Journal of Applied Biology & Biotechnology Vol. 8(02), pp. 6-11, March-April, 2020
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2020.80202

Partial purification and characterization of antimicrobial peptide from the hemolymph of cockroach *Periplaneta americana*

Reymend Encily Martin*, Yogita Raosaheb Channe
Department of Zoology, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, 431004, India

ARTICLE INFO

Article history:
Received on: October 08, 2019
Accepted on: November 27, 2019
Available online: March 26, 2020

Key words:
Purification, characterization, antimicrobial activity, *Periplaneta americana*

ABSTRACT

The partial purification and characterization of antimicrobial peptide (AMP) from the hemolymph of cockroach, *Periplaneta americana*, was studied. Hemolymph was drawn from cockroach and the AMP was purified on a sephadex G-75 gel filtration column. The gel filtration showed two peaks, I and II, and only peak II showed five active fractions, namely, 12, 13, 14, 15, and 16, of antimicrobial activity. Fraction 13 showed the highest microbial Inhibition concentration (MIC) and a single protein band on SDS-PAGE(sodium dodecyl sulfate–polyacrylamide gel electrophoresis) with a molecular mass of 60.2 kDa. Purified antimicrobial protein exhibited the highest antimicrobial activity against *Escherichia coli* at 30°C, pH 6.0, and 5 mM calcium ion. The AMP showed higher activity of MIC against lipopolysaccharides and β-1,3 glucan during 5 hours of exposure. The study concludes that the AMP from hemolymph was effective against microbes or was able to recognize the molecular pattern of microorganisms.

1. INTRODUCTION

Antimicrobial peptides (AMPs) are evolutionarily highly conserved molecules and constitute an important component of insect immunity. It is observed in all organisms of the domain of life, from bacteria to humans. The major family of AMPs, which has been characterized, is the defensin [1]. During the past two decades, several AMPs were isolated and purified from a wide range of animals, including both vertebrates and invertebrates, and have been reviewed by Martin [2]. These peptides show a broad spectrum of activity against a range of microorganisms including Gram-negative and Gram-positive bacteria, protozoa, yeast, fungi, and viruses. They have the potential to overcome bacterial resistance, thus making them a promising candidate for therapeutic drugs [3].

The cockroaches are seen in habitats that are always exposed to potential pathogenic microorganisms and parasites, but only a few encounters resulted in infection [4], because insects, on recognizing the infections, often initiate a complex genetic cascade and produce AMPs into the hemolymph [5,6]. Some insects synthesize inducible AMPs, such as lysozyme, a constitutive molecule like lipopolysaccharide (LPS) binding protein, which have been isolated from the hemolymph of the American cockroach, *Periplaneta americana* [7,8]. This protein also acts as an opsonin [9–11].

The American cockroaches mostly live in sewage and sewer pipe, which harbor high density of bacteria or other microbes that are potentially pathogenic. Hence, it is most likely possible that these insects may defend foreign microbes invading its body by means of AMPs. The AMPs are usually synthesized in fat body or hemolymph to eliminate microorganisms [12–14]. This work was carried out to partially purify and characterize hemolymph AMP from *P. americana.*

2. MATERIAL AND METHOD

2.1. Insects Rearing

The cockroaches *P. americana* were collected from godowns and reared in the laboratory in dark containers at room temperature 27°C ± 1°C and 12:12 light:dark (L:D) cycle. All experiments were conducted at room temperature unless specified.

2.2. Collection of Hemolymph From Non-Induced and Induced Microbes

For collecting hemolymph, the cockroaches were divided into four groups of 30 cockroaches. Group I as control (non-induced

*Corresponding Author
Reymend Encily Martin, Department of Zoology, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, 431004, India.
E-mail: martinbamu@gmail.com

with *Escherichia coli*), group II with induced *E. coli*, group III as control (non-induced with β-1,3 glucan), and group IV with β-1,3 glucan induced.

2.2.1. Collection of hemolymph for AMP assay after treatment with β-1,3 glucan and LPS

Before collecting the hemolymph from the non-induced (control) group, adult cockroaches were topically sterilized with 70% ethanol. The control cockroach was injected with 10 µl of Carlson's saline as described by Jomori and Natori [9] in the abdominal cavity, whereas, for induced, 10 µl of *E. coli* (0.01 µg/10 µl) (10^6 cells /ml) or β-1,3 glucan (Sigma) (0.01 µg/10 µl) was injected in the abdominal cavity using 190 µl Carlson saline as a medium. The hemolymph of control and induced *E. coli* or β-1,3 glucan was collected after 1, 3, and 5 hours from the coxal membrane of legs, using a sterile syringe from all groups. The collected hemolymph was centrifuged at 1200× g for 15 minutes and the supernatant was stored at 4°C for further use. The resulting supernatant was used as a source of AMP and assayed. The protein content of all samples was determined by Lowry's method [15] or by UV (ultra violet) spectrophotometer using BSA(Bovine Serum Albumin) as standard protein.

2.2.2. The bacterial and yeast strains

For screening, the AMP from hemolymph compounds was based on evidence as mentioned in earlier studies [16,17]. We used *E. coli* ATCC25972, which was susceptible to all antibiotics. The β-1,3 glucan used in the present study was obtained from Sigma.

2.3. Purification Procedure

The hemolymph collected was analyzed for AMP. The collected hemolymph was centrifuged at 700× g for 10 minutes. The supernatant was loaded on a gel filtration column of Sephadex G-75 (1.5 cm × 13 cm), which was previously equilibrated with 20 mM ammonium acetate buffer (pH 6.4). The aliquot was collected at a flow rate of 0.5 ml/min, a volume of 3 ml was collected as described by Kyung et al. [18] with minor modification. The protein content in aliquot was assayed at 280 nm absorbance, and the AMP activity of each fraction was measured according to the procedure described in the following section.

2.4. Antibacterial Assay

The antibacterial assay was carried out by the agar well diffusion method as described by Perez et al. [19]. For control, paper soaked with 20 µl of non-induced hemolymph was used. The plates were incubated overnight at 37°C, and the diameter of the clear zone was measured.

2.4.1. Effect of pH

The effect of pH was studied by varying pH ranges pH 3.0, 4.0, 5.0, 6.0, and 7, respectively. The reaction mixture contains 50 µl AMP aliquot added with 50 µl phosphate buffer of different pH, well mixed and incubated for 15 minutes at room temperature, and assayed for antibacterial assay.

2.4.2. Effect of temperature

The effect of temperature was studied by incubating the aliquot to different temperatures, namely, 10°C, 20°C, 30°C, and 40°C in a heat block for 20 minutes. A 50 µl AMP aliquot was mixed with 50 µl 20 mM Phosphate Buffered Saline (pH 7.8) and kept for 5 minutes, followed by heating on a heating block at different temperatures for 10 minutes; 50 µl AMP aliquot from each incubated temperature was assayed for antibacterial assay.

2.4.3. Effect of metal ion on AMP

50 µl of the purified AMP sample was mixed with 50 µl of different concentrations of calcium, namely, 3, 4, 5, and 6 mM were added and gently mixed well and incubated for 20 minutes. The sample was poured in petri dishes ,and antimicrobial assay was carried out.

2.5. Determination of Microbial Inhibition Concentration (MIC)

MIC was determined by the broth dilution method [20]. About 0–120 µl/ml of AMP was incubated into the nutrient broth and incubated for 24 hours at 37°C. MIC was defined as "the lowest concentration of the peptides inhibiting visible growth." After incubation, absorbance was measured at 620 nm.

2.6. SDS-PAGE

The SDS-PAGE of the isolated peptide was carried out on 12% separating gel according to the procedure described by Laemmli [21]. The electrophoresis was carried out at a constant voltage of 100 V for 3 hours. The molecular weight of the protein band was determined using standard medium-range molecular markers: 97.4 KDa phosphorylase-b, 66 KDa-Bovine serum albumin, 43 KDa-Ovalbumin, 29 KDa-Carbonic anhydrase, 20.1 soybean trypsin inhibitor, and 14.3 KDa-lysozyme.

3. RESULTS AND DISCUSSION

3.1. Chromatographic Profile of Peptide

The purification of the peptide is shown in Table 1, and the elution pattern on gel filtration is shown in Figure 1. The elution fraction showed two peaks of which only fraction 2 showed antimicrobial activity against *E. coli*. The five fractions are 12, 13, 14, 15, and 16. In this study, these five fractions showed a clear zone of inhibition against *E. coli*. A similar result was reported by Seraj et al. [17]. They observed fractions F13, F16, F17, F18, F19, and F20 with antimicrobial activity to *Bacillus megatorium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus* sp. F13 showed antimicrobial activity against *E. coli*. The *Musca domestica* larvae showed five peaks where only the second peak showed an AMP activity against *E. coli* [22].

3.2. Effect of Different Temperatures, pH, and Calcium Ions

The effect of different temperatures on the purified peptides (peak II, F13) against *E. coli* is shown in Figure 2. The peptide showed the zone of inhibition (mm) as 3, 4, 6, and 4 mm for temperatures 10°C,

Table 1: The purification table of Antimicrobial Peptide.

Step	Volume (ml)	Protein A_{280nm}	Total Protein (mg/ml)	Total activity (AU)	Specific activity (U/mg)	Yield (%)	Purification (fold)
HLS	7.2	0.538	64.8	336.96	5.2	100	1
Gel Filtration (F-13)	3.0	0.358	43.2	129.6	3.0	38.46	0.57

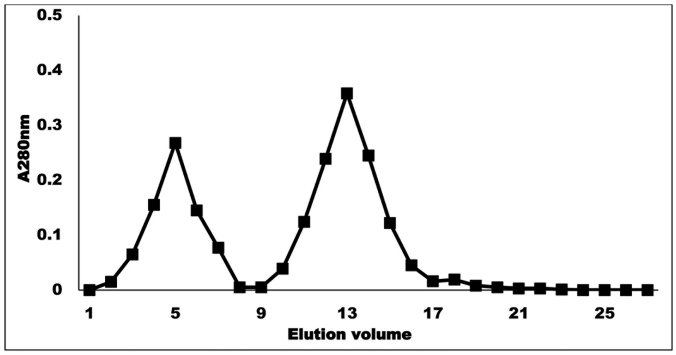

Figure 1: Elution profile of the AMP on gel filtration.

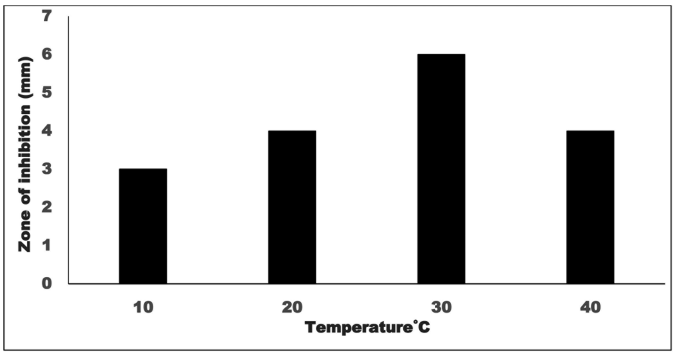

Figure 2: Effect of various temperatures on the anti-microbial activity against *E coli.*

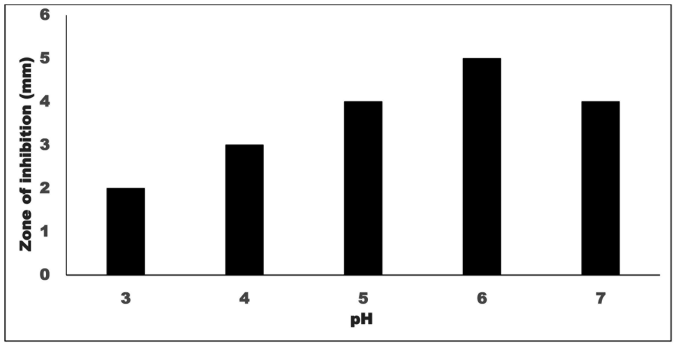

Figure 3: Effect of various pH on the antimicrobial activity of peptide against *E. coli.*

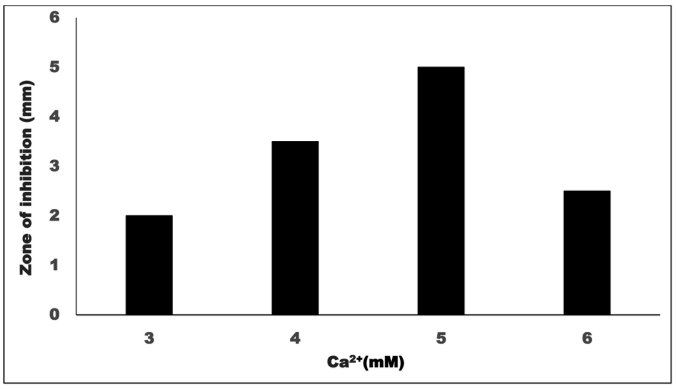

Figure 4: Effect of calcium ion concentration on the antimicrobial activity of peptide against *E. coli.*

20°C, 30°C, and 40°C, respectively. A decline in the zone of inhibition was observed at 40°C. Dang et al. [23] and Lu et al. [24] reported thermostable antibacterial peptides in *Bactrocera dorsalis* and *Musca domestica* (Diptera). However, in *Oryctes rhinoceros* (Coleoptera), peptide was only stable up to 37°C against *Micrococcus luteus* [25].

The effect of different pH on the antimicrobial activity of purified peptides (peak II, F13) was determined against *E. coli* (Fig. 3). It was observed that with a change in pH, the antimicrobial activity of peptide also changed. The AMP activity was observed between pH 3–7, maximum activity was observed at pH 6, and thereafter a decrease was observed. The result showed that the activity of purified peptide is pH dependent and varies for *E. coli*. The study showed a broad pH range of 3–7, and no pH activity was observed below pH 3. It appears that the peptide possibly requires an acidic environment for exhibiting its optimum antimicrobial activity. Rabeeth et al. [25] reported antimicrobial activity at pH 7 in rhinoceros beetle, *Oryctes rhinoceros* (L.), at pH 6.2 in *Brevibacillus laterosporus* [26], at pH 6.8 in bovine Lactophoricin [27]. Some higher or lower pH values have also been reported: pH 5.2 for *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Enterobacter sakazakii* [28]; pH 5.0 for *Clitocybe sinopica* [29]; pH 7.0 for *Bacillus megaterium* [30]; and pH 7.0 for *Bacillus subtilis* [31]. The peptide was found to be highly sensitive to pH because at high or low pH resulted in the loss of antibacterial activity.

We observed that at 5 mM calcium ion, the antibacterial activity showed the highest activity against *E. coli* (Fig. 4). Sakthivel and Palani [32] reported that Ca^{2+} and Mn^{2+} ions enhanced the antimicrobial activity from *Bauhinia purpurea*. Wen et al. [33] reported the presence of an anionic AMP in *Bombyx mori* and in the wax moth *Galleria mellonella* [34], which causes surface alterations in *E. coli* and the death of this bacterium along with lysozyme [34]. A higher concentration is reported to destroy the structure of the cell membrane through electrostatic interaction with LPS [35].

3.3. Effect of Purified Peptide on Apparent Proliferation of *E. coli*

The active purified peptide chromatographic fraction was used to determine MIC in culture media. The MIC increased with an increase in microbial peptide concentration (Fig. 5). The highest value of MIC was observed in F13 (Fig. 6). The *E. coli* growth

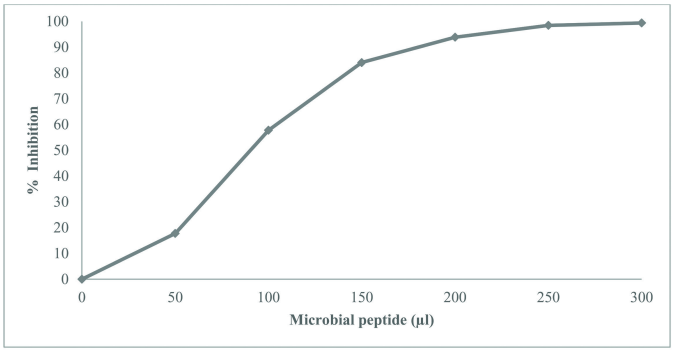

Figure 5: Effect of concentration of MIC on percentage inhibition of *E. coli* in a culture media.

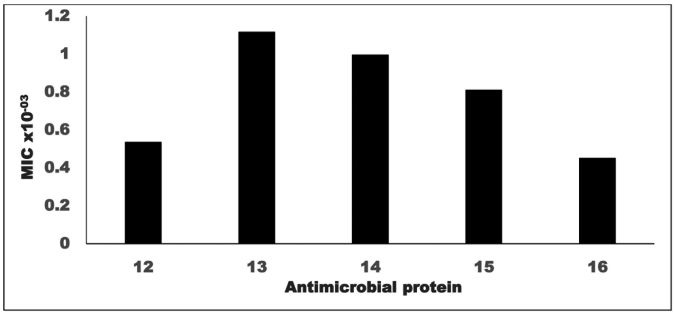

Figure 6: Effect of different fractions of AMP on MIC of *E coli.*

reduced to 57.05% at 100 µg and to 93.86% by 200 µg. The percentage inhibitory activity of purified protein against *E. coli* showed that the peptide was active against *E. coli* at a lower concentration of 4.5×10^{-03} mM in F16 and a higher concentration of 1.115×10^{-03} mM in F13. In *G. mellonella*, immune hemolymph defensing-like peptide inhibited bacterial growth at a concentration of 2.9 and 1.9 µM [36]. Liu et al. [37] reported inhibition of bacterial growth at a concentration of 50–200 µM depending on the isoforms.

3.4. Effect of LPS and b-1,3 Glucan on MIC

The MIC for the control hemolymph before injection of β-1,3 glucan and LPS was $1.17 \times 10^{-3}$and 1.190×10^{-3} respectively. However, after 1, 3, and 5 hours after injection with β-1,3 glucan, the values observed were 1.585×10^{-3}, 1.68×10^{-3}, and 1.75×10^{-3}, and for LPS, the values were 1.64×10^{-3}, 1.79×10^{-3}, and 1.8×10^{-3}, respectively (Fig. 7). The inhibitory activity was measured against *E. coli*. A similar report for AMP was obtained for *B. mori* [38]. In sand fly, *Phlebotomus duboscqi*, bacteria injection resulted in the release of defensin family peptides with antibacterial activity in their hemolymph [39]. Andrejko and Mizerska-Dudka [40] reported that the antimicrobial cecropin proteins remain active even after 18 hours of infection by *E. coli* in *G. mellonella*, and the peak activity remains active till 48 hours in the immune system of the insect. In other insects, the maximum antimicrobial activity after injection was observed for 12 hours [41]. Erler et al. [42] reported that *E. coli* is an effective inducer of insect humoral immune responses. The insect hemocytes being a repository of AMPs, therefore, may involve in the activation of a complex innate immune system

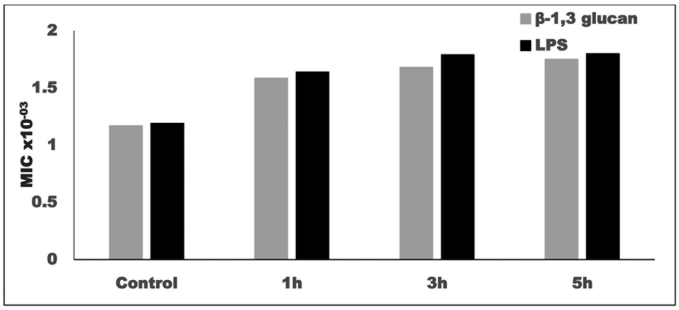

Figure 7: The effect of β-1,3, glucan and LPS a induced microbial peptides on MIC against *E coli*.

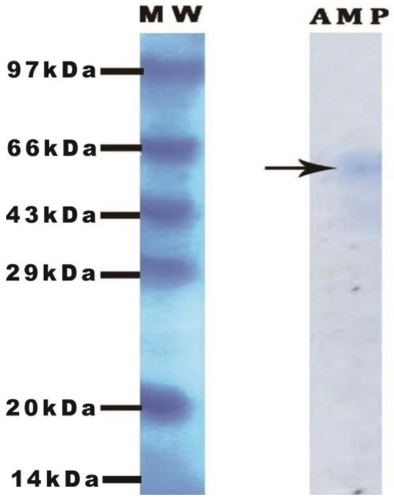

Figure 8: Molecular weight of AMP calculated by SDS-PAGE. Lane 1 shows Standard molecular weight markers, and lane 2 shows the purified AMP molecular weight.

involving cellular and humoral responses [43,44]. We observed that apart from *E. coli*, β-1,3 glucan too showed a higher MIC, suggesting AMP may have both antibacterial and antifungal activity. However, how exactly the, β-1,3 glucan is influenced by AMP is not known.

3.5. SDS-PAGE

The pattern of separation of purified protein by gel filtration showed a single band of 60.2kDa molecular weight (Figure 8). Seraj et al. [17] reported a molecular weight of 61 kDa antibacterial protein from the American cockroach, *P. americana*. Similarly, Rethna Priya [45] reported a 77 kDa antibacterial protein molecule in *Clibanarius clibanarius*. Morishima et al. [46] reported a peptide of 6 1 kDa in *Bombyx mori*, and a 64 kDa in *Hyalophora cecropia* [47].

4. CONCLUSION

The AMP purified from *P. americana* exhibited a potential antimicrobial activity against *E. coli*. The purified protein was effective at an optimum pH 6.0 and at temperature 30°C. At 5 mM calcium ion, the antibacterial activity was higher. The activity of the purified protein was influenced by the changes in pH, temperature, and concentration of calcium ions. The molecular

weight of the purified protein was determined as 60.2 kDa by SDS-PAGE. The study showed that the MIC increased with an increase in microbial peptide concentration. The AMP showed an increased MIC activity against *E. coli* and β-1,3 glucan, till 5 hours. The study concludes that the AMP from hemolymph may be effective against microbes or will have a specific recognition pattern against microbial infection. It is not surprising that cockroaches carry harmful pathogenic microorganisms that are contagious between humans and animals. However, these microbes do not have a pathogenic effect on cockroaches; surely, the answer may lie in their complicated specific immune defense mechanisms, which are now being studied in detail to understand their mechanisms for the benefit of mankind.

REFERENCES

1. Zasloff, M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–95.
2. Martin ER. Biological peptides/proteins derived from living resources. In Physiology and ecotoxicology. In Bio-resources for Rural Livelihood (Vol II). Kulkarni GK, Pandey BN & Pandey PN (Eds.), Narendra Publishing House, Delhi, pp 39–58, 2010.
3. Bals R. Epithelial antimicrobial peptides in host defense against infection. Resp Res 2000;1:141–50.
4. Gillespie JP, Kanost MR, Trenczek T. Biological mediators of insect immunity. Ann Rev Entomol 1997;42:611–43.
5. Eleftherianos I, Marokhazi J, Millichap PJ, Hodgkinson AJ, Sriboonlert A, Ffrench-Constant RH, et al. Prior infection of *Manduca sexta* with non-pathogenic *Escherichia coli* elicits immunity to pathogenic *Photorhabdus luminescens*: roles of immune-related proteins shown by RNA interference. Insect Biochem Mol Biol 2006;36:517–25.
6. Eleftherianos I, Gokcen F, Felfoldi G, Millichap PJ, Trenczek TE, Ffrench-Constant RH, et al. The immunoglobulin family protein Hemolin mediates cellular immune responses to bacteria in the insect *Manduca sexta*. Cell Microbiol 2007;9:1137–47.
7. Ha Lee J, Hee Lee I, Noda H, Mita K, Taniai K. Verification of elicitor efficacy of lipopolysaccharides and peptidoglycans on antibacterial peptide gene expression in *Bombyx mori*. Insect Biochem Mol Biol 2007;37:1338–47.
8. Fiolka MJ. Immuno-suppressive effect of cyclosporin an on insect humoral immune response. J Invert Pathol 2008;98:287–92.
9. Jomori T, Natori S. Function of the lipopolysaccharide-binding protein of *Periplaneta americana* as an opsonin. FEBS Lett 1992;296:283–6.
10. Hashimoto Y, Tabuchi Y, Sakurai K, Kutsuna M, Kurokawa K, Awasaki T, et al. Identification of lipoteichoic acid as a ligand for raper in the phagocytosis of *Staphylococcus aureus* Drosophila hemocytes. J Immunol 2009;183:7451–60.
11. Kim CH, Shin YP, Noh MY, Jo YH, Han YS, Seong YS, et al. An insect multiligand recognition protein functions as an opsonin for the phagocytosis of microorganisms. J Biol Chem 2010;285:25243–50.
12. Brivio MF, Moro M, Mastore M. Down regulation of antimicrobial peptide synthesis in an insects model induced by the body surface of an entomoparasite (*Steinernema feltiae*). Develop Comparat Immunol 2006;30:627–38.
13. Yu Y, Park JW, Kwon HM, Hwang HO, Jang IH, Masuda A, et al. Diversity of innate immune recognition mechanism for bacterial polymeric meso-diaminopimelic acid type peptidoglycan in insects. J Biol Chem 2010;285:32937–45.
14. Yakovlev AY. Induction of antimicrobial peptide synthesis by the fat body cells of maggots of *Calliphora vicina* R.-D. (Diptera: Calliphoridae). Zh Evol Biokhim Fiziol 2011;47(6):461–8.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265–75.
16. Jomori T, Kubo T, Natori S. Purification and characterization of lipopolysaccharide binding protein from hemolymph of American cockroach *Periplaneta americana*. Eur J Biochem 1990;190:201–6.
17. Seraj MU, Hoq MI, Anwar MN, Chowdhury S. A 61 kDa Antibacterial Protein isolated and purified from the hemolymph of the American cockroach *Periplaneta americana*. Pakistan J Biol Sci 2003;6(7):715–20.
18. Kyung T, Chung D, Ourth A. Viresin, A. A novel antibacterial protein from immune hemolyph of *Heliothis virenscens pupae*. Eur J Biochem 2000;267:677–83.
19. Perez C, Pauland M, Bazerque P. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp 1990;15:113–5.
20. Wang FS. Effect of antimicrobial proteins from porcine leukocytes on *Staphylococcus aureus* and *Escherichia coli* in comminuted meats. Meat Sci 2003;65:615–21.
21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 1970;227:680–5.
22. Hou L, Yonghui S, Zhai P, Le G. Inhibition of food-borne pathogens by Hf-1, a novel antibacterial peptide from the larvae of the housefly (*Musca domestica*) in medium and orange juice. Food Control 2007;18:1350–7.
23. Dang XI, Tian JHJH, Yi HY, Wang WX, Zheng M, Li YF, et al. Inducing and isolation of antimicrobial peptides from oriental fruit fly, *Bactrocera dorsalis* Hendel. Insect Sci 2006;13:257–62.
24. Lu J, Zhong Y, Liu L, Fu K, Chen ZW. Optimization of extraction technique for antibacterial peptides from *Musca domestica* larvae. Acta Entomologica Sinica 2007;50:106–12.
25. Rabeeth M, Muthukumar M, Balaji Rajkumar M, Kesava Kumar H. Isolation, evaluation and purification of antibacterial peptides from rhinoceros beetle, *Oryctes rhinoceros* (L.). GERF Bull Biosci 2012;3:16–22.
26. Zhao J, Guo L, Zeng H, Xiong Y, Chen M. Purification and characterization of a novel antimicrobial peptide from *Brevibacillus laterosporus* strain A60. Peptide 2012;33:206–11.
27. Kim JS, Jeon JH, KimY. Design and engineering of antimicrobial peptides based on lpcin-yk3,an antimicrobial peptide derivative from bovine milk. J Microbiol Biotechnol 2018;28(3):381–90.
28. Yoo S, Kim JY, Park SC, Choi DY, Seo CH, Hahm KS, et al. Effect of acidic pH on antibacterial action of peptide isolated from Korean pen shell (*Atrina pectinata*). J Peptide Sci 2011;17:353–7.
29. Zheng S, Liu Q, Zhang G, Wang H, Ng TB. Characterization of purification of lectin from *B. mori.* Acta Biochem Poly 2010;57:43–8.
30. Abdullah S, Al-Thubiani A, Yahia A, Maher B, Adel F, Mohammed A, et al. Identification and characterization of a novel antimicrobial peptide compound produced by *Bacillus megaterium* strain isolated from oral microflora. Saudi Pharm J 2018;26:1089–97.
31. Lettieri M, Teixeira A, Rosa D, Brandelli A. Characterization of an antimicrobial peptide produced by *Bacillus subtilis* showing inhibitory activity towards *Haemophilus parasuis*. Microbiology 2013;159:980–88.
32. Sakthivel M, Palani P. Isolation, purification and characterization of antimicrobial protein from seedlings of *Bauhinia purpurea* L. Int J Biol Macromol 2016;86:390–401.
33. Wen H1, Lan X, Cheng T, He N, Shiomi K, Kajiura Z, et al. Sequence structure and expression pattern of a novel anionic defensin-like gene from silkworm (*Bombyx mori*). Mol Biol Rep 2009;36(4):711–6; doi:10.1007/s11033-008-9233-4. Epub 2008 Mar 22.
34. Zdybicka-Barabas A, Mak P, Klys A, Skrzypiec K, Mendyk E, Fiolka MJ, et al. Synergistic action of *Galleria mellonella* anioic peptide 2 and lysozyme against Gram negative bacteria. Biochem Bio Acta 2012;1818(11):2623–35
35. Hancock RE, Chapple DS. Peptide antibiotics. Antimicrobial Agents Chemother 1999;43:1317–23.
36. Miyashita M, Sakai Y, Matsushita N, Hanai Y, Nakagawa Y, Miyagawaa H. Novel amphipathic linear peptide with both insect toxicity and antimicrobial activity from the venom of the scorpion *Isometrus maculatus*. Biosci Biotechnol Biochem 2010;74(2):1–6.
37. Liu L, Xu K, Wang H, Tan J, Fan W, Venkatraman S, et al. Self-assembled cationic peptide nanoparticles as an efficient antimicrobial agent. Nature Nano-Technol 2009;4:457–63.

38. Abraham EG, Nagaraju J, Salunke D, Gupta HM, Datta RK. Purification and partial characterization of an induced antibacterial protein in silkworm, *Bombyx mori*. L. J Invertebrate Pathol 1995;65:17–24.
39. Boulanger N. Characterization of a defensin from the sand fly *Phlebotomus duboscqi* induced by challenge with bacteria or the protozoan parasite *Leishmania major*. Infect Immunol 2004;72:7140–6.
40. Andrejko M, Mizerska-Dudka M. Analysis of *Galleria mellonella* hemolymph proteins profile after metalloproteinase immune challenge. Ann UMCS, Biol 2011;135–42.
41. Milad L, Mohammad Y, Aref S, Mansour N, Ali RB, Amir HZ. The antibacterial effect of American cockroach hemolymph on the nosocomial pathogenic bacteria. J Clin Microbial Infect 2015;2(1):17795–23017.
42. Erler S, Popp M, Lattorff HG. Dynamics of immune system gene expression upon bacterial challenge and wounding in a social insect (*Bombus terrestris*). PLoS One 2011;6:18126.
43. Lee SY, Soderhall K. Early events in crustacean's innate immunity. Fish and Shellfish Immunol 2002;12:421–37.
44. Iwanaga S, Lee BL. Recent advances in the innate immunity of invertebrate animals. Biochem Mol Biol 2005;38:128–50.
45. Rethna Priya E, Ravichandran S, Ezhilmathi R. Bioactive proteins from pipefishes. J Costal Life Med 2015;1(1):1–5.
46. Morishima I, Yamada K, Ueno T. Bacterial peptidoglycan as an elector of antibacterial protein synthesis in larvae of the silkworm *B. mori*. Insect Biochem Mol Biol 1992;22:363–7.
47. Gudmundsson CG, Lidholn DA, Asling B, Gan R, Boman HG. The cecropin locus: cloning and expression of a gene cluster encoding three antibacterial peptides in *Hyalophora cecropia*. J Biol Chem 1991;266:11510–17.

How to cite this article:
Martin RE, Channe YR. Partial purification and characterization of antimicrobial peptide from the hemolymph of cockroach *Periplaneta americana*. J Appl Biol Biotech 2020;8(02):6–11. DOI: 10.7324/JABB.2020.80202