ANTIMICROBIAL POTENCY SCREENING OF CLERODENDRUM INFORTUNATUM LINN.

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 America. 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 diseases. It was also reported that tribal use C. inerme as an antidote of poisoning from fish, crabs and toads. C. phlomidis, C. colebrookianum, C. calamitosum and C. trichotomum have been reported to have antidiabetic, antihyperpertensive and sedative properties. C. cyrtophyllum and C. chinense were used for the treatment of fever, jaundice, typhoid and syphilis. Roots, leaves and fresh juice of leaves of C. infortunatum were used in eliminating ascacids and tumors and also as a laxative. 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 Organization, 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 care. Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, insect etc. Natural products have been a consistently successful source in drug discovery and sily offer more opportunities to find antimicrobial drugs or lead compounds. 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...
Preparation of Culture Medium and Inoculums

Disc Diffusion Assay

Antimicrobial activity was determined as diameter of inhibition zone using disc diffusion method\(^6\). Nutrient agar (NA) and Saboraud 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 also10 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 \(C.\) \textit{infortunatum}. The activity was tested against three gram positive and three gram negative bacterial strains; \(S.\) \textit{aureus}, \(B.\) \textit{subtilis}, \(S.-β\) haemolyticus, \(S.\) \textit{typhi}, \(E.\) \textit{coli} and \(K.\) \textit{pneumoniae} employing the Nutrient broth medium using by a serial dilution technique\(^6\). 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 each 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 \(C.\) \textit{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 when compared to tetracycline 14 mm in diameter against \(A.\) \textit{flavus}, and \(P.\) \textit{vulgaris} (gram negative, Figure 1-2) in ethanolic extract on the other hand 7 mm in diameter against \(S.\) \textit{aureus} (gram positive, Figure 3); \(P.\) \textit{vulgaris} and \(P.\) \textit{aeruginosa} (gram negative, Figure 4) in ethyl acetate extract when compared to tetracycline 14 mm in diameter against \(S.\) \textit{sonnei}.

Antifungal activity

Antifungal activity was also observed significantly 11 mm against \(A.\) \textit{niger}, \(A.\) \textit{flavus} and \(C.\) \textit{albicans} for the highest inhibition zone in both type of extractions and 8mm against \(A.\) \textit{niger}, \(A.\) \textit{flavus}, \(C.\) \textit{albicans}, \(Mucor\) sp. and \(F.\) \textit{oxyosporum} 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 \(A.\) \textit{fumigatus} and \(Mucor\) sp. and 13 mm against \(F.\) \textit{vasinfectum} and \(F.\) \textit{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 \(B.\) \textit{subtilis} (gram positive), \(S.\) \textit{typhi} and \(K.\) \textit{pneumoniae} (gram negative); 128 µg/ml against \(S.\) \textit{aureus}, \(S.-β\)-haemolyticus (gram positive) and \(E.\) \textit{coli} (gram negative) in the Figure 7 as relatively good antibacterial compounds. Similar results were found\(^6\) in some medicinal plants used in folklore remedies in South Western core, in some Allium Species from Hamedan Iran\(^9\). This result of antimicrobial activity is in accord with formers studies executed on \(C.\) \textit{viscosum}\(^9\).
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
This finding reveals also coincides with the study\textsuperscript{20,21} 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 posses 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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REFERENCES

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