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RESEARCH ARTICLE

A Potential Insect Antimicrobial of Black Soldier Fly Larvae (*Hermetia illucens*) against Pathogenic Bacteria

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ABSTRACT:

Antimicrobial activity of black soldier fly larvae extract against gram-positive and gram-negative pathogenic bacteria has been discovered. Water-soluble extracts have the most potential and effectiveness as antimicrobials, with MIC values ranging from 12.5 to 25 mg/mL for both gram-positive and gram-negative bacteria. BSF larvae also create peptides called defensins and cecropins, which have antibacterial properties. Defensins were the best and most effective peptides in inhibiting bacterial growth, inhibiting both gram-positive and gram-negative bacteria (MIC value = > 29.97 µM), while cecropins were more effective on gram-negative bacteria (MIC value= 0.52 – 2.07 µM) than gram-positive bacteria (MIC value not detected).

KEYWORDS: Insect antimicrobial, BSF Larvae, pathogenic bacteria.

INTRODUCTION:

Antibiotics have successfully prevented or treated infections, not only saving patients' lives, but also assisting in major medical and surgical advancements. By therapeutically modifying the outcome of bacterial infections, it helped to extend projected life spans. Despite efforts to improve antibiotics, bacteria continue to change in reaction to the medications they are exposed to, and many environmental species already have antimicrobial resistance[1]. Several reasons for the promotion of resistent bacteria include the overuse of antibiotics[2] and the use of inappropriately prescribed antibiotics[3]. Now, antibiotic resistance is becoming a global public health concern, as is the development of novel antibiotics, which is still in the research stage[4]. Several bacteria that are known to be resistant to antibiotic include MRSA (*Methicillin resistant staphylococcus aureus*)[5], Pathogenic ESBL (Extended Spectrum ß-lactamases) producing *E. coli*[6], and Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis[7].

Several research have examined the possibility of antibiotics made from natural substances as an alternative. This is due to the fact that antibiotics derived from materials can prevent resistance to antibiotics developed from chemicals. Tea leaves[8], *Bryonia seabra*[9], flowers extract from *Cassia auriculata*[10], *Allium Kurrat*, and *Eruca sativa*[11] are some of the natural substances that are commonly used as a source of novel antibiotics, while the antimicrobial from extract of insects are still limited.

Several researchers have been concerned about antimicrobial peptides (AMPs), which are considered natural anitbiotics. Several researchers have been studying the potential use of this chemical in a variety of fields, including the pharmaceutical business. In general, this chemical does not cause bacterial resistance to develop. AMPs are abundant in insects, and they have antibacterial activity by destroying the bacterial cell

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membrane. Gram-positive bacteria's peptidoglycan contains lipoteichoic and teichoic acids, while Gram-negative bacteria's lipopolysaccharide contains anionic phospholipid and phosphate. When a peptide attaches to the cytoplasmic membrane, it is incorporated into the bilayer phospholipid of the cytoplasmic membrane, causing the membrane structure of bacteria to change. The penetration of AMPs into a cell has the potential to influence nucleic acid and protein synthesis, which could explain their involvement in antibiotic-resistant bacteria[12]. Defensins, cecropins, attacins, and diptericins are the four main groups of insect AMPs, with defensins being the biggest. Many insect species from the orders Diptera, Hymenoptera, Coleoptera, Lepidoptera, Hemiptera, Isoptera, and Odonata have been found to have Defensins[13].

The black soldier fly (*Hermetia illucens*), a non-pest insect that lives in tropical places with mild temperatures that reduce the concentration of animal dung and other biological substances, is one of the Diptera order's species[14-16]. This species is also known as an ecological decomposer since it lives in areas with high concentrations of hazardous microbes including bacteria and fungi[17]. As a result, the insects' ability to protect themselves from dangerous microbial infections is critical to their survival. BSF larvae have also been found to express a variety of AMPs, including defensins, cecropins, attacins, and diptericins[18], and are capable of reducing the amount of bacteria in substrates such as *Escherichia coli*[19].

The purpose of this review is to discuss the potential use of AMPs of BSF larvae as an alternative to antibiotics for pathogenic bacteria. The discussion covers the knowledge of BSF larvae and its preparation for further examination, the biochemical contents, and the antimicrobial activities against pathogenic bacteria.

Black Soldier Fly (BSF) Larvae (*Hermetia illucens*)

Insect description:

Hermetia illucens, also known as the black soldier fly (BSF) larvae, is a member of the Order Diptera, Family Stratiomyidae, Genus Hermetia[20], and may be found in practically every temperate and tropical region of the world[21]. Adults are medium to large, measuring 15-20 mm long and slender, and resembling bees (wasplike)[22,23]. They simply require water to survive, having reserves of nutrients for reproduction accumulated during the larvae stage[24,25]. Female *H. illucens* are commonly observed ovipositing near organic waste, such as decomposing animal or plant waste[26]. The female *H. illucens* has a blue-black body, whilst the male's abdomen is brown. The tips of the legs are white in both sexes, and the wings are black and gray when resting[22]. The abdomen is extended at the top and narrows towards the bottom, with a translucent portion visible in the first two segments[27]. While the C vein does not entirely surround the wing, the wing venation is tightly packed towards the ribs and more pigmented than the rear[27].

Distribution of insect:

These insects are almost universally distributed, with tropical, subtropical, and temperate regions accounting for the majority of their distribution[28-30]. The first documented occurrences from Asia date from the 1940s (for example, Malaysia), but no data from China have been confirmed until 1960[30]. These insects have been found in Nepal, India, Sri Lanka, Thailand, Malaysia, Vietnam, Japan, Taiwan, Indonesia, and the Philippines, among other Asian countries. Hermetia illucens populations have been reported in Indonesia's Java, Sulawesi, and Papua provinces, but the species is now found practically everywhere in the country.

Larvae collection:

Egg traps composed of three layers of double-faced corrugated cardboard glued together and cut into 2.5 by 5-cm blocks can be used to gather larvae from the attack colony initially[31]. The egg trap was then placed 2-5cm above the wet Gainesville House Fly Diet, which was made up of 50% wheat bran, 30% alfalfa meal, and 20% maize meal, in a 5 liter plastic bucket[32]. A oviposition attractant or larval development medium was made by mixing equal parts of this dry media and water. H. illucens ovipositing on wet substrates was less appealing[31]. To enhance oviposition in the dry cardboard egg traps, water was added to the substance used as an oviposition attractant until it reached near saturation.

At 30°C and 80% humidity, eggs and larvae were kept in an insectery. Until the eggs hatched, they were placed in a 460-ml plastic cup (MC160 Sweetheart Cup, Chicago, IL) with a cover. In a 460-ml squat plastic cup, neonate larvae were fed 80g of moist Gainesville house fly meal. Fungus was considered to be the reason of low hatch and neonatal survival when eggs were placed on media. High humidity caused by a paper towel kept securely over the cup with a rubber band to contain wandering neonates resulted in fungal development. When larvae were placed on fresh media, fungus formation was infrequent[33].

Larvae process and extraction:

The following procedure was used to obtain BSF larvae extract. 1500 larvae were sterilized and stored at -80°C after being rinsed three times with sterile water. 100g of freeze-dried larvae The mixture was agitated for 12 hours at 4°C and then filtered through filter paper to remove the solid residues. To separate the solution layer containing the larval extract, the mixture was

centrifuged for 20 minutes at 13,000g. After that, a sterilized 0.45m membrane filter was used to filter the separated layer (Advantec MFS, Dublin, CA, USA). The material was kept at -20 degrees Celsius until needed[34].

Two-week-old H. illucens larvae were prepared. H. illucens larvae (600g) were initially rinsed with distilled water three times to remove all impurities. The larvae were immunized with probiotics Lactobacillus casei using a needle as previously described[35]. Small scissors were used to open the rear part of the larva one by one. Afterwards, 10µl of hemolymph from each larva was collected and a total of 2ml from 200 larvae were collected for use in the next step. The hemolymphs were fractionated into seven portions using C18 cartridges (Waters Co., USA), and subsequently evaporated under reduced pressure using a rotary evaporator at 50°C as indicated previously[36,37]. The seven column fractions were collected and each fraction was tested in vitro for antimicrobial effect before subjecting to further high performance liquid chromatography (HPLC) analysis.

The bacteria were incubated in 10 mL MH agar at 37°C and 30°C (*S. lutea* and *B. subtilis*) for 24 h. 10mL of this bacterial culture was inoculated into a fresh MH agar, which was incubated for 24 h. They were used for the experiment once the optical density (OD) 550 reached 0.5. Cell counts were determined by counting the colonies after Petri dish plates (Ø 87mm) were incubated at 37°C and 30°C for 24 h.

We investigated zone of growth inhibition of bacteria on the antibacterial effects of the extracts of *H. illucens* larvae. The respective bacteria were incubated in 10mL of MH agar at 37°C for 24 h, which were adjusted to a density of 2 x 10^6 bacteria/mL in fresh LB broth. Petri dishes with nutrient agar were inoculated with 40mL of bacterial culture (a final concentration of 2 x 10^6 bacteria/mL). The culture was evenly mixed and immediately distributed on Petri plates. Then, 45ml of the extract solution and antibiotics were added to each 6 mm disc, which were dried at room temperature for 30 min. The discs were placed onto the surface of agar plates and the agar plates were incubated at 37°C. Penicillinstreptomycin (10U/mL and 10mg/mL/disc) was used as the antibacterial positive control group. The diameters of bacterial inhibition zone surrounding the discs were measured in 12 and 24 h, respectively. The diameter of the zone of inhibition was measured in mm and the result was recorded. All experiments were performed in triplicate.

Biochemical Studies:

BSF larvae had 17.5% crude protein, 14% fat, 3.5 ash, 3 % acid detergent fiber, 0.8% nitrogen free extract, and 61.2% moisture, according to proximate analysis (Table 1). The amount of crude protein in BSF larvae is relatively significant. BSF larvae protein content varies based on the meal ingested during the growing period. Crude protein content in larvae increased immediately after hatching and then steadily dropped throughout the next 4–12 days of larval development, with a minimum concentration of 38% crude protein (CP) in the larval phase and a further increase of 39.2% in mature larvae on day 14. The concentration of CP increased steadily throughout the development phases, peaking at 46.2% CP in the early pupa stage. CP content was found to be the highest at the post-mortem adult stage (57.6%)[38].

BSF larvae are known to include a variety of minerals and vitamins, the most abundant of which are calcium 0.93% and choline 0.11% (Table 2). The fact that the outer layer of the larvae's epidermis releases a deposit of calcium carbonate (CaCO3), which may lead to the high calcium and ash content, could also explain such diversity[39,40]. Despite its prominence as a calcium-rich feeder insect, black soldier fly (BSF) larvae have received a lot of attention in the animal feed industry. However, there has been little research on non-production species like reptiles. Calcium serves a variety of functions in the human body. Cell signaling pathways, neurotransmitter release, muscle contractions, cardiac health, bone strength, enzyme function, and blood clotting are all impacted by it[41]. When calcium or phosphorus intake is either low or too high, the body attempts to maintain blood homeostasis by diverting or removing calcium that should be stored in bones. Bones become mushy or brittle as a result of this. Long bones are prone to pathological fractures, while the jaw bones deteriorate and become fibrous[41,42].

Lauric acid constituted a significant component among the SFAs (5.12%). Furthermore, linoleic acid (1.69%) and palmitic acid (1.61%) accounted for a significant portion of the fatty acids found in the larvae (14%). Lauric acid (C12:0) was found in significant concentrations (13–52%) in all larvae, but only in trace amounts (0–1%) in their meals. As noted by Spranghers *et al.*[43], this strongly suggests that it is synthesized by the larvae (2017). The larvae may also produce lauric acid from the carbohydrates in the substrate, according to those authors. This may explain why the largest quantities of lauric acid were seen in BSFL fed on bread (79% carbohydrates) (28% on dry matter basis). There was a positive association between larval weight and % age of lauric acid (C12:0) and total SFA in the larvae, indicating that these fatty acids accumulate the greatest as the larvae gain weight. BSFL at later phases of development had larger % ages of SFA and lauric acid[44]. It has been shown that BSFL differs from other insect larvae due to its high SFA concentration[45]. As adult BSF is not fed by the larvae, fatty acids are most likely a way

of store energy for this later stage of life[46]. The cause of energy storage under SFA in particular could be, as suggested by Ushakova et al.[47], because these fatty acids are less susceptible to oxidization.

Aspartic acid and glutamic acid were the most abundant amino acids in the larvae. BSFL protein hydrolysate amino acid composition has been analyzed with HPLC instruments. Glutamic acid was the most prevalent amino acid in BSFL hydrolysate (18.4%). Janssen et al.[48] and Müller et al.[49] reported the dominance of glutamic acid in BSFL. Also glutamic acid is the most prevalent amino acid in the prepupa of the BSF sample[50]. Glutamic acid is also the most prevalent amino acid with a composition of up to 19% from other insect species such as Tropical banded grickets (*Gryllodes sigillatus*)[51].

Table 1: Proximate Analysis, Mineral, and Vitamin Content of BSF Larvae[52]

Proximate analysis			**Mineral content**			**Vitamin Content**		
Composition	**Value**	**(%) per kg**	**Composition**	**Value**	**(%) per kg**	**Composition**	**Value**	**(%) per kg**
Moisture (g/kg)	612	61.20	Calcium (mg/kg)	9,340	0.93	Choline (mg/kg)	1,100	0.11
Crude Protein (g/kg)	175	17.50	Potassium (mg/kg)	4,530	0.45	Carnitine (mg/kg)	83.8	0.01
Crude Fat (g/kg)	140	14.00	Phosphorus (mg/kg)	3,560	0.36	Niacin (mg/kg)	71.0	0.01
Ash (g/kg)	35	3.50	Magnesium (mg/kg)	1,740	0.17	Pantothenic acid (mg/kg)	38.5	< 0.01
Acid Detergent Fiber (g/kg)	30	3.00	Chloride (mg/kg)	1,160	0.12	Riboflavin (mg/kg)	16.2	< 0.01
Nitrogen Free Extract (g/kg)	8	0.80	Sodium (mg/kg)	887	0.09	Vitamin C (mg/kg)	< 10.0	< 0.01
			Iron (mg/kg)	66.6	0.01	Thiamin (mg/kg)	7.7	< 0.01
			Manganese (mg/kg)	61.8	0,01	Vitamin E (mg α-tocopherol/kg)	6.2	< 0.01
			Zinc (mg/kg)	56.2	0.01	Pyridoxine (mg/kg)	6.01	< 0.01
			Copper (mg/kg)	4.03	< 0.01	Folic acid (mg/kg)	2.70	< 0.01
			Selenium (mg/kg)	0.32	< 0.01	Biotin (mg/kg)	0.35	< 0.01
			Iodine (mg/kg)	0.26	< 0.01	Vitamin A (µg retinol/kg)	< 300	< 0.01
						Vitamin B12 (µg/kg)	55.8	< 0.01
						Vitamin D3 (IU/kg)	100	< 0.01
						Vitamin D2 (IU/kg)	< 80	< 0.01

Table 2: Amino Acid and Fatty Acid Content of BSF Larvae[52]

Amino acid content			**Fatty acid content**		
Composition	**Value**	**(%) per kg**	**Composition**	**Value**	**(%) per kg**
Glutamic acid (g/kg)	19.7	1.97	Lauric 12:0 (g/kg)	51.2	5.12
Phenylalanine + tyrosine (g/kg)	19.66	1.97	Linoleic 18:2 (g/kg)	16.9	1.69
Aspartic acid (g/kg)	16.5	1.65	Palmitic 16:0 (g/kg)	16.1	1.61
Valine (g/kg)	12.9	1.29	Oleic 18:1 (g/kg)	15.6	1.56
Arginine (g/kg)	12.3	1.23	Myristic 14:0 (g/kg)	12.0	1.20
Alanine (g/kg)	12.2	1.22	Palmitoleic 16:1 (g/kg)	4.96	0.50
Leucine (g/kg)	12.1	1.21	Stearic 18:0 (g/kg)	2.45	0.25
Tyrosine (g/kg)	12.1	1.21	0.01Capric 10:0 (g/kg)	0.69	0.07
Lysine (g/kg)	11.9	1.19	Linolenic 18:3 (g/kg)	0.65	0.07
Proline (g/kg)	10.2	1.02	Myristoleic 14:1 (g/kg)	0.50	0.07
Glycine (g/kg)	9.14	0.91	Heptadecanoic 17:0 (g/kg)	0.20	0.05
Isoleucine (g/kg)	7.62	0.76	Arachidic 20:0 (g/kg)	0.16	0.02
Phenylalanine (g/kg)	7.56	0.76	Pentadecanoic 15:0 (g/kg)	0.12	0.01
Serine (g/kg)	7.02	0.70	Eicosadienoic 20:2 (g/kg)	< 0.10	< 0.01
Threonine (g/kg)	6.82	0.68	Benhenic 22:0 (g/kg)	0.09	< 0.01
Histidine (g/kg)	5.94	0.59	Eicosenoic 20:1 (g/kg)	< 0.08	< 0.01
Methionine + cystine (g/kg)	4.39	0.44	Arachidonic 20:4 (g/kg)	< 0.08	< 0.01
Methionine (g/kg)	3.37	0.34	Heptadecenoic 17:1 (g/kg)	< 0.08	< 0.01
Tryptophan (g/kg)	3.00	0.30			
Cystine (g/kg)	1.02	0.10			
Taurine (g/kg)	0.1	0.01			

Antimicrobial Activities:

Antimicrobial activities of several extract fraction of BSF Larva:

Several types of extracts from BSF larvae indicated antibacterial activity against both Gram positif and Gram negative bacteria at certain concentrations. The results of several studies showed that the water-soluble extract of BSF larvae was more potential compared to other types of extracts. This was indicated by the lower average of MIC value of the extract were in a similar range from 12.5 to 25mg/mL. Several results show that the water soluble fraction of the entire body extract had

considerably stronger antibacterial activity against MRSA and indicate the presence of more than one "antibacterial" product acting in synergy to enhance the action. Furthermore, the aqueous extract of *H. illucens* is highly solid, resilient to a number of freezing cycles and lyophilization and stable, as a frozen preparation, which all are key elements for the pharmaceutical development of a product. It was recently discovered that the presence and generation of a new class of tiny, maybe semi-peptidergic antimicrobial chemicals is not limited to the hemolymph or fat body, and that preparing a whole-body extract could be more efficient when attempting to isolate those compounds[17].

Furthermore, this sort of water-soluble extraction is highly recommended since the extraction method is simple, quick, and easy to use[53], and the protein content in the extraction is not destroyed but reduced by 17.2%. The decreased protein concentration is most likely due to the difficulty of extracting proteins from insects or the hydrophobicity of proteins, which are difficult to dissolve in aqueous buffer systems and remain bonded to the solid cellular components[54].

Table 3: Antimicrobial activities of several extract fraction of BSF Larvae against pathogenic bacteria

Bacteria Group	Type of extract	Bacteria tested	MIC	References
Gram-positive bacteria	Water-soluble extract	Methicillin-resistant *Staphylococcus aureus* (MRSA) † (clinically isolated)	25 mg/ml	[17]
		Staphylococcus aureus KCCM 40881	> 100 mg/ml	
		Staphylococcus aureus KCCM 35494	50 mg/ml	
		Staphylococcus epidermidis KCCM 35494	50 mg/ml	
		Kocuria rhizophila KCCM 11236	25 mg/ml	
		Micrococcus luteus KCCM 11326	25 mg/ml	
		Bacillus subtilis KCCM 11316	12.5 mg/ml	
	Methanol extract	*Bacillus subtilis* KCTC 1325	ND	[14]
		Streptococcus mutans KCTC 3289	ND	
		Sarcina lutea ATCC 9341	ND	
	Hexadenolic acid	MRSA	137.37 µg/ml	[55]
		Staphylococcus aureus (ATCC 25923	140.38 µg/ml	
	Ethyl acetate	*Staphylococcus epidermidis* KCCM 35494	25 mg/ml	[14]
		Staphylococcus aureus KCCM 40881	> 100 mg/ml	
		Bacillus subtilis KCCM 11316	25 mg/ml	
Gram-negative bacteria	Water-soluble extract	*Escherichia coli* KCCM 11234	12.5 mg/ml	[17]
		Enterococcus aerogenes KCCM 12177	25 mg/ml	
		Pseudomonas aeruginosa KCCM 11328	12.5 mg/ml	
	Methanol extract	*Klebsiella pneumoniae* KCTC 2253	44.74 mg/ml	[14]
		Neisseria gonorrhoeae	43.98 mg/ml	
		Shigella sonnei KCTC 2581	43.96 mg/ml	
		Escherichia coli	160 mg/ml	[56]
		Salmonella sp.	160 mg/ml	
		Salmonella typhimurium	325 mg/ml	[57]
		Pseudomonas aeruginosa	325 mg/ml	
	Hexadenolic acid	*Klebsiella pneumoniae* (ATCC 13883	139.12 µg/ml	[17]
		Shigella dysenteriae (ATCC 9750)	139.70 µg/ml	
		Klebsiella pneumoniae	1.25 mg/ 50µL	[36]
	Ethyl acetate	*Escherichia coli* KCCM 11234	> 100 mg/ml	[14]

Potential of Antimicrobial Peptides (AMPs) of BSF Larvae:

Due to their minimal toxicity to eukaryotic cells and broad spectrum of action against bacteria, mycobacteria, fungus, viruses, and cancer cells, AMPs are interesting prospects as alternatives to conventional antibiotics[58]. AMPs can kill bacteria in a variety of ways, including disrupting membranes, targeting intracellular components, and interfering with bacterial metabolism[59-61]. Moreover, most AMPs are cationic, with a positive net charge that promotes electrostatic interaction with bacterial membranes which are negatively charged[62]. Every living organism produces AMPs where the high biodiversity and extremely diverse living conditions make insects one of the richest sources. As it is possible to observe in other species like in Eristalis tenax, the H. illucens immune system is highly developed because this species feeds on decaying substrates and manure extremely rich in pathogenic microorganists. In fact, 20 AMPs of the Diptera E. tenax have been identified that have been able to adapt to different aquatic habitats with heavy microbial load (sewage tanks and manure pits)[63]. An essential part of immunological protection is AMPs which synthesize the fat body and hemocytes and then secrete into the hemolymph[64,65].

The defensins and cecropins of AMPs from BSF larvae extract have potential as antimicrobials against bacteria. Insect defensins can inhibit the growth of both gram-positive and gram-negative bacteria, but they are more

effective against gram-positive bacteria. In comparison, insect cecropins are more active and effective at inhibiting gram-negative bacteria development. The MIC value of insect defensis against gram-positive bacteria is in the range 0.01 – 14.99µM, while for gram-negative bacteria is about > 29.97µM. Six conserved cysteines generate three intramolecular disulfide bonds in the insect defensins, a class of tiny (4 kDa) cysteinerich cationic peptides. The insect defensin family shares a lot of similarities with mammalian defensins. An N-terminal loop is the most common structure of insect defensins[66]. By interacting with phospholipids, insect defensins target the cytoplasmic membrane of microbes, producing channels that cause membrane permeabilization and, ultimately, microbial cell death[66-68].

MIC values of insect cecropins from BSF larvae extract could not be detected in gram-positive bacteria, but only detected in gram-negative bacteria with MIC values of 0.52 – 2.07 µM. The tiny cationic cecropin peptides have antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as fungi[66]. The amidation of the C-terminus of the peptide, which is necessary for the interaction with liposomes of the attacking bacterium, has also been linked to cecropin antibacterial activity[69,70]. In a hydrophobic environment, cecropins are bactericidal as helical forms (e.g. the bacterial cell membrane or lipopolysaccharides). Cecropins are made up of an N-terminal amphipathic -helix and a C-terminal hydrophobic -helix joined by an intramolecular hinge region[71].

Table 4: Potential of Antimicrobial Peptides (AMPs) of BSF Larvae against Gram-positive Bacteria

Antimicrobial peptide		Antimicrobial peptide	MIC	Preference
Name	**Class**			
Defensin-like peptide 2 (DLP2)	Defensins	*Staphylococcus aureus* ATCC25923	0.01 µM	[72]
		Staphylococcus aureus ATCC43300	0.12 µM	
		Staphylococcus aureus ATCC6538	0.12 µM	
		Staphylococcus aureus CICC546	0.23 µM	
		Streptococcus suis CVCC606	0.93 µM	
		Listeria ivanovii ATCC19119	0.12 µM	
Defensin-like peptide 3 (DLP3)	Defensins	MRSA (methicillin-resistant *Staphylococcus aureus* → clinical isolated)	5 µg/mL	[73]
		Staphylococcus aureus KCCM 40881	5 µg/mL	
		Staphylococcus aureus KCCM 12256	10 µg/mL	
		Staphylococcus epidermidis KCCM 35494	10 µg/mL	
Defensin-like peptide 4 (DLP4)	Defensins	MRSA	0.59-1.17 µM	[34]
		Staphylococcus aureus KCCM 40881	0.59-1.17 µM	
		Staphylococcus aureus KCCM 12256	1.17-2.34 µM	
		Staphylococcus epidermidis KCCM 35494	0.59-1.17 µM	
		Bacillus subtilis KCCM 11316	0.02-0.04 µM	
		Staphylococcus aureus ATCC25923	0.01 µM	[72]
		Staphylococcus aureus ATCC43300	0.23 µM	
		Staphylococcus aureus ATCC6538	0.47 µM	
		Staphylococcus aureus CICC546	0.47 µM	
		Streptococcus suis CVCC606	1.88 µM	
		Listeria ivanovii ATCC19119	0.12 µM	
		Staphylococcus aureus CVCC 546	3.75 µM	[74]
		Staphylococcus epidermidis ATCC 12228	14.99 µM	
		Streptococcus pneumoniae CVCC 2350	7.50 µM	
		Streptococcus suis CVCC 3928	3.75 µM	
ID13	Defensins	*Staphylococcus aureus* CVCC 546	0.95 µM	[74]
		S. epidermidis ATCC 12228	1.91 µM	
		Streptococcus pneumoniae CVCC 2350	0.05 µM	
		Streptococcus suis CVCC 3928	0.95 µM	
Cecropin-like peptide 1 (CLP1)	Cecropins	*Staphylococcus aureus* KCCM 40881	ND	[75]
		Staphylococcus aureus KCCM 12256	ND	
		Staphylococcus epidermidis KCCM 35494	ND	
Trx-stomoxynZH1	Cecropins	*Staphylococcus aureus*	27-54 µg/ml	[76]

Table 5: Potential of Antimicrobial Peptides (AMPs) of BSF Larvae against Gram-negative Bacteria

Antimicrobial peptide		Antimicrobial peptide	MIC	Preference
Name	**Class**			
Defensin-like peptide 2 (DLP2)	Defensins	*Escherichia coli* CVCC1515	>29.97 µM	[72]
		Escherichia coli CICC21530 (serotype O157:H7)	>29.97 µM	
		Salmonella typhimurium ATCC14028	>29.97 µM	
		Salmonella enteritidis CMCC50336	>29.97 µM	
Defensin-like peptide 3 (DLP3)	Defensins	*Escherichia coli* KCCM 11234	10 µg/mL	[73]
		Pseudomonas aeruginosa KCCM 11328	40 µg/Ml	

Defensin-like peptide 4 (DLP4)	Defensins	*Escherichia coli* CVCC1515	>29.98 μM	[72]
		Escherichia coli CICC21530 (serotype O157:H7)	>29.98 μM	
		Salmonella typhimurium ATCC14028	>29.98 μM	
		Salmonella enteritidis CMCC50336	>29.98 μM	
		Escherichia coli ATCC 25922	>29.98 μM	[74]
		Escherichia coli K88	>29.98 μM	
		Salmonella pullorum CVCC 533	>29.98 μM	
		Salmonella enteritidis CVCC 3377	>29.98 μM	
ID13	Defensins	*Escherichia coli* ATCC 25922	> 30.50 μM	[74]
		Escherichia coli K88	> 30.50 μM	
		Salmonella pullorum CVCC 533	> 30.50 μM	
		Salmonella enteritidis CVCC 3377	> 30.50 μM	
Cecropin-like peptide 1 (CLP1)	Cecropins	*Escherichia coli* KCCM 11234	0.52–1.03 μM	[75]
		Enterobacter aerogenes KCCM 12177	1.03–2.07 μM	
		Pseudomonas aeruginosa KCCM 11328	1.03–2.07 μM	
Trx-stomoxynZH1	Cecropins	*Escherichia coli*	15-30μg/ml	[76]

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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