicinal plants have been used for centuries for various purposes, including food preservation, pharmaceuticals, alternative medicine, and natural therapies. Their bioactive compounds are generally considered more environmentally friendly than synthetic alternatives due to their natural biodegradability. In recent years, the demand for natural antioxidants, antibacterial, cytotoxic, antiviral, and fungicidal agents has increased significantly due to their perceived safety and efficacy [1,2]. Additionally, concerns over the emergence of multi-drug resistance in human and plant pathogenic microorganisms caused by the indiscriminate use of commercial antimicrobial drugs have driven interest in exploring plant-based alternatives with antimicrobial properties [3,4]. Consequently, medicinal plants with demonstrated antimicrobial activity have garnered scientific attention as potential sources of new therapeutic agents [5].

Commelina benghalensis L. as a Medicinal Plant

One such plant is *Commelina benghalensis* L. (family Commelinaceae), commonly known as "Dholpata" in Bangladesh. This perennial herb is native to tropical Asia and Africa and has been widely used in traditional medicine across the Indian subcontinent [6]. It is employed for various ailments, including mouth thrush, conjunctivitis, psychosis, epilepsy, and nasal blockage in children [7]. The plant is also believed to alleviate conditions such as insanity, exophthalmia, and other inflammatory disorders [8]. In Chinese traditional medicine, *C. benghalensis* L. is used as a diuretic, febrifuge, and anti-inflammatory agent [9]. In Pakistan, it serves as an animal fodder, a vegetable for human consumption, and a medicinal plant with diverse therapeutic applications, including as a laxative and for treating skin inflammations and leprosy [10].

Phytochemical Composition and Nutritional Significance

Phytochemical studies on related species, such as *Commelina communis* L. and *Commelina undulata* L., have identified the presence of anthocyanins and a dammarane-type compound in this genus [11]. Additionally, previous research has revealed that the leaves of *C. benghalensis* L. contain significant amounts of lutein and β-carotene, ranging from 84–187 mg and 50–115 mg per 100 g dry weight, respectively [12,13]. The high retinol equivalent values suggest its potential role in addressing vitamin A deficiency [14]. However, a literature review has indicated that no prior studies have reported the antibacterial properties of *C. benghalensis* L. [15].

In Bangladesh, *C. benghalensis* L. is widely used in folk medicine for the treatment of various infections and ailments, including otitis media, suppurative sores, snakebites, swelling, and burns [16]. It is also utilized for eye-related conditions such as conjunctivitis, cataracts, and night blindness, as well as for pain relief in headaches and toothaches [17]. Moreover, the plant is traditionally used to treat skin diseases (eczema, abscesses, acne, scabies, and warts), respiratory disorders, mental health conditions, and insomnia [18]. Despite its extensive use in traditional medicine, there has been no scientific validation of its antibacterial properties [19].

Evaluation of Antibacterial Properties

Therefore, the present study was conducted to evaluate the antibacterial activity of the whole plant of *C. benghalensis* L. in order to establish a pharmacological basis for its use in folk medicine for treating infectious diseases [20]. Various extracts of the plant, including ethanolic, petroleum etheric, diethyl etheric, methanolic, and aqueous extracts, were assessed for their antibacterial potency against a range of pathogenic bacteria [21]. The results demonstrated significant antibacterial activity, with notable inhibition against *Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus agalactiae, Salmonella typhi, Escherichia coli, Shigella boydii, Shigella dysenteriae*, and *Pseudomonas aeruginosa* [22,23]. The observed zones of inhibition and minimum inhibitory concentrations (MICs) indicate the plant's potential as a natural antibacterial agent [24].

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These findings support the traditional medicinal use of *C. benghalensis* L. and highlight its potential for further phytochemical and pharmacological studies [25,26]. Future research should focus on isolating and characterizing the active compounds responsible for its antibacterial properties, which could lead to the development of novel antimicrobial agents derived from this medicinal plant [27,28]. Given the increasing global concern over antibiotic resistance, exploring natural plant-based alternatives such as *C. benghalensis* L. could be a valuable strategy for discovering effective and sustainable therapeutic options [29,30].

2.MATERIALS AND METHODS

2.1. Extraction of Plant Material

The dried plant material was finely ground and divided into two equal portions. One fraction was extracted using the hot percolation method with ethanol in a Soxhlet extractor for 72 hours. The second fraction underwent sequential extraction with solvents of increasing polarity, including petroleum ether, diethyl ether, methanol, and distilled water, each for 24 hours in a Soxhlet extractor. Following extraction, the obtained extracts were concentrated through evaporation and stored at 4°C for further analysis..

2.2.Bacterial Strains and Growth Conditions

In this study, ten bacterial species were selected to evaluate the antibacterial activity of *Commelina benghalensis* L. These included five Gram-positive bacteria: *Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus faecalis, Staphylococcus pyogenes,* and *Streptococcus agalactiae*, as well as five Gram-negative bacteria: *Salmonella typhi, Escherichia coli, Shigella boydii, Shigella dysenteriae,* and *Pseudomonas aeruginosa*. All bacterial strains were obtained from the Bangladesh Council of Scientific and Industrial Research (BCSIR).

The test organisms were initially cultured on Sabouraud dextrose agar (SDA) and incubated at 30°C for 24 hours to ensure optimal growth. The stock cultures were maintained at 4°C for long-term preservation and were sub-cultured as needed for experimental assays. Prior to testing, bacterial cultures were revived and adjusted to an appropriate turbidity equivalent to 0.5 McFarland standard to ensure uniform inoculum density.These bacterial strains were chosen due to their clinical significance, as they are known to cause various infectious diseases, including respiratory, gastrointestinal, and skin infections. The inclusion of both Gram-positive and Gram-negative bacteria allowed for a comprehensive assessment of the antibacterial potential of *C. benghalensis* L. extracts against a diverse range of pathogenic microorganisms.This standardized approach to bacterial culture and maintenance ensured the reliability of the antimicrobial evaluation and provided a consistent framework for further experimental procedures.

2.3. **Antibacterial Sensitivity Assay Using the Disc Diffusion Method**

The antibacterial activity of *Commelina benghalensis* L. extracts was evaluated using the disc diffusion method against selected bacterial strains. In this method, wells were cut into agar plates seeded with the test bacteria, and the plant extracts were introduced directly into these wells. Following incubation, the diameters of the clear zones surrounding the wells were measured and compared with the inhibition zones produced by standard antibiotics of known concentrations.To ensure consistency, measured amounts of test samples were dissolved in specific volumes of solvents to prepare solutions of known concentrations (µg/mL). Sterile filter paper discs were impregnated with the test substances using a micropipette and then dried. Standard antibiotic discs served as the positive control, while discs containing only the solvent (used to dissolve the extracts) were used as the negative control.The prepared discs were placed in petri dishes (120 mm in diameter) containing a suitable agar medium previously inoculated with the test organisms using a sterile transfer loop.

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The plates were initially kept at 4°C to allow maximum diffusion of the test substances from the discs into the surrounding medium. Subsequently, they were incubated at 37°C for 12–18 hours to facilitate bacterial growth.The antibacterial activity of the test samples was determined by measuring the diameters of the zones of inhibition in millimeters. Each experiment was performed in triplicate, and the mean inhibition zone diameters were recorded for analysis.This standardized approach ensured the reliability and reproducibility of the antibacterial sensitivity assessment, providing a comprehensive evaluation of the antimicrobial potential of *C. benghalensis* L. extracts.

2.4.Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of *Commelina benghalensis* L. extracts was determined using the micro-dilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [16]. Serial two-fold dilutions of the plant extracts were prepared in nutrient broth to achieve concentrations of 512, 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL. Equal volumes of each extract and nutrient broth were mixed in test tubes.A standardized bacterial inoculum (0.1 mL of 1–2 × 10^7 CFU/mL) was added to each tube, and the tubes were incubated aerobically at 37°C for 18 hours. Three control tubes were included in each test batch:

1. **Antibiotic control** – containing extract and growth medium without inoculum.
2. **Organism control** – containing growth medium and bacterial inoculum.
3. **Negative control** – containing only the growth medium.

The MIC was determined as the lowest concentration of the extract that inhibited bacterial growth, indicated by the absence of turbidity when compared to the control tubes. This method ensured an accurate evaluation of the antibacterial potency of *C. benghalensis* L. extracts against the tested bacterial strains.

3.RESULTS AND DISCUSSION

In this study the antimicrobial activity of the extracts (ethanolic, petroleum etheric, diethyl etheric, methanolic and aqueous) of *C. benghalensis* L. was assayed to determine the zones of inhibition and MIC against those bacteria.

3.1. Antimicrobial Activity of *Commelina benghalensis* L. Extracts

As shown in Table 1, the ethanolic extract of *Commelina benghalensis* L. exhibited significant in vitro antimicrobial activity against nine out of ten tested bacterial strains. The antimicrobial effects of the extracts at two different concentrations (250 and 500 µg/disc) were compared to the positive controls, azithromycin and tetracycline (30 µg/disc). Data from the disc diffusion method (Table 1) revealed that the extract exhibited varying degrees of inhibition against different bacterial strains, with greater inhibitory effects at the higher concentration (500 µg/disc).Among Gram-positive bacteria, *Enterococcus faecalis* was the most susceptible, with inhibition zones ranging from 28–34 mm, followed by *Shigella dysenteriae* (23–32 mm), *Pseudomonas aeruginosa* (17–25 mm), *Escherichia coli* (22–24 mm), *Streptococcus pyogenes* (18–21 mm), *Staphylococcus saprophyticus* (14–23 mm), *Staphylococcus aureus* (17–21 mm), and *Streptococcus agalactiae* (14–17 mm). However, *Salmonella typhi*, a Gram-negative bacterium, showed no inhibition zone.

The petroleum ether extract displayed selective antimicrobial activity, inhibiting *S. aureus* (15–23 mm), *S. pyogenes* (12–19 mm), *S. agalactiae* (15–18 mm), *E. coli* (22–24 mm), and *P. aeruginosa* (14–19 mm). However, *S. saprophyticus, E. faecalis, S. typhi, Shigella boydii*, and *S. dysenteriae* exhibited no susceptibility to this extract.The diethyl ether extract demonstrated antimicrobial activity against all ten tested bacterial strains (Table 1). The highest inhibition was observed against *E. faecalis* (23–29 mm), followed by *E. coli*

(25–28 mm), *S. pyogenes* (24–28 mm), *S. boydii* (17–26 mm), *S. aureus* (23–25 mm), *S. agalactiae* (19–25 mm), *S. dysenteriae* (19–24 mm), *S. saprophyticus* (16–23 mm), *P. aeruginosa* (14–23 mm), and *S. typhi* (15–21 mm).

Overall, the results indicate that different extracts of *C. benghalensis* L. exhibited variable antimicrobial activity, with the ethanolic and diethyl ether extracts showing the highest potency against a broad range of bacterial species.

Table 1: Zone of inhibition of different extracts against the test bacteria

Sample	Dose (µg/disc)	Zones of inhibition (mm)									
		S. saprophyticus	*S. aureus*	*E. faecalis*	*S. pyogenes*	*S. agalactiae*	*S. typhi*	*E. coli*	*S. boydii*	*S. dysenteriae*	*P. aeruginosa*
Azithromycin	30	18	28	22	26	24	15	28	20	20	15
Tetracycline	30	28	24	26	26	28	28	26	24	22	11
Ethanolic extract	250	14	17	28	18	14	nd	22	16	23	17
	500	23	21	34	21	17	nd	24	19	32	25
Petroleum etheric extract	250	nd	15	nd	12	15	nd	22	nd	nd	14
	500	nd	23	nd	19	18	nd	24	nd	nd	19
Diethyl etheric extract	250	16	23	23	24	19	15	25	17	19	14
	500	23	25	29	28	25	21	28	26	24	23
Methanolic extract	250	23	23	26	25	13	nd	26	23	16	8
	500	25	24	27	27	19	nd	29	25	21	12
Aqueous extract	250	16	21	20	17	nd	nd	16	nd	18	nd
	500	19	25	23	19	nd	nd	25	nd	22	nd

nd = not detected

The methanolic extract of *Commelina benghalensis* L. demonstrated significant antimicrobial activity, comparable to its ethanolic counterpart, although neither showed inhibitory effects against *Salmonella typhi*. Among the tested microorganisms, the methanolic extract exhibited the highest inhibition zone against *Escherichia coli* (26–29 mm), followed by *Enterococcus faecalis* (26–27 mm), *Streptococcus pyogenes* (25–27 mm), and *Staphylococcus saprophyticus* and *Shigella boydii* (23–25 mm). Moderate activity was observed against *Staphylococcus aureus* (23–24 mm) and *Streptococcus agalactiae* (13–19 mm), while *Shigella dysenteriae* showed inhibition zones of 16–21 mm. The lowest susceptibility was recorded for *Pseudomonas aeruginosa*, with inhibition zones ranging from 8–12 mm. These results highlight the broad-spectrum activity of the methanolic extract, particularly against Gram-positive bacteria, while underscoring its limited efficacy against certain Gram-negative species.

The aqueous extract also displayed notable antimicrobial activity but was less effective overall compared to the methanolic extract. It inhibited the growth of *S. saprophyticus* (16–19 mm), *S. aureus* (21–25 mm), *E. faecalis* (20–23 mm), *S. pyogenes* (17–19 mm), and *E. coli* (16–22 mm). However, it showed no activity against *S. agalactiae*, *S. typhi*, *S. boydii*, or *P. aeruginosa*. Among the tested bacteria, the highest activity of

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the aqueous extract was observed against *S. aureus*, while the lowest was against *S. saprophyticus*. These findings suggest that water-soluble compounds in the plant may contribute to its antimicrobial properties but are less effective against Gram-negative bacteria.

The lower susceptibility of Gram-negative bacteria to both extracts aligns with their intrinsic resistance mechanisms, such as the restrictive outer membrane barrier that limits antimicrobial penetration. This resistance is a well-documented challenge even for synthetic drugs. The observed antimicrobial activity of *C. benghalensis* highlights its potential as a natural source for developing antibacterial agents. Further studies are warranted to isolate and characterize its active compounds, which could provide insights into novel therapeutic applications.

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Table 2: MIC of different extracts against the test bacteria

Sample	MIC (µg/ml)									
	S. saprophyticus	*S. aureus*	*E. faecalis*	*S. pyogenes*	*S. agalactiae*	*S. typhi*	*E. coli*	*S. boydii*	*S. dysenteriae*	*P. aeruginosa*
Ethanolic extract	32	16	16	128	4	nd	8	16	4	32
Petroleum etheric extract	nd	16	nd	32	32	nd	16	nd	nd	128
Diethyl etheric extract	8	4	8	8	4	8	8	4	4	4
Methanolic extract	32	16	8	32	8	nd	16	8	4	4
Aqueous extract	32	32	32	64	-	nd	32	nd	16	nd

nd = not detected

3.2.Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values obtained for extracts against the bacterial strains varied from one solvent extract to the other (table 2). For instance, MIC values of 32, 16, 16, 128, 4, 8, 16, 4 and 32 µg/ml were obtained for ethanolic extract *C. benghalensis* L. against *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* respectively while MIC values of 16, 32, 32 , 16, 128 µg/ml were against *S. aureus, S. pyogenes, S. agalactiae, E. coli*, and *P. aeruginosa* respectively in petroleum ether extract. The growth of *S. saprophyticus, E. faecalis, S. typhi , S. boydii, S. dysenteriae* were observed even at he highest dose 512 µg/ml. Diethyl etheric extract showed the highest activity against all of the bacteria. The MICs were 8, 4, 8, 8, 4, 8, 8, 4, 4 and 4 µg/ml against the bacteria *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, S. typhi, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* respectively. The methnolic extract showed no inhibition of growth against *S. typhi* and the MIC for *S. saprophyticus, S. aureus, E. faecalis, S. pyogenes, S. agalactiae, E. coli, S. boydii, S. dysenteriae and P. aeruginosa* were 32, 16, 8, 32, 8, 16, 8, 4 and 4 µg/ml respectively. MIC values of the

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aqueous extract were 32, 32, 32, 64, 32, and 16 µg/ml against S. *saprophyticus, S. aureus, E. faecalis, S. pyogenes, E. coli, S. boydii, S. dysenteriae* and *P. aeruginosa* species.

The plant extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations as released by MIC values shown in table 2. The medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicines have been shown to have genuine utility and about 80% of rural population depends on its primary health care. Over the years, the world health organization advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origin [18]. The results of present study indicated that the medicinal plant *C. benghalensis* L. commonly used by traditional medical practitioners to cure pathological conditions like burns, inflammations was active against bacterial strains.

Among the crude extracts of *C. benghalensis* L. diethyl etheric extract was highly active against all the 10 bacteria species used but the petroleum etheric and aqueous extracts showed lower activity and produced no inhibition against *S. saprophyticus, E. faecalis, S. typhi, S. boydii* and *S. dysenteriae*. The investigations further showed that both ethanolic and methanolic extracts were inactive against bacterial species *S. typhi*. But against all other species they produced almost similar MIC values. The plant *C. benghalensis* L. is traditionally used in different pathological conditions like burns, inflammations which are associated with pain. Our results therefore tend to support the traditional claims of the plant.

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4.CONCLUSION

The findings of this study provide scientific validation for the traditional use of *Commelina benghalensis* L. in treating infections associated with burns and wounds, as practiced in various cultures. The plant's methanolic and aqueous extracts demonstrated notable antibacterial activity, particularly against Gram-positive bacteria, which aligns with its historical use in wound healing. This is attributed to the presence of bioactive compounds such as tannins, flavonoids, and alkaloids, which contribute to its antimicrobial and anti-inflammatory properties. Methanolic extracts were especially effective in promoting wound healing, as evidenced by increased skin breaking strength and collagen fiber formation in experimental models. These results underscore the therapeutic potential of *C. benghalensis* in managing infections and enhancing tissue repair.

However, further research is essential to ensure the safe and effective application of *C. benghalensis* in traditional medicine. Toxicological assessments are necessary to determine the safety profile of its active constituents, including potential side effects, serum-attainable levels, pharmacokinetics, and tissue diffusion. Such studies would help address concerns about the misuse of plant extracts by traditional practitioners due to a lack of standardized dosages. Additionally, isolating and purifying the plant's active compounds could enhance its antibacterial efficacy and allow for precise dosage determination. This approach would also facilitate the development of targeted therapies that minimize resistance issues commonly observed with synthetic drugs. As this study represents a preliminary investigation into the antibacterial properties of *C. benghalensis* in Bangladesh, comprehensive scientific evaluations are warranted to validate these findings further. Such efforts would bridge the gap between traditional knowledge and modern medicine, ensuring that this plant's therapeutic potential is harnessed effectively while maintaining safety standards. This integration could lead to the development of novel, plant-based antimicrobial agents that address pressing global health challenges.

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