Research Article

ANTIMICROBIAL POTENCY SCREENING OF *CLERODENDRUM INFORTUNATUM* LINN.Talukdar Muhammad Waliullah^{1*}, Akter Mst Yeasmin¹, Alam Ashraful², Islam Md Wahedul³, Hassan Parvez³¹Molecular and Cell Biology Laboratory, Bioscience Department, GSST, Shizuoka University, Shizuoka, Japan²FIQC Laboratory, Department of Fisheries, Matshya Bhaban, Ramna, Dhaka, Bangladesh³Institute of Biological Sciences University of Rajshahi, Rajshahi, Bangladesh

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ABSTRACT

Plants play a major role in all the traditional system of medicine. Plants contain the rich source of natural products like vitamins, minerals and other immune-modulators. Most of which have been used for human welfare specially to cure disease caused by pathogenic microorganisms. The present study was conducted to determine the antimicrobial activity of *C. infortunatum* (Verbenaceae) with two different solvents viz; ethanol and ethyl acetate against medically important pathogens such as six gram positive bacterial strains, nine gram negative bacterial strains and seven fungal strains. The *in vitro* screening for antimicrobial activity was carried out by using disc diffusion and micro broth dilution techniques. All the extracts showed significant inhibitory activity over the bacteria and fungus comparable to the standard drug tetracycline and fluconazole. The maximum diameter zone of inhibition was recorded to bacterial strains against *B. megaterium* and *B. subtilis*; *S. typhi*, *S. shiga* and *K. pneumoniae* and to fungi against *A. niger*, *A. flavus* and *C. albicans*. The MIC values of ethanol leaf extract were determined 64 µg/ml to *B. megaterium*, *S. typhi* and *K. pneumoniae*; 128 µg/ml to *S. aureus*, *S.-β-haemolyticus* and *E. coli* according to the serial dilution technique. Most of the extracts of root, leaf and stem of *C. infortunatum* are effective against both organisms; bacteria and fungi, especially leaf extract > root extract > stem extract. Thus, *C. infortunatum* appears to be an effective material for development of antimicrobial drugs.

Keywords: *C. infortunatum*, Bacterial strains, Fungal strains, Antimicrobial activity, Zone of inhibition, MIC.

INTRODUCTION

C. infortunatum Linn. (Verbenaceae) is generally used in folk, Hill tracts and rural slum dwellers as indigenous medicine, food supplement, decoration of home appliances and firewood. *Clerodendrum* is a very large and diverse genus and till now five hundred and eighty species of the genus have been identified and are widely distributed in Asia, Australia, Africa and America¹. Roots and leaf extracts of *C. indicum*, *C. phlomidis*, *C. serratum*, *C. trichotomum*, *C. chinense* and *C. petasites* have been used for the treatment of rheumatism, asthma and other inflammatory diseases²⁻³. It was also reported that tribals use *C. inerme* as an antidote of poisoning from fish, crabs and toads⁴. *C. phlomidis*, *C. colebrookianum*, *C. calamitosum* and *C. trichotomum* have been reported to have antidiabetic, antihypertensive and sedative properties⁵⁻⁷. *C. cyrtophyllum* and *C. chinense* were used for the treatment of fever, jaundice, typhoid and syphilis⁸⁻⁹. Roots, leaves and fresh juice of leaves of *C. infortunatum* were used in eliminating ascarids and tumors and also as a laxative¹⁰. Microorganisms are often a cause of existing diseases, regarding a solemn public health issue in a major part of the population as revealed by either personal or authorized health care systems. According to the World Health Organization¹¹, about three-quarters of the world population rely on plants for the treatment of many illnesses. The economic crisis, high cost of industrialized medicines, insufficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care¹². Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect etc¹³. Natural products have been a consistently successful source in drug discovery and silly offer more opportunities to find antimicrobial drugs or lead compounds¹⁴. More than 70 % of all medicinal

compounds have been derived from a small fraction of the World's biodiversity. Keeping this in view, the present study was undertaken to screen out *in vitro* antimicrobial activity of *C. infortunatum* to use as a possible source for new antimicrobial substances against important pathogens of agricultural and veterinary importance.

MATERIALS AND METHODS

Plant Collection and identification

The plant specimen was collected from Rajshahi University campus, Bangladesh in 2009. Identification of voucher specimen was confirmed at the taxonomical section, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of Extracts

Root, leaf and stem were dried in shade and stored in cotton bags and then finely powdered (100 g) separately with the help of a grinder. Each ground material was soaked in 500 ml ethyl alcohol and ethyl acetate separately for 24-72 h and filtered (Whatman no 1). The filtered was then allowed to evaporate in rotary evaporator until completely dried and kept in a refrigerator. Then (100 mg and 50 mg) dried extract, for further study, was weighed and dissolved in 10 ml of respected solvents for dilution. The concentration of the final extract was 100 µg/10 µl and 50 µg/10 µl.

Microorganisms with strains no

Six gram positive bacterial strains, viz., *Staphylococcus aureus* (ATCC-259233), *Sercinia lutea* (QL-166), *Bacillus subtilis* (QL-40), *Bacillus megaterium* (QL-38), *Bacillus cereus* (ATCC-14603) and *Streptococcus-β-haemolyticus* (ATCC-10389), nine gram negative bacterial strains, viz., *Salmonella typhi* (ATCC-14028), *Shigella dysenteriae* (AL-35587), *Escherichia coli* (FPFC-1407), *Shigella shiga* (ATCC-26107), *Shigella boydii* (AL-17313), *Shigella sonnei*

(AJ-8992), *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (ATCC-27853) and seven fungal strains, viz., *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans* (ATCC-2091), *Fusarium vasinfectum*, *Mucor* sp. And *Fusarium oxysporum* were employed in this test. These species were obtained from the mother stock of the Enteric Microbiology Laboratory, ICDDR, B Dhaka and the Molecular Biology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Medium

Nutrient broth, Nutrient agar and Sabouraud dextrose agar medium were used. All are the products of Mast Diagnostics, Mast Group Ltd. Merseyside. UK.

Disc Diffusion Assay

Antimicrobial activity was determined as diameter of inhibition zone using disc diffusion method¹⁵. Nutrient agar (NA) and Saboroud Dextrose agar (SDA) were distributed in sterilized petridishes for bacteria and fungi respectively. This was accomplished by placing 10 µl of the extract on a small (6 mm) filter paper disc. This disc was placed on an agar growth medium containing a confluent lawn of microorganism. The concentration of the organism was also 10 µl/petridish. The absence of bacterial and fungal growth around the disc indicated that the plant extract contains antimicrobial properties against that particular organism.

Minimum Inhibitory Concentration

The test was performed at ten concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml) of the ethyl alcohol leaf extracts of *C. infortunatum*. The activity was tested against three gram positive and three gram negative bacterial strains; *S. aureus*, *B. subtilis*, *S.-β haemolyticus*, *S. typhi*, *E. coli* and *K. pneumoniae* employing the Nutrient broth medium using by a serial dilution technique¹⁶. Each test was replicated three times.

Preparation of the Standard

Standard disc of tetracycline (30 µg/disc) and fluconazole (25 µg/disc) obtained from Mast Diagnostics, Mast Group Ltd. Merseyside. UK was used as positive control

Preparation of Culture Medium and Inoculums

Nutrient agar and sabouraud dextrose agar were prepared by dissolving 2.8 g and 6.2 g powder of agar respectively in 100 ml water. About 25 ml of medium was poured into a petridish. The inoculum was prepared by culturing a large number of organisms in a tube containing 10 ml liquid medium for bacterial strains and incubating over night at 37°C. On the other hand, the inoculum of fungal strains was transferred directly into petridish and incubating at 27°C for 72 h. The agar plates of the assay were prepared by labeling them with the date, the name of the microorganism and the name (code) of the discs. The inoculums of bacteria were transferred into petridish containing solid nutrient medium of agar using a sterile swab. The swab was used to spread the bacteria on the medium in a confluent lawn. It was done by rotating the petridish at 90°C and continuing the spread of bacteria. One swab was used for one species of bacteria.

Placing Test Discs

Dried test discs were transferred on bacterial lawn under aseptic conditions using spirit-flame sterilized forceps each time. Each disc was placed gently on the agar surface, and platted with the forceps so that it sticks. The petridish was incubated upside down at 37°C for 24 h. Resulting zones of inhibition were observed and measured in millimeters. Tests were repeated in triplicate and were performed to insure reliability of the results.

RESULTS AND DISCUSSION

In continuation of our search for substances of plant origin with pharmacological effects, we have screened out root, leaf and stem of *C. infortunatum*, extracted into ethyl alcohol and ethyl acetate. A correlation was found between the antibacterial activity observed by agar disc diffusion assay and MIC determination. It is interesting to note that these plant extracts showed more or less similar activity against a number of standard bacteria and fungi compared to the standard antibiotic tetracycline and antifungal fluconazole.

Antibacterial activity

We found the highest antibacterial activity as diameter of zone of inhibition 12 mm and then 11, 10, 9, 8 and 7 mm respectively against gram positive and gram negative bacterial strains in both type of extraction of ethanol and ethyl acetate either root, leaf or stem. The maximum zone of inhibition was observed 12 mm against *B. megaterium* (gram positive) in the Figure 1 and 3; *S. typhi*, *S. dysenteriae*, *S. shiga*, *S. sonnei* and *K. pneumoniae* (gram negative) Figure 2 in both type of extractions while the standard antibiotic tetracycline showed 18 mm in diameter against *S. lutea*, *S. β-haemolyticus* and *S. typhi* (Figure 1 and 3) and the lowest zone of inhibition was recorded 8 mm in diameter against *S. aureus*, *B. subtilis* (gram positive); *P. vulgaris* (gram negative, Figure 1-2) in ethanolic extract on the other hand 7 mm in diameter against *S. aureus* (gram positive, Figure 3); *P. vulgaris* and *P. aeruginosa* (gram negative, Figure 4) in ethyl acetate extract when compared to tetracycline 14 mm in diameter against *S. sonnei*.

Antifungal activity

Antifungal activity was also observed significantly 11 mm against *A. niger*, *A. flavus* and *C. albicans* for the highest inhibition zone in both type of extractions and 8mm against *A. niger*, *A. flavus*, *C. albicans*, *Mucor* sp. and *F. oxysporum* in both type of extracts (Figure 5-6) for the lowest zone of inhibition in diameter showed in case of fungi while the inhibition zone of the standard fluconazole was 16 mm against *A. fumigatus* and *Mucor* sp. and 13 mm against *F. vasinfectum* and *F. Oxysporum* (Figure 5-6).

Minimum inhibitory concentration activity

The MIC test was also showed a potential activity. From the ten concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml) we observed the MIC activity 64 µg/ml against *B. subtilis* (gram positive), *S. typhi* and *K. pneumoniae* (gram negative); 128 µg/ml against *S. aureus*, *S.-β-haemolyticus* (gram positive) and *E. coli* (gram negative) in the Figure 7 as relatively good antibacterial compounds. Similar results were found¹⁷ in some medicinal plants used in folklore remedies in South Western core, in some Allium Species from Hamedan Iran¹⁸. This result of antimicrobial activity is in accord with formers studies executed on *C. viscosum*¹⁹.

Figure 1: Antibacterial activity of *C. infortunatum* extracts and antibiotic Tetracycline

Figure 2: Antibacterial activity of *C. infortunatum* extracts and antibiotic Tetracycline

Figure 3: Antibacterial activity of *C. infortunatum* extracts and antibiotic Tetracycline

Figure 4: Antibacterial activity of *C. infortunatum* extracts and antibiotic Tetracycline

Figure 5: Antifungal activity of *C. infortunatum* extracts and antifungal Fluconazole

Figure 6: Antifungal activity of *C. infortunatum* extracts and antifungal Fluconazole

Figure 7: Minimum inhibitory of concentration

This finding reveals also coincides with the study²⁰⁻²¹ who investigated the antimicrobial activity of the same species of *Clerodendron*. Thus, *C. infortunatum* appears to be an effective material for the development of antimicrobial drugs.

CONCLUSION

The above findings clearly indicate promising antibacterial and antifungal properties of *C. infortunatum* against life threatening pathogens. Leaf possesses quite potent activity than root and stem specially leaf extract > root extract > stem extract. Implications of these results for bioactivity and drug discovery potential of herbal products are discussed. This study serves as basis for further research on this plant.

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