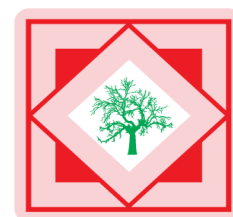




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Antibacterial activity of different fractions of *Commelina benghalensis* L.

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ABSTRACT

In Bangladesh the herb *Commelina benghalensis* L. is used for otitis media, suppurative sores, burns, conjunctivitis and skin diseases (eczema, abscesses, acne, scabies and warts) although the compounds responsible for the medicinal properties have not been identified. In this study the plant was evaluated for antibacterial activity and the extracts (ethanolic, petroleum etheric, diethyl etheric, methanolic and aqueous) were found to possess maximum potency against infectious pathogens *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus pyogenes*, *Streptococcus agalactiae*, *Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*. The zone of inhibition was observed with almost all bacteria with some exceptions. Minimum inhibitory concentrations of the extracts were found to be significant.

Key words: Antibacterial activity, *Commelina benghalensis* L., Minimum inhibitory concentration.

INTRODUCTION

Some medical plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily and therefore be more environmentally acceptable. Thus, natural antioxidants, antibacterial, cytotoxic, antiviral, fungicidal agents and nutrients have gained popularity in recent years, and their use and positive image among consumers are spreading. In recent years, multiple drug resistance in both human and plant pathogenic microorganisms have

been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [1, 2]. In order to find new therapeutic agents, plants that have antimicrobial activity have attracted attention [3, 4, 5].

Commelina benghalensis L. (family Commelinaceae), locally known as Dholpata, is a perennial herb native to tropical Asia and Africa. It is used in the Indian subcontinent as a folk medicine for the treatment of variety of ailments. The plant is used for mouth thrush [6], inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children [7], insanity [8] and exophthalmia. In China, *C. benghalensis* L. is used medicinally as a diuretic, febrifuge and anti-inflammatory [9]. It is used as an animal fodder, eaten by humans as a vegetable in Pakistan, also used there medicinally, but with different purported effects, including as a laxative and to cure inflammations of the skin as well as leprosy [10]. Some previous phytochemical screenings on *Commelina communis* L. and *Commelina undulata* L. revealed the presence of anthocyanins and a dammarane type compound in this genus [11]. In a research it was found that leaves of *C. benghalensis* L. contain higher levels of both lutein and β -carotene in the range of 84–187 and 50–115 mg /100 gm dry weight respectively. The values of retinol equivalents were higher in it [12]. Literature search revealed no previous report on antibacterial activity of *C. benghalensis* L. In Bangladesh the plant is used for otitis media, suppurative sores, snakebites, swelling and burns. It is also used for conjunctivitis, cataracts, night blindness, pain (headaches and toothaches), skin diseases (eczema, abscesses, acne, scabies, and warts), respiratory tract and mental disorders and insomnia [13]. Therefore, the present study was designed to investigate the antibacterial activity of the whole plant of *C. benghalensis* L. in order to examine the pharmacological basis of the use of the plant in folk medicine for the treatment of infectious diseases.

MATERIALS AND METHODS

Plant material and reference standards

The dried plant material was grinded into fine powder and the total mass was divided into two halves. One fraction was extracted by hot percolation method with ethanol in soxhlet extractor for 72 hrs. And another fraction was subjected to sequential extraction using solvents of increasing polarity viz. petroleum ether, diethyl ether, methanol and distilled water in Soxhlet extractor. Each solvent extraction step was carried out for 24 hrs and after extraction the extracts were concentrated by evaporation and stored at 4°C for further study. Azithromycin and tetracycline were used as standard drugs.

Bacteria and growth medium

Ten bacterial species; five of gram positive - *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus pyogenes* and *Streptococcus agalactiae* and five of gram negative - *Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* were used as test organisms obtained from Bangladesh Council of Scientific and Industrial Research. These organisms were cultured on Sabouraud dextrose agar at 30°C for 24 hrs and the stock culture was maintained at 4°C and then sub-cultured as needed.

Antibacterial sensitivity assay by disc diffusion method

Antibacterial sensitivity of the extracts was tested using disc diffusion method against the bacterial strains. In the agar diffusion method, wells were cut in seeded agar and the test sample was then introduced directly into these wells. After incubation the diameter of the clear zone around the well were measured and compared against zones of inhibition produced by solutions of known concentrations of standard antibiotics. Measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration ($\mu\text{g/ml}$). Then sterile filter paper discs were impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent (used to dissolve the samples) was adsorbed and dried. Then the dried discs were used as positive and negative control respectively. These discs were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for antibacterial screening. The plates were then kept at 4°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates were then kept in an incubator (37°C) for 12-18 hrs to allow the growth of the microorganisms. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments were carried out three times and the mean of the reading were recorded [14, 15].

Determination of minimum inhibitory concentration

MIC was determined by micro-dilution method using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards [16]. MIC values of the extracts were determined by dilution of the extracts of various concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 $\mu\text{g/ml}$ respectively. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1ml of standardized inoculums ($1-2 \times 10^7$ cfu/ml) was added in each tube. The tubes were incubated aerobically at 37°C for 18hrs. Three control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media with out inoculum), organism control (tube containing the growth medium and the inoculum) and negative control (tube containing the growth medium only). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

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.

Antibacterial sensitivity assay by disc diffusion method

As shown in table 1, the ethanolic extract of the plant had great *in vitro* potential of antimicrobial activities against 9 species of the 10 strains tested. In this study, the antimicrobial activity of the extracts at two different concentrations of 250 and 500 $\mu\text{l/discs}$ were compared with those of positive control such as azithromycin and tetracycline at the dose of 30 $\mu\text{g/disc}$. The data obtained from the disc diffusion method (table 1) indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. The inhibitory effect increased with increase of the extract concentration from 250 to 500 $\mu\text{g/disc}$. Gram-positive *E. faecalis* was the

most sensitive strain with the strongest inhibition zones (28-34 mm) followed by *S. dysenteriae* (23-32 mm), *P. aeruginosa* (17-25 mm), *E. coli* (22-24 mm) *S. pyogenes* (18-21 mm) *S. saprophyticus* (14-23 mm) *S. aureus* (17-21 mm) and *S. agalactiae* (14-17 mm). But in case of gram-negative bacteria, *S. typhi* it displayed no zone of inhibition.

From the table 1, it was observed that the petroleum etheric extract of the plant showed different degree of antimicrobial activity against *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) among the 10 bacteria species tested. The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. But the bacterial species *S. saprophyticus*, *E. faecalis*, *S. typhi*, *S. boydii* and *S. dysenteriae* showed no susceptibility to petroleum etheric extract.

The diethyl etheric extract of the plant showed antimicrobial activity against all of the 10 bacterial strains tested (table 1). The extract produced different degree of zones of inhibition against the bacteria. Highest activity was observed against *E. faecalis* (23-29mm) 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) *S. typhi* (15-21 mm). The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains.

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

Sample	Dose (µg/disc)	Zones of inhibition (mm)									
		<i>S. saprophyticus</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>S. typhi</i>	<i>E. coli</i>	<i>S. boydii</i>	<i>S. dysenteriae</i>	<i>P. aeruginosa</i>
Azithromycin	30	18	28	22	26	24	15	28	20	20	15
Tetracycline	30	28	24	26	26	28	28	26	24	22	11
Ethanol 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 methnolic extract produced similar activity of ethanol extract. As like ethanol extract methanolic extract showed no zone of inhibition against *S. typhi*. The highest zone of inhibition

was observed against *E. coli* (26-29 mm) followed by *E. faecalis* (26-27 mm), *S. pyogenes* (25-27 mm), *S. saprophyticus* and *S. boydii* (23-25 mm), *S. aureus* (23-24 mm), *S. agalactiae* (13-19 mm) and *S. dysenteriae* (16-21 mm) and *P. aeruginosa* (8-12 mm). *P. aeruginosa* showed the lowest susceptibility (table 1).

From the table 1, the aqueous extract of the plant showed antimicrobial activity against *S. saprophyticus* (16-19 mm), *S. aureus* (21-25 mm), *E. faecalis* (20-23 mm), *S. pyogenes* (17-19 mm), *E. coli* (16-22 mm) *S. dysenteriae* among the 10 bacteria species tested. The data indicated that the extract displayed a variable degree of antimicrobial activity on different tested strains. The highest activity was against *S. aureus* and the lowest was against *S. saprophyticus*. But he extract showed no zone of inhibition against *S. agalactiae*, *S. typhi*, *S. boydii* and *P. aeruginosa*.

Gram-negative bacteria exhibited low susceptibility to the extract which is in accordance with the fact that those have high level of intrinsic resistance to virtually all known antimicrobials and antibiotics due to a combination of a very restrictive outer membrane barrier, and it is highly resistant even to synthetic drugs [17].

Table 2: MIC of different extracts against the test bacteria

Sample	MIC (µg/ml)									
	<i>S. saprophyticus</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>S. typhi</i>	<i>E. coli</i>	<i>S. boydii</i>	<i>S. dysenteriae</i>	<i>P. aeruginosa</i>
Ethanollic 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

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 the 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 methanolic 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 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.

CONCLUSION

The present results therefore offer a scientific basis for traditional use of the plant *C. benghalensis* L. against infection by burns or wounds. But *in vivo* studies on the medicinal plant are necessary and should seek to determine toxicity of active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. The antibacterial activity could be enhanced if active components are purified and adequate dosage is determined for proper administration. It goes a long way in curbing administration of inappropriate concentration, a common practice among many traditional practitioners. This represents a preliminary report on the antibacterial activity of the medicinal plant *C. benghalensis* L. in Bangladesh. And for rational use of the traditional plant it requires further scientific study as necessary on it.

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