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A Novel Antimicrobial Peptide Derived from the Insect *Paederus dermatitis*

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Abstract Much research has been focused on antimicrobial peptides (AMPs) derived from insect immune defense reactions due to their potential in the development of new antibiotics. In this study, a new AMP from the insect *Paederus dermatitis,* named sarcotoxin Pd was identified and purified using gel filtration and reverse-phase high-performance liquid chromatography. Our results showed that this peptide has broad-spectrum inhibitory effects on examined microbes. Sarcotoxin Pd is composed of 34 amino acids and its molecular weight was estimated to be 3613.26 ± 0.5 Da. Minimum inhibitory concentration (MIC) values of sarcotoxin Pd against Gram-negative bacteria were lower than Gram-positive bacteria and fungi. The identified peptide showed the highest antimicrobial effect against *Klebsiella pneumonia* and *Escherichia coli*. This peptide did not reveal significant hemolytic activity against human red blood cells particularly in the range of MIC values. Confirming the potential antimicrobial activities of synthetic peptide, this paper addresses the role of sarcotoxin Pd in the treatment of systemic microbial illnesses.

Keywords *Paederus dermatitis* · Antimicrobial peptides · Gel filtration · RP-HPLC · MIC · Hemolysis assay

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Introduction

Today, the widespread antibiotic consumption both inside and outside of medicine is playing a significant role in the emergence of antibiotic-resistant bacterial strains. (Hancock and Sahl 2006; Toke 2005). Thus, the development of new antimicrobial compounds is an extremely active field in biological researches. It has been recognized that antimicrobial peptides (AMPs) are suitable alternatives to classic antibiotics (Boman 1995; Montaño et al. 2011; Zasloff 2002). These peptides have been isolated from various sources, such as plants (Manners 2009), invertebrates (Hwang et al. 2009; Kang et al. 2011), vertebrates (Boman 2003; Wang et al. 2009; Zairi et al. 2009), eggs (Memarpoor-Yazdi et al. 2012) and humans (Bensch et al. 1995; Cunliffe and Mahida 2004; Godballe et al. 2011; Silverstein et al. 2007; Zasloff 2002). Insects secrete such peptides as a part of immune defense reactions. Active AMPs developed by insects to rapidly eliminate infectious pathogens are considered as a component of the defense weapons (Dürr and Peschel 2002; Hoffmann et al. 1999; Kang et al. 2008; Taguchi et al. 2009). With respect to their high abundance, many antimicrobial peptides such as cecropin (Hoffmann et al. 1993), stomoxyn, spinigerin (Esteves et al. 2009; Kulkarni et al. 2011), sarcotoxin IA (Matsuyama and Natori 1990) and etc. have been isolated from different types of insects. *Paederus* is a genus of small beetles of the family *Staphylinidae*. It was named *Paederus dermatitis* because of toxins in the hemolymph of many species within this genus (Godballe et al. 2011). *Paederus* species are widely distributed around the world. Due to their preference for moist soil, large numbers of *Paederus* beetles may be attracted to irrigated farmland where they provide some benefit by eating herbivorous insects. However, they can cause problems for people

working in fields or grassy areas (Armstrong and Winfield 1969; Gnanaraj et al. 2007; Nicholls et al. 1990). *Paederus dermatitis* is abundant in meadow of Golestan, Mazandaran and Gilan provinces in north of Iran (Gelmetti and Grimalt 1993). This insect has complete metamorphosis (holometabolous), thus, AMPs are rapidly synthesized by the fat body and secreted into the hemolymph, as found in some other insects (Bullet et al. 1999). The aim of this study was to isolate antimicrobial peptides from *Paederus dermatitis* and also to characterize the most active peptides.

Materials and Methods

Materials

Methanol, ethanol, TFA (trifluoroacetic acid), acetonitrile, acetic acid, sodium chloride, formaldehyde, sodium hydroxide, TCA (tricloroacetic acid), acetone, chloridric acid and glycerol were obtained from Merck and Company Inc. (Whitehouse Station, NJ, USA). Mueller–Hinton Broth (MHB), Trypticase soy broth (TSB) and blood agar culture media were purchased from Himedia Laboratories. Analytical and semi-preparative columns were purchased from Macherey–Nagel GmbH Co. All other used chemicals, including agar, methyl green, agarose, bromophenol blue, Triton X-100 (Dow Chemical Company, Midland, MI, USA), EDTA (ethylenediaminetetraacetate) and Coomassie Brilliant Blue R-250, were of analytical grades.

Extraction

Between June and July, five hundred numbers of adult *Paederus dermatitis* were collected from different parts of Golestan province in the north-east of Iran. Collected insects were kept in small cages under normal laboratory conditions at 25 (±2) °C. To isolate antimicrobial compounds, the insects were frozen in liquid nitrogen and then reduced to a powder in the presence of liquid nitrogen. The powder was acidified with distilled water containing 1 % TFA and incubated for 30 min in an ice-cold water bath under gentle shaking. After centrifugation at 15, 000×*g* for 30 min, the supernatant was transferred to fresh tubes and lyophilized for the isolation of antimicrobial peptides.

Purification of Antimicrobial Peptides

To isolate antimicrobial peptides, the lyophilized extract (50 mg) was dissolved in 1 mM phosphate buffer (5 ml, pH 6.0) containing 5 mM EDTA. The sample was applied to a Sephadex G-50 gel filtration column (1 × 100 cm), previously equilibrated with phosphate buffer (0.1 M, pH 6.0). Elution was performed using the same buffer with a flow rate of 0.4 ml/min. The absorbance of the eluted peaks was monitored at 280 nm using a UV detector. All fractions were concentrated, lyophilized and assayed to determine the major fractions containing antimicrobial peptides. For further purification, the fractions containing antimicrobial activity were dissolved in the smallest possible volume of distilled water and purified using reverse-phase high-performance liquid chromatography (RP-HPLC) on a semi-preparative C_{18} column (10 × 250 mm). The injected amount was 400 µl each time. The mobile phase consisted of eluent A (0.1 % TFA in distilled water (v/v)), and eluent B (0.098 % TFA in acetonitrile). The elution was conducted using a linear gradient of 5–65 % eluent B with a flow rate of 1 ml/min for 70 min. According to the absorbance at 220 nm, major peaks were collected, lyophilized and evaluated for their antimicrobial activity. The most active fractions were further purified using the same procedure except that the elution was conducted using a 0.5 % per minute increasing gradient of solution B. To evaluate purity, a small amount of each peak was checked on the analytical C_{18} column (4.6 × 250 mm) using RP-HPLC.

The Assay of Antimicrobial Activity

Radial Diffusion Assay (RDA)

A radial diffusion assay (RDA) was used to detect antimicrobial effects of the collected peptides, as described in Asoodeh et al. (2012). For primary assays, microorganisms such as *Bacillus subtilis* (PTCC4533), *Escherichia coli* (PTCC2433) and *Penicillium lilacinum* were used. An aliquot of bacteria with a titer of 4×10^6 CFU was mixed with 10 ml of medium containing 1 % agarose and 0.03 % TSB and was poured into a plate. Creating holes in the medium using a punch, the peptide sample was loaded into the wells and the plates were incubated for 3 h at 37 °C. After 3-h incubation, secondary medium, enriched with 1 % agarose and 6 % TSB was poured into the plates, and the plates were incubated for 18 h at 37 °C. Subsequently, the plates were stained for 24 h using a solution containing 37 % formaldehyde, 15 ml; methanol, 27 ml; water, 63 ml; and Coomassie brilliant blue R-250, 2 mg. The plates were destained for approximately 10 min with an aqueous solution of 2 % dimethylsulphoxide and 10 % acetic acid. Neomycin antibiotic (15 µg) was used as a positive control. For assessment of antifungal activity, one ml of fungal suspension was inoculated in 20 ml of potato dextrose agar and poured into the germ culture plates. The holes were then created in the medium using a punch, and were filled by the insect extract. The plates were incubated for 7 days at 30–35 °C and the results were recorded during this period.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value was defined as the peptide concentration at which bacterial growth was inhibited after 24 h of incubation at 37 °C (Amiri et al. 2012; Asoodeh et al. 2012; Zardini et al. 2012). At first, each bacterium was grown separately in MHB for 18 h at 37 °C. Approximately 1 ml of culture was transferred to 9 ml of broth medium and incubated for 15 h at 37 °C, cell concentration was then adjusted to obtain a final concentration of 10^6 CFU/ml using MHB. 100 µl of bacterial suspension (1×10^6 CFU/ml) and 80 µl l of MHB medium were poured into the microplate. Stock serial dilutions of 1–32 mg/ml of the fractions were prepared and 20 µl of the fraction stocks were added to the above mentioned MHB to yield a final concentration of 10^5 CFU/ml in each well. The microplate was incubated for 24 h at 37 °C. Then, the absorbance of each well was read at 630 nm using an enzyme-linked immunosorbent assay (ELISA) reader, and the results were compared to the control samples. To determine the fungi-associated MIC, 180 µl of Sabouraud Dextrose Agar medium with 10 µl of fungal suspension (10^6 CFU/ml) and 10 µl of serial concentrations of the insect extract were poured into the microplates and incubated for 24 h at 37 °C. Six bacterial species, including Escherichia *coli* PTCC2433, *Klebsiella pneumonia* PTCC4231, *Pseudomonas aeruginosa* PTCC2834, *Bacillus subtilis* PTCC4533, *Staphylococcus aureus* PTCC1442, and *Bacillus cereus* PTCC1435 as well as three fungal species, *Aspergilus niger*, *Aspergilus fumigates* and *Candidate albicans* were used for MIC determination. All experiments were carried out in triplicate.

Determination of Purity on Tricine–SDS–PAGE

To evaluate the sample purity, 50 µl of each peak (0.5 µg/µl) was checked on SDS-PAGE. The resultant fractions from gel filtration and RP-HPLC column were lyophilized and redissolved in distilled water. Evaluation of the electrophoretic pattern of the F6 fraction and purified peptides (peptide 5, 9, 10) was performed using Tricine–SDS–PAGE (Schagger 2006). A 6.5 % (w/v) stacking gel and a two-part (10 and 15 % (w/v)) separating gel were employed.

Peptide Sequencing

Tandem mass spectrometry (MS–MS) is used for peptide sequencing [Astbury Centre for Structural Molecular Biology, Astbury Building, The University of Leeds]. The first analyzer is used to select user-specified sample ions arising from a particular component. These chosen ions pass into the collision cell, are bombarded by the gas molecules which cause fragment ions to be formed, and these fragment ions are analyzed i.e. separated according to their mass to charge ratios, by the second analyzer. All the fragment ions arise directly from the precursor ions specified in the experiment, and thus produce a fingerprint pattern specific to the compound under investigation. Purified peptides were reconstituted with 10 µL of 0.1 % TFA (v: v). An aliquot (1 µL) of each peptide solution was applied to a ground steel MALDI target plate, followed immediately by an equal volume of a freshly-prepared 5 mg/mL solution of 4-hydroxy-α-cyano-cinnamic acid (Sigma-Aldrich, St. Louis, MO, USA) in 50 % aqueous (v: v) acetonitrile containing 0.1 % TFA (v: v). Bruker flex Analysis software (version 3.3) was used to perform the spectral processing and peak list generation for both the MS and MS/MS spectra. De novo sequencing was performed by hand, allowing for a maximum mass error of 0.5 Da for any given fragmentation ion. Deduced b-ion and y-ion series were overlaid onto their fragmentation spectra using the Bruker flex analysis software (version 3.3).

Phylogenetic Analysis

Thirteen amino acid sequences of peptides from different insects were obtained from the antimicrobial peptide database (http://aps.unmc.edu/AP/main.php). These sequences, along with those for sarcotoxin Pd, were aligned using the program Blast. Then, the alignment was adjusted manually. A phylogenetic tree was obtained from the CLC main workbench Ver.5.5 software (CLC bio A/S, Denmark) by using the neighbor-joining method. Bootstrap analysis with 100 replications was performed on the phylogenetic tree to evaluate the reproducibility of the tree topology.

Hemolytic Assay

The hemolytic activity of sarcotoxin Pd was determined using fresh human erythrocytes, according to the method of Asoodeh et al. (2012). Briefly, fresh human blood (5 ml) was added to a heparinized tube and then centrifuged at $7{,}000 \times g$ for 5 min. Isolated red blood cells (RBCs) were washed five times using 4 ml sterile PBS (phosphate buffered saline) and centrifuged at $7{,}000 \times g$ until the solutions became clear. Finally, RBCs were diluted in PBS buffer (20 ml). Hemolytic activity was evaluated using hemoglobin release in blood agar plates, indicated by a clear zone around the wells. For assessment of hemolytic activity, serially diluted peptide samples were added to microfuge tubes containing 190 µL of diluted RBC (10 % cells). After incubation for 30 min at 37 °C, the tubes were centrifugated at 8, $000 \times g$ for 5 min. An aliquot of the supernatant solution (100 µL) was removed and then diluted with PBS buffer to a final volume of 1 ml. After all, the absorbance was recorded at 567 nm. The hemolytic

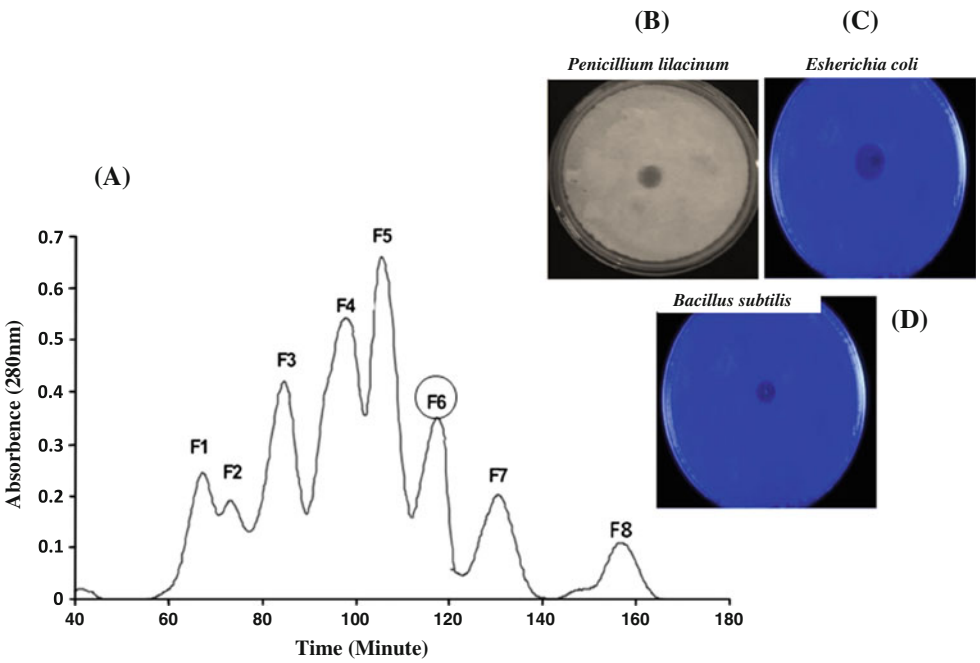

Fig. 1 **a** Sephadex G-50 gel filtration of lyophilized powder prepared from *paederus dermatitis*. The filtration was applied on a Sephadex G-50 column equilibrated with phosphate buffer (0.1 M, pH 6.0). The most potent antimicrobial fraction was named the F6 fraction showed by a *circle*. The antimicrobial activity of the fraction F6 against *Penicillium lilacinum* (**b**), *Escherichia coli* (**c**), and *Bacillus subtilis* (**d**)

effect appeared as a clear zone around the wells. Triton X-100 was used as a positive control to compare the hemolytic effect of the identified peptides.

Peptide Synthesis

The identified peptides were synthesized by Fmoc solid-phase using an Applied Biosystems Model 432A Synergy peptide synthesizer. The synthetic peptides were purified using RP-HPLC on a C_{18} semi-preparative column (10 × 250 mm). The column was developed at a flow rate of 2 ml/min by a linear gradient of acetonitrile (5–45 % for 40 min) containing 0.1 % TFA.

Results

Purification and Antimicrobial Assay

From 500 adult *Paederus dermatitis*, 50 mg of lyophilized powder was obtained. The first step of fractionation of the lyophilized powder on a gel filtration column allowed the effective separation of eight fractions (Fig. 1a). The antimicrobial activity of each fraction was assayed using RDA method. As shown in inset of Fig. 1b, c and d, the F6 fraction exhibited antimicrobial activity against three pathogens (*P.lilacinum*, *E.coli*, and *B. subtilis,*). The purity of this fraction which mainly contained proteins and peptides of molecular masses below 18 kDa (Fig. 2) was evaluated on SDS-PAGE. The F6 fraction was more purified using RP-HPLC and eleven common fractions were obtained (Fig. 3a). The fractions were numbered sequentially (Fig. 3a). The results of the antimicrobial assays indicated that the obtained fractions are effective against nine pathogens (Fig. 4). The antimicrobial activities of these fractions are much more than that of the 15 μg of neomycin used as a control. The antimicrobial activities of the F8*, F9 and F10 fractions are more than that of other fractions. According to the results of Tricine–SDS–PAGE, the F8* fraction was found to be completely pure, while the F9 and F10 fractions contained two and three peptides, respectively (Fig. 2). Consequently, the F9 and F10 fractions were more purified using the same conditions, except for the use of a gradient of 0.5 % eluent B per minute. The most active peptides are indicated with an arrow (Fig. 3b, c).

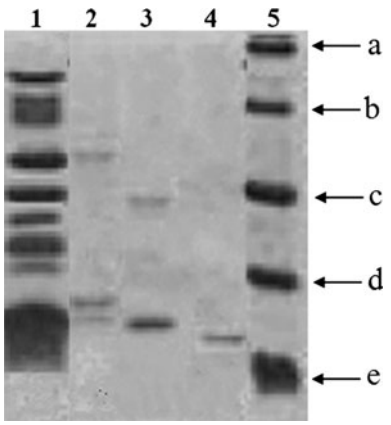

Fig. 2 Tricine–SDS–PAGE of F6 fraction from gel filtration(1), F10 fraction (2), F9 fraction (3) and F8* fraction (4) from C18 RP-HPLC column and size marker (5). *a*, 35 KD; *b*, 18 KD; *c*, 14 KD; *d*, 5.8 KD and *e*, 2.2KD

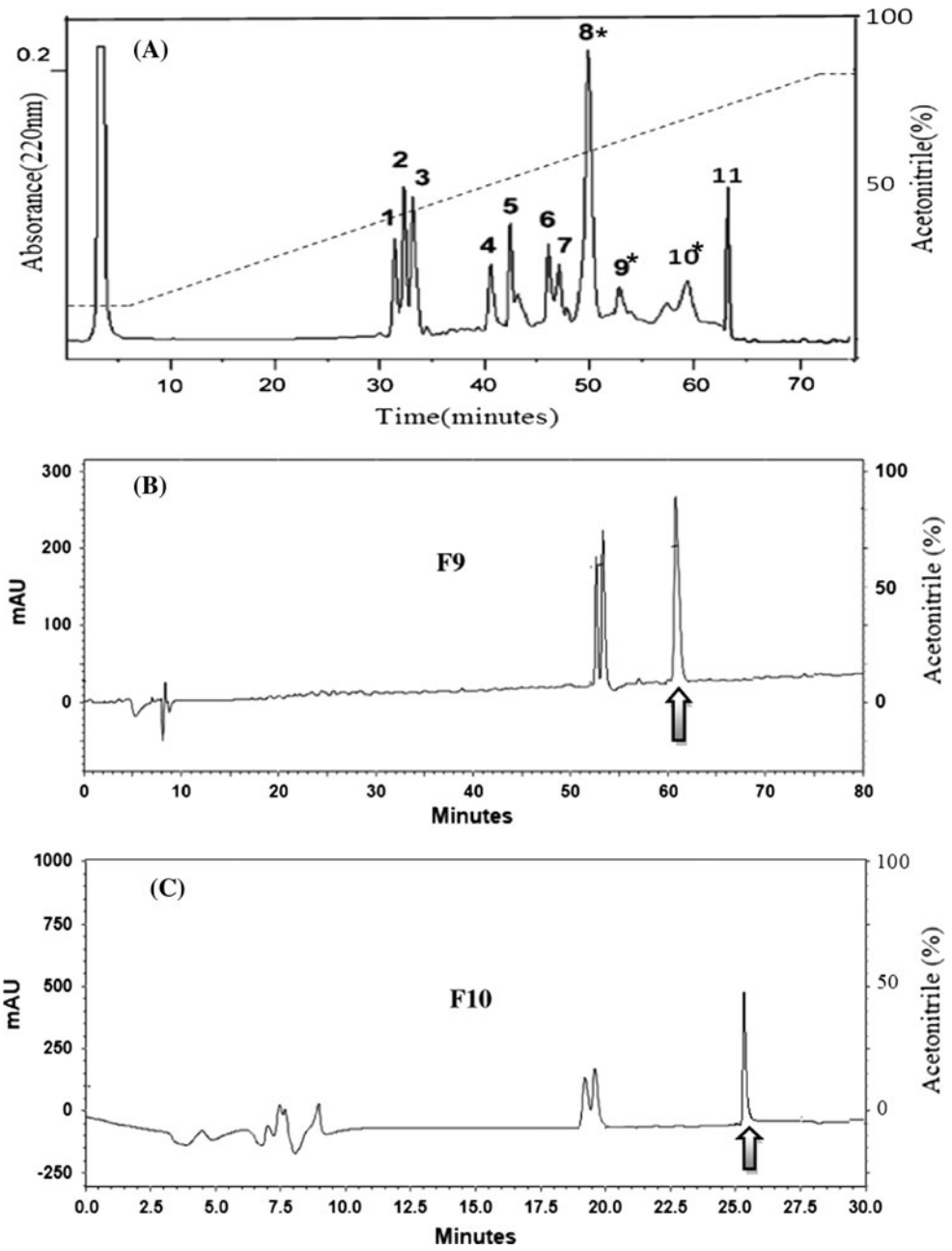

Fig. 3 **a** RP-HPLC chromatogram of the F6 fraction obtained from Sephadex G-50 gel filtration. An aliquot of filtrated extract (400 µl) from gel filtration was loaded onto a semipreparative C18 reverse-phase column. The active fractions (F8*, F9 and F10) are indicated by an *asterisk*. The most active fractions, F9 and F10, more purified using RP-HPLC (**b** and **c**). The most active peptides are indicated by the *arrow*

Structural Characterization

Three antimicrobial peptides, marked by an asterisk in Fig. 3a, were subjected to amino acid sequence analysis using MS/MS spectrometry analysis. Figure 5 shows the MS/MS spectrometry analysis of the F6 fraction. The estimated amino acid sequences, theoretical and experimental molecular masses as well as calculated isoelectric points of the purified peptides are summarized in Table 1. Comparison of the peptide sequences and other published AMP sequences revealed that the F8* and F10 peptides were identical to cecropin C and cecropin D, respectively. While the F9 peptide exhibited no sequence homology to any AMP in the database, suggesting that this peptide is a novel AMP. The newly identified AMP was named sarcotoxin Pd based on a systematic nomenclature for AMPs (J. Michael 2008). The observed mass of sarcotoxin Pd was found to be 3613.26, which was consistent with the theoretical mass (3613.265 Da). The amino acid composition of this peptide was identified as GWLKKIGKKIERVGQHTRGLGIAQI AANVAATAR (Table 1). There are multiple basic amino acids in the sequence of sarcotoxin Pd as found in other

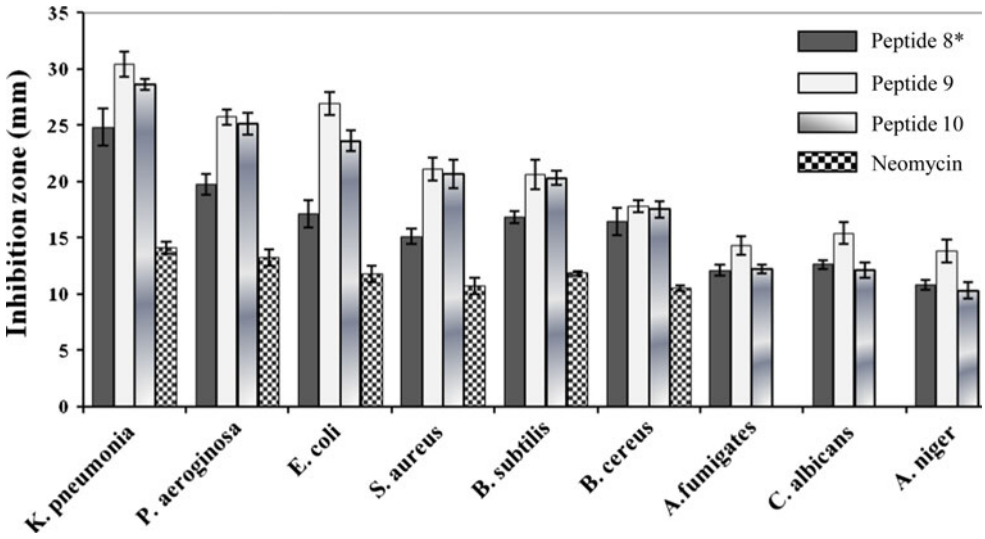

Fig. 4 Antimicrobial activity of the purified peptides against bacteria and fungi using the RDA method (inhibition zone). The diameter of the non-growth halo was measured and showed as a *diagram*. The antimicrobial activities of these peaks are much more than that of the 15 μg of neomycin

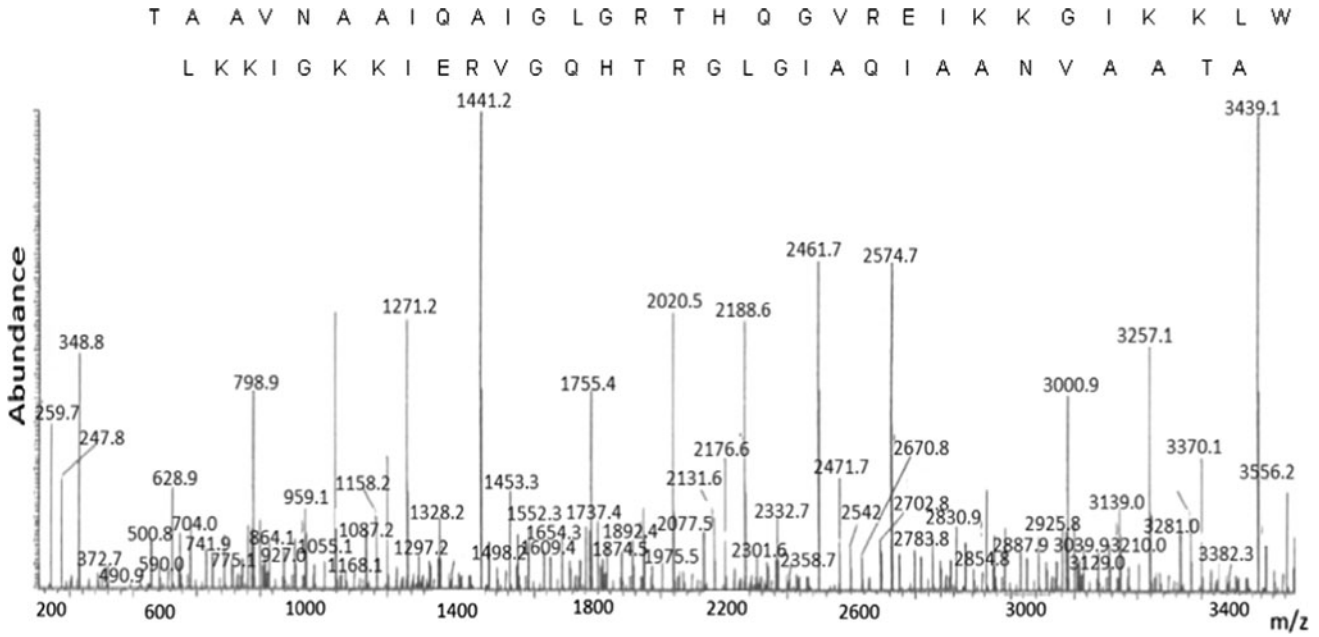

Fig. 5 Identification of the molecular mass and amino acid sequence of the F6 peptide using MALDI-TOF (Matrix-assisted laser desorption/ionization time of flight) spectrometer. MS/MS spectra of the F6 peptide and the interpretation of the obtained spectra

Table 1 Primary structure, molecular weight and pI values of the peptides identified from *Paederus dermatitis*

Peptides	Sequence	Mass (Da)	pI	Homology peptide
F8*	GGLKKLGKKLEGAGKRVFNAAEKALPVVAGAKALG	3448.11	10.81	Cecropin C
F9	GWLKKIGKKIERVGQHTRGLGIAQIAANVAATAR	3613.26	11.8	Non found
F10	WNPFKELERAGQRVRDAIISAGPAVATVAQATALAK	3807.32	10.41	Cecropin D

AMPs. Analysis using the ExPASy MW/pI tool (http://www.expasy.ch/tools/pi-tool.html) exhibited that sarcotoxin Pd had the predicted pI (isoelectric point) of 11.8. Thus, sarcotoxin Pd, as a novel AMP, was selected for MIC determination and the hemolysis assay.

Determination of MIC Value

Analysis of the antimicrobial activity by means of MIC value evaluation was carried out on fungi, Gram-positive and Gram-negative bacteria (Table 2). Sarcotoxin Pd

Table 2 The MIC values of sarcotoxin Pd against various microbes

Microorganisms	MIC (μg/ml)[a]
Escherichia coli	6.12 (±0.08)
Klebsiella pneumonia	6.25 (±0.1)
Psodomonas aeroginosa	10.35 (±0.11)
Staphylococcus aureus	19.24 (±0.12)
Bacillus subtilis	18.62 (±0.12)
Bacillus cereus	16.22 (±0.15)
Candidate albicans	18.62 (±0.16)
Asperjilus niger	25.26 (±0.18)
Asperjilus fumigates	22.3 (±0.15)

[a] The concentration of an antimicrobial peptide to inhibit of bacterial or fungal growth after 24 h at 37 °C

showed a higher efficacy against Gram-negative than Gram-positive bacteria and fungi. Among the tested microorganisms, *Klebsiella pneumonia* and *Escherichia coli* were the most sensitive strains to sarcotoxin Pd. The MIC values of sarcotoxin Pd against *K. pneumonia* and *E.coli* were 6.25 and 6.12 μg/ml, respectively. This peptide was also active against studied phytopathogenic fungi, so that total inhibition of fungal growth for *Aspergilus niger*, *Aspergilus fumigates* and *Candidate albicans* occurred at the concentrations of 25.2, 22.3 and 18.62 μg/ml, respectively.

Phylogenetic Analysis

Multiple sequence alignments of sarcotoxin Pd were carried out along with 13 reference AMPs obtained from the antimicrobial peptide database. The results of the BLAST revealed that sarcotoxin Pd shares a high homology to the sarcotoxin IA from *Drosophila melanogaster* (Fig. 6a). Phylogenetic analysis of the amino acid sequences was carried out by constructing a phylogenetic tree. On the basis of the length of lines in the phylogenetic tree, sarcotoxin IA from *D. melanogaster* and sarcotoxin IC from *Sarcophaga peregrina* exhibited the highest similarity to sarcotoxin Pd (Fig. 6b). These peptides demonstrated 84.61 % and 79.48 % identity to sarcotoxin Pd, respectively. Furthermore, with 36.82 % and 35.54 % similarity,

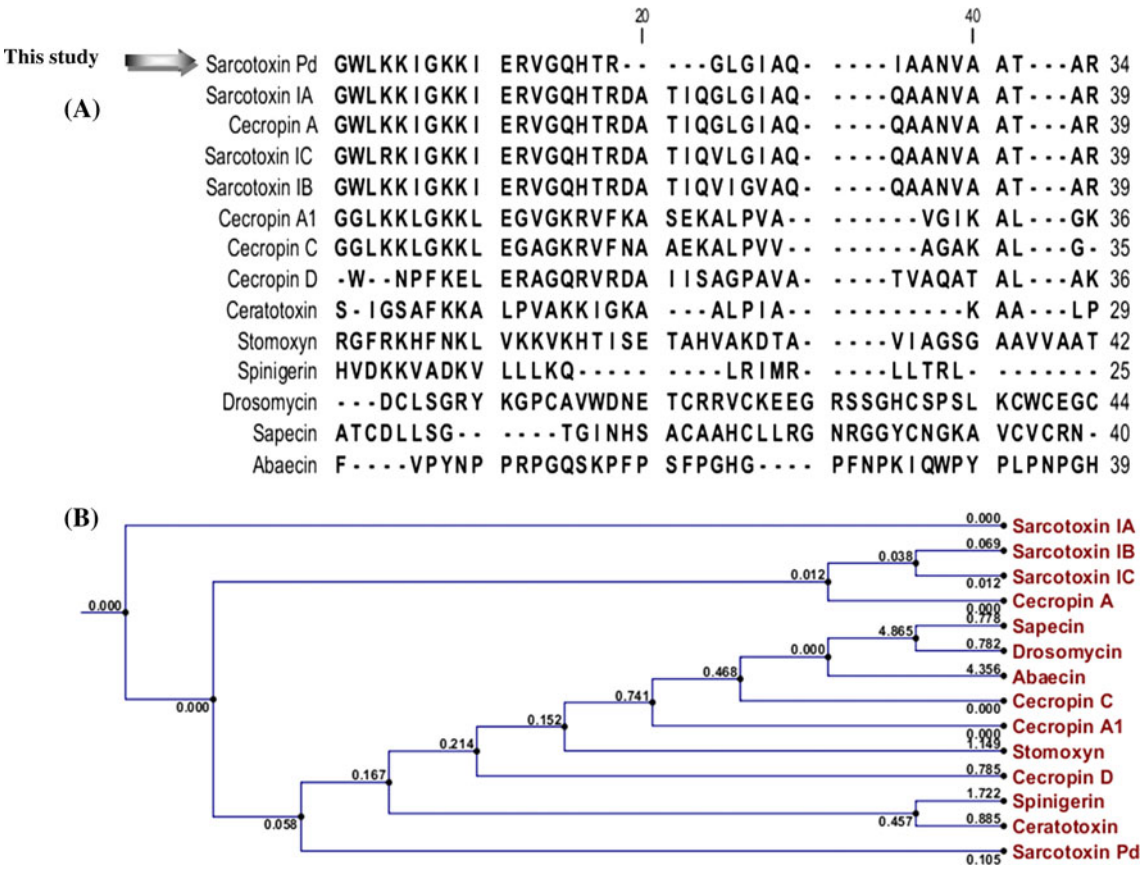

Fig. 6 **a** Alignment of sarcotoxin Pd with other antimicrobial peptides from various insects. The alignment was carried out with CLC Main Work Bench Ver.5.5 software. **b** Phylogenetic tree of sarcotoxin Pd. Amino acid sequences of 13 reference peptides obtained from the antimicrobial peptide database were incorporated into the tree using the neighbor-joining method. The name of each sequence is typed at the end of the corresponding branch. Reliability of the tree was assessed by bootstrap analysis with 100 replications. The substitutions per amino acid position are typed above each branch

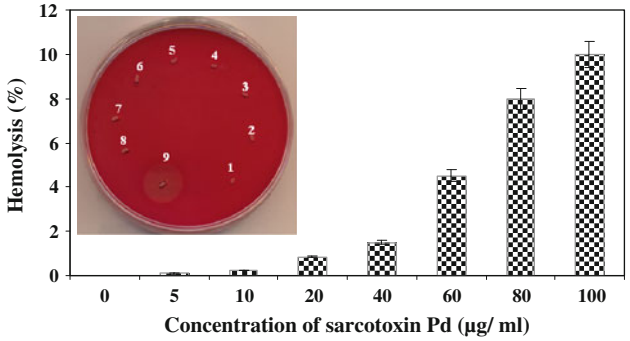

Fig. 7 Hemolytic activities of sarcotoxin Pd isolated from *paederus dermatitis*. The *inset*, indicates the hemolytic activity assay of sarcotoxin Pd in the range of 5–100 µg/ml based on the method of RDA (*1* PBS, *2* 5 µg/ml, *3* 10 µg/ml, *4* 20 µg/ml, *5* 40 µg/ml, *6* 60, *7* 80 µg/ml, *8* 100 µg/ml and *9* Triton X-100)

spinigerin and ceratotoxin had the lowest homology to sarcotoxin Pd. Despite these similarities, sarcotoxin Pd can be considered as a novel member of the cecropin family.

Assessment of Hemolytic Activity

For future therapeutic applications, the hemolytic activity of sarcotoxin Pd toward human RBCs was tested. Sarcotoxin Pd had no significant hemolytic effect in the concentration range (5–100 µg/ml) used for RDA test (Fig. 7, inset). As shown in Fig. 7, sarcotoxin Pd showed a moderate hemolytic activity by inducing only 10 % human RBC hemolysis at the concentration of 100 µg/ml. It was observed that sarcotoxin Pd had a little hemolytic effect around its MIC values ($\leq$ 30 µg/ml), indicating a weak interaction between the peptide and phospholipids of RBCs.

Peptide Synthesis

The identified peptides were synthesized by Fmoc solid-phase using an Applied Biosystems Model 432A Synergy peptide synthesizer. 5 mg of peptide were obtained using this synthetic manner. The synthetic peptide also shows appropriate antimicrobial activity as shown in natural peptide. These data are not shown.

Discussion

Antimicrobial peptides (AMPs) are key factors in insect immune response against invading microorganisms. AMPs are typically 12–50 amino acids in length with a net positive charge owing to the excess of basic amino acid residues (Hancock and Chapple 1999; Hancock and Diamond 2000). The most abundant family of linear AMPs of insect origin are the cecropins, including sarcotoxins, spodopsin, hyphancin, enbocin, and cecropin-like molecules (Bullet et al. 2003; Bullet et al. 1999). In this study, a novel AMP from *Paederus dermatitis* was purified and characterized. Previous structure–activity relationship (SAR) studies suggest that the antimicrobial activity of AMPs is remarkably dependent on their α-helical structure, which is affected by charge, size, helicity, hydrophobic moment and hydrophobicity (Tossi et al. 2000). The identified AMP consists of 34 residues with a net charge of +7 (clustered at positions 4, 5, 8, 9, 12, 18 and 34). The total hydrophobic ratio of this peptide is 45 % (Boman 1991; Lu et al. 2006; Rees et al. 1997; Shao et al. 2004). Sequence alignment of AMPs revealed striking similarities between parts of this hydrophobic-cationic peptide and sarcotoxin IA (Katarina et al. 2002), sarcotoxin IC (Kulkarni et al. 2011) and sarcotoxin IB (Lauth et al. 1998), as shown in Fig. 6a. Moreover, it showed sequence similarity to the amphibian antimicrobial family of cecropins. Therefore, the identified peptide is a member of cecropin family and it has given its name to sarcotoxin Pd according to nomenclature recommendation by Conlon (2008). Cecropins are originally found in *Hyalophora cecropia* (Steiner et al. 1981). Sarcotoxin Pd has a broad antimicrobial activity against Gram-negative and Gram-positive bacteria as well as fungi. It has been previously identified that cecropins contained two α-helices which lead to the emergence of their antimicrobial activity against Gram-negative bacteria. Like other cecropin family members, sarcotoxin Pd is more efficient against Gram-negative bacteria (Yang et al. 1999). According to these observations and in agreement with previous studies, sarcotoxin Pd was deduced to have an α-helical structure (Bullet et al. 1999). The hydrophobic nature of the sarcotoxin Pd being justified by its positive hydropathicity average plays an important role in the interaction with microbial membrane. It has been found that sarcotoxin IA contained two amphiphilic helices possessing two hydrophilic and hydrophobic faces (Iwai et al. 1993). As shown in Fig. 8, the comparison of amino acid sequence of sarcotoxi Pd and sarcotoxin IA suggested sequence and thus the conformation similarity of the N-terminal region of both peptides (Iwai et al. 1993). The peptide structure was predicted based on the structure of sarcotoxin IA. In this predicted structure, its side chains are oriented in an amphiphilic arrangement, with all charged residues separated to one face of the helix (Fig. 8). This new peptide may form α-helices with at least 14 residues on the same hydrophobic surface. The MIC values of the sarcotoxin Pd were compared with other AMPs purified from different insects. The MIC values of sarcotoxin Pd are lower than thanatin (Romanelli et al. 2011), drosocin (Bullet et al. 1996), defensin (Lauth et al. 1998), and Gm cecropin D-like peptide (Hwang et al. 2009). Furthermore,

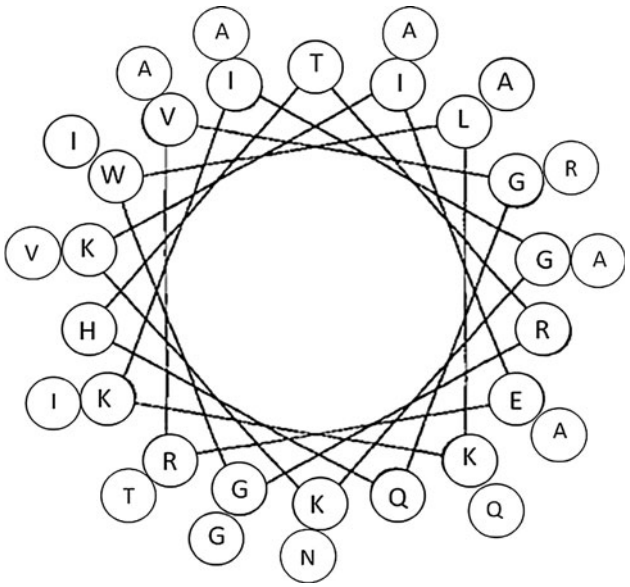

Fig. 8 The predicted helical wheel plot of sarcotoxin Pd

the hemolytic activity of darcotoxin Pd (10 %) was lower in comparison to some identified AMPs, such as decoralin (15 %) (Baek et al. 2011) and jelleins (11 %) (Romanelli et al. 2011). It is noteworthy that the identified peptide with low hemolytic and wide-spectrum antimicrobial activity may be beneficial in the treatment of pathogenic diseases. However, more detailed researches are still required as in vivo testing its antimicrobial activity.

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