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# PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS FROM *CLERODENDRUM VISCOSUM*

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## ABSTRACT

The present study deals with the preliminary phytochemical screening and determination of antibacterial and antioxidant activity of various extracts from different parts of *clerodendrum viscosum*. The results revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, saponins, tannins and steroids in the extracts. All the extracts exhibited antibacterial effect against the tested bacteria with the diameter of inhibition zone ranging from 9.0 to 13 mm, however, highest antibacterial activity was observed by methanol extracts. In some cases, the organic extracts exhibited almost similar activity compared with standard sample streptomycin. The antioxidant potential of the samples was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Among the extracts, the strongest activity was exhibited by the ethyl acetate extract of steam with an $IC_{50}$ value of 12.29±0.9 µg/ml. The preliminary studies on *clerodendrum viscosum* extracts exhibited their antibacterial and antioxidant potential which could be exploited further as future antimicrobials for pharmaceutical treatment, natural therapies, food preservation and cosmetic applications.

**KEYWORDS:** *Clerodendrum viscosum*, phytochemicals, antibacterial activity, DPPH, antioxidant activity.

## INTRODUCTION

Plants are important sources of potentially useful substances for the development of new therapeutic agents. Various phytochemical compounds as secondary metabolites have been implicated in plants as the conferment of antibacterial activities.[1-2] Now a days, there is a renewed interest in traditional medicine. This revival of interest in plant-derived drug is mainly due to the current wide spread belief that the 'green medicine' is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. The medicinal action of plants is unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct to other species.[3] Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents.[4]

In Bangladesh, about 500 plant species have been identified as medicinal plants because of their therapeutic properties. Approximately hundreds of traditional medicines have been developed in the form of Ayurvedic and Unaani formulations in Bangladesh. About 400 herbal industries have been established in this country for producing Ayurvedic and Unani medicines and marketed herbal products of 500-crore taka worth annually.[5] Proper scientific evaluation of the pharmacological properties of these plants, used in different formulations, would carry enormous potential and promise for the 21st century.

*Clerodendrum viscosum* (Traditional name: Ghetu; Family: Verbenaceae) is a medicinally important plant bitter in taste that widely grow in Bangladesh. This plant has been reported to have antioxidative and analgesic activities.[6-7] The leaf and root have been used in traditional medicine for the treatment of asthma, fever, bronchitis, skin diseases, epilepsy, inflammation, tumors, worm infestation and snake bite.[8-9] The fresh leaf juice is used as vermifuge, bitter tonic, febrifuge in malaria fever, especially in children. The leaves of this plant is also used for chest complaints and cough.[10]

There is currently enormous surge of significance in the utilization, progress and preservation of the medicinal plants throughout the world. The presence of these phytoconstituents make the plant useful for treating different ailments and have a potential of providing

useful drug for human use.[11] Therefore, this study aims to evaluate the phytoconstituents of plant extract and their antibacterial and antioxidant activities.

## MATERIALS AND METHODS

### *Plant materials*

Fully matured fresh leaves, stems and roots of *Clerodendrum viscosum* were collected from Kumarkhali of Kushtia district, Bangladesh in the month of April 2018. The plant samples were then grinded in a fine powder form and then stored in air-tight container with marking for identification and kept in cool, dark, and dry place for future use.

### *Preparation of extracts*

Briefly 100 g of each powdered plant materials is submerged in suitable solvents of increasing polarity as chloroform, ethyl acetate and methanol subsequently in an air-tight separating funnel for 5 days at room temperature with occasional shaking and stirring. The obtained extract was filtered by using Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till a viscous mass was obtained. Finally, the prepared extracts were stored at 4$^{o}$C for further analyses.

### *Phytochemical screening*

The extracts of *Clerodendrum viscosum* (20 mg) were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant.

### *Test for Alkaloids*

Chloroform, ethyl acetate, methanol extract of each part of *Clerodendrum viscosum* were warmed separately with 2% $H_2SO_4$ for two minutes. It was filtered and few drops of Dragendroff's reagent was added and a red precipitation indicated the presence of alkaloids.

### *Test for Flavonoids*

A small quantity of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration confirmed the presence of flavanoids.

### *Test for Saponins*

A small quantity of different extracts was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable foam indicated the positive test.

### *Test for Steroids*

2 ml of acetic anhydride and 2 ml $H_2SO_4$ were added to the extracts. The color changed from violet to blue or green indicated the presence of steroids.

### *Test for Terpenoids*

Each extract was mixed with 2 ml of chloroform followed by concentrated $H_2SO_4$ (3 ml) was carefully added to form a layer. A reddish brown coloration in the interface indicated positive result for the presence of terpeniods.

### *Test for Tanins*

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration confirmed the presence of tannins.

### *Bioactivity screening*

### *Microbial strains*

The antimicrobial properties of *Clerodendrum viscosum* were investigated against three gram positive bacterial strains; *Bacillus cereus*, *Bacillus subtilis*, *S. aureus* and three gram negative bacterial strains; *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*. A total of six pathogenic microorganisms were collected as pure cultures from the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria-Bertani (LB) broth medium at 37$^{o}$C for 24 h. Cultures of each bacterial strains were maintained on LB agar medium at 4$^{o}$C.

### *Antibacterial activity assay*

The dried extracts were dissolved in the same solvent used for their extraction to a final concentration of 30 µg/µL and sterilized by filtration by 0.45 µm Millipore filters (Millipore Corp., Bedford, MA, USA). The antibacterial test was then carried out by agar disc diffusion method[12] using 100 µL of standardized inoculums suspension containing $10^7$ CFU/mL of bacteria. 10 µL of 30 µg/µL of each organic extract (300 µg/disc) was applied on the filter paper discs (6 mm diameter) and placed on the inoculated LB agar. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard reference antibiotics streptomycin (10 µg/disc, each from Sigma-Aldrich Co., St. Louis, MO, USA) was used as positive controls for the tested bacteria. The plates were incubated at 37$^{o}$C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria.

### *Antioxidant activity*

### *DPPH assay*

The hydrogen atoms or electrons donation ability of the corresponding extracts was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent.[13] Various concentrations of test extracts were added to 2.9 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm. $IC_{50}$ values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation. Synthetic antioxidant reagent, L-ascorbic acid was used as reference positive controls. Inhibition free

radical DPPH in percent (*I%*) was calculated in following way:

$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$

Where, $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$ is the absorbance of the test compound. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the linear regression algorithm of the graph plotted inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used. Tests were carried out in triplicate. Values are presented as means ± S.E.M. of three parallel measurements.

## RESULTS AND DISCUSSION

### *Phytochemical screening*

Phytochemicals are non-nutritive plant chemicals that have disease preventive properties.[14] The investigation of chloroform, ethyl acetate and methanol extracts of *Clerodendrum viscosum* showed differences in their phytoconstituents. Methanol extract yielded better results. Ethyl acetate and chloroform extracts showed moderate results. This reveals that solubility of each constituent in each solvent is different.

According to the Table 1, terpenoids are found in chloroform and methanol extracts of leaf and roots, while for stem it was in ethyl acetate and chloroform extracts. Terpenoids are aromatic compounds found in plant species, which is responsible for flavour and fragrance.[15] Plant terpenoids have also some bioactivities like antibacterial, antiparasite, antiviral, anticancer and anti-inflammatory.[16] Saponins are found only in methanol extract of stem. Saponins are steroid or triterpenoid glycosides characterised by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells.[17] Tanins are found in methanol extract of leaf and roots. Tannins possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial ftinction, as well as inhibition of angiogenesis and cell proliferation activities.[18] On the other hand, ethyl acetate and methanol could have extracted more polyphenols such as flavonoids better than other solvents, which explains why the ethyl acetate and methanol extract of *C. viscosum* tested positive for flavonoid. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties.[19] Alkaloids are mostly found in methanolic extracts of *Clerodendrum viscosum*. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities.[20-21] The presence of steroids was found in n-hexane and methanol, while tannins and saponins in methanolic extract of *Clerodendrum viscosum* Steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties. [22]

**Table 1: Phytochemical screening of *Clerodendrum viscosum.***

| Plant Parts | Solvent | Tested groups | | | | | |
|---|---|---|---|---|---|---|---|
| | | Terpenoids | Saponins | Tannins | Flavonoids | Alkaloids | Steroids |
| Leaf | Chloroform | + | - | - | + | - | - |
| | Ethyl acetate | - | - | - | + | - | + |
| | Methanol | + | - | + | + | + | + |
| Stem | Chloroform | + | - | - | - | - | + |
| | Ethyl acetate | + | - | - | + | - | + |
| | Methanol | - | + | - | + | - | - |
| Roots | Chloroform | + | - | - | - | - | + |
| | Ethyl acetate | - | - | - | + | - | - |
| | Methanol | + | - | + | + | + | - |

### *In vitro antibacterial activity assay*

The *in vitro* antibacterial activity of various extracts (chloroform, ethyl acetate and methanol) of *C. viscosum* against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones.

According to the results given in Table 2, a total of six pathogenic and spoilage bacteria, including three gram-positive and three gram-negative bacteria were tested. The extracts exhibited antibacterial activity against most of the tested bacteria at a concentration of 300 µg/disc. Some of the extract exhibited a noticeable antibacterial effect against the tested bacteria, with diameter of inhibition zones ranging from 8 to 15 mm and compared with standard samples streptomycin (10µg/disc).

The results showed that the chloroform extract of the leaf, steam and root of *Clerodendrum viscosum* had the considerable antibacterial activity. Chloroform extract of root showed the moderate antibacterial effect against all tested bacteria with their respective diameter zones of inhibition from 10mm to 12mm, whereas inhibition zone for chloroform extract of leaf and stem found in between 8mm to 11mm. Ethyl acetate extract of all tested samples showed intermediate antibacterial effect against all tested bacteria with their respective diameter zones of inhibition from 8mm to 13mm.

**Table 2: Antibacterial activity of various extracts of leaf, stem and root from *C. viscosum*.**

| Plant Parts | Solvent | Diameter of Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram positive bacteria | | | Gram negative bacteria | | |
| | | *Bacillus cereus* | *Bacillus subtilis* | *S. aureus* | *Pseudomonas aeruginosa* | *Salmonella typhi* | *E. coli* |
| Leaf | Chloroform | 11 | 10 | 10 | 9 | 10 | 8 |
| | Ethyl acetate | 12 | 11 | 11 | 10 | 10 | 8 |
| | Methanol | 14 | 12 | 12 | 10 | 11 | 10 |
| Stem | Chloroform | 11 | 10 | 9 | 8 | 9 | - |
| | Ethyl acetate | 11 | 11 | 10 | 11 | 10 | 9 |
| | Methanol | 13 | 11 | 12 | 11 | 10 | 9 |
| Roots | Chloroform | 11 | 12 | 10 | 10 | - | - |
| | Ethyl acetate | 12 | 13 | 12 | 11 | 10 | 9 |
| | Methanol | 15 | 13 | 13 | 11 | 10 | 10 |
| Streptomycin | | 19 | 18 | 16 | 15 | 14 | 12 |

Diameter of inhibition zones of various extracts including diameter of disc 6 mm (tested at a volume of 10 µL/disc with concentration 300 µg/disc). Standard antibiotics: streptomycin (10 µg/disc).

Methanol extract of leaf showed intermediate antibacterial effect with inhibition zones in the range of 10–14mm, whereas, stem extract showed the effect with inhibition zones in the range of 9–13mm. On the other hand, methanol extract of root showed the strongest effect with inhibition zones in the range of 10–15mm. Thus according to our investigation *Clerodendrum viscosum* has antibacterial potential and can be used as a potent antibacterial agent for human pathogenic and spoilage bacteria.

***Scavenging activity of DPPH radical***

The DPPH free radical is a stable free radical, which has been widely used as tool to estimate free radical-scavenging activity of antioxidants. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character.[13] The DPPH-radical-scavenging activity of the plant extracts are shown in Fig-1. Lower $IC_{50}$ value indicates higher antioxidant activity. Polar extracts exhibited stronger activity than non-polar extracts. DPPH-radical scavenging capacity of the extracts was compared to L-ascorbic acid (Fig-1). Of all samples studied, the ethyl acetate and methanol extract of stem had the remarkable free radical-scavenging activity with an $IC_{50}$ value of 12.29 ± 0.8 µg/ml and 17.45 ± 1.2 µg/ml, respectively. The root extracts showed moderate DPPH-radical-scavenging activity in the range of $IC_{50}$ = 30.26 ± 1.8 µg/ml to 41.78 ± 0.6 µg/ml, while leaf extract showed the scavenging activity in the range of $IC_{50}$ = 26.29 ± 1.2 µg/ml to 68.19 ± 0.6 µg/ml. However, its activity was lesser than Aascorbic acid (6.34 ± 0.9 µg/ml).

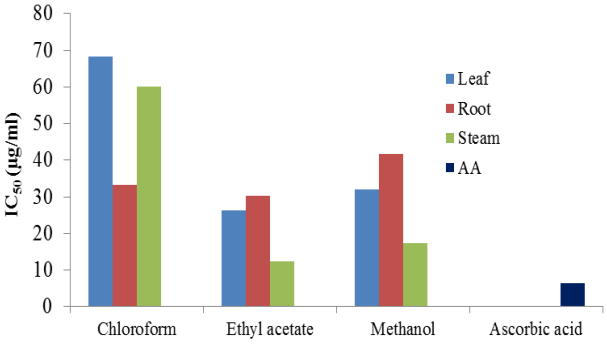


**Figure 1: Antioxidant activity of different extracts of *Clerodendrum viscosum*.**

## CONCLUSION

The phytochemical screening of the medicinal plants is of prime importance for the verification of effectiveness and safety of folk medicines. The plant parts under study could be a potential source of active antimicrobial agents and may help in the detailed assessment of them *in vivo* potencies and toxicological profile. The results obtained in the present study thus suggest that the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

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