Abstract:
The aim of this study was to evaluate the antibacterial and cytotoxic activities of the methanolic extract of *Callistemon citrinus* leaves. Disc diffusion method was carried out to evaluate antibacterial activity using kanamycin as standard. The methanolic leaf extract showed potential antibacterial activity against both gram positive and gram negative pathogenic bacteria. This extract showed highest activity against *Bacillus megaterium* and *Escherichia coli* with 29 mm and 27 mm zone of inhibition. The minimum inhibitory concentration (MIC) of the extract was determined that showed potential efficacy against the bacteria. The extract exhibited MIC with 64 µg/ml and 64 µg/ml for *B. megaterium* and *E. coli* respectively. The cytotoxicity of the leaf extract was investigated by brine shrimp lethality bioassay using vincristine sulfate as standard. The extract showed significant cytotoxicity with LC$_{50}$ value of 9.84 µg/ml as compared with LC$_{50}$ value of 6.05 µg/ml of standard.

Keywords: *Callistemon citrinus*, Antibacterial and Cytotoxic activity.

Introduction

The number of resistant microorganisms developed against antibiotics due to overuse, incorrect diagnosis, unnecessary prescriptions, improper use by patients. So the use and search of natural antibacterial agents from medicinal plants increases day by day. Traditional healers use plants in infectious diseases as plants are rich in secondary metabolites which possess antibacterial activity and cytotoxic activity. Traditionally many plants are used in cancer possessing cytotoxic activity. Antibacterial and cytotoxic activities of medicinal plants contribute to a great importance in health practice. The current study protocol was designed based on the medicinal plant named *Callistemon citrinus*.
that *C. citrinus* possesses some pharmacological and biological properties. The crude methanolic extract of fruits of *C. citrinus* showed significant relaxant activity through voltage operated calcium channels [2]. Twenty-four constituents were identified and quantified in the oil of *C. citrinus*, the major components are 1,8-cineole, α-pinene and β-pinene [3], [4]. All these components showed significant antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumonia*, *Bacillus* species and can be used as a therapeutic agent[3],[5]. The plant is enriched with relaxant constituents and possesses cytotoxic property [6]. Anti-mycobacterium tuberculosis property of *C. citrinus* have been reported against Mycobacterium tuberculosis [7]. The essential oils of the plant showed significant antifungal activity against *Phaeoramularia angolensis* [8]. The review on the plant shows the main purpose of the study.

**Materials and methods**

**Drugs and chemicals**

DMSO (dimethylsulfoxide) was purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh. Vincristine sulfate was collected from Alfa Asear Ltd. USA.

**Collection and identification of the plant**

The fresh leaves of *C. citrinus* were collected in April 2012 from Mirpur, Dhaka and authenticated at Bangladesh National Herbarium, where a voucher specimen No. DACB 37500 has been deposited.

**Preparation of the plant extract**

The collected fresh leaves were sun dried for seven days. The dried plant parts were ground into small powder by a grinder machine. Then 50 gm of powder of leaves was extracted separately by cold extraction process using methanol (300 ml) with daily shaking and stirring for 7 days at room temperature. After 7 days the extract was filtered through cotton followed by filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was filtered through filter paper (Double filter paper 102, 11.0 cm) and dried. The dried discs were placed on plates (Petri dishes, 120 mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms.

**Antibacterial screening**

The leaf extract of *C. citrinus* was subjected to antibacterial screening by the standard disc diffusion method [9]. In this method, the test solutions of known concentration (500 µg/disc) were made by dissolving measured amount of the samples (50 mg) in 1 ml of methanol. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substance and dried. The dried discs were placed on plates (Petri dishes, 120 mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with methanol followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4 °C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37 °C) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test samples was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

**Determination of MIC**

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of the leaf extract at which it shows the highest activity against microorganisms. Nutrient broth medium and culture media were prepared following the standard methods [10]. Kanamycin (30 µg/disc) was used as standards disc. Crude methanolic extract of the leaves of the plant was transferred in separate vials containing 2% DMSO solution (2 ml). This was mixed well to achieve sample solutions having concentration 1024 µg/ml. Nine sterilized test tubes containing 1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml sample solutions were prepared by serial dilution. Three test tubes containing media (C_m), media plus sample (C_mS) and media plus inoculums (C_mI) were also maintained. Diluted inoculums (10 µl) was added to each of the nine test tubes and mixed well. One ml of the sample was added to C_m and mixed well. 10 µl of inoculums was added to C_mS to observe the growth of the organisms in the media. C_m containing media was used the check the sterility of the solution. The test tubes were incubated at 37.5°C for 24 h. The lowest concentration
of the extracts which inhibited microbial growth was recorded as the MIC.

**Evaluation of Cytotoxicity**

Brine shrimp lethality bioassay was carried out to evaluate the cytotoxic activity [11]; [12] of the extract *C. citrinus*. The eggs of brine shrimp (*Artenia salina*) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37 °C with constant air supply. The test samples were prepared by dissolving the extract in dimethylsulfoxide (DMSO) not more than 50 µl in 5 ml solution and solutions of varying concentrations (20, 40, 60, 80 and 100 µg/ml) were prepared by the serial dilution process using simulated seawater and a vial containing 50 µl DMSO diluted to 5ml was used as a control. Then 10 live brine shrimp nauplii were added to each of the experimental vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. Vincristine sulphate was used as positive control. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated.

**Results and Discussion**

**Antibacterial activity**

Antibacterial Screening of the leaf extract of *C. citrinus* was assessed against five gram positive *S. aureus, S. lutea, B. cereus, B. subtilis, B. megaterium* and six gram negative *S. dysentriae, P. aeruginosa, S. paratyphi, V. mimicus, V. parahaemolyticus, E. coli* like pathogenic bacteria. All bacteria were extremely sensitive to the leaf extract shown in Table 1.

The Minimum Inhibitory Concentration of the extract was determined by serial dilution technique using nutrient broth media against the sensitive organisms. The methanolic leaf extract of *C. citrinus* was subjected to serial dilution with *B. megaterium, E. coli, V. mimicus* and *B. subtilis* and the MIC of antibacterial property against the bacteria are 64 µg/ml, 64 µg/ml, 128µg/ml, 128µg/ml respectively. The extract exhibited very good antibacterial activity. This extract showed highest activity against gram positive *B. megaterium* and gram negative *E. coli* with 29 mm and 27 mm of zone of inhibition. This extract showed significant sensitivity against the other bacteria with average zone of inhibition of 18-25 mm. Previous report of antibacterial study on this plant has been verified properly through the results. The plant possesses natural antibacterial activity focusing new bioactive compounds.

**Cytotoxic activity**

The lethality of the extracts of *C. citrinus* to brine shrimp was determined and the results (% mortality at different concentrations and LC₅₀ values) were shown in Figure 1. An approximate linear correlation was observed when logarthim of concentration versus percentage of mortality [3] was plotted on the graph paper and the values of LC₅₀ were calculated using Microsoft Excel 2003. The percent mortality increased with an increase in concentration. The extract showed significant cytotoxicity with LC₅₀ value of 9.84 µg/ml as compared with LC₅₀ value of 6.05 µg/ml of vincristine sulfate. This property would be taken under consideration in case of cancer and tumor.

**Conclusion**

The study results rationale the traditional uses and previous report on biological properties of *C. citrinus*. The methanolic extract of this plant can be used as a natural antibacterial and cytotoxic agent in various infectious diseases caused by gram positive, gram negative pathogenic bacteria and in cancer. Review work and present study necessitate the future investigation on this plant for more bioactive compounds with potential activities.

**Acknowledgement**

The authors wish to acknowledge the phytochemical research laboratory of Southeast University of Bangladesh and Bangladesh National Herbarium, Dhaka, Bangladesh.
Table 1: Antibacterial Screening of C. citrinus leaves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Organisms</th>
<th>Diameter of Zone of inhibition in mm</th>
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<tr>
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<td>Leaves 500µg/disc</td>
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<td>29</td>
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<tr>
<td>Gram positive bacteria</td>
<td>B. subtilis</td>
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<tr>
<td></td>
<td>S. lutea</td>
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<td></td>
<td>B. cereus</td>
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<tr>
<td></td>
<td>S. aureus</td>
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<tr>
<td></td>
<td>B. megaterium</td>
<td>29</td>
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<td>S. paratyphi</td>
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<td></td>
<td>V. parahaemlyticus</td>
<td>25</td>
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<td></td>
<td>V. mimicus</td>
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<td></td>
<td>S. dysenteriae</td>
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<td></td>
<td>E. coli</td>
<td>27</td>
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<td>P. aeruginosa</td>
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Figure 1: Determination of LC₅₀ values for standard and methanolic extract of leaves of Callistemon citrinus from linear correlation between logarithms of concentration versus percentage of mortality

References