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New bioactive peptides from the venom gland of social hornet *Vespa velutina*

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ABSTRACT

Bacterial resistance to drugs is a global problem requiring the urgent development of new antibiotics. Antimicrobial peptides (AMPs) are excellent candidates for the design of novel antibiotics to combat microbial resistance. In this research, we identified four new peptides (U-VVTX-Vp1a, U-VVTX-Vp1b, U-VVTX-Vp2a, and U-VVTX-Vp2b, respectively) from the venom of *Vespa velutina*, and tested their antimicrobial, antioxidant, and hemolytic effects. All four peptides showed scavenging ability against DPPH, $ABTS^{+}$, and •OH free radicals. Of note, Vp1b strongly inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria at concentrations of 60 and 120 μM. Due to their low hemolytic activity, all four peptides could be utilized in the development of new antioxidants and as candidates for the design of novel antimicrobial agents.

1. Introduction

The excessive use of antibiotics and subsequent selection of microorganisms that can develop resistance have resulted in the emergence of antibiotic-resistant bacteria and genes, which can have considerable impact on human health and environmental stability (Kennedy, 2013; Pruden et al., 2006; Rysz and Alvarez, 2004). At present, more than 700 000 people die each year from drug-resistant diseases and infections, a figure that could increase to 10 million by 2050 if global efforts to control antimicrobial resistance are not expanded (IACG, 2019). As such, new antibiotics and antimicrobial agents are urgently needed.

Antimicrobial peptides (AMPs), which are small active compounds produced in bacteria, plants, insects, and vertebrates, can resist infection by pathogenic bacteria. These peptides range from 10 to 40 amino acids in size and show cationic and amphiphilic characteristics (Fjell et al., 2012; Park et al., 2011). Cationic AMPs interact with negatively charged bacterial outer membranes, thereby damaging the membrane structure (Hancock and Annett, 2002). In addition, they exhibit strong selectivity, killing speed, and antibacterial activity, without drug resistance. Therefore, AMPs may be a potential source of novel anti-infective agents (Nijnik and Hancock, 2009). To date, thousands of AMPs have been identified in viruses, bacteria, fungi, fish, birds, insects, amphibians, mollusks, and mammals (Wang et al., 2016). At present, a variety of AMPs have been approved for clinical application and food storage (Jiang et al., 2021). For example, tigecycline (Wyeth, formerly GAR-936), licensed by the FAD in 2005, works against Gram-positive and Gram-negative bacterial activity by inhibiting protein synthesis (Livermore, 2005).

Given their lack of antibodies, thymus glands, and immune cells, insects rely on various innate immune defenses to resist microbial infection from environmental sources. In particular, the rapid synthesis of AMPs is one of the most important ways for insects to resist microbial invasion (Gillespie et al., 1997; Kuhn-Nentwig, 2003; Otvos, 2000). Wasp venom glands are highly specialized organs that produce venom for protection against other insects and small vertebrates, even large mammals under multiple sting attacks (Piek, 1986). In recent years, wasp venom has attracted increasing research attention as a rich source of pharmacologically active peptides (Baek and Lee, 2010).

The *Vespa velutina* hornet, which belongs to the order Hymenoptera,

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Table 1
Antibacterial activity of tested peptides against five kinds of bacteria. Data are mean ± standard deviation (SD) of three independent experiments (n = 9). Bacteriostatic rates >0.50 and > 0.90 indicate strong and very strong antibacterial effects, respectively.

Peptide	Peptide concentration (μM)	Gram-positive bacteria			Gram-negative bacteria	
		Staphylococcus aureus	Bacillus subtilis	*Enterococcus faecalis*	*Escherichia coli*	*Klebsiella pneumoniae*
Vp1a	120	0.410 ± 0.045	0.354 ± 0.006	0.269 ± 0.030	0.250 ± 0.039	0.313 ± 0.022
	60	0.217 ± 0.034	0.340 ± 0.030	0.163 ± 0.056	0.239 ± 0.006	0.180 ± 0.038
	30	0.131 ± 0.035	0.333 ± 0.023	0.134 ± 0.019	0.213 ± 0.015	0.097 ± 0.040
	15	NA	0.304 ± 0.029	0.133 ± 0.050	0.176 ± 0.027	0.130 ± 0.042
	7.5	NA	0.301 ± 0.080	NA	0.174 ± 0.006	0.078 ± 0.013
	3.25	NA	0.301 ± 0.028	NA	0.117 ± 0.041	0.047 ± 0.012
Vp1b	120	0.998 ± 0.003	0.707 ± 0.001	0.106 ± 0.062	1.000 ± 0.001	0.317 ± 0.042
	60	0.413 ± 0.016	0.533 ± 0.005	NA	0.244 ± 0.038	0.273 ± 0.049
	30	0.173 ± 0.005	0.404 ± 0.033	NA	0.236 ± 0.019	0.194 ± 0.044
	15	0.145 ± 0.032	0.347 ± 0.010	NA	0.216 ± 0.010	0.145 ± 0.034
	7.5	NA	0.328 ± 0.014	NA	0.193 ± 0.038	0.121 ± 0.051
	3.25	NA	0.325 ± 0.011	NA	0.136 ± 0.019	0.076 ± 0.014
Vp2a	120	0.566 ± 0.020	0.473 ± 0.030	0.358 ± 0.022	0.464 ± 0.013	0.685 ± 0.000
	60	0.517 ± 0.040	0.444 ± 0.042	NA	0.373 ± 0.015	0.864 ± 0.036
	30	0.449 ± 0.032	0.403 ± 0.005	NA	0.340 ± 0.042	0.783 ± 0.009
	15	0.468 ± 0.019	0.397 ± 0.023	NA	0.345 ± 0.042	0.475 ± 0.019
	7.5	0.375 ± 0.008	0.377 ± 0.041	NA	0.296 ± 0.007	0.323 ± 0.038
	3.25	0.373 ± 0.033	0.371 ± 0.014	NA	0.189 ± 0.050	0.267 ± 0.022
Vp2b	120	0.237 ± 0.039	0.271 ± 0.007	0.351 ± 0.016	NA	0.533 ± 0.029
	60	0.203 ± 0.033	0.261 ± 0.038	NA	NA	0.400 ± 0.035
	30	0.126 ± 0.042	0.256 ± 0.020	NA	NA	0.342 ± 0.034
	15	NA	0.254 ± 0.018	NA	NA	0.209 ± 0.010
	7.5	NA	0.272 ± 0.041	NA	NA	0.118 ± 0.038
	3.25	NA	0.285 ± 0.029	NA	NA	0.122 ± 0.022

is primarily distributed in southern and eastern China, but also occurs in neighboring South Korea and Japan (Choi et al., 2011; Kishi and Goga, 2017), as well as in France, Spain, and Portugal (Bertolino et al., 2016; Budge et al., 2017; Grossosilva and Maia, 2012; López et al., 2011; Villemant et al., 2006), where it is considered a serious invasive pest. As such, most previous studies have focused on its biology and control, with research on novel venom-derived peptides still limited.

In this report, we identified potential AMPs from *V. velutina* venom using cDNA library screening, then tested their antibacterial, antioxidant, and hemolytic properties. The peptides identified in this research may be potential candidates for the design and development of new antimicrobial agents.

2. Materials and methods

2.1. Animal sample collection

V. velutina workers were collected whilst hawking outside the entrance to several beehives located at the Yunnan Agricultural University (Yunnan, China) and were immediately placed in liquid nitrogen. After removal from liquid nitrogen, the venom sacs of the wasps were removed, dissected, and placed in microcentrifuge tubes and stored in liquid nitrogen for later use.

2.2. RNA extraction

RNA was extracted with a TransZol Up Plus RNA Kit (TransGen, Beijing, China) following the manufacturer's protocols and then stored at −80 °C.

2.3. Construction of cDNA library

Construction of a cDNA library from the wasp venom was performed using previously reported methods (Ji et al., 2011; Loit et al., 2008). cDNA was synthesized using a SMARTTM cDNA Construction Kit (Clontech, Canada) following the manufacturer's protocols. cDNA synthesized by polymerase chain reaction (PCR) was used as a template to screen for cDNA-encoding AMP precursors. Primers S1 (5′-CATCATGAAGAACACGAT-3′) and S2 (5′-ATGAAGTATATTAGTTTG-3′) were designed and used for screening against known hornet AMPs found in the National Center for Biotechnology Information (NCBI) database. The PCR products were recovered using a Fast Pure Extraction Mini Kit (Vazyme, Nanjing, China) and PMD19-T vector (TaKaRa, Dalian, China) and then cloned into *Escherichia coli* DH5 cells and sequenced (Shuoyang Technology Co., Ltd., Yunnan, China). Vector fragments and target gene fragments were extracted and identified, with peptides synthesis based on gene fragments using solid phase synthesis by Shuoyang Technology Co., Ltd. (Yunnan, China). Four peptides (i.e., Vp1a, Vp1b, Vp2a, and Vp2b) were synthesized.

2.4. Detection of peptide activity

2.4.1. Antimicrobial activity assays

Table 1 lists the gram-positive and -negative bacteria used in the antibacterial tests. The experimental data were the results of three independent experiments. All strains were obtained from the Kunming Medical College (Yunnan, China). Bacteria were cultured to an optical density of 1 (OD600 = 1) and diluted 3 000 times in Luria-Bertani (LB) medium. We added 50 μL of diluted bacterial solution and 50 μL of treatment solution to a 96-well plate, which was then cultured at 37 °C for 16–18 h. Optical density (OD600) was then measured to determine if the various treatments inhibited bacterial growth. We tested different concentrations (3.25, 7.5. 15, 30, 60, and 120 μM) of each peptide (MP-2, MP-3, VP-2, and VP-3). DD water as the peptide solvent was used as a blank control. The above concentrations were selected based on previous studies, which reported minimum inhibitory concentrations (MICs) of wasp AMPs against bacterial strains of 5–200 μM (Ji et al., 2011). The following equation was used to measure the bacteriostatic rate:

$$Bacteriostatic\ rate = (A_{blank} - A_{sample}) \times 100 \,/\, A_{blank} \quad (1)$$

2.4.2. Antioxidant assays

We measured the antioxidant effects of the peptides by detecting their ability to clear different free radicals. Each experiment required three replicates of the independent experiment.

(1) DPPH radical scavenging ability

A

Mastoparan-VT1

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ATGAAGAACACAATCTTAATTTTATTCACCGCTTTCATCGCACTTTTGGGATTCTTCGGA   60
 M  K  N  T  I  L  I  L  F  T  A  F  I  A  L  L  G  F  F  G    20
ATGAGTGCCGAAGCTTTAGCTGACCTAAAAGCTGATCCATTAGCTGGTCCAAATCCTGAT   120
 M  S  A  E  A  L  A  D  L  K  A  D  P  L  A  G  P  N  P  D    40
GCTGATCCAGAAGCAATAAACCTGAAGGCTATTGCAGCATTGGCTAAGAAACTATTAGGT   180
 A  D  P  E  A  I  N  L  K  A  I  A  A  L  A  K  K  L  L  G    60
TAACATATCGTTTATTCCGAAAATGAAATATATGTTCTTGATGAAATGTAAAAGTTATAT   240
 *                                                              60
TGAGTTATGATATTATAAATTGTTGTGCACATTAAAAAAAAAAAAAAAAAAAAAAAAAA   300
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Vp1a

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ATGAAGAACACGATTTTAATTTTATTCCCCGCTTTCATCGCACTTTTGGGATTTTTCGGA   60
 M  K  N  T  I  L  I  L  F  P  A  F  I  A  L  L  G  F  F  G    20
ATGAGTGCTGAAGCTTTAGCTGACCCAGTAGCTGACCCATTAGCTGGTCCAAATGCTGAA   120
 M  S  A  E  A  L  A  D  P  V  A  D  P  L  A  G  P  N  A  E    40
GCTGATCCAGAAGCCATAAACTGGAAGGGTATTGCAGCAATGGGTAAGAAACTACTAGGT   180
 A  D  P  E  A  I  N  W  K  G  I  A  A  M  G  K  K  L  L  G    60
TAACATATCGTTTATTCCGACAATAAATTATATGTTTTTGATGAAATGTAAAAGTTATAT   240
 *                                                              60
TGAGTTATGATATTATAAATTGTTGTGCACGGAAAAAAAAAAAAAAAAAAAAAAAA      296
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B

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Mastoparan-VT1   MKNTILILFTAFIALLGFFGMSAEALADLKADPLADLKADPLAGPNPDADPEAINLKAIAALAKKLLG
Mastoparan-VT2   MKNTILILFTAFIALLGFFGMSAEALADLKADPLADLKADPLAGPNPDADPEAINLKAIAALAKKLLG
Mastoparan-VT3   MKNTILILFTAFIALLGFFGMSAEALADLKADPLADLKADPLAGPNPDADPEAINLKAITALAKKLLG
Mastoparan-VT4   MKNPILILFTAFIALLGFFGMSAEALADPKADPLADLKADPLAGPNPDADPEAINLKAIAPLAKKLLG
Vp1a             MKNTILILFPAFIALLGFFGMSAEALADPVA--------DPLAGPNAEADPEAINWKGIAAMGKKLLG
Vp1b             MKNTILILFTAFIALLGFFGMSAEALADPVA--------DPLAGPNAEADPEAINWKGIAAMAKRLLG
VCP-VT1          MKYNIVFLFAIIASLACLQLTFAAPAASPLANPGASPDAAPNADPLADPFLPIIGKLLSGLLGKK
Vp2a             MKYNIVFLFAIIASLACLQL----------------------NADPSADPFFPIIAKLLGGFLGKK
Vp2b             MKYNIVFLFAIIASLACLQLAFAALAAFPLDNLGPSPDAAPNADPSADPFLPIIDKLLGGLLGKK
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Fig. 1. Amino acid sequences of four new peptides identified in this study, together with other homologous peptides. (A) Nucleotide and amino acid sequences of Mastoparan-VT1 and Vp1a. Stop codons of each sequence are indicated by asterisks. Mature AMP sequences were identified by sequence comparison with the peptides of the same family through Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and shown in bold type. (B) Comparison of four new peptides with known peptides in same general family. Mature AMP sequences are shown in bold type.

We added 190 μL of DPPH solution (100 μM) to a 96-well plate, followed by the addition of 10 μL of peptide solution (3, 6, 12, 30, 60, and 120 μM, respectively) and incubation at 37 °C for 30 min. Absorbance was then measured at 517 nm. In addition, DPPH (alone), vitamin C (120 μM), and methanol solutions were used as the blank, positive, and negative controls, respectively. The following equation was applied to measure the DPPH radical scavenging rate:

$$DPPH\ radical\ scavenging\ rate = (A_{blank} - A_{sample}) \times 100 / A_{blank} \quad (2)$$

(2) ABTS $^{+}$ radical scavenging ability

Methanol was used as a solvent to prepare the ABTS^{+} (7.4 mM) and K2S2O8 (2.6 mM) reserve solutions. We mixed 5 mL of ABTS^{+} reserve solution and 88 μL of K2S2O8 reserve solution. After standing for 16 h, the ABTS^{+} working solution was prepared with 40 × dilution in methanol. Absorbance was determined at 734 nm after adding 200 μL of ABTS^{+} solution and 10 μL of peptide solution to each well of a 96-well plate for 6 min. In addition, ABTS^{+} (alone), vitamin C (120 μM), and

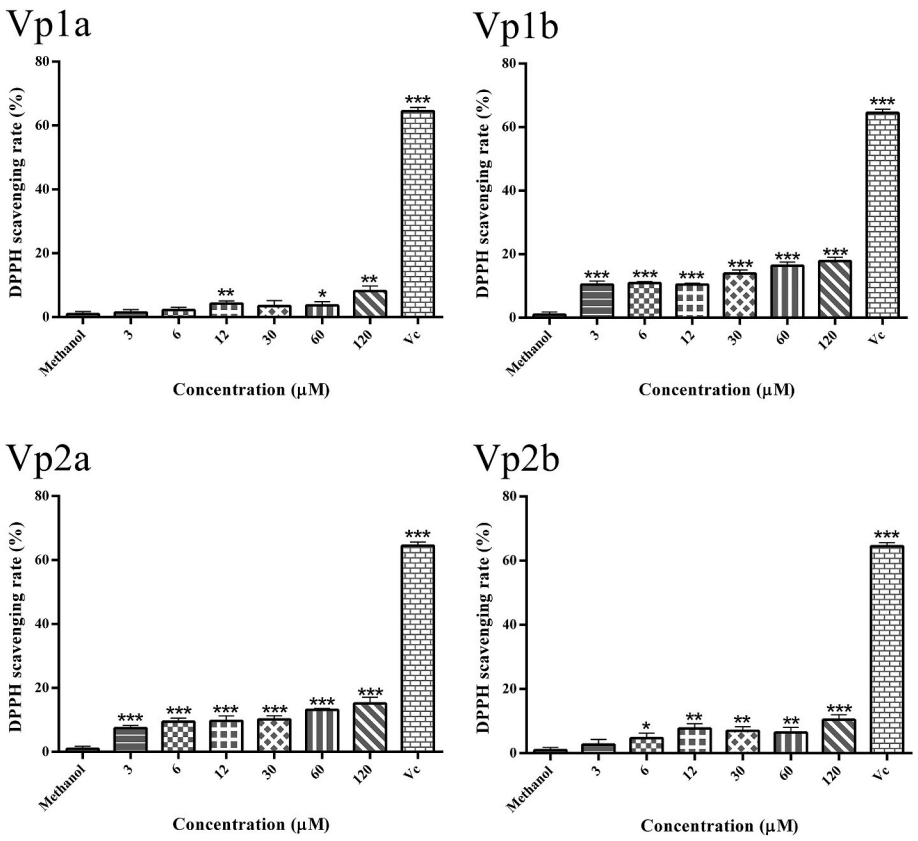

Fig. 2. DPPH radical scavenging rates of four *V. velutina* venom peptides tested at different concentrations in methanol. Vc is vitamin C (120 μM). Data are mean ± SD of three independent experiments (n = 9). Statistical significance of differences was determined by *t*-test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significantly different from negative control, methanol was used as a negative control and compared with each group).

(2) Scavenging activity against $ABTS^+$ radicals

methanol solutions were used as the blank, positive, and negative controls, respectively. The following equation was employed to measure the $ABTS^+$ radical scavenging rate:

$$ABTS^+ \ radical \ scavenging \ rate = (A_{blank} - A_{sample}) \times 100 / A_{blank} \quad (3)$$

(3) •OH radical scavenging ability

We added peptide solution (3, 6, 12, 30, 60, and 120 μM, respectively), 70 μL of distilled water, 10 μL of salicylic acid-methanol solution (9.1 mM), and 10 μL of $FeSO_4$ solution (9 mM) to a 96-well plate, then added 10 μL of 30% H_2O_2 solution. Absorbance A_1 was measured at 510 nm. Absorbance A_2 was measured with distilled water instead of $FeSO_4$ solution. Absorbance A_3 was measured with distilled water instead of the sample. In addition, vitamin C (120 μM) and methanol solutions were used as the positive and negative controls, respectively. The following equation was used to measure the •OH radical scavenging rate:

$$OH \ radical \ scavenging \ rate = [1 - (A_1 - A_2) / A_3] \times 100 \quad (4)$$

2.5. Hemolytic activity assays

Red blood cells from mice were used to measure the hemolytic activity of each peptide (Bignami, 1993). In total, 1 mL of red blood cells was washed twice with 500 μL of phosphate-buffered saline (PBS) and then diluted with 5% PBS, followed by the addition of each peptide (120 μM) and incubation in a 37 °C water bath for 30 min. Samples were then centrifuged at 3 000 rpm for 3 min at 4 °C. The absorbance of the resulting supernatant was measured at 540 nm. We added 1 μL of Triton 100-x to the blood cells to determine the maximum hemolysis rate. The experiment required three replicates of independent experiments.

3. Results

3.1. cDNA library

We used DNAMAN v10 to analyze the sequencing data. The targeted base sequences were translated into peptide sequences and then run through the NCBI database. Two *V. velutina* peptides corresponding to primer S1 were from the mastoparan (MP) family (Fig. 1), which we named U-VPTX-Vp1a (Vp1a, GeneBank accession number: MZ147005): INWKGIAAMGKKLL and U-VPTX-Vp1b (Vp1b, GeneBank accession number: MZ147006): INWKGIAAMAKRLL. All G after the mature peptide sequences are the amidation recognition sites (Fig. 1), these are cleaved before yielding the mature peptides in the venom. The third peptide corresponding to primer S1 was previously reported in the venom of *Vespa xanthoptera* (Hirai et al., 1979) and named Mastoparan-X (MP-X).

Two peptides corresponding to primer S2 were in the vespid chemotactic peptide (VCP) family (Fig. 1), which we named U-VPTX-Vp2a (Vp2a, GeneBank accession number: MZ147007): FFPIIAKLLGGFLGKK and U - VPTX-Vp2b (Vp2b, GeneBank accession number: MZ147008): FLPIIDKLLGGLLGKK. The third peptide corresponding to primer S2 was previously isolated from the venom of *Vespa magnifica* (Xu et al., 2006) and named VCP-5E.

An overall comparison with the same general family of peptides (VT1, VT2, VT3, and VT4) identified by Yang et al. (2013) showed that

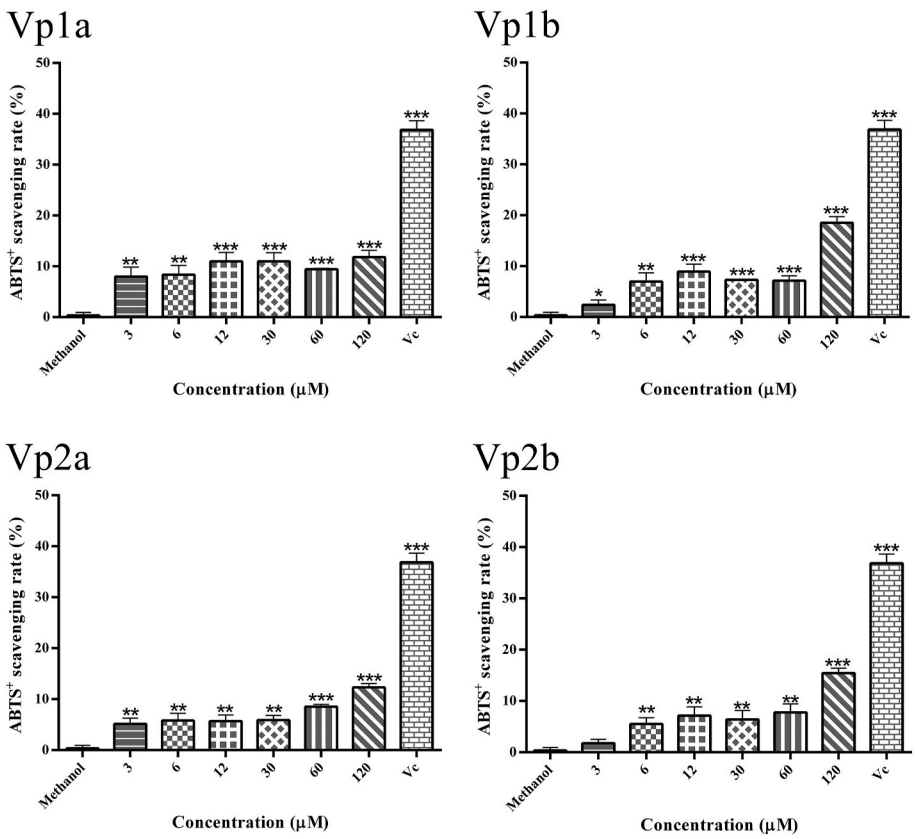

Fig. 3. ABTS $^{+}$ radical scavenging rates of four *V. velutina* venom peptides tested at different concentrations in methanol. Vc is vitamin C (120 μM). Data are mean ± SD of three independent experiments (n = 9). Statistical significance of differences was determined by *t*-test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significantly different from negative control, methanol was used as a negative control and compared with each group).

(3) Scavenging activity against •OH radicals

the four new peptides (Vp1a, Vp1b, Vp2a, and Vp2b) differed from other known peptides by two to four amino acids (Fig. 1B). Based upon their amino acid sequences, Vp1a, Vp1b, Vp2a, and Vp2b were then synthesized, resulting in four 20-mg samples with purities of 98% for the biological activity tests.

3.2. Detection of peptide activity

3.2.1. Antimicrobial activity

The four peptides differed in their antibacterial effects (Table 1). Neither Vp1a nor Vp2b exhibited any strong inhibitory effects on bacterial strains at concentrations of 120 μM and below. However, Vp1b exhibited very strong inhibitory effects on *Staphylococcus aureus* and *Escherichia coli* bacteria at 120 μM, and inhibited *Bacillus subtilis* at 60 and 120 μM Vp2a inhibited *S. aureus* at concentrations of 60 and 120 μM and *Klebsiella pneumoniae* at 15, 60, and 120 μM. However, Vp1a showed no strong inhibitory effects on any of the tested strains at any of the tested concentrations.

3.2.2. Antioxidant assays

(1) Scavenging activity against DPPH radicals

Both Vp1b and Vp2a exhibited excellent antioxidant abilities against DPPH radicals, with highly significant ($P < 0.001$) scavenging rates at concentrations of 3–120 μM (Fig. 2). Vp2b exhibited relatively good scavenging ability against DPPH radicals at concentrations of 6–120 μM Vp1a showed significant scavenging ability at concentrations of 12, 60, and 120 μM, but not at 30 μM.

At concentrations of 6 to 120 μM, all four peptides exhibited significant antioxidant properties against ABTS^{+} radicals (Fig. 3).

At concentrations ranging from 30 to 120 μM, all four peptides showed significant antioxidant abilities against •OH radicals (Fig. 4). Notably, Vp1b showed significant antioxidant ability, even at the relatively low concentration of 12 μM.

3.3. Hemolytic activity

The potential toxicity of the peptides was assayed by measuring their hemolytic activity against mouse red blood cells. At the highest concentration of 120 μM, the hemolysis rates of Vp1a, Vp1b, Vp2a, and Vp2b were 2.37%, 4.08%, 3.46% and 2.12%, respectively. The absorbance of the four peptides at 540 nm was not significantly different from that of the control. As expected, the positive control, Triton-100x, had a highly significant hemolytic effect ($P < 0.001$, n = 9, *t*-test). At 120 μM, none of the tested peptides exhibited hemolytic activity against mouse blood cells, with activities all well below 5%.

4. Discussion

Small peptides with molecular weights of 1.4–7 kDa account for ~70% of the main components of wasp venom (Baek, 2013; Monsalve and Lu, 1999; Yoon et al., 2015), and include mastoparan, kinin, and chemotactic peptides (Habermann, 1972). In the past few decades, an increasing number of small peptides have been found in wasp species (Argiolas and Pisano, 1984; Bignami, 1993; Chen et al., 2012; Hirai et al., 1979; Wu et al., 2018; Vaara, 2009); however, reports on small peptides from *V. velutina* remain scarce.

In the current study, we identified four new peptides in the venom of *V. velutina*. Based on peptide sequence similarities with known AMPs, we

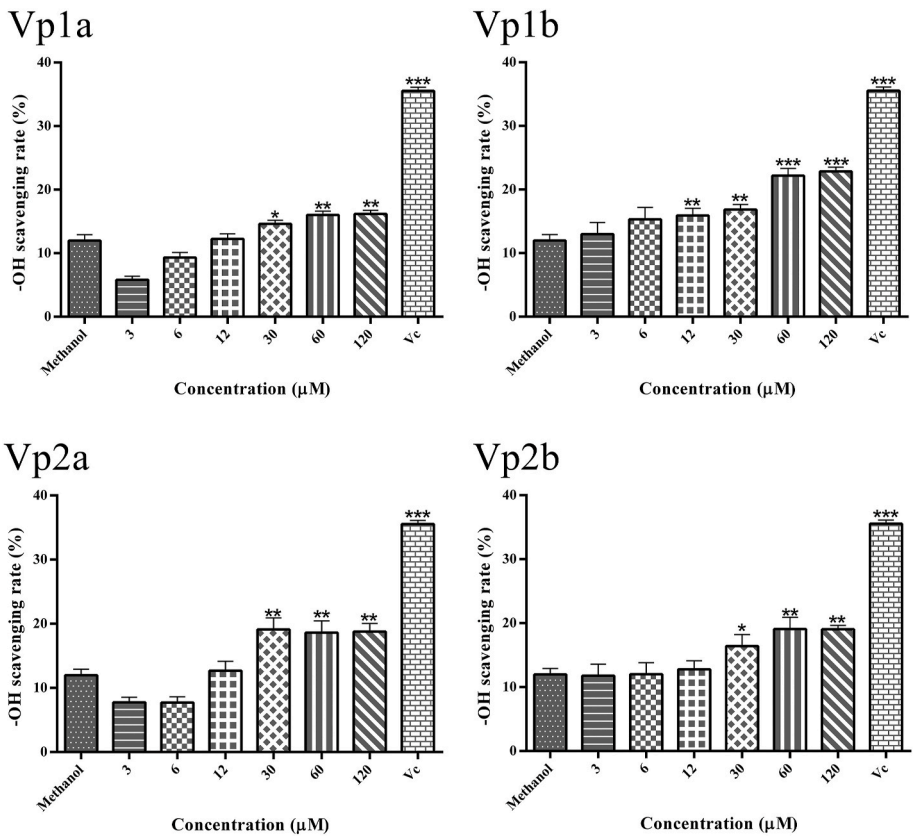

Fig. 4. •OH radical scavenging rates of four *V. velutina* venom peptides tested at different concentrations in methanol. Vc is vitamin C (120 μM). Data are mean ± SD of three independent experiments (n = 9). Statistical significance of differences was determined by *t*-test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significantly different from negative control, methanol was used as a negative control and compared with each group).

investigated their antimicrobial properties. Results showed that Vp1b and Vp2a significantly inhibited bacterial growth at concentrations above 60 μM, with the precise effect dependent on the bacterial species. Compared with other hornet venom AMPs (Xu et al., 2006; Yang et al., 2013), Vp1a, Vp2a, and Vp2b did not exhibit strong antibacterial effects, even at relatively high concentrations. Vp1b demonstrated good inhibitory effects against several experimental strains and therefore shows promise as a new natural antibiotic agent. However, Vp1b showed good antibacterial effect against bacteria such as *Staphylococcus aureus* at 120 μM, while VT1-7 (Yang et al., 2013), which are also members of the MP family, can achieve the same effect at concentration of about 3–60 μM. High concentration is a potential challenge for future clinical use of Vp1b. Additionally, the four new peptides had almost no inhibitory effect on *Enterococcus faecalis*, probably due to its good aggregation and fluidity, which made the external bacteria contact AMP first and die, while the internal bacteria were protected (Butler et al., 2010). As a common infection-causing bacterium, *Enterococcus faecalis* may not be suitable for AMP.

Our study adds to the growing list of AMPs identified from wasp venom that show good anti-microbial activity. For example, the VCP peptides, i.e., OdVP1, OdVP2, EpVP1, EpVP2a, and EpVP2b, from *Orancistrocerus drewseni* and *Eumenes pomiformis* venom (Baek and Lee, 2010; Baek et al., 2013), mastoparan AMPs from *Vespula lewisii* venom (Ji et al., 2011), and VesP-VB1 and MP-VB1 peptides from *Vespa bicolor* venom (Chen et al., 2008) all exhibit good anti-bacterial activity against various standard and drug-resistant strains at MIC of 5–200 μM.

There is growing interest in the antioxidant abilities of natural peptides, and therefore we also studied this property in the identified peptides. All four peptides demonstrated significant antioxidant effects at concentrations between 30 and 120 μM against the three different free radicals. All peptides showed good scavenging ability against $ABTS^{+}$, although all scavenging rates were lower than those achieved by the positive control (vitamin C). In addition, the scavenging rates of free radicals at 120 μM did not exceed 30%, and thus the antioxidant properties of our tested compounds were not high in comparison to other natural antioxidant peptides (Cao et al., 2018). However, our peptides may exhibit synergistic antioxidant activity when combined with other compounds, a potential avenue for future research. Moreover, as Vp1a, Vp1b, Vp2a, and Vp2b showed low hemolytic activity, they may be useful as safe antioxidant drugs. The combined activity of these compounds as antimicrobials and antioxidants warrants further study, particularly their synergic activity with other compounds and against antibiotic-resistant biofilms and microbes.

Due to the long-term overuse of antibiotics, bacterial resistance to drugs has become an urgent problem requiring the discovery and development of new antimicrobial agents (Kennedy, 2013). Given their good antimicrobial properties and low drug resistance, AMPs are excellent candidates for drug development (Nijnik and Hancock, 2009). Here, we identified four peptides from two families from the venom glands of *V. velutina*. Antimicrobial experiments indicated that several of these peptides showed good antibacterial effects, thus providing potential candidates for the design of effective antimicrobial agents.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2021.06.002.

Author contribution

Yichuan Meng: Investigation, Writing – original draft; Xianggui Mo: Investigation; Tiantian He: Investigation; Xinxin Wen: Software; James C Nieh: Writing – review & editing; Xinwang Yang: Supervision; Ken Tan: Resources.

Ethical statement

The animals are cared for and treated in accordance with the requirements of the Ethics Committee of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

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