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Antibacterial Efficacy of *Ricinus communis* Against *Bacillus cereus* and *Escherichia coli*

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Abstract: The antibacterial activity of *Ricinus communis* leaf was studied for its *in vitro* antimicrobial activities through the disc diffusion method. The leaf extract of the castor plant significantly inhibited the growth of *Bacillus cereus* ATCC 14579, *Bacillus cereus* ATCC 10987, *Bacillus cereus* 10876, *Escherichia coli* 43889, *Escherichia coli* 35150 and *Escherichia coli* 43890. Various types of zone of inhibition were found against the selected bacteria using the *R. communis* leaf extract with three selected solvents. Methanolic extract showed the highest zone of inhibition (2.00 cm) against *E. coli* ATCC 43890 and minimum (1.33 cm) against *B. cereus* ATCC 14579. In case of petroleum ether and control, no zone of inhibition was found. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic extract ranged from (62.5-125 µg mL^{-1}). In cell viability assay, methanolic extract completely inhibited the bacterial growth after 200 min expusure. The results of the present study suggest that *R. communis* can be used in food industry to control different food borne diseases.

Key words: *Ricinus communis*, antibacterial effect, foodborne, *Bacillus*, *Escherichia*.

Introduction

The research of natural product is a fast-moving field whose continuous developments have far-reaching implications for world health. From prehistoric times, plants have been used as medicines by means for cure and management of different plant as well as human diseases. The World Health Organization has also realized that an effective health agenda for developing countries can be achieved by traditional herbal medicine as an alternative to chemical and also urged developing countries to utilize medicinal plant resources to achieve the goal of primary health care [1]. The resistance to antibiotics and high recurrence rates are the serious health threats associated with various infections. Many bacteria are present in the environment of hospitals in which majority of them are resistant to ampicillin and co-trimoxazole [2]. Various phytochemicals are well known for their antimicrobial properties and can be of great significance in therapeutic treatments. However, a vast amount of medicinal plant species were studied by many researchers for antibacterial, antifungal and antioxidant activity [3,-7].

Ricinus communis (castor plant), is a flowering plant under the family of Euphorbiaceae. It is a tall glabrous shrub or almost small tree, 2-4 m high, a tropical plant and commonly known as castor bean, the palm of Christ or Palma Christi [8]. Leaves, root, stem, flowers, fruits, the complete aerial parts as well as the whole plant are used for medicinal purposes. This plant is cultivated mainly for leaf and flower colors and for oil production. Leaves are use to relieve headache and

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as a poultice for boils [9]. In the treatment of rheumatism, headache, dropsy (edema), abscesses; ringworms and warts leaf Juice of the castor plant is used [10]. The Phytochemical properties presences in *R. communis* are of steroids, saponins, alkaloids, flavonoids, and glycosides [11]. In Bangladesh few works have been carried out with *Ricinus communis* against bacterial pathogene. Therefore, the present study was aimed to evaluate the antibacterial efficacy of *Ricinus communis* leaf extract against several foodborne bacteria.

Materials and methods

Preparation of leaf extract

R. communis plant leaves were collected and washed properly using distilled water to minimize the contaminants. Then the collected leaves were oven dried at 60°C for 24 h 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 use. Fifty gram of powdered leaves of *R. communis* were macerated by using different solvents including distilled water, methanol, and petroleum ether at a ratio of 1:3 in 1 L capacity glass beaker and kept in an electrical shaker for 24 h. The extracts were then filtered by Whatman filter paper No.1 and dried by using a rotary evaporator at a temperature not exceeding 65°C and were kept at 4°C until further use.

Antibacterial assay

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 in this study. Evaluation of antibacterial activity of the prepared leaf extracts was done by the paper disc diffusion method [12]. At a definite temperature, all the isolated bacterial strains were cultured in nutrient broth media for overnight and 100 µL of bacterial suspensions containing 1×10^8 CFU mL^{-1} were spread on Nutrient agar plates. After removing the excess bacterial suspension, the plate was allowed to dry for 5 mins. The paper discs of Whatman No. 1 filter paper containing plant extracts of 100 µL (150 mg/mL) were placed in each plate. Control was composed of only solvents following the same procedure. Plates were kept for incubation at 37°C for 24 h[13]. For each bacterial strain triplicate plates were maintained and the antimicrobial activity was evaluated by measuring the zone of inhibition in cm scale.

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

The MIC of the selected plant extract was measured by standard two-fold dilution method [14]. MIC was done with the best solvent resulted from zone of inhibition and the plant extracts with methanol solvent were diluted to the highest concentration (500 µg/mL) and then serial dilutions were made to achieve 500, 250, 125, 62.5 and 31.25 µg/mL concentrations. Then, each of the Eppendorf tube containing 500 µl methanol extract and 500 µL bacterial suspensions were poured. The control tubes were maintained with only bacterial suspension. Then the Eppendorf tubes were incubated at 37°C for 24 h in an electric shaker. The MIC was calculated as the lowest concentration of the plant extract showed the inhibition of visible growth of bacteria. Then 10 µl of the culture medium from each of the Eppendorf tube (in the MIC assay) appearing no visible growth was spread on to NA medium and incubated at 37°C for 24 h for the determination of MBC. By observing the complete absence of growth on the media surface in the lowest concentration of sample MBC was calculated.

Cell viability assay

To count the viable bacterial cell, approximate bacterial suspension (10^7 CFU/mL) was inoculated with 125 µg/mL concentration of the plant extract and incubated at 37°C. After that, in 900 µL NB media 100 µL re-suspended culture was diluted, 100 µL sample of the cultured suspension was spread on the surface of NA plate at a time intervals of 0, 40, 80, 120, 160 and 200 mins. The cultures were incubated at 37°C for 2-3 days and the viable cells were counted.

Statistical analysis

All the experiment in this study was conducted

in triplicate, and the average values were calculated with standard deviation. By using on way ANOVA ($p<0.05$), data were analyzed with R language statistical package.

Results and discussions

This study was done in *in vitro* with the aim of evaluating the activity of different solvent extracts of *R. communes* against six foodborne bacteria. The activity of solvents extracts was measured by the zone of inhibition in cm. scale against the bacteria.

Among the three strains of *E. coli,* the highest zone of inhibition (2.0 cm) with methanol extract and the lowest zone of inhibition (1.67 cm) with aqueous extract were found against *E. coli* ATCC 43890 (Table 1). In case of *Bacillus* strains, aqueous extract showed the highest zone of inhibition (1.83 cm) against *B. cereus* ATCC 10987 and *B. cereus* ATCC 14579 where methanol extract showed the lowest zone of inhibition (1.33 cm) (Table 1). Petroleum ether extract and control did not show any antibacterial efficacy against all the tested foodborne bacteria (Table 1).

In MIC and MBC test, the methanol extract of *R. communis* leaf showed potent inhibitory activity against six foodborne bacteria. The MIC and MBC values of methanol extract against all the tested bacteria were ranged from 62.5- 125 µg/mL, where, *E. coli* ATCC 43890 and *B. cereus* ATCC 10876 found less sensitive to methanol extract as compared to others (Table 2).

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

Table 1. Antibacterial efficacy of *R. communis* leaf extract against food borne bacteria

Name of the bacteria	Zone of inhibition (cm)			
	Methanol	Water	Petroleum ether	Control
E. coli ATCC 43889	1.7±0.12	1.9±1.28	NZ	NZ
E. coli ATCC 35150	1.93±0.027	1.73±0.178	NZ	NZ
E. coli ATCC 43890	2.0±0.047	1.67±0.027	NZ	NZ
B. cereus ATCC 14579	1.33±0.190	1.47±0.072	NZ	NZ
B. cereus ATCC 10987	1.5±0.047	1.83±0.027	NZ	NZ
B. cereus ATCC 10876	1.4±0.047	1.8±0.082	NZ	NZ

NZ= No. zone of inhibition

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *R. communis* against six bacterial isolates

Name of the bacteria	MIC (µg/mL)	MBC (µg/mL)
E. coli ATCC 43889	62.5	125.0
E. coli ATCC 35150	62.5	62.5
E. coli ATCC 43890	125.0	62.5
B. cereus ATCC 14579	62.5	62.5
B. cereus ATCC 10987	62.5	62.5
B. cereus ATCC 10876	125.0	125.0

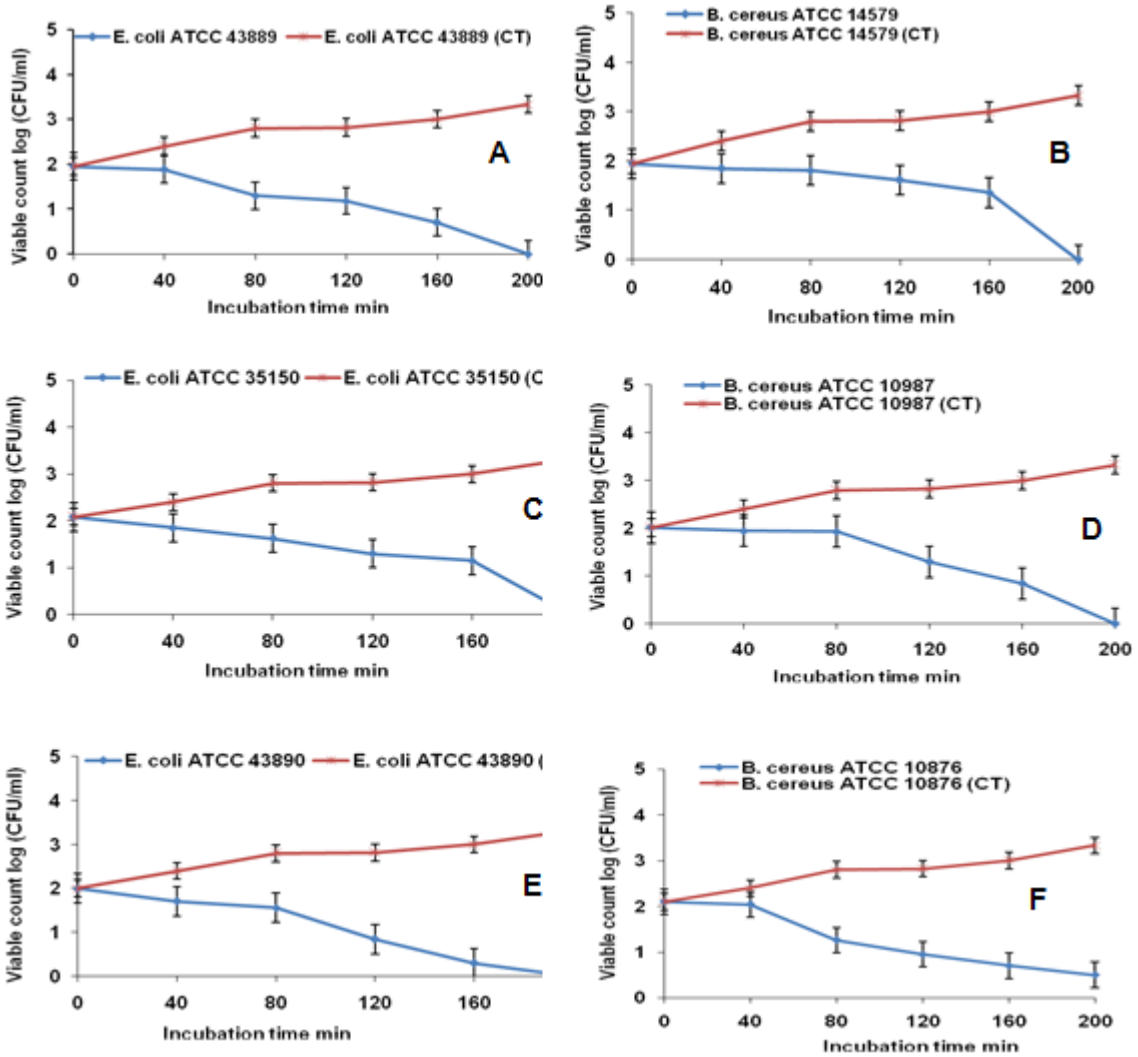

Fig. 1. Effect of methanolic extract of *R. communis* on different gram-negative (A. *E. coli* ATCC 43889, B. *E. coli* ATCC 35150 and C. *E. coli* ATCC 43890) and gram-positive (D. *B. cereus* ATCC 14579, E. *B. cereus* ATCC 10987 and F. *B. cereus* ATCC 10876) food borne bacteria

The common property of flavonoids is to reinforce capillary walls, improving the exchange of nutrients and oxygen between the blood and the tissues. However, the flavonoids have been reported to possess anti-inflammatory, anti-oxidant and anti-bacterial activities [15]. Tannins are metal chelators and can form complexes with macromolecules. Through this process, essential substrates and enzymes of micro-organisms are depleted leading to cell death. Our results are similar to a study in which methanol leaf extract is more effective against pathogenic bacterial strains than ethanol or water extracts [16]. Aqueous leaf extract of *R. communis* also found to show the antibacterial efficacy in terms of inhibition of bacterial growth including *Staphylococcus aureus* and *Escherichia coli*.

Conclusion

The methanolic extract of *R. communis* showed better antibacterial efficacy over aquous extracts. However, aqueous extracts also showed potential antibacterial efficacy in compare with control which may facilitate the cost effective management of bacterial infection. In addition, further in depth study with *R. communis* might offer a rich and sustainable source for traditional medicine and food industry.

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