**Reprint** **ISSN 1997-2571 (Web Version)**

# Journal of Innovation & Development Strategy (JIDS)

*(J. Innov. Dev. Strategy)*

**Volume: 6** **Issue: 1** **April 2012**

*J. Innov. Dev. Strategy 6(1):69-72(April 2012)*

## INVESTIGATION ON ANTIMICROBIAL ACTIVITIES OF THE PLANT *Clerodendrum viscosum* Vent.

M.N. HASAN, N. ARA, N. SULTANA, M.A. RABBI AND T.K. BARAI

GGF
Nature is Power

An International Scientific Research Publisher
Green Global Foundation©
Publication and Bibliography Division
100 Leeward Glenway
Apartment # 1601
M3c2z1, Toronto, Canada
E-mails: publication@ggfagro.com, editor@ggfagro.com
**http://ggfagro.com/ejournals/current issues**

**ISSN-1997-2571 (Online)**
*J. Innov. Dev. Strategy 6(1):69-72(April 2012)*

# INVESTIGATION ON ANTIMICROBIAL ACTIVITIES OF THE PLANT *Clerodendrum viscosum* Vent.

M.N. HASAN[1*], N. ARA[2], N. SULTANA[3], M.A. RABBI[4] AND T.K. BARAI[5]

[1]Department of Genetic Engineering and Biotechnology, Jessore Science & Technology University, Jessore 7408, Bangladesh; [2] Pharmacy Department, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh; [3]Department of Microbiology, Jessore Science & Technology University, Jessore 7408, Bangladesh; [4]Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh; [5]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh.

[*]Corresponding author & address: Md. Nazmul Hasan, E-mail: nazmul_bt19@yahoo.com
Accepted for publication on 18 March 2012

**ABSTRACT**

Hasan MN, Ara N, Sultana N, Rabbi MA, Barai TK (2012) Investigation on antimicrobial activities of the plant *Clerodendrum viscosum* Vent. *J. Innov. Dev. Strategy.* 6(1), 69-72.

The present study deals with the antimicrobial activity of the ethanolic extract of the leaves of *Clerodendrum viscosum* Vent. using agar diffusion method against human pathogens, such as *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Proteus vulgaris, Staphylococcus aureus, Shigella flexneri, Shigella sonnei, Pseudomonas* spp., *Enterococcus faecalis* and *Shigella dysenteriae*. In the present investigation, the extract was found to be effective against nine human bacterial species, except *Shigella sonnei* which showed sensitive to the plant extract. The study suggests that the extract of the plant parts possesses potential broad spectrum antimicrobial activity. The results of antibacterial evaluation of *C. viscosum* Vent. showed that EtOH extract of *C. viscosum* Vent. inhibited the growth of 9 out of 10 (90%).

***Key words:*** *Clerodendrum viscosum* Vent., *Medicinal plants, antimicrobial activity, plant extracts, growth inhibition, disc diffusion method*

## INTRODUCTION

The capacity of the substances to inhibit microbial growth is called antimicrobial activity. The use of medicinal plants and their extracts for the cure of localized and specific diseases in human as well as animal is an age-old practice from the time immemorial. In search of anti-microbial agents a number of surveys have been carried out by different workers, all of which point to the wide distribution of anti-microbial principles in marine algae and in higher plants. The use of bioassay-directed fractionation methodology is responsible for the rapid progress in the field of anti-microbial agents from higher plants. The isolated compounds from higher plants that are having anti-microbial properties are different in chemical structures; they are essential oils, alkaloids, flavonoids, anthraquinones etc.

In vitro antibacterial activity of plants can be detected by observing the growth response of various microorganisms to those plant extracts or their solvent fractions that are placed in contact with them. This can be done in three ways (diffusion, dilution, and bioautographic methods) and a great number of factors viz. the extraction method, inocula volume, culture medium composition (Bauer *et al.* 1966), pH (Leven *et al.* 1979), and incubation temperature (Lorian 1991) can influence the results. Disk diffusion technique is widely acceptable for the preliminary screening of antimicrobial activity. It is essentially a qualitative or semi-quantitative test indicating the sensitivity or resistance of microorganisms to the test materials. However, no distinction between bacteriostatic and bacteriocidal activity can be demonstrated by this method (Roland 1982).

### Test of Antimicrobial Activity by Disc Diffusion Method

Antimicrobial activity of the ethanolic extract of *Clerodendrum viscosum* Vent. was determined by disc diffusion method. (Bauer *et al.* 1966; Ahmed *et al.* 2003)

### Principle

In this method-measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile Matricel (BBL, Cocksville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried are used as positive and negative control, respectively. These discs are then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening. The plates are then kept at $4^0$C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator ($37^0$C) for 12-18 hour to allow the growth of the microorganisms.

If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments are carried out three times and the mean of the reading are recorded (Bauer *et al.* 1966).

**Microorganisms used for the activity test**

Both gram positive and gram-negative bacterial strains were taken for the test. The bacterial strains used for the investigation are listed in Table 1. These organisms were collected from the Microbial Biotechnology Lab., Biotechnology and Genetic Engineering Discipline, Khulna University Khulna.

Table 1. List of bacteria used for screening of antimicrobial activity

| Gram negative | Gram positive |
|---|---|
| 1. *Escherichia coli* – Dirrhoeal disease<br>2. *Shigella dysenteriae*- Dysentry<br>3. *Shi-gella sonnei*- Shigelosis<br>4. *Salmonella typhi*- Typhoid fever<br>5. *Salmonella paratyphi*- Fever<br>6. *Shigella flexneri*- Shigelosis<br>7. *Pseudomonas* spp.- Pneumonia<br>8. *Proteus* spp.– Urinery tract disease | 1. *Staphylococcus aureus* - Impetigo<br>2. *Enterococcus faecalis* – Enteric Disease |

**Culture media**

A mixture of nutrients used in the laboratory to support growth and multiplication of a culture (a population of microorganisms) is called culture medium (Pelczar *et al.* 1996). The nutritional requirements of bacteria vary widely; there are great differences in the chemical compositions own in a medium containing only inorganic compounds, whereas others require a medium containing organic compounds (amino acids, sugars, purines, or pyrimidines, vitamins, or coenzymes).

In addition to specific nutrients, each kind of organism also requires specific physical conditions for growth. For example, some bacteria cannot grow below 40°C, some cannot grow above 20°C and some require a temperature close to that of the human body (i.e., 37°C). Light may be another important physical condition. The successful cultivation of bacteria requires an awareness of all of these factors (Pelczar *et al*. 1996).

Each kind of microorganism grows in a characteristic manner. On solid media, microbes grow as colonies-distinct, compact masses of cells that are macroscopically visible. Colonies are characterized by their size, shape, texture, consistency, color, and other notable features (Pelczar *et al.*1986).

The following media are used normally to demonstrate the antibacterial activity and to make subculture of the test organisms: Nutrient agar media, Nutrient broth media, Muellar-Hinton agar media, Tryptic soy broth (TSB).

Among these, the first one is most frequently used which was also used for the present anti-microbial screening. Composition of Nutrient agar media (Mast Diagnostics, Mast Group Ltd., Merseyside)

| Ingredients | Amounts (gram/liter) |
|---|---|
| Peptone A | 6.0 |
| Yeast extract | 2.0 |
| Beef extract | 1.0 |
| Sodium chloride | 5.0 |
| Agar A | 14 |
| $P^H$ | 7.3(approx) |

**METHODOLOGY**

**Preparation of media**

Nutrient agar media was prepared by adding water to a dehydrated product that contains all the ingredients. Practically all media are available commercially in powdered form (Pelczar *et al.* 1986).

Media of the nutrient agar type was prepared by compounding the required individual ingredients or, more conveniently, by adding water to a dehydrated product which contains all the ingredients. Practically all media are available commercially in powdered form. The following steps were involved in the preparation of bacteriological media.

- Definite amounts of nutrient agar were accurately weighed.
- It was taken in a volumetric flask containing distilled water (half of the required volume).
- A clear medium was obtained by thorough dissolving agar over a water bath with occasional shaking.
- Then the final volume was adjusted.
- The medium was then transferred in 16 ml and 5 ml volume respectively, to prepare plates and slants, in a number of test tubes.
- The test tubes were then plugged with cotton and sterilized in an autoclave at a temperature of 121°C and pressure of 15-lbs/sq inch for 20 minutes.

**Sterilization of different equipments and media**

Media, Petri - dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq inch for 15 minutes. Blank discs kept in a covered petridishes; loop and forceps were subjected to dry heat sterilization at160°C for 1 hour. Later they were kept in a laminar hood under UV light for 30 minutes. UV light was switched on before one hour working in a laminar hood to avoid accidental contamination.

**Preparation of sub-culture**

- ✓ These slants were used for fresh culture of micro-organisms which in tern would be used for sensitivity tests.
- ✓ With the help of an inoculating loop, the test organisms from the pure cultures were transferred to the agar slants in an aseptic condition using laminar air hood.
- ✓ The inoculated slants were then incubated at 37°C for 18-24 hours to assure the growth of test organisms. This culture was used within two days.

**Preparation of the seeded test plates**

Each of the test organisms were transferred from the subculture to the test tube containing 16 ml autoclaved media with the help of the sterilized inoculating loop at 45°C in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of organism. The bacterial suspensions were immediately transferred to the sterile petridishes aseptically. The petridishes were rotated several times, first clockwise and then anticlockwise, to assure homogeneous distribution of the test organisms. The medium was poured into petridishes in such a way as to give a uniform layer of depth of approximately 4 mm. after the medium became cooled to room temperature, it was stored in a refrigerator (4°C). In this way 16 discrete media in petridishes were prepared for 16 bacterial strains used for screening & was properly marked.

**Preparation of test sample**

500mg of the crude extract of the accurately measured by the electronic balance & taken in a 10 ml volumetric flask. Then small amount of ethanol was added & was triturated in unidirectional manner, after proper mixing the volume (10ml) was adjusted by ethanol & the concentration became 50µg/µl.

**Preparation of disc**

Three types of discs were used for antibacterial screening: a) Sample discs, b) Standard discs and, c) Blank discs

**Sample discs**

20 sterile filter paper discs (5 mm in diameter) were taken in a blank petridish. 10µl of the test sample solution was applied on the discs (500 µg/disc) with the help of a micropipette in an aseptic condition under the laminar air flow. These discs were left for several hours (4-6) in aseptic condition under the laminar air flow for complete removal of solvent as the solvent (ethanol) has some antimicrobial activity.

**Standard discs**

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that produced by test samples. In this investigation Kanamycin (30 µg/disc) standard discs was used as the reference.

**Blank discs**

These were used as negative control. They ensure that the residual solvents (left over the discs even after air drying) and the filter paper were not active themselves.

**Application of discs**

Sample impregnated discs, standard antibiotic discs (Kanamycin discs) and negative control discs (blank discs) were placed gently on the 10 discrete solidified agar plates, freshly seeded with the test organisms with the help of a sterile forceps to assure complete contact with medium surface.

The spatial arrangement of the discs was such that the discs were no closer than 15mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in refrigeration for about 4 hours at 4°C. This was sufficient time for the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 12-18 hours.

**Determination of zone of inhibition**

After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a digital slide calipers.

**RESULT AND DISCUSSION**

The experiment was conducted only with ten species of bacteria. The results of antibacterial evaluation of *C. viscosum* Vent. showed that EtOH extract of *C viscosum* Vent. inhibited the growth of 9 out of 10 (90%). The

results presented in the figure 1, showed that EtOH extract of *C. viscosum* Vent. showed antibacterial activity, which inhibited the growth of all the bacteria tested except *Shigella sonnei.* The Further study should be done with other species of bacteria for more scientific evidence.

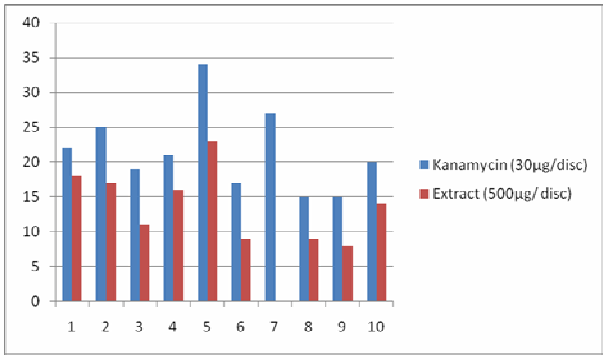


Fig. 1. Comperative study of Zone of inhibition against Kanamycin and Extract among different bacterial strains

Chronology of bacterial species on the chart: 1) *Escherichia coli*, 2) *Salmonella typhi*, 3) *Salmonella paratyphi* 4) *Proteus* spp., 5) *Staphylococcus aureus*, 6) *Shigella flexneri*, 7) *Shigella sonnei*, 8) *Enterococcus faecalis*, 9) *Pseudomonas* spp., 10) *Shigella dysenteriae.*

Fig. 2. Zone of Inhibition Test

## CONCLUSION

The present study was aimed to evaluate the anti-bacterial properties of the leaves of *Clerodendrum viscosum* Vent. From the above research study, it was found that this plant parts (leaf) has highly potent antibacterial activity which may explain the scientific basis of the traditional uses of the plant.

## REFERENCES

Ahmed F, Das PK, Islam MA, Rahman KM, Rahman MM, Selim MST (2003) Antibacterial activity of *Cordyline terminalis* Kunth Leaves. *J. Medical Sci*; 3, 418-422.

Bauer AW, Kirby, Sherris WMM, Turck JC (1966) Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol*; 45 (4), 493-496.

Leven M, Vannen Berghe DA, Mertens F (1979) Medicinal Plants and its importance in antimicrobial activity. *J. Planta Med*; 36, 311-321.

Lorian V, McGinnis MR, Rinaldi MG (1991) Determination of Antifungal MICs by a Rapid Susceptibility Assay. *Journal of Clinical Microbiology*; 13, 81–85.

Pelczar MJ, Chan JR, Noel ECS, Krieg R (1986) Microbiology, Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Pelczar MJ, Chan JR, Noel ECS, Krieg R (1996) Microbiology, Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Roland R (1982) Antibiotics: An Introduction. F. Hoffman La Roche and Co. Basle, Switzerland. pp. 70-71.