Available online at www.scientiaresearchlibrary.com

Scientia Research Library

ISSN 2348-0416
USA CODEN: JASRHB

Journal of Applied Science And Research, 2014, 2 (6):1-5

(*http://www.scientiaresearchlibrary.com/arhcive.php*)

Antibacterial activity of the venom protein from *Periphylla periphylla* against human pathogens

T. Aravindhan *, S. Saravanan, S. Mahendran and P. Seedevi

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

---

ABSTRACT

The aim of this work was to the venom protein extract from P. Periphylla and characterized the molecular weight of 48 and 26 kDa protein respectively. Further the venom protein was evaluated for the antibacterial activity against 10 human clinical pathogens like Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae, Klebsiella oxytoca, Escherichia coli, Salmonella paratyphi, Proteus mirabilis, Vibrio parahaemolyticus and Streptococcus pyogenes. From this research the venom protein found to have the antibacterial activity against 7 bacterial strains, of the 10 pathogens tested. The venom protein showed the highest levels of antibacterial activity with 100 µg/ml. Based on the result present investigation suggested that venom protein from P. periphylla can be used to protect human bacterial diseases.

Keywords: *P. periphylla*, venom protein, SDS-PAGE, Antibacterial activity.

INTRODUCTION

The bacterial pathogens caused often series health problem in human, and other living organism all over the world. Moreover the bacterial diseases showing difference in different species, an instance, *Bacillus subtilis* is accountable for causing food borne gastroenteritis. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* caused mastitis, abortion and upper respiratory difficulties, whereas *Salmonella* sp. Causes diarrhoea and typhoid fever (Jawetz *et al.*, 1995). Even the *P. aeruginosa*is a most important and widespread pathogen be on fire patients able to causing life-threatening diseases, the uses of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria (Boyd, 1995).

Recently researchers all over the world are concerne with the endemic properties of these bacteria in terms of infections that can be considered community-acquired or hospital-acquired and can potentially cause death and become resistant to almost all the available antibiotics (Danial, 1988). Infectious diseases affect approximately one-half of deaths in tropical countries severely (Iwu *et al*., 1999). The WHO estimated globally that about 1500 people die each hour from infectious diseases, half of these are children under five years of age (Meylears *et al*., 2002).

The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produced. These limitations demand for improved pharmacokinetics properties, which necessitate continued research for new antimicrobial compounds for the development of drugs (Alhaj *et al.*, 2009). All the interest in marine organisms as a potential and promising source of pharmaceutical products has increased during recent years (Kim and Lee 2008). Hence the present study has been made to investigate the antibacterial efficacy of the venom protein isolated from *P. periphylla* against human pathogenic bacterial strains.

MATERIALS AND METHODS

Collection of animals

P. periphylla was collected during the low tide period from the intertidal region (up to 0.5 to 2 m depth) from the coastal area of Parangipettai coast **(**Lat. 11^{o} 29' N; Long. 79^{o} 46' E) in Tamil Nadu, Southeast coast of India.

Isolation of crude venom

For the isolation of venom protein from the tentacle of *P. periphylla* using homogenization buffer which contains 50 mM Tris Hydrochloride, 120 mM Sodium chloride, 5 mM Potassium chloride, 1 mM Magnesium chloride and 2 mM Calcium chloride. Further homogenized with 2 ml of buffer in a manual tissue homogenizer and sonicated three times for 50 sec per cycle (10sec on, 20sec off). During sonication the vessel was cooled with an ice bath. The mixture was centrifuged at 17,200 g for 10 min at 4°C. The supernatant was retained and stored at -20°C for further use, which was considered to be venom protein extract (McIntosh *et al.*, 1995).

Molecular weight determination through SDS-PAGE analysis

The molecular weight of the protein was separated using (SDS-PAGE) and was performed following the protocol described by Sambrook and Russell (2001). The bands were observed under gel documentation system and the molecular weight was determined in comparing with the molecular standard BSA and also by using the total lab software (Version 1.11).

Bacterial strains

Ten human clinical pathogens selected for the present study, namely *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae, Klebsiella oxytoca, Escherichia coli, Salmonella paratyphi, Proteus mirabilis, Vibrio parahaemolyticus* and *Streptococcus pyogenes* were obtained from Microbial Culture Maintaining Laboratory, Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Tamil Nadu, India.

Inoculum Preparation

Nutrient broth was prepared in the test tubes and autoclaved at 15 lbs pressure for 15 min. All the 10 bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37 for 24 h.37°C

Antibacterial activity

Agar well diffusion method

The antibacterial activity was evaluated using agar well diffusion method (Seedevi, 2013). The 24 h old cultures were swabbed in nutrient agar plates by using a sterile cotton swab aseptically. The wells were punched on swabbed plates using a sterile 5 mm well cutter. The stock solution of venom protein extract was prepared at 1 mg/ml concentration in 10% dimethylsulfoxide (DMSO). Four different concentrations such as 25, 50, 75 and 100 µg/ml were used. The standards tetracycline (1mg/ml dissolved in 10% DMSO) and 10% DMSO were loaded into the labelled wells respectively. The plates were incubated at 37^{o}C for 24 h in upright position. The results were obtained by measuring the diameter of inhibition zone for each well and expressed in millimeter.

RESULTS AND DISCUSSION

Molecular weight of venom protein

The profile of the SDS-PAGE demonstrated the molecular weight of the venom protein from *P. periphylla* (Fig. 1). The molecular weight of venom protein was found as 48 and 26kDa respectively (Lane 1), compared with the standard BSA (66 kDa) (Lane 2).

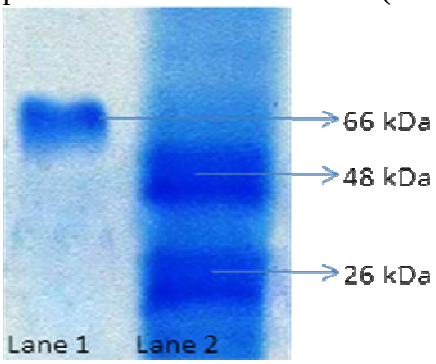

Fig. 1. The profile of the SDS-PAGE from the venom protein of *P. periphylla*

Antibacterial activity of venom protein

The venom protein showed the antibacterial activity against 7 clinical bacterial strains, of the 10pathogens tested. The highest levels of antibacterial activity was observed in 100 µg/ml (Table 1).

In 100µg/ml concentration of the venom protein showed a maximum of 17 mm of inhibition zone against *S. pyogenes*, the lowest inhibition zone of 10 mm observed against *K. pneumoniae* and *P. mirabilis*. Whereas 75 µg/ml concentration, venom protein showed the highest activity with 14 mm against *S. pyogenes*. The lowest activity of venom protein with 9mm of inhibition zone was observed against *K. pneumonia.* At 50 µg/ml concentration, venom protein showed the highest inhibition zone of 13mm against *S. pyogenes*. The lowest activity of 8 mm inhibition zone was recorded against *K. pneumonia.* In 25 µg/ml concentration, venom protein showed 11 mm of inhibition zone against *S. pyogenes.* The lowest activity with 8 mm of inhibition zone was observed for the venom protein against *K. pneumonia.*

Table 1. Antibacterial activity of the venom protein from *P. periphylla* against human pathogens

S. No	Name of the strains	Zone of inhibition (mm)					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	+ve	-ve
1	*S. typhi*	9	11	12	14	24	-
2	*S. aureus*	9	10	10	12	26	-
3	*K. pneumoniae*	8	8	9	10	26	-
4	*V. cholera*	-	-	-	-	22	-
5	*K. oxytoca*	9	12	12	14	26	-
6	*E. coli*	10	10	11	12	24	-
7	*S. paratyphi*	-	-	-	-	19	-
8	*P. mirabilis*	9	9	10	10	27	-
9	*V. parahaemolyticus*	-	-	-	-	21	-
10	*S. pyogenes*	11	13	14	17	27	-

DISCUSSION

Marine organisms are rich source of structurally novel and biologically active drug substances. So for chemically unique compounds of marine organisms' origin with different biological activity have been isolated and many numbers under investigation and are being developed as new pharmaceuticals (Faulkner, 2000). In the present study, the venom protein was extracted from *P. periphylla,* the venom showed the molecular weight of 48 and 26 kDa respectively. In the present study, the protein venom molecular weight was higher when compared the purified protein from *Heteractis magnifica* showed 17 kDa (Gunasundari*et al*., 2013). Nagai *et al*. (2000) reported the two liable hemolytic toxins from tentacle of *Carybdearastoni,* molecular weight being 43 kDa and 46 kDa. Radwan *et al*. (2005) screened the low molecular weight protein (<10kDa) from the jellyfish of *Cassiopea xamachanai.*

Antibacterial activity varies with the bioactive compounds of different species and pathogenic bacterial strains. Diluting extract usually weakens their antimicrobial activity. For the first time an attempts to study the antimicrobial activity with marine organisms were initiated around 1950s (Jensen *et al*., 1996). Since this first record, a large number of marine organisms from a broad range of phyla have been screened for their antimicrobial activity (Rinhart *et al*., 1981). There are only few studies carried out until now on the antibacterial activity of the venom protein of jelly fish. In the present study, the antibacterial activity of venom protein from *P. periphylla* was found to be higher at 100µg/ml concentrations. The venom protein has showed the antibacterial activity against 7 clinical bacterial strains, among the 10 pathogens tested. The venom protein showed a maximum of 17 mm of inhibition zone against *S. pyogenes,* the lowest inhibition zone of 10 mm observed against *K. pneumoniae* and *P. mirabilis*. On the other hands the 25, 50 and 25 µg/ml concentration showed the lower activity. In the present study, concluded that the venom protein from *P. periphylla* has good antibacterial effect against human pathogens.

REFERENCES

[1]. Jawetz, E., J.L. Mellnick and E.A. Adelberg, **1995**. Review of medical microbiology, (20th Edition). Applellation Lange Norwalk, Connecticut, 139-218.

[2]. Boyd, R.C., **1995**. Basic medical microbiology (5th edition). Little Brown Company, Boston.

[3]. Danial, W.W., **1988**. Biostatistics a foundation for analysis in health sciences, (4th edition). *John Wilely and Sons Inc*.

[4]. Iwu, M.W, A.R. Duncan, C.O. Okunji, **1999**. New antimicrobials of plant origin. In: J Janick (Ed.), Perspectives on new crops and new uses. ASHS Press Alexandria VA. 457-462.

[5]. Meylears, K., A. Cerstiaens, E. Vierstraete, G. Baggerman, C.W. Michiels, A. De Loof and L. Schoofs, **2002**. Antimicrobial compounds of low molecular mass are constitutively present in insects: characterisation of B-alanyl-tyrosine. *Curr. Pharm. Des.*, 8: 99–110.

[6]. Alhaj, N.A., N.I. Mashan and M.N. Shamudin, **2009**. Antibacterial activity in marine algae *Eucheuma denticulatum* against *Staphylococcus aureus* and *Streptococcus pyogenes*. *Res J Biol Sci.*, 4(4): 519-524.

[7]. Kim, I.H. and J.H. Lee, **2008**. Antimicrobial activity against methicillin resistant *Staphylococcus aureus* from macroalgae. *J. Ind. Eng. Chem.*, 14: 568-572.

[8]. McIntosh, J.M., F. Ghomaschi, M.H. Gelb, D.J. Dooley, S.J. Stoehr, A.B. Giordani, S.R. Naisbitt and B.M. Olivera, **1995**. Conodipine-M, a novel phospholipase A2 isolatedfrom the venom of the marine snail Conus magus J. *Biol. Chem.* 270, 3518–3526.

[9]. Sambrook, J. and D.W. Russell, **2001**. *Molecular Cloning Laboratory, Manuals.* (3rd Edition) Cold Spring Harbor Laboratory Press, Cold spring Harbor, New York, No.1-3.A. 8.40-A.8.49.

[10]. Seedevi, P., **2013**. Studies on biological activities of α-L-Rhamnose from *Grateloupia lithophila* (borgesen –c.a. agardh, 1822). Ph. D., Thesis, Annamalai University. 79-104.

[11]. Faulkner, D.J., **2000**. Highlights of marine natural products chemistry (1972– 1999). *Nat. Prod. Rep.*, 17: 1 – 6.
[12]. Gunasundari, V., T.T. Ajith Kumar, Swagat Ghosh and S. Kumaresan, **2013**. An ex vivo Loom to Evaluate the Brewer's Yeast Saccharomyces cerevisiaein Clownfish Aquaculture with Special Reference to Amphiprionpercula(Lacepede, 1802). *Turkish Journal of Fisheries and Aquatic Sciences*, 13: 389-395.
[13]. Nagai, H., K. Takuwa, M. Nakao, E. Ito, M. Miyake, M. Noda and T. Nakajima, **2000**. Novel Proteinaceous Toxins from the Box Jellyfish (Sea Wasp) *Carybdearastoni*. *Journal of Biochemical and Biophysical Research Communications*, 275(2): 582-588.
[14]. Radwan, F.F.Y., L.G. Roman, K. Baksi and J.W. Burnett, **2005**. Toxicity and mAChRs binding activity of *Cassiopea xamachana* venom from Puerto Rican coasts. *Toxicon* 45(1): 107-112.
[15]. Jensen, P.R., C.D. Harvell, K. Wirtz and W. Fenical, **1996**. Antimicrobial activity of extracts of caribbean gorgonian corals. *Mar. Biol.*, 125: 411-419.
[16]. Rinhart, K.L., P.D. Shaw, L.S. Shield, J.B. Gloer, G.C. Harbour, M.E.S. Koker, D. Samain, R.E. Schwartz, A.A. Tymaik, E.G. Swynenberg, D.A. Stringfellow, J.J. Vavva, J.H. Coats, G.E. Zurenko, S.L. Kuentzel, L.H. Li, G.J. Bakus, R.C. Brasca, I.I. Craft, D.N. Young and J.L. Connot, **1981**. Marine natural products as a source of antiviral, antimicrobial and antineoplastic agents. *Pure Applied Chem*., 53: 795-809.