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In vitro Antimicrobial Efficacy of *Calotropis procera* (Ait) Against Some Foodborne Bacteria

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Abstract: The antimicrobial activity of methanol, aqueous and petroleum ether extracts of leaves of *Calotropis procera* against six foodborne pathogenic bacteria, namely *Escherichia coli* ATCC 43889, *Escherichia coli* ATCC 35150, *Escherichia coli* ATCC 43890, *Bacillus cereus* ATCC 14579, *Bacillus cereus* ATCC 10987 and *Bacillus cereus* ATCC 10876 was determined using paper disk methods. The growth of six bacterial isolates inhibited by the leaf extract with methanol and water where petroleum did not show any effect. Methanol extract gave the highest zone of inhibition (2.4 cm) against *Bacillus cereus* ATCC 10987 followed by water extract (1.70 cm) against *E. coli* ATCC 43889; whereas, petroleum ether extracts did not show any antibacterial activity. The minimum inhibitory concentration and minimum bactericidal concentration were ranged from 33.75-67.5 µg/ml. Cell viability assay showed the complete inhibition of all the tested microorganism within 200 min. This study revealed that the *C. procera* leaf extract demonstrated strong inhibitory effect on the test organisms that could be a good support for the use of *C. procera* in traditional medicine.

Key words: *Calotropis procera*, Bacteria, Foodborne, Antibacterial Efficacy.

Introduction

Microorganisms like bacteria cause serious infectious disease in human being. To combat the bacterial disease, antibiotics discovered in the 20th century to provide a resolution of bacterial diseases [1]. However, due to indiscriminate and inappropriate use of antibiotics, some strains of bacteria developed resistance by generating substances to reduce the efficacy of antibiotics or change their ability to move to the cell [2]. Hence, the microbe used to cause disease are becoming more resistant to the drug which creating a huge public health problem. To avoid the problems related to the development of resistance or environmental pollution, many of modern and effective control measures are generating from traditional fold medicinal plant [3]. According to The World Health Organization, about 80 % of people in some Asian and African countries depend on herbal medicines for their primary health care [4]. Plants have the ability to synthesize aromatic substances, including phenols and their derivatives that have therapeutic tendencies to treat diseases [5].

Nowadays, medicinal plant extracts are also used in food as a natural antimicrobial due the containment of potential health benefit compound [6,7]. However, the most effective bioactive compound occurred in plants are flavonoids, alkaloids,

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tannins and phenolic compounds [8,9]. The phytochemicals are natural bioactive compounds which also take part in the plant defense system.

Calotropis procera belongs to the family Asclepiadaceae commonly known as milkweed or swallow-wort. The plant is latex bearing which consists of alkaloids, tannins, gum, sugars, starch, resins and protein [10]. Latex and extracts of leaves, roots, stem and flowers of *C. procera* have the properties of antiasthmatic, stomachic, bechic, analgesic activity, tuberculosis, leprosy, syphilitic ulceration, diarrhea etc. [11,12,13]. Latex and extracts of the leaves also showed the antimicrobial activity against different bacteria and fungi, including *Staphylococcus, Pseudomonas,* and *E. coli*.[14]. In Bangladesh, *C. procera* is widespread and grown plentiful and used traditionally in folk medicine. However, antimicrobial activities of *C. procera* against foodborne bacteria were not studied much. Therefore, the present study was undertaken to find out the antimicrobial activities of *C. precera* against some gram-positive and gram-negative foodborne bacteria in *in vitro*.

Materials and methods

Plant material

Leaves of *C. procera* plant were collected from Hajee Mohammad Danesh Science and University (HSTU) campus, Dinajpur, Bangladesh during June, 2016. The collected leaves were brought to the laboratory of Plant Pathology, HSTU and identified with the help of the Department of Horticulture of the same university.

Preparation of plant sample

The collected leaves were washed properly with distilled water to minimize the contaminants. The leaves were then oven-dried for 24 h at 60°C and uniformly powdered by using an electric blender at the speed of 500 rpm. Powdered samples were maintained in tightly closed container at 4°C until further processing.

Preparation of leaf extract

Fifty gram of powdered leaves of *C. procera* were macerated in 1 L capacity glass beaker by using distilled water, methanol and petroleum ether at a ratio of 1:3 and kept in an electrical shaker for 24 h. The extracts were filtered by Whatman filter paper No. 1 and dried by using a rotary evaporator at a temperature not exceeding 65°C. The extracts were kept at 4°C until further study.

Test microorganisms

The following six gram positive and gram negative bacterial strains, namely *Bacillus cereus* ATCC 14579*, B. cereus* ATCC 10987, *Bacillus cereus* 10876, *Escherichia coli* 43889*, E. coli* 35150 and *E. coli* 43890 were used for this study.

Antibacterial assay

The antibacterial activity of the prepared crude extracts was evaluated by paper disc diffusion method [15]. All the bacterial isolates were prepared by culturing in nutrient broth media at a definite temperature for overnight. The subculturing of the bacteria were done to adjust the bacterial concentration to 1×10^8 CFU ml^{-1} by using a spectrophotometer[16]. 100 µL bacterial suspensions were seeded on NA plates and the excess suspension was removed to dry the NA plate. In each of these plates, paper disc supplemented with 100 µL (150 mg/mL) plant extract or without plant extracts using methanol, petroleum ether and, water was placed. Plates were incubated for 24 hours at 37°C. Triplicate plates were maintained for each bacterial strain and the antimicrobial activity was evaluated by measuring the zone of inhibition (cm).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was measured by standard two-fold dilution method [17]. In brief, various concentrations of the extracts were prepared by dissolving crude extracts in the solvent (methanol). Extracts were diluted to the highest concentration (500 µg/ml) and then two fold dilutions were made to achieve 500, 250, 125, 62.5 and 31.25 µg/ml concentration. Then, 500 µl bacterial suspension was poured on each of the Eppendorf tube containing 500 µl methanol extract where, the control tubes were maintained with only bacterial suspension. Eppendorf tube was then kept at 37°C for 24 h with shaking. Following incubation, MIC was

calculated as the lowest concentration of the extract inhibiting the visible growth of bacteria. MBC was determined by applying of 0.1 mL of the culture medium from each tube (in the MIC assay) observing no apparent growth and sub-culturing it on fresh NA medium and incubated at 37°C for 24 h. The MBC was measured as the least concentration of the plant extracts showing no visible growth on NA subculture.

Cell viability assay

For viable cell count assay, bacterial suspension (approximately 10^7 CFU/ml) was inoculated with 125 µg/ml concentration of the crude extract, and incubated at 37°C. Following incubation, 1 ml of the re-suspended culture was diluted into 9 ml NB media, thereby diluting it 10-folds. 0.1 mL sample of each treatment was diluted and spread on the surface of NA plate. The colonies were counted after 2-3 days of incubation at 37°C. Samples for viable cell counts were taken out at 0, 40, 80, 120, 160 and 200 min time intervals

Results and discussions

Our aim was to evaluate the efficacy of different solvent extracts of *C. procera* against different foodborne pathogenic bacteria *in vitro*. The efficacy of different solvent extracts was measured by the zone of inhibition against the specific bacteria.

However, in case of different strain of *Bacillus*, the highest zone of inhibition (2.4 cm) was found in the methanol extract against *B. cereus* ATCC 10987 and *B. cereus* ATCC 10876 where, the lowest (1.53 cm) was observed in aqueous extracts against *B. cereus* ATCC 14579. In case of the strains of *E. coli*, the highest inhibition zone (2.17 cm) were recorded with the methanol extract against *E. coli* ATCC 43890 where, lowest zone of inhibition (1.53 cm) found in aqueous extract against *E. coli* ATCC 43890, and the leaf extracts with water solvent did not show any effect against *E. coli* ATCC 35150. Likewise, control, petroleum ether extracts did not show any effect against all the tested foodborne pathogenic bacteria (Table 1).

The methanol extract of *C. Procera* showed a potent inhibitory effect as the MIC and MBC values against all the tested isolated of foodborne bacteria. The MIC and MBC values of the methanol extract of *C. procera* against all tested bacteria were found to range from 33.75-67.5 µg/ml (Table 2). The isolate *B. cereus* ATCC 10987 was found to less sensitive to the methanol extract as compared to other isolates.

The cell viability assay was carried out to evaluate the antibacterial effect of the methanol extract of *C. procera* on the viable counts of the tested bacterial isolates of *B. cereus* ATCC 14579, *B. cereus* ATCC 10987, *B. cereus* 10876, *E. coli* 43889, *E. coli* 35150 and *E. coli* 43890. The effect of the *C. procera* extract on the growth of the tested bacterial isolates demonstrated the reduced viability at the concentration used. As shown in the (Fig. 1) at 160 min exposure, near 80 % inhibition of all the tested isolates was observed. An exposure of 200 min of the methanol extract revealed complete inhibition of CFU number against *B. cereus* ATCC 14579, *B. cereus* ATCC 10987, *B. cereus* 10876, *E. coli* 43889, *E.*

Table 1. Antibacterial activity of *C. procera* leaf extract against foodborne pathogens

Name of the bacteria	Zone of inhibition (cm)			
	Methanol	Water	Petroleum ether	Control
E. coli ATCC 43889	1.97±0.109	1.70±0.047	NZ	NZ
E. coli ATCC 35150	1.87±0.166	NZ	NZ	NZ
E. coli ATCC 43890	2.17±0.072	1.60±0.047	NZ	NZ
B. cereus ATCC 14579	2.03±0.119	1.53±0.027	NZ	NZ
B. cereus ATCC 10987	2.40±0.047	1.57±0.054	NZ	NZ
B. cereus ATCC 10876	2.40±0.124	1.67±0.054	NZ	NZ

NZ= No zone of inhibition

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. procera* against six foodborne pathogens

Name of the bacteria	MIC (µg/ml)	MBC (µg/ml)
E. coli ATCC 43889	33.75	33.75
E. coli ATCC 35150	33.75	33.75
E. coli ATCC 43890	33.75	67.5
B. cereus ATCC 14579	33.75	33.75
B. cereus ATCC 10987	67.5	33.75
B. cereus ATCC 10876	33.75	33.75

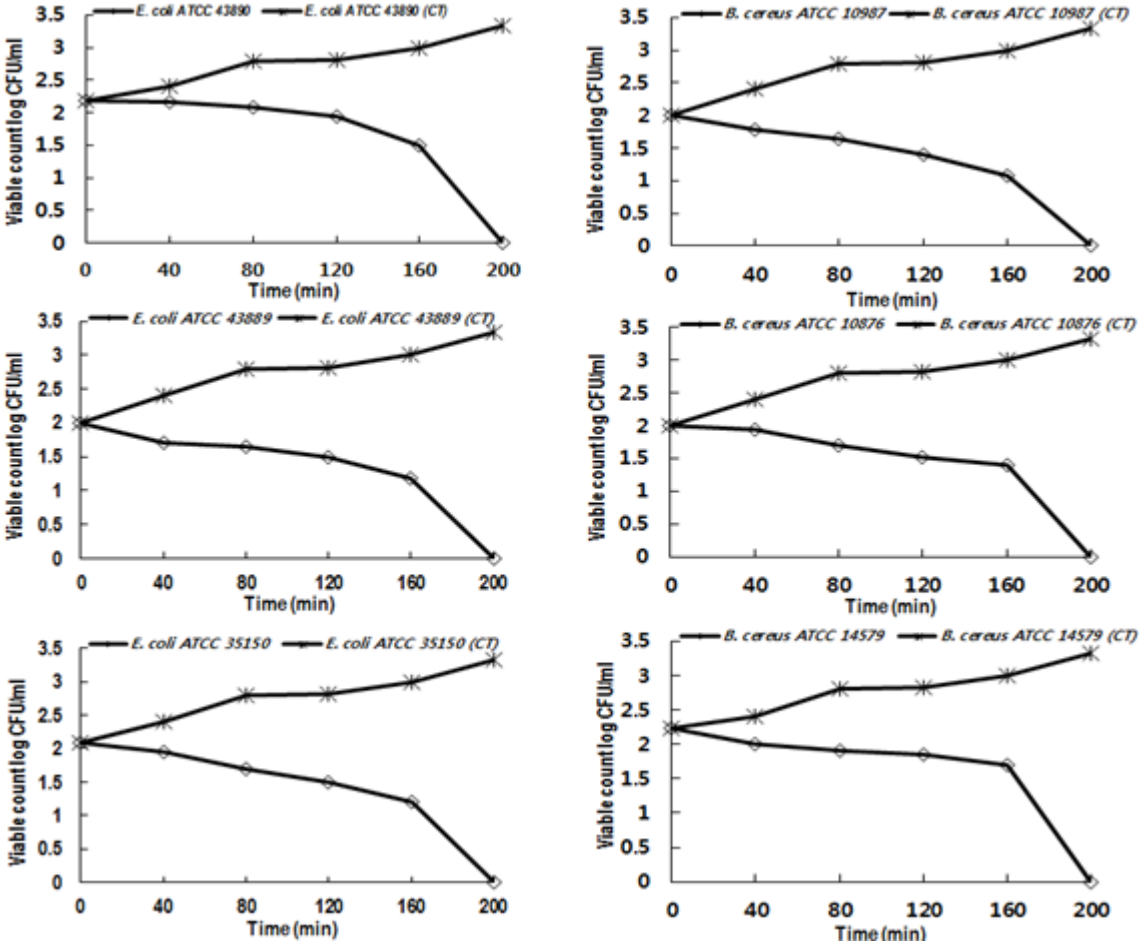

Fig. 1. Effect of methanolic extract of *C. procera* on different gram-positive and gram-negative foodborne bacteria

coli 35150 and *E. coli* 43890 (Fig. 1).

Our results showed that, methanol extracts showed better antibacterial activity followed by water, while no antibacterial efficacy was found with petroleum ether. Similar to our results, highest antibacterial activity against different bacteria, including *Staphylococcus aureus, Bacillus subtilis, Micrococcus, E. coli, Pseudomonas, Salmonella* etc. was also reported with methanol extracts of *C. procera* in comparison to other solvent used [14,18].

The antimicrobial activity of the plant extracts

is due to the chemical constituents, hence the efficacy of the medicinal plant may not be due to a single active constituent, but due to the combined action of many components present in the plant extracts [19]. The antimicrobial action may be due to the synergistic or even, the colorant effects among these components. We also observed that, gram positive bacteria were more susceptible than the gram negative one, where Nenaah [14] also reported the similar observation with the same plant extracts. The difference occurred may be due to the cell wall composition; Gram-positive bacterial composed of a single layer where, Gram-negative bacterial composed of a multi-layered with complex structured cell wall. The methanolic extracts showed better antibacterial efficacy due to the fact that, methanol extracted flavonoids quercetin-3-O-rutinoside from *C. procera* [14]. Flavonoids disrupts the cell peptidoglycan and alter the permeability of the bacterial membrane [20]. It is also used as inhibitors for vital enzymatic pathways, including cytochrome-P450 dependent oxidase, in which they precisely block steroid hydroxylase enzymes [21]. However, compounds with a phenolic nature like flavonoids, which consists free hydroxyl groups are considered as active antimicrobial agents [22]. Hydroxyl groups might affect the antimicrobial potentiality by binding to the active site of enzymes, creating hydrogen bonds with enzymes and modify their metabolism [23]. Several flavoinds including quercetin, kaempferol, isoharmentin etc. have been found to show antimicrobial activities [24].

Conclusion

Methanol and water extracts of *C. procera* showed their efficacy against the tested gram-positive and gram-negative bacteria. However, it will be early to make a direct correlation between the observed efficacy of the tested plant extracts in *in vitro* and efficacy in natural environmental condition. Therefore, it is obvious that the plant species which have shown the growth inhibiting efficacy against the tested microorganism, further investigation is needed to evaluate the practical significance. Additional research is also recommended to find the active compound responsible for the pharmacological testing.

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Conflict of interest

The authors declare no conflict of interest.

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