Bangl. J. Vet. Med. (2021). 19 (1): 69–73 ISSN: 1729-7893 (Print), 2308-0922 (Online)

Received: 30-05-2021, Accepted: 01-06-2021 DOI: https://doi.org/10.33109/bjvmjj2021vph3

ORIGINAL ARTICLE

Evaluation of antibacterial potentials of *Parthenium hysterophorus* L. leaf extract against some pathogenic and nonpathogenic bacteria

M. A. Kallol[1], N. J. Pinky[1], Assaduzzaman[1], B. K. Dash[1], M. Rahman[2]*

[1]Department of Genetic Engineering and Biotechnology, Jashore University of Science and Technology, Jashore -7408, Bangladesh.

[2]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Background: The herb, *Parthenium* (*P.*) *hysterophorus* L. has been used in folk medicine for many years to treat neurological disorders, inflammation, fever, and malaria. This study aimed to evaluate the antibacterial activity of *P. hysterophorus* L. leaf extracts against some pathogenic and nonpathogenic bacterial strains.

Methods: Methanol and chloroform extracts of *P. hysterophorus* L. leaf were applied against pathogenic *Vibrio* (*V.*) *parahaemolyticus* ATCC 17802, *Escherichia coli 0157* ATCC 43894, *Sarcina* (*S.*) *lutea* IFO 3232) and nonpathogenic *Bacillus* (*B.*) *subtilis* IFO 3026 strains by agar well diffusion assay. Visible zones of inhibition were measured.

Results: The methanol and chloroform extracts of *P. hysterophorus* leaf exhibited remarkable antibacterial activity against all three pathogenic (*V. parahaemolyticus*, *E. coli 0157*, *S. lutea*) and nonpathogenic (*B. subtilis*) bacterial strains. Chloroform solvent extract of *P. hysterophorus* leaves showed highest zone of inhibition (23mm) against gram-positive pathogenic *S. lutea*and methanolic leaf extract showed the highest zone of inhibition (26mm) against *B. subtilis*. The lowest zone of inhibition was observed for gram-negative pathogenic *V. parahaemolyticus* in the response of methanolic (9mm) and the chloroform (10mm) leaf extract.

Conclusion: The findings of this study suggest that, leaf extracts of *P. hysterophorus* L. could be a potential source of antibacterial agents to cope with antibiotic resistance and new drug discovery.

Keywords: Antibacterial, disorders,inflammation,well diffusion assay, zone of inhibition.

*Correspondence: marzia_micro@bau.edu.bd All right reserved 0468/2021

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Introduction

The current world is standing on a tremendous danger slope of antibiotic resistance threat. A large number of bacterial strains are being resistant gradually to antibiotics (Gould, 2009). Human civilizations have been used plants as depots of medicines, therapeutics and treatment of diseases for thousands of years (Dar et al., 2017). As the effectiveness of antibiotics is decreasing, plants are being considered as alternative sources of new antibiotics (Sher, 2009, Zihadi et al*.,* 2019). Leaf extracts of different medicinal plants have showed antibacterial, anti-fungal, anti-viral, antipyretic and cytotoxic activity (Hasan et al., 2015). *Parthenium hysterophorus* L. is a member of Asteracea family and a common weed in many countries in the world. Traditionally, it is used for the treatment of neurological disorders, urinary infections, diarrhoea, fever and malaria (Fazal et al., 2011). This study aims to investigate the antibacterial activity of *Parthenium hysterophorus* L. leaf extract against three pathogenic and one nonpathogenic bacterial strains.

Materials and methods

Plant material

Vigorous and fresh leaves of *P. hysterophorus* were collected from the campus area of Jashore University of Science and Technology, Jashore -7408, Bangladesh. The plant was identified by Regional Agricultural Research Station, BARRI, Jashore, Bangladesh.

Crude extract preparation

Collected leaves of *Parthenium hysterophorus* L. were washed thoroughly with running tap water and sterile distilled water. The washed leaves were air dried on a paper towel at room temperature for two days and further dried in a dryer machine at 55°C for 15 hours. Complete powder of dry leaves was prepared by a grinder. The powder was stored in an air tight container and kept in a cool dark and dry place. Then the powder was soaked in methanol and chloroform solvent completely into two separate flat-bottomed containers. The containers were sealed and placed into a shaking incubator at 37°C for two days. After incubation, the extracts were filtered through Whatman no.1 filter paper. Semi solid extracts of the leaf were prepared from the filtrates by an evaporator (Sturt, UK) and preserved in labeled air tight glass bottle at 4 °C for experimental anti-microbial activity investigation.

Preparation of the test bacteria

Vibrio parahaemolyticus ATCC 17802, *Escherichia coli 0157* ATCC 43894, *Sarcinalutea* IFO 3232 and *Bacillus subtilis* IFO 3026 were collected from the Department of Microbiology, Jashore University of Science and Technology, Jashore -7408, Bangladesh to perform the test. Collected pure bacterial strains were revived on nutrient agar and observed under light microscope to check the purity. The pure single colony was allowed to grow in nutrient broth to obtain stationary phase to perform the antibacterial activity of the plant leaf extracts.

Preparation of culture media for antibacterial activity test

Muller Hinton agar (Thermo Fisher scientific, USA) was used to perform the test. Adequate amount of media was suspended in distilled water, frequently agitated and heated into microwave oven to dissolve completely. Autoclave was performed at 121°C for 15 minutes and then the media was poured into petri-plates and cooled at room temperature in the Biosafety cabinet (ESCO, Singapore).

Antibacterial activity testing

The investigation of antibacterial activity of *P. hysterophorus* leaf extracts was performed by agar well diffusion assay (Mummed et al., 2018). 1mg/ml of chloroform and methanolic leaf extracts were prepared as stock solutions. Then, the stock solutions were diluted into 500 µg/ml and 250 µg/ml concentrations. The day before the test, pure cultured bacterial strains were grown overnight to the stationary phase in nutrient broth; subsequently the cultured bacterial strains

were inoculated separately on the surface of whole surface area using sterile cotton swabs. About 30 minute later, wells of 6mm diameter were made in a balanced distance on the solid agar using sterile glass borer. About 50µl of the diluted[500 µg/ml and 250 µg/ml] leaf extract solutions were poured into the selective wells. Methanol and chloroform were used as negative control. About 1 hour later, the plates were placed into an incubator at 37°C for 24 hour. After 24 hours incubation, the zones of inhibition were measured by a millimeter indicating scale.

Results and discussion

This study showed remarkable antibacterial activity against two gram negative (*V. parahaemolyticus* and *E. coli 0157*) and two gram positive (*S. lutea*and *B. subtilis*) bacterial strains among which, *V. parahaemolyticus*, *E. coli 0157*, *S. lutea*are pathogenic and *B. subtilis* is nonpathogenic.

The chloroform and methanolic leaf extracts of *Partheniumhysterophorus* have demonstrated Muller Hinton agar plates and streaked on the remarkable antibacterial activity against *V. parahaemolyticus*, *E. coli* 0157, *S. lutea* and *B. subtilis* (Figure1). Methanol and chloroform solvent extract showed highest zone of inhibition against B. subtilis (26mm) and S. lutea (23mm) respectively at the concentration of 500 µg/ml. But the lowest zone of inhibition was observed against *V. parahaemolyticus*measuring 9mm for methanol solvent extract and 10mm for chloroform solvent extract at concentration of 250 µg/ml. The antibacterial activity of *P. hysterophorus* leaf extracts against *S. lutea* was also noticeable at the concentration of 500 µg/ml where the zones of inhibition were 24mm for methanol solvent extract and 18mm for chloroform solvent extract. *E. coli* 0157 also showed susceptibility to the leaves extracts. No zones of inhibition were found in the case of negative control. Findings of the antibacterial activity of chloroform and methanolic leaf extracts of *P. hysterophorus* leaf extracts are presented in Table 1.

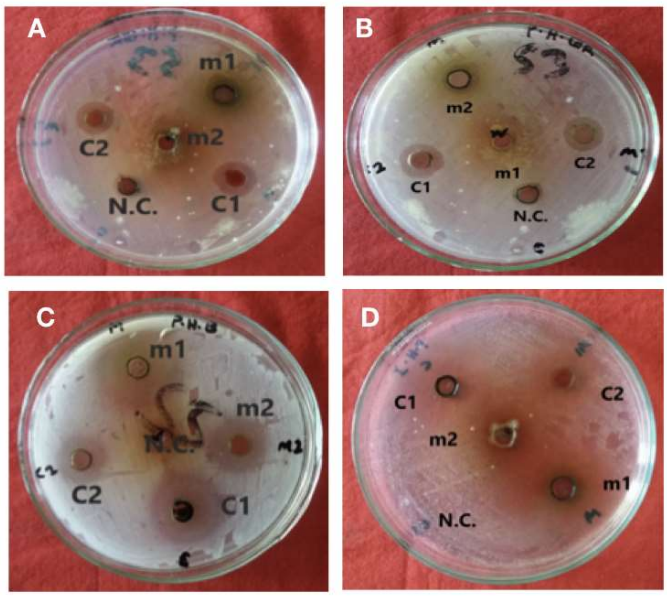

Figure1: Antibacterial activity of chloroform and methanolic leaf extract of *P. hysterophorus*. Here, A = *V. parahaemolyticus*, B = *E. coli* 0157, C = *B. subtilis*, D =*S. lutea*. C1 =500 µg/ml chloroform

solvent extract and C2 = 250 µg/ml chloroform solvent extract, m1 = 500 µg/ml methanolic extract, m2 = 250 µg/ml methanolic extract. N.C. = Negative Control (methanol and chloroform).

Table 1. Antibacterial activity of methanol and chloroform solvent extracts of*Partheniumhysterophorus* L. leaves.

Bacterial strains	Zone of inhibition (mm)				
	Methanol extracts		Chloroform extracts		N.C.
	500 µg/ml	250 µg/ml	500 µg/ml	250 µg/ml	
Vibrio parahaemolyticus	10	9	12	10	0
Escherichia coli 0157	13	11	14	11	0
Bacillus subtilis	26	19	23	16	0
Sarcinalutea	24	15	18	13	0

N.C. =Negative Control (Methanol and Chloroform)

Among the biochemical compositions of plant compounds some are antimicrobials, which are effective against viruses, bacteria and fungi in several defense mechanisms (González-Lamotheet al., 2009). 1- Nonadecene is one of the top ten most abundant compounds of *Partheniumhysterophorus* L. leaves (Ahmad et al., 2018). Studies report that, 1- Nonadecene is antibacterial, antifungal (Khan and Javaid, 2019) and anticancer agent (Amudha, 2018). Clearly, 1-Nonadecene is one of the bioactive compounds present in the *Partheniumhysterophorus* L. leaves which acts against bacteria along with other compounds. *P. hysterophorus* L. leaves also contains numerous identified and non-identified constituents which are the possible causes of antibacterial activity of this plant.

Conclusion

In this *in vitro* study the chloroform and methanolic leaf extract of *P. hysterophorus*exhibited antibacterial activity against all the tested bacteria. The findings of this study suggest that, leaf extracts enable to restrict the growth of both gram positive and gram negative bacteria. Moreover, the leaf extracts could be a potential source of antibacterial agents to cope with antibiotic resistance and new drug discovery. More investigations are needed immensely and pharmacological test using *in vivo* models are necessary to confirm efficiency of the extracts in living system.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgement

The authors are grateful to the Department of Genetic Engineering and Biotechnology and the Department of Microbiology, Jashore University of Science and Technology, Jashore -7408, Bangladesh.

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