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## Qualitative evaluation of antibacterial and DNA binding activity of four local ethnomedicinal plants available in Barishal, Bangladesh

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**Abstract:** Screening of antibacterial activities of plants due to secondary metabolites is an authentic basis to look for a new drug. As a result of increasing demand for traditional treatments, scientists have look forward to search the efficacy of these so called ethnomedicinal plants and have to exchange with pharmacologists. An initiative of this, the antibacterial activity of *Eclipta prostrata* (L.) Mant. *Azadirachta indica* A. Juss., *Aloe vera* (L.) Burm. f. and *Catharanthus roseus* (L.) G. Don was studied against *Escherichia coli* and *Staphylococcus aureus*. Among four ethnomedicinal plants subjected to test antibacterial activities, *Eclipta prostrata* was found to act against both strains of bacteria whereas *Azadirachta indica* was effective against *Escherichia coli* only. DNA binding affinity of these medicinal plants was also studied by viscometry. No affinity between each medicinal plant extracts and isolated human cheek cell's DNA was observed.

**Keywords:** ethnomedicinal plant; antibacterial activity; DNA binding; viscometry; human cheek cell; Barishal

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### 1. Introduction

Bangladesh, specially Barishal has rich floral diversity of traditional ethnomedicinal plants and the traditional medical system which is several hundred years old including Unani and Ayurveda. Besides, from time immemorial primitive people living in close contact about utilization of ethnomedicinal plants by way of trial and error. World health organization (WHO) has estimated that 80% of the world population depends on traditional herbal medicines (WHO, 2001). Physiologically bioactive compounds are contributed from medicinal plant species for the remedy of numerous ailments (Adebanjo *et al.*, 1983; Natarajan *et al.*, 2005) which are infectious or non-infectious. As a consequence of sharing this resource of knowledge from one generation to next without any written documents, it is getting lost day by day. So, it is essential that the knowledge now available among local and tribal people to be documented and integrated with modern scientific values before it gets lost in Bangladesh.

Synthetic antibiotics discovered in the twentieth century can treat only of the infectious diseases. However, due to the incessant use of these antibiotics over the years, pathogens have become resistant to synthetic medicines (Ahmad and Beg, 2001) as a result of bacteria's genetic ability and mutation. Thereby, herbs have been emphasized as traditional medicines containing medicinal ingredients because of their curative potentiality (Bhalodia and Shukla, 2011); thus they are used in the further development of drugs. Majority of plants species in different locations around the world have been examined for their pharmacological properties and they are distinguished to possess numerous secondary metabolites such as flavonoids, steroids, tannins, terpenes, saponins (Kamali and Myel, 2010; Lalitha *et al.*, 2010; Sen and Batra, 2012) and different investigations have been published on describing the pharmacological properties. Antibacterials of plant origin have immense therapeutic potentials and are effective in the treatment of infectious diseases than synthetic antimicrobials

which have adverse effects (Brandi *et al.*, 2006). Out of the total 225000 plants species, only less than 10% have been studied so far for their medicinal properties (Widjaja and Lester, 1978; Kelmanson *et al.*, 2000). The practice of complementary and alternative medicinal treatment is now increasing in developing countries in response to World Health Organization (WHO) directives and has provided the scientific basis for the effectiveness of traditional medicinal practices. The role of traditional medicines in the solution of health problems is invaluable on a global level and as a result, research on medicinal plants has intensified and information on these plants has been exchanged worldwide. Moussaoui *et al.* (2016) studied the antibacterial activity and synergistic effect between antibiotics and the essential oils of some medicinal plants. Furthermore, the combination of essential oils of plants with antibiotics was found effective against the emerging microbial drug resistance. Cagri-Mehmetoglu *et al.* (2017) studied the antibacterial properties and minimum inhibition concentration of *Microdesmis puberula*, *Hypoestis verticillaris*, *Icacina tricantha*, and *Enterolobium cyclocarpum* against 21 different bacteria and reported the antibacterial activity and phenolic contents of four medicinal plants of Nigeria. Meghla *et al.* (2016) studied the antibacterial activity of some medicinal plants against *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus*. Valle *et al.* (2015) studied the antibacterial activities of ethanol extracts of Philippine medicinal plants against multi drug resistant bacteria. Valli *et al.*, (2014) also studied the anti-inflammatory, analgesic and DNA binding studies of manjanathi fruit extract. They reported that manjanathi fruit extract have a binding affinity to calf thymus DNA. Therefore, in this study the antibacterial effect of the Ethanol extract of *Eclipta prostrata*, *Azadirachta indica*, *Aloe vera* and *Catharanthus roseus* were studied against *Escherichia coli* and *Staphylococcus aureus* as Uddin *et al.* (2017), Hossain and Rahman (2018), Alam and Haider (2018), Das *et al.* (2013) reported these species as ethnomedicinal plants along with high citation frequency, fidelity level and available in University of Barishal campus as wild, exotic and cultivated. So the objectives of present study are to find out the modern scientific basis of antibacterial properties by disk diffusion method in which the diameter of inhibition zone is directly proportional to bacterial susceptibility (Suhag *et al.*, 2014). In addition, binding between DNA from human cheek cells and plant extracts was performed by viscometric analysis to find out the interaction.

## 2. Materials and Methods

### 2.1. Collection of plant specimens

The plants i.e. *Eclipta prostrata*, *Azadirachta indica*, *Aloe vera* and *Catharanthus roseus* had been collected from their natural habitat, from different locations of University of Barishal, Bangladesh. The plant specimens were identified and authenticated in the Department of Botany, University of Barishal, Bangladesh (Figure 1) using standard literature (Ahmed *et al.*, 2008-2009). Then the plant specimens had been subjected to *ex-situ* conservation at the net house of mentioned University for future uses.

### 2.2. Preparation of extraction

Collected medicinal plants leaves were washed with tap water, ethanol and distilled water; and dried in sunshine and at oven (50°C temperature). Then the dried samples were ground by mortar and pestle (Meghla *et al.*, 2016; Ramchandran *et al.*, 2012). The conical flasks were sterilized in the oven for 1 hour. 5 g of powder from each of the samples was poured into the conical flask. Following this, 50 mL of ethanol (Merck, Germany) and 50 mL of methanol (Merck, Germany) was added to each flask separately and gently mixed it perfectly. Then solutions were shook for 3 days at room temperature at orbital shaker (Model No-JSOS-300). The preparation of each solution was repeated and kept for 3 days at static conditions at room temperature. The extracts were filtered, centrifuged and evaporated (Meghla *et al.*, 2016; Ramchandran *et al.*, 2012) and were kept for further uses.

### 2.3. Collection of bacterial strain

Isolated pathogenic bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were collected from Popular Diagnostic Centre and Hospital, Sylhet. The collected strains were incubated in an incubator (Model No-Binder RL-53) and inoculated on sterile, analytical nutrient agar medium and stored at 4°C until further use.

### 2.4. Study of antibacterial activities

Antibacterial activity of medicinal plants was studied by the disk diffusion method (Suhag *et al.*, 2014; Bell *et al.*, 2009). At first nutrient broth medium was prepared by beef extract (Sigma-Aldrich, India), yeast extract (Sigma-Aldrich, India), peptone (Sigma-Aldrich, India) and NaCl (Sigma-Aldrich, India) in distilled water and autoclaved for 15 minutes. Then a single colony of *Escherichia coli* and *Staphylococcus aureus* bacteria was added in 20 mL broth separately and incubated at 37°C for 24 hours. After that, a stock solution of 50 mg mL<sup>-1</sup> was made by dissolving ethanol extract of medicinal plants in distilled water. 10 µL of stock solution (500 µg

sample) was soaked in each sterilized filter paper (Whatman, 0.45 micro pore) disk of uniform diameter (5 mm) and thickness (1 mm). Then 25  $\mu\text{L}$  of activated bacterial solution was placed onto the surface of an agar plate (agar plate was prepared by 35  $\text{gL}^{-1}$  of bacteriological nutrient agar) with the help of micropipette with sterilized tips and spread evenly over the surface by using a sterilized bent glass rod. Finally, sample disks and antibiotic disks of ciprofloxacin (CONDA, Spain) were applied in freshly prepared agar plates and incubated at 37°C for 24 h. The zone of inhibition (diameter) was then measured (in mm) around the sample and standard antibiotic disks. Antibiotic ciprofloxacin (CIP) was used as a standard antibiotic disk to make a comparison with plants extracts. The antibacterial results of the extracts were compared with the standard antibiotic.

### 2.5. Isolation of DNA

DNA was isolated from human cheek cells by general salt extraction process (by using sodium chloride and detergent solution), purified by chloroform and precipitated in ice-cooled ethanol (Garbieri *et al.*, 2017). Purity of DNA was evaluated by a UV-Vis spectrophotometer (Lambda-365).

### 2.6. DNA binding studies

The qualitative viscosity experiments were carried out by using Ostwald viscometer at room temperature. The relative viscosity of solutions was measured at a fixed concentration of DNA (25  $\mu\text{M}$ ) and different concentrations of plant extracts (50-500 ppm) with respect to pure DNA solution (25  $\mu\text{M}$ ) in water (Ramachandran *et al.*, 2012 and Prasad *et al.*, 2011).

## 3. Results and Discussion

### 3.1. Extraction of medicinal plants at different physico-chemical conditions

Medicinal plants were extracted by ethanol and methanol separately. It was observed that extracted ability of ethanol to medicinal plants is significantly higher than methanol. Plants were also extracted at static and shaking conditions separately. It was observed that negligible amount of extracts were found at static condition. So plant extracts by ethanol at shaking conditions have been chosen for antibacterial and DNA binding studies.

### 3.2. Antibacterial activity

Antibacterial activity of experimental medicinal plants was tested against *Escherichia coli* and *Staphylococcus aureus* and compare with antibacterial activity of antibiotics ciprofloxacin (CIP) against corresponding bacteria. *Azadirachta indica* showed highest zone of inhibition against *E. coli* among ethanol extract of *Eclipta prostrata*, *Azadirachta indica*, *Aloe vera* and *Catharanthus roseus*. Besides this, both of the bacterial strain showed highest susceptibility and resulted a landmark zone of inhibition against standard antibiotic disks named ciprofloxacin (Table 1; Figure 2A, 2B; and Figure 3). Detail description for each specific plant species are:

#### 3.2.1 *Eclipta prostrata*

This medicinal plant species showed both gram-positive and negative reactions against *Staphylococcus aureus* and *Escherichia coli* respectively showing almost the same diameter of inhibition zone (Table 1 and Figure 2A, 2B; Figure 3A and 3B). Rahman and Rashid (2008), Kaisar *et al.* (2011), Ripa *et al.* (2012), Uddin *et al.* (2010) reported the antibacterial properties of this species against different of both gram-positive and gram-negative bacterial species including *E. coli* and *S. aureus*.

#### 3.2.2. *Azadirachta indica*

Only gram-negative reaction has been observed against *E. coli*. But no antibacterial properties against *S. aureus* had been seen (Table 1; Figure 2A and Figure 3C). Islam *et al.* (2012) reported the antibacterial properties of this species against different of both gram-positive and gram-negative bacterial species including *E. coli* and *S. aureus* but the plant extraction was prepared in Hexane, Butanol.

#### 3.2.3. *Aloe vera*

Both of the bacterial strains showed no antibacterial activity (Table 1). Molla *et al.* (2010) reported the antibacterial properties of this species against different strain of both gram positive and gram negative bacterial species including *E. coli* and *S. aureus* with ethanol extracts but minimum inhibitory concentration (MIC) was many times higher than ours.

### 3.2.4. *Catharanthus roseus*

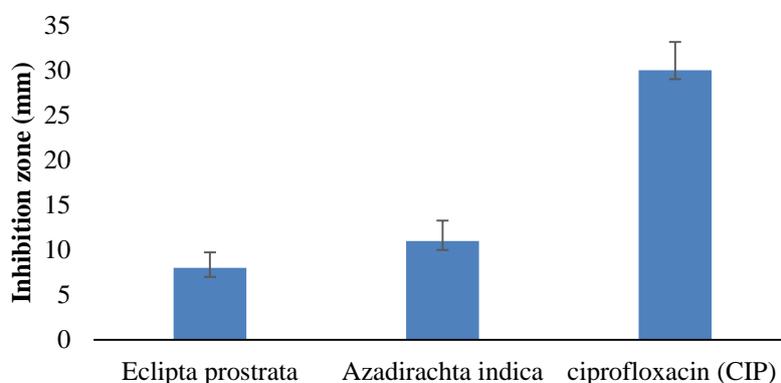
No antibacterial activity for both *E. coli* and *S. aureus* (Table 1) was observed. Molla *et al.* (2010) reported the antibacterial properties of this species against different of both gram-positive and gram-negative bacterial species including *E. coli* and *S. aureus* with ethanol extracts but MIC was many times higher than ours.

### 3.3. DNA binding studies

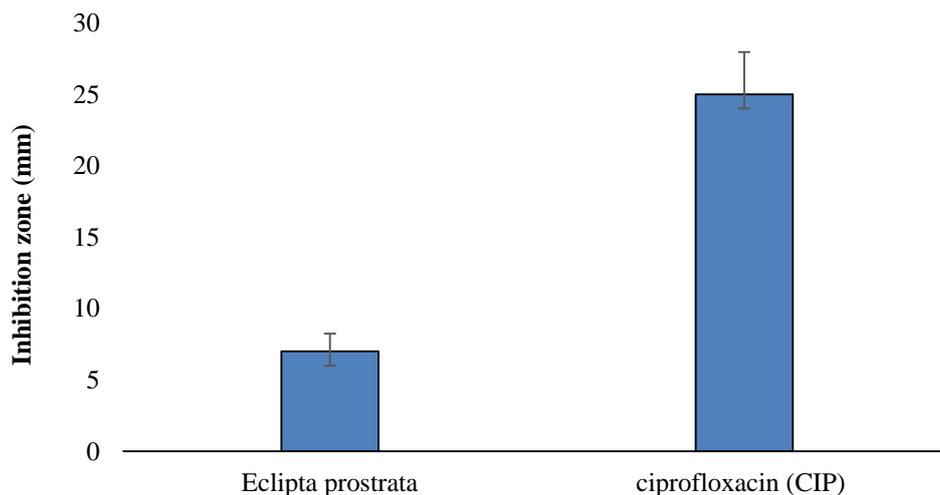
The binding of four experimental medicinal plants extract to the DNA was investigated by viscometric studies. It was observed that there is no significant increase in the viscosity of DNA on the addition of plants extracts, which indicates that there were no binding interactions of DNA with these medicinal plants.



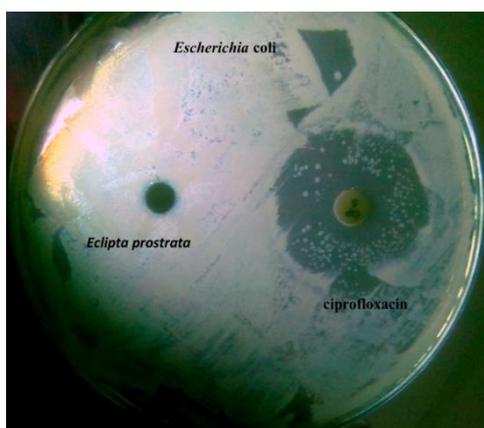
**Figure 1.** Specimen of collected medicinal plants (A) *Eclipta prostrata*, (B) *Azadirachta indica*, (C) *Aloe vera* and (D) *Catharanthus roseus*.



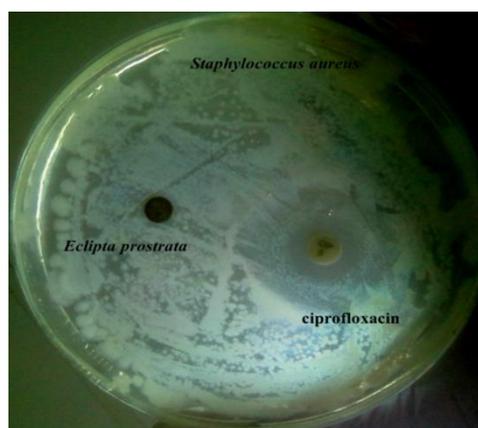
**Figure 2A.** Comparative bar diagram of inhibition diameter from plant extracts and antibiotic against *E. coli*. The above bar indicates standard error.



**Figure 2B.** Comparative bar diagram of inhibition diameter from plant extracts and antibiotic against *S. aureus*. The above bar indicates the standard error.



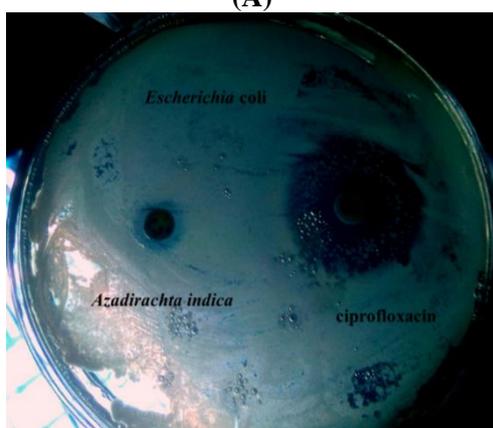
(A)



(B)

**Figure 3A.** Antibacterial activity of *Eclipta prostrata* and ciprofloxacin (CIP) against *Escherichia coli*.

**Figure 3B.** Antibacterial activity of *Eclipta prostrata* and ciprofloxacin (CIP) against *Staphylococcus aureus*.



(C)

**Figure 3C.** Antibacterial activity of *Azadirachta indica* and ciprofloxacin (CIP) against *Escherichia coli*.

**Table 1. Antibacterial activity data for medicinal plants extract.**

Bacteria	Gram -reaction	Inhibition zone diameter (mm)				
		<i>E. prostrata</i>	<i>A. indica</i>	<i>A. vera</i>	<i>C. roseus</i>	Antibiotic
<i>E. coli</i>	Negative	8±1.73	11±2.27	NA	NA	30±3.14
<i>S. aureus</i>	Positive	7±1.24	NA	NA	NA	25±2.96
Comments		++	+	-	-	++

++ indicates antibacterial property for both of the bacterial strain.

+ indicates antibacterial property for only single bacterial strain.

- indicates no antibacterial property for both of the bacterial strain

NA indicates no activity

#### 4. Conclusions

Ethanol extract of *Eclipta prostrata* showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*; whereas *Azadirachta indica* showed antibacterial activity against *Escherichia coli* only. *Aloe vera* and *Catharanthus roseus* did not show antibacterial activity against these subjected pathogens. Extract of *Eclipta prostrata*, *Azadirachta indica*, *Aloe vera* and *Catharanthus roseus* plants did not show any binding affinity to isolated DNA of human cheek cells. From this experimental result, *E. prostrata* and *A. indica* may provide an authentic basis to search for a new drug that will be used infectious ailments while remaining plant materials subjected to experiment will be investigated further for both extraction methodologies and antibacterial activities.

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#### Conflict of interest

None to declare.

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