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Short Communication

ANALYSIS OF *Provespa barthelemyi* VENOM AND EVALUATION FOR ANTICANCER, ANTIBACTERIAL ACTIVITIES OF THE PURIFIED TOXINS

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Abstract

Background: *The venom of wasp contains various biological activities. It is a potentially important drug. It is a mixture of several hundred different components and lethal toxins. Due to their presence, the venom shows potent antimicrobial, antifungal and antioxidant activity which can be used in the treatment of different infectious diseases. The consideration of the use of wasp venom is increasing nowadays.* ***Objectives:*** *The present study is an attempt to analyze the venom of Provespa barthelemyi and to check whether the toxin has any anticancer, antibacterial activity, or not.* ***Materials and Method:*** *Live Provespa barthelemyi species were collected and the venom was extracted for further experiment. For total protein concentration, Lowry's method was used. For the antibacterial activity, theagar disc diffusion method was considered. The evaluation of anticancer activity, the process is undergoing.****Result and Discussion:*** *Fromthecurrent study, it was found that the amount of venom extracted from 10 samples is approximately 0.5 ml- 0.6 ml. The protein concentration of one sample was found to be 0.18 µg/µl. As for antibacterial activity, the venom shows some activity against E. coli. Anticancer activity is still undergoing.****Conclusion:*** *The finding shows the protein content of the venom may have some antibacterial activity and if the venom extract has any anticancer activity, it would be a valuable addition to the medical field.*

Keywords: Hymenoptera; *Provespa barthelemyi*; Wasp venom; Protein; Antibacterial; Anticancer.

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Introduction

Hymenoptera, an extremely diverse group where wasps are present [1]. Hymenoptera is collectively most important to humans as a pollinator of wild and cultivated flowering plants, as parasites of destructive insects and as makers of honey and beeswax [2].The envenomation of toxins present in the venom has medical as well as ecological significance [3].The venom of wasp can also cause scanty micturition, generalization, swelling and respiratory distress in the body of the prey [4]. During a wasp sting attack, from simple allergic reactions to severe systemic reactions can be observed [5].

The venom of wasp serves as a defensive mechanism during the gaining of food which they use as a weapon to paralyze the victim. Hymenopteran venoms are secreted from poison glands present in the last segment of the abdomen where the sting apparatus is attached [6]. They inflict venom cause of self-defence. Chemically the protein present in the venom of wasps is a mixture of biologically active substances of high – medium and small molecular weight with a variety of physiological functions [7].

Vespid or wasp venom is more variable in terms of composition among the other species. Primarily made up of protein, the wasp venom is composed of a complex mixture of powerful allergens and pharmacologically active compounds [8]. The venom toxins which generally proteinaceous and are composed of few peptides and mixed with certain elements and acids [9]. The components are- (i) high molecular weight proteins (phospholipases (PLA), hyaluronidases, antigen 5, etc.), (ii) low molecular weight proteins (mastoparans, wasp kinins and chemotactic peptides) and (iii) bioactive molecules which include histamine, serotonin, tyramine etc. [10]

Hyaluronidase, phospholipase and antigen 5 are three major venom proteins that are isolated and characterized from vespids which can act as an allergen and a wide variety of vasoactive amines and peptides and the most important allergen in the case of wasp venom is Antigen 5 [8].

For this study, the considered wasp species is *Provespa barthelemyi* which is a nocturnal species [11].From the above paragraphs, it is quite obvious that all those peptides mentioned are present in almost all species of wasp venom and those peptides containsome really important biological activity and those activities are diverse from one another.These wasps venom peptides possess activities like antibacterial, antioxidant, antifungal, etc. So it can be assumed

that the wasp species which is taken into consideration for study have these biological activities also. Through research, it was found that there is no such work done on this species, that's why it was taken into consideration.

The present study was conducted to investigate *Provespa barthelemyi*analysis and evaluation of antibacterial activity and anticancer activity of the venom.

Materials And Methods

Collection and Identification of the species

Provespa barthelemyi samples were collected in February 2020 from the areas of Pathsala which is located at 26.5 °N and 91.18 °E. The wasp samples were immobilized by keeping them at -20ºC for further use. The authentication of the species was done by Dr P. Girish Kumar, Scientist-C, Zoological Survey Of India, Kozhikode, Kerala (No.F.36/25/17-18/Tech./, dated:28th February 2020)

Chemicals: All the chemicals used were procured commercially and were of analytical grade. Those were used as such without testing and purification.

Extraction of venom from the species

At the time of extraction, the venom gland was taken out by cutting the last two segments of the abdominal region and with the help of the forceps the venom gland comes out easily. After that, they were homogenized in phosphate buffer saline (pH 7.4) in cold condition otherwise the peptides present in the venom may get denatured. The homogenate was made by crushing the venom gland in PBS in cold conditions with a crusher in the Eppendorf tube. Then the homogenate was centrifuged at 4°C at 10000 rpm for 10 minutes. After the centrifugation, the supernatant was taken in a different tube. This supernatant was further used as a crude venom for the experiment [12].

Estimation of Protein content

For the estimation of protein content, venom extract was collected at first. The venom was collected at once from 6 species. After that, the extract was mixed with 1ml cold PBS and homogenized in that solubilising buffer agent. Then the homogenate was centrifuged at 4°C at 10000 rpm for 10 minutes. After that, the supernatant was taken out. The venom protein was present in

the supernatant and the total protein concentration that presents in the supernatant was estimated by Lowry's method. Bovine Serum Albumin (BSA) was taken as a standard protein for the method [12, 13].

Purification of the venom protein

The extracted venom was purified using a syringe filter. The extracted supernatant was filtered by the filter which has a PTFE (Polytetrafluoroethylene) membrane and pore size of 0.45 μm, lyophilised and stored in -20ºC for further use [14].

Preparation of Microbial Culture

For the microbial culture, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *B. cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *L. monocyte* (ATCC BA751) were maintained in the laboratory in Luria broth (2% w/v) and kept at 4ºC. For the subculture, a solid agar medium was prepared and kept at 4ºC for further use[12].

Antibacterial Assay

For the antimicrobial activity tests, the agar disc diffusion method was considered. In this method, 6 mm sterile filter paper discs (Whatman No. 1) were made which were supposed to coat with different concentrations of venom toxins which were prepared in phosphate buffer saline (pH 6.9). The inoculum sizewas adjusted to 10^6 colony-forming units (CFU/ ml). It was spread evenly on the agar plate surface. Each toxin was assayed. Sterile distilled water was used as a negative control. Ampicillin and Chloramphenicol were supposed to be used for comparison. The incubation period for the plates was 24 hours at 7°C and the diameters of inhibition zone were to be measured and MIC values to be found[15].

Anticancer Assay

The supernatant was taken for consideration which was prepared in phosphate buffer saline (pH 6.9). Three samples were sent for the detection of anticancer activity present on the venom of the *Provespa barthelemyi*. No specific cancer cells were not mentioned.

Results and Discussion

In extraction, at once 10 venom sacs were collected from the species. The weight of 10 venom sacs was approximately between 0.6 gm-0.7 gm and the

weight of 1 sac of venom was approximately 0.05 gm. So if we converted it to ml, in case 10 sac it would be 0.6ml – 0.7 ml and in the case of 1 sac it would be 0.05 ml. In that 10 sacs, 1 ml of the buffering agent (PBS) was added. The homogenate was then centrifuged and the amount of supernatant was recovered from the homogenate was approximately between 0.5 ml -0.6 ml. So this amount of venom extract can be recovered from 10 samples. So the amount of actual venom present in that supernatant would be approximately 0.25-0.3 ml in the case of 10 samples and for 1 sample it would be approximately 0.02 ml.

In protein estimation, the standard Lowry's Method was used where BSA was taken as a standard. One blank, five test tubes namely S_1, S_2, S_3, S_4, S_5 and two unknown samples E_1 and E_2 were taken into consideration for the estimation. Their absorbance was taken at 600 nm. After putting the absorbance value at graph, the equation is $y= 0.0015x+0.0091$, $R^2=0.9851$. So for E_1,the concentration of the sample would be 1187.27 µg/ml or 1.19 µg/µl and in the case of E_2, the concentration of the sample would be 183.93 µg/ml or 0.184 µg/µl.

For the antibacterial assay, one agar plate was cultured with Escherichia coli, where one paper disc was coated with S3 dilution of filtered venom, and Chloramphenicol was added for comparison (Fig 2).

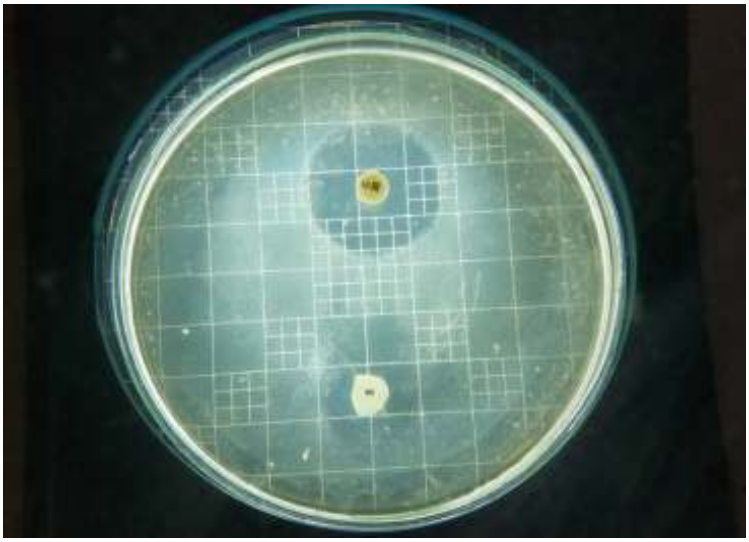

Figure 2: Plate cultured with Escherichia coli and filter paper coated with filtered venom (S3) compared with Chloramphenicol (C 30)

In the plate (Fig 2), it clearly shows some antibacterial activity. Available literature suggests that most of the wasp venom possesses some antimicrobial activity, so from considered species, it was also expected to be the same.Wasp venom shows antibacterial activity towards both gram-positive and gram-negative bacteria. Anoplin is a significant peptide from wasp toxin that shows various antimicrobial activity [12].It shows strong antifungal activity against plant pathogens *Leptosphaeria maculans* and it shows the protection of *Brassica napus* plants from diseases [16]. They also act as broad-spectrum antiviral drugs [17].The anticancer activity study is still undergoing. In literature, it is found that wasp venom contains a mastoparan likea peptide named MP1 AMP which has highly selective antitumor activities against different types of cancer cells likeprostate and bladder cancer cells andleukemic cells which are resistant to multidrug [18, 19].

Conclusion

The present study was conducted to detect if there is any antibacterial and anticancer activity present in the venom of *Provespa barthelemyi*. The venom shows antibacterial activity towards gram-negative bacteria*Escherichia coli.* However, the present work is still in progress to analyze antibacterial activity against other bacteria and anticancer activity of the venom.If we can find any significant therapeutic activities like antibacterial, anticancer, etc., it will be a great contribution to the health sector.

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References

1. Lee SH, Baek JH, Yoon KA. Differential Properties of Venom Peptides and Proteins in Solitary vs. Social Hunting Wasps. Toxins (Basel), 2016; 8(2): 32.

2. Fitzgerald KT, Flood AA. Hymenoptera Stings. Clinical Techniques in Small Animal Practice, 2006; 21: 194-204.
3. Ennik F. Deaths from Bites and Stings of Venomous Animals. The Western Journal of Medicine, 1980; 133: 463-468.
4. Jesmin T, Muinuddin G, Hossain MM, Rahman MH, Mamun AA. Acute renal failure following wasp sting. Mymensingh Med J., 2013; 22(3): 609-12.
5. Dongol Y, Paudel YP, Shrestha RK et al. Acute Renal Failure Following Multiple Hornet Stings; Clinical Kidney Journal, 2012; 5: 158-161.
6. Hardy MC, Cochrane J, Allavena RE. Venomous and Poisonous Australian animals of veterinary importance: a rich source of novel therapeutics. Biomed Research International, 2014: 671041:12.
7. Ciszowski K, Mietka Ciszowski A. Toxicology of Hymenoptera venoms. Przegl Lek, 2012; 69(8): 519-527.
8. Fitzgerald KT, Flood AA. Hymenoptera Stings. Clinical Techniques in Small Animal Practice, 2006; 21: 194-204.
9. Prajapati KK, Upadhay RK. Wasp Venom Generated Toxic Effects, Allergic Immune Responses and Immunotherapy. World Journal Of Pharmaceutical Research, 2016; 5(5), doi: 10.20959/wjpr20165-6125.
10. Dongol Y, Dhananjaya BL, Shrestha RK, Aryal G. Pharmacological and Immunological Properties of Wasp Venom. IntechOpen, 2013; doi: 10.5772/57227.
11. Dorji P, Gyeltshen T, Klein W, Nidup T. New records of social wasps (Hymenoptera: Vespinae: Vespa and Provespa) from Bhutan. Journal of Threatened Taxa, 2017; 9(4): 10102–10108.
12. Prajapati KK, Upadhayay RK. Yellow Wasp Polistes Flavus Venom Protein, Its Purification, Solubilization and Antimicrobial Activity. Biomed J Sci & Tech Res, 2017;1(1): 154-159.
13. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Phenol reagent. J Biol Chem, 1951; 193(1): 265-275.

14. King TP, Alagon AC, Kuan J, Sobotka AK, Lichtenstein LM. Immunochemical studies of yellow jacket venom proteins. Mol Immunol, 1983, 20 (3): 297-308.

15. NCCLS (National Committee for clinical Laboratory Standards) (1993) Performed standard for Antimicrobial Disc susceptibility tests, approved Standard, National committee for Clinical Laboratory Standards, villanova, PA Publication M2-A5, USA.

16. Jindřichová B, Burketová L, Novotná Z. Novel properties of antimicrobial peptide anoplin. Biochem Biophys Res Commun, 2014; 444(4): 520-524.

17. Gortzak K, Gortzak RA. Wasp and bee stings. Ned Tijdschr Tandheelkd, 2013; 120(7-8): 373-6.

18. Wang KR, Zhang BZ, Zhang W, Yan JX, Li J. Antitumor effects, cell selectivity and structure-activity relationship of a novel antimicrobial peptide polybia-MPI. Wang R Peptides, 2008; 29(6):963-8.

19. Wang KR, Yan JX, Zhang BZ, Song JJ, Jia PF. Novel mode of action of polybia-MPI, a novel antimicrobial peptide, in multi-drug resistant leukemic cells. Wang R Cancer Lett, 2009; 278(1): 65-72.

20. Lee SH, Baek JH, Yoon KA. Differential Properties of Venom Peptides and Proteins in Solitary vs. Social Hunting Wasps. Toxins (Basel), 2016; 8(2): 32.

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