Jagannath University Journal of Life and Earth Sciences, **8**(1) pISSN 2414-1402, eISSN 2791-1845

Research Article JnUJLE

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *CLERODENDRUM VISCOSUM* LEAF EXTRACTS

Farjana Islam Penu[1†], G M Al Amin[2†], Fatema Akhter[1] and Mohammad Saydur Rahman[1*]

[1]*Department of Pharmacy, Jagannath University, Dhaka-1100*

[2]*Department of Botany, Jagannath University, Dhaka-1100*

[†]*These authors contributed equally to this work*

Received: 11 August, 2022, Accepted: 02 December 2022

ABSTRACT

The methanolic extracts of *Clerodendrum viscosum* leaves were screened for phytochemical constituents and antimicrobial functions. The phytochemical constituents were identified using a qualitative phytochemical screening procedure. Qualitative analyses revealed the presence of tannins, saponins, flavonoids, and alkaloids in the crude extracts. The disc diffusion method was used to test the antimicrobial activity of leaf tissue soluble fractions such as methanolic extracts soluble fraction (MESF), dichloromethane soluble fraction (DCMSF), aqueous soluble fraction (AQSF), carbon tetra chloride soluble fraction (CTCSF), and petroleum-ether soluble fraction (PESF) against 11 microbes, including *Bacillus cereus*, *Escherichia coli*, *Vibrio parahemolyticus*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Salmonella typhi*, *Bacillus subtilis*, *Aspergillus niger*, *Candida albicans*, and *Vibrio mimicus*. Among the fractions, DCMSF, CTCSF, and AQSF, significant antimicrobial activity has been observed. We are constantly looking for new antimicrobial agents due to the drawbacks of current antimicrobials as well as the ongoing emergence of bacterial resistance.

Keywords: *Clerodendrum viscosum, Phytochemical constituents, Antimicrobial, Crude extracts*

Introduction

Medicinal plants have evolved into a significant component of the basic medical system in the modern world because of the vast quantities of good natural products. As a result, the study of natural product chemistry has experienced an increased wave of interest (Akhter *et al.*, 2021; Cartwright, 2010). Natural products are essential in the research and development of new medicines. They have been used as a preliminary step and foundation for drug discovery initiatives (Gray *et al.*, 2012).

[*] **Correspondence:** *saydur_pr@yahoo.com*

Plants are a rich source of secondary metabolites with diverse biological actions (Pandit *et al.*, 2022). Therefore, natural products offer more chances to find antimicrobial drugs or lead compounds than synthetic ones, which have historically been successful sources of drug discovery (Atanasov *et al.*, 2021). Plants and herbs (oregano, garlic, parsley, sage, coriander, rosemary, and lemongrass), spices (cinnamon, clove), oils (citral), or organic compounds (vanillin) have been used alone for their antimicrobial and antioxidant properties or in combination with other techniques for food preservation (Quinto *et al.*, 2019).

Our research focuses on the phytochemical constituents and antimicrobial activity of various leaf extracts of *Clerodendrum viscosum.* This plant, also known by its common name, Ghetu, is a member of the Verbenaceae family of plants. It is widely obtainable in torrid and subtorrid zones. In all districts of Bangladesh, it often grows in squatter areas and cemeteries (Ghani, 1998). It also grows frequently in waste places in all regions of India and Burma. It is obtainable in India's evergreen, semi-evergreen, and mixed deciduous forests. It is a small tree that grows to be about 1-2 m tall but has the potential to grow much taller (Nandi and Lyndem, 2016). The leaves of this plant have long been used to treat tumors, asthma, and a few skin conditions due to their acrid tonic, antipyretic, and anthelmintic properties. Leaf extract possesses laxative and cholagogue properties (Panigrahi *et al.,* 2015). A methanolic extract of *C. viscosum* is screened for phytoconstituents including carbohydrates, proteins, amino acids, glycosides, flavonoids, and alkaloids in a preliminary phytochemical analysis (Nandi and Lyndem, 2016; Srivastava *et al.*, 2021). The specimen is of great importance due to its many therapeutic applications. This plant might be used as a reliable and consistent source of bioactive ingredients, making it an important source of raw materials for the production of pharmaceuticals. Therefore, efforts were made to look into the phytochemical and antimicrobial potential of *C. viscosum* leaves.

Materials and Methods

***The plant material collection and its preparation*:** The whole plant of *C. viscosum* was collected from the National Botanical Garden, Dhaka, Bangladesh, and identified with the help of the Bangladesh National Herbarium, Dhaka, Bangladesh, and Accession No. DACB 66330. The specimen is conserved at the Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka, for further study. The leaves were air-parched for a few days and then stove-parched for 24 hours at notably less heat (not exceeding 40°C) for better pounding. Then the parched leaves were pounded to an indelicate grind using high powered pounding instruments in the laboratory of the BCSIR, Dhaka.

***Extraction of Plant Materials*:** Pulverized leaves (250 gm) had been placed in a clean, amber-colored reagent flask (5 L) and drenched in 2.0 L of methanol. The basket was sealed with the closure of a bottle and kept for 10 days with casual stirring and shaking (Asif *et al.*, 2014; Konyak *et al.*, 2021). After that, the entire composition was refined by using a pure cotton cork and, at the end, a Whatman No. 1 filter document. The refinement was gradually approved to vaporize at room temperature until almost 70% of the diluent had vaporized. Then, the extracts were carefully weighed and kept in the refrigerator (Hossain *et al.*, 2019; Khan *et al.*, 2013). A modified Kupchan method of liquid-liquid extraction was used for partitioning the crude extract (Kupchan

et al., 1973). The soluble fractions in methanolic extract (MESF), aqueous soluble fraction (AQSF), dichloromethane soluble fraction (DCMSF), carbon tetra chloride soluble fraction (CTCSF), and pet-ether soluble fraction (PESF) of plant tissues were tested for phytochemical screening and antimicrobial activity (Asif *et al.,* 2014).

Test organisms: The bacterial and fungal filtrations adopted in the research were obtained as fresh instructions from Jagannath University, Dhaka. Three gram-positive (*Staphylococcus aureus*, *Bacillus subtilis, and Bacillus cereus*), five gram-negative (*Vibrio parahemolyticus, Vibrio mimicus, Shigella dysenteriae, Salmonella typhi, and Escherichia coli*), and three fungi (*Candida albicans, Saccharomyces cerevisiae*, and *Aspergillus niger*) were used in the screening of anti-microbial activity (Rahman *et al.,* 2009).

***Test samples*:** Methanolic extract soluble fraction (MESF), aqueous soluble fraction (AQSF), chloroform soluble fraction (DCMSF), carbon tetrachloride soluble fraction (CTCSF) and petroleum ether soluble fraction (PESF).

Culture medium: Nutrient Agar Medium (MAM), Nutrient Broth Medium (NBM), Mueller-Hinton Broth (MHB), and Tryptic Soya Broth Medium (TSBM).

Phytochemical screening methodology: Standard phytochemical methods were used to identify the presence of various plant metabolites in *Clerodendron viscosum* leaf crude extracts (Panigrahi *et al.,* 2015).

Test for reducing sugar

*Benedict's test :*Test samples were included in a test tube with 2.5 mL of Benedict's commentary . Then it was warmed in a water bath for five minutes.

Test for tannins

Lead acetate test: 1-3 ml of 10% direct acetate accomplishments were added to a portion of each essence (50 mg). The presence of tannins is indicated by a bulky white or buff coloration (light yellow color) or precipitate.

Test for alkaloids

Mayer's test: The few drops of Meyer's reagent (potassium mercuric chloride solutions) were included to 1 ml of each sample solution. A cream-colored precipitate may indicate the presence of alkaloids (Saha and Bandyopadhyay, 2019).

Test for flavonoids

Alkaline reagent test: A liquid dissolution of each essence was medicated with 5 ml of sleazy ammonia treatment, followed by the composition of consolidated H_2SO_4. A yellow coloration suggested the existence of flavonoids. The yellow coloration faded on standing (Edeoga *et al.,* 2005; Saha and Bandyopadhyay, 2019).

Test for saponins: 1-2 mg of each extract was adopted through a test tube. Shaken with 5 ml of distilled water. The observation of foams revealed the existence of saponins. (George *et al.,* 2010; Kamal *et al.,* 2019).

***Disc diffusion method*:** Antibiotics diffuse throughout the nutrients in agar gels from a confined source, resulting in a concentration gradient. A filter paper disc (6 mm in diameter) comprising a known amount of test specimen is settled on nutrient agar medium that has been consistently sown with the test microorganisms. The standard antibiotic (ciprofloxacin) and vacant specimens are adopted as neutral and opposite controls, respectively. These platters are set up at a lower temperature (4°C) for 24 hours to allow the highest radiation of the test ingredients to reach the inclusive medium. After that, the platters are overturned and twirled at 37°C for 24 hours to allow the organisms to grow to their full potential. Antimicrobial samples interdict microbial aggrandizement in the media neighboring the discs, resulting in a transparent, unique circle of prohibition. The antimicrobial operation of the test doer is ascertained by standardizing the diameter of the circle of prohibition in millimeters (Hoque *et al.,* 2011).

The antimicrobial activity of unrefined essence was assessed using the disc diffusion method in this study. The experiment is repeated three times, and the approximate mean of the exercises is required.

Results and Discussion

Phytochemical analysis of the methanolic extract of the leaves of *C. viscosum* revealed the presence of reducing sugars, tannins, saponins, flavonoids, and alkaloids (Table 1). Tannins exhibit their biological effects as a non-absorbable (high MW) complex structure with binding capabilities that may induce local effects in the gastrointestinal tract and as absorbable tannins whose metabolites impose systemic effects (Ou and Gu, 2014; Rodríguez *et al.,* 2014). Tannins appear capable of exerting antimicrobial, anticarcinogenic, anti-inflammatory, anti-allergic, vasodilatory, and antioxidant activities (Smeriglio *et al.,* 2017). Flavonoids inhibit a variety of enzymes, including xanthine oxidase, aldose reductase, phosphodiesterase, lipoxygenase, cycloxygenase, etc. In addition, they regulate hormones such as estrogens, thyroid hormone, and androgens. They also showed antioxidant, cytotoxic, anti-inflammatory, anti-allergic, antimicrobial, and anticarcinogenic properties (Rathee *et al.,* 2009). The major effect of saponins is to inhibit the protozoa in the rumen (Patra and Saxena, 2009). The medicinal worth of the plant is due to the presence of certain chemical elements that have a specific physiological activity on the human body. In addition, the most significant bioactive plant components contain tannins, flavonoids, alkaloids, and saponins, among others (Edeoga *et al.,* 2005).

Table 1. Chemical tests of the crude extracts

Test for constituents	Result	Comment
Reducing Sugars	-	Reducing Sugars absent
Tannins	+	Tannins present
Alkaloids	+	Alkaloids present
Flavonoids	+	Flavonoids present
Saponin	+	Saponin present

The methanol essence of leaves of *Clerodendrum viscosum* (MESF) and various partitions, i.e., aqueous (AQSF), carbon tetrachloride (CTCSF), dichloromethane (DCMSF), and petroleum ether (PESF), liquefiable partitions of the methanol essence of leaves of *C. viscosum*, were each subjected to antimicrobial screening at a pace of 200 mg/disc. The outcomes are presented in Table 2.

The dichloromethane soluble fraction (DCMSF) inhibited microbial growth the best, with a circle of prohibition ranging from 10.0 mm to 20.0 mm. The highest circle of prohibition created by DCMSF was reflected to be 20.0 mm against *Sacharomyces cerevacae* (fungi), with other inhibition of 16.0 mm against *Aspergillus niger* (fungi), *Staphylococcus aureus* (+), and *Bacillus subtilis* (+). This partition also demonstrated endurable antibacterial operation, with a circle of prohibition of 15.0 mm opposite *Bacillus cereus* (+), *Vibrio mimicus* (-), and *Shigella dysenteriae* (-), However, *Candida albicans* had a zone of inhibition of 14.0 mm, while *Salmonella typhi* (-) and *Escherichia coli* (-) had circles of prohibition of 12.0 mm .The DCMSF also inhibited *Vibrio parahemolyticus* (-) development with a zone of inhibition of 10.0 mm (George *et al.,* 2010; Oladipo *et al.*, 2010).

The carbon tetra-chloride soluble fraction (CTCSF) had the second maximum prohibition opposite to microbial aggrandizement, with a circle of prohibition ranging from 7.0 mm to 18.0 mm. The highest circle of prohibition created by CTCSF was reflected to be 18.0 mm opposite *Staphylococcus aureus* (+), followed by 7.0 mm against *Aspergillus niger* (fungi), and against 10.0 mm *Saccharomyces cerevacae* (fungi). This partition also demonstrated medium antibacterial operation opposite to *Vibrio parahemolyticus* (-), with a circle of prohibition of 15.0 mm, *Shigella dysenteriae* (-), with a circle of prohibition of 12.0 mm, and other *Escherichia coli* (-), microbial aggrandizement with a circle of prohibition of 13.0 mm (George *et al.*, 2010).

The aqueous soluble fraction also inhibited microbial growth, with a circle of prohibition ranging from 13.0 to 17.0 mm. The highest circle of prohibition created by AQSF was reflected to be 17.0 mm opposite *Aspergillus niger* (fungi). The partitions also demonstrated medium antibacterial aspiration against fungi, *Saccharomyces cerevisiae* (circle of prohibition = 16.0 mm) and *Candida albicans* (circle of prohibition = 15.0 mm). The AQSF also inhibited *Shigella dysenteriae* growth with a 13.0 mm circle of prohibition. The zone of inhibition for methanolic extract soluble fraction (MESF) expanded from 8.0 mm to 16.0 mm.

In vitro microbial screening of *C. viscosum* leaf revealed that DCMSF, CTCSF, and AQSF have excellent antimicrobial activities, which may be further investigated to find potential antimicrobial doers. PESF was discovered to have no antimicrobial activity. In addition, each of these test subjects exhibits a notable level of function opposite that of both gram-positive and gram-negative bacteria and fungi.

Table 2. Antimicrobial activity of the various methanol extracts (10 mg/ml) of *Clerodendrum viscosum* leaf

Different test microbes	Zone of inhibition* (mm ± SEM) in different extracts					Ciprofloxacillin
	MESF	CTCSF	PESF	DCMSF	AQSF	
Gram positive bacteria						
Bacillus cereus	10.17±0.26	16.30±0.06	-	15.17±0.09	-	45.03±0.12
Bacillus subtilis	10.07±0.15	14.00±0.10	-	16.03±0.12	-	46.17±0.07
Staphylococcus aureus	10.30±0.31	18.13±0.03	-	16.37±0.09	14.03±0.09	48.03±0.12
Gram negative bacteria						
Escherichia coli	09.20±0.06	13.20±0.15	-	12.13±0.09	-	45.17±0.07
Salmonella typhi	08.20±0.09	10.20±0.12	-	12.00±0.15	-	46.23±0.03
Shigella dysenteriae	16.30±0.12	12.10±0.15	-	15.13±0.20	13.23±0.12	50.13±0.09
Vibrio mimicus	08.17±0.12	10.20±0.12	-	15.30±0.06	-	44.13±0.03
Vibrio parahemolyticus	-	15.07±0.18	-	10.20±0.10	-	43.30±0.12
Fungi						
Candida albicans	-	10.20±0.12	-	14.17±0.12	15.10±0.06	46.30±0.12
Aspergillus niger	-	07.00±0.15	-	15.93±0.07	17.07±0.15	45.17±0.19
Sacharomyces cerevacae	08.07±0.12	10.13±0.09	-	20.30±0.06	16.17±0.12	47.03±0.13

*The diameter of zones of inhibition of different leaf extracts of *C. viscosum*. Standard antibiotic Ciprofloxacillin (200 μg/disc) was employed as a positive control. Values are presented as standard error mean (SEM) of 3 independent experiments, "-" indicate no zone of inhibition.

Conclusion

C. viscosum is a plant that is said to be abundant in bioactive components and has undergone some scientific study. This study has performed several phytochemical screening tests to detect the presence of the secondary metabolites of this plant. The phytochemicals found in the screening tests are tannins, flavonoids, alkaloids, saponins, etc. In addition, the methanolic extract and its partitions from this plant were also screened for antimicrobial activity. Most bacterial and fungal species tested showed that the different fractions of the crude extract had very high levels of antimicrobial activity. Therefore, considering the potentiation, the different parts of this plant can be a profound source of many essential lead compounds. Further research can be conducted in more detail to perceive their undisclosed potency.

References

Akhter, F., Rahman, M. S., Al Amin, G. M., Miah, M. I., and Koh, Y. S. (2021). Beneficial Therapy with Natural Anti-Inflammatory Agents and Supplements. *Journal of Bacteriology and Virology*, 51(4): 149–162.

Asif, F., Boby, A. Z., Alam, N., Taraquzzaman, M., Chowdhury, S. R., and Rashid, M. A. (2014). Canvassing of thrombolytic, cytotoxic, and erythrocyte membrane-stabilizing attributes in in vitro screening of *Gynocardia odorata*. *Journal of Analytical Science and Technology*, 5(1): 1–7.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., and Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug discovery*, 20(3): 200–216.

Cartwright, R. (2010). Calcium and Magnesium in Drinking-Water: Public health Significance. *Perspectives in Public Health*, 130(5): 239.

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7), 685–688.

George, N. J., Obot, I. B., Ikot, A. N., Akpan, A. E., and Obi-Egbedi, N. O. (2010). Phytochemical and antimicrobial properties of leaves of *Alchonea cordifolia*. *Journal of Chemistry*, 7(3): 1071–1079.

Ghani, A. (1998). Medicinal plants of Bangladesh: chemical constituents and uses: *Asiatic society of Bangladesh.*

Gray, A. I., Igoli, J. O., and Edrada-Ebel, R. (2012). Natural products isolation in modern drug discovery programs. *In. Natural Products Isolation*. 515–534, Springer.

Hoque, T., Sikder, M. A. A., Kaisar, M. A., Chowdhury, A. A., and Rashid, M. A. (2011). Biological screenings of two araceous plants growing in Bangladesh. *Dhaka University Journal of Pharmaceutical Sciences*, 10(2): 131–135.

Hossain, M. M., Aka, T. D., Rahman, M. S., Uddin, A. H. M. M., Rahman, N., and Rashid, M. M. O. (2019). Neuropharmacological activity of the crude ethanolic extract of *Syzygium aromaticum* flowering bud. *Discovery Phytomedicine*, 6(4): 191–198.

Kamal, S., Rony, S. R., Sharmin, S., Laboni, F. R., and Sohrab, M. H. (2019). Phytochemical and pharmacological potential of *Enhydra fluctuans* available in Bangladesh. *Journal of Pharmaceutical Research International*, 29(4): 1–11.

Khan, M. S. S., Syeed, S. H., Uddin, M. H., Akter, L., Ullah, M. A., Jahan, S., and Rashid, M. H. (2013). Screening and evaluation of antioxidant, antimicrobial, cytotoxic, thrombolytic and membrane stabilizing properties of the methanolic extract and solvent-solvent partitioning effect of *Vitex negundo* Bark. *Asian Pacific Journal of Tropical Disease*, 3(5): 393–400.

Konyak, P. L., Jamir, T. T., Kichu, A., and Imsong, I. (2021). Preliminary phytochemical screening of a medicinal tree plant *Gynocardia odorata* found in the native of mon district, Nagaland India. *Journal of Pharmacognosy and Phytochemistry*, 10(3): 288–291.

Kupchan, S. M., Tsou, G., and Sigel, C. W. (1973). Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from Datisca glomerata. *The journal of organic chemistry*, 38(7): 1420–1421.

Nandi, S., and Mawkhlieng Lyndem, L. (2016). *Clerodendrum viscosum*: traditional uses, pharmacological activities and phytochemical constituents. *Natural Product Research*, 30(5): 497–506.

Oladipo, I. C., Adeleke, D. T., and Adebiyi, A. O. (2010). The effect of pH and chemical preservatives on the growth of bacterial isolates from some Nigerian packaged fruit juices. *Pakistan Journal of Biological Sciences*, 13(1): 16–21.

Ou, K., and Gu, L. (2014). Absorption and metabolism of proanthocyanidins. *Journal of Functional Foods*, 7: 43–53.

Pandit, M. A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., . . . and Kaur, J. (2022). Major Biological Control Strategies for Plant Pathogens. *Pathogens*, 11(2): 273.

Panigrahi, B. K., Mishra, S. K., and Sahu, S. K. (2015). Antidiabetic effects of *Clerodendrum viscosum*, vent. *World Journal of Pharmaceutical Sciences*, 3(9): 1944–1948.

Patra, A. K., and Saxena, J. (2009). The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. *Nutrition Research Reviews*, 22(2): 204–219.

Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-Silleras, B., and Redondo-Del-Río, M. P. (2019). Food safety through natural antimicrobials. *Antibiotics*, 8(4): 208.

Rahman, S., Akbor, M. M., Howlader, A., and Jabbar, A. (2009). Antimicrobial and cytotoxic activity of the alkaloids of Amlaki (*Emblica officinalis*). *Pakistan Journal of Biological Sciences*, 12(16): 1152–1155.

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., and Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: A Review. *Inflammation & Allergy-Drug Targets*, 8(3): 229–235.

Saha, M., and Bandyopadhyay, P. K. (2019). Green biosynthesis of silver nanoparticle using garlic, *Allium sativum* with reference to its antimicrobial activity against the pathogenic strain of *Bacillus* sp. and *Pseudomonas* sp. infecting Goldfish, *Carassius auratus*. In Proceedings of the Zoological Society, 72: 180–186, Springer India.

Smeriglio, A., Barreca, D., Bellocco, E., and Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*, 174(11): 1244–1262.

Srivastava, M., Maurya, P., and Shanker, K. (2021). *Clerodendrum viscosum*: A critical review on phytochemistry, pharmacology, quality assurance, and safety data. *Medicinal Chemistry Research*, 30(12): 2145–2167.

Velderrain-Rodríguez, G. R., Palafox-Carlos, H., Wall-Medrano, A., Ayala-Zavala, J. F., Chen, C. O., Robles-Sánchez, M., . . . and González-Aguilar, G. A. (2014). Phenolic compounds: Their journey after intake. *Food & Function*, 5(2): 189–197.