Received: 1 November 2023 | Revised: 4 April 2024 | Accepted: 27 April 2024

DOI: 10.1002/hsr2.2120

NARRATIVE REVIEW

Health Science Reports Open Access WILEY

Black soldier fly (*Hermetia illucens* L.): A potential small mighty giant in the field of cosmeceuticals

Ashley Sean Lai-Foenander[1] | Giva Kuppusamy[2] | Janaranjani Manogoran[2] | Tengfei Xu[3] | Yong Chen[3] | Siah Ying Tang[4] | Hooi-Leng Ser[5] | Yoon-Yen Yow[5] | Khang Wen Goh[6] | Long Chiau Ming[7] | Lay-Hong Chuah[1] | Wei-Hsum Yap[8,9] | Bey-Hing Goh[1,3,10,11]

[1]Biofunctional Molecule Exploratory Research Group, School of Pharmacy, Monash University Malaysia, Bandar Sunway, Malaysia

[2]Laboratory of Molecular Biology, Department of Research and Development, GK Aqua Sdn Bhd, Port Dickson, Negeri Sembilan, Malaysia

[3]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China

[4]Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia

[5]Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, Malaysia

[6]Faculty of Data Science and Information Technology, INTI International University, Nilai, Malaysia

[7]Department of Medical Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, Malaysia

[8]School of Biosciences, Taylor's University, Subang Jaya, Selangor, Malaysia

[9]Centre for Drug Discovery and Molecular Pharmacology (CDDMP), Faculty of Health and Medical Sciences (FHMS), Taylor's University, Subang Jaya, Selangor, Malaysia

[10]Sunway Biofunctional Molecules Discovery Centre (SBMDC), School of Medical and Life Sciences, Sunway University, Sunway, Malaysia

[11]Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, NSW, Australia

Correspondence
Bey-Hing Goh, Biofunctional Molecule Exploratory Research Group, School of Pharmacy, Monash University Malaysia, Bandar Sunway 47500, Malaysia.
Email: goh.bey.hing@monash.edu

Abstract

Background and Aims: Natural products are widely used in the pharmaceutical and cosmetics industries due to their high-value bioactive compounds, which make for "greener" and more environmentally friendly ingredients. These natural compounds are also considered a safer alternative to antibiotics, which may result in antibiotic resistance as well as unfavorable side effects. The development of cosmeceuticals, which combine the cosmetic and pharmaceutical fields to create skincare products with therapeutic value, has increased the demand for unique natural resources. The objective of this review is to discuss the biological properties of extracts derived from larvae of the black soldier fly (BSF; *Hermetia illucens*), the appropriate extraction methods, and the potential of this insect as a novel active ingredient in the formulation of new cosmeceutical products. This review also addresses the biological actions of compounds originating from the BSF, and the possible association between the diets of BSF larvae and their subsequent bioactive composition.

Methods: A literature search was conducted using PubMed and Google Scholar to identify and evaluate the various biological properties of the BSF.

Results: One such natural resource that may be useful in the cosmeceutical field is the BSF, a versatile insect with numerous potential applications due to its nutrient content and scavenging behavior. Previous research has also shown that the BSF has several biological properties, including antimicrobial, antioxidant, anti-inflammatory, and wound healing effects.

Conclusion: Given the range of biological activities and metabolites possessed by the BSF, this insect may have the cosmeceutical potential to treat a number of skin pathologies.

KEYWORDS

black soldier fly, cell culture, chemical analysis, cosmeceutical, microbiology, natural product

1 | INTRODUCTION

The use of natural products in disease treatment and in cosmetics has been ubiquitous throughout human history. Mesopotamian civilization possessed the earliest known records of natural products used to relieve ailments such as inflammation, colds, and coughs, utilizing oils from cypress (*Cupressus sempervirens*) and myrrh (*Commiphora* sp.); the Ancient Egyptians extracted henna from *Lawsonia inermis*, a flowering shrub tree, and used it to dye their hair, fingernails, and toenails; the Chinese have long been practising traditional medicine using natural products, its records carefully documented in the Chinese Materia Medica (Wu Shi Er Bing Fang), Shennong Herbal, and the Tang Herbal.[1,2] To this day, natural products remain an attractive commodity owing to their diverse range of bioactive compounds with unique chemical structures compared to their synthetic counterparts, generating opportunities for researchers to find novel candidates for the pharmaceutical and cosmetic industries.[2,3] On top of that, there is no denying the emerging overlap between both the pharmaceutical and cosmetic sectors, giving rise to the term "cosmeceutical" as coined by an American dermatologist, Albert Kligman, defined as cosmetic products that can be used for both cosmetic and therapeutic purposes.[4–6] Despite the lack of recognition of the term by the Federal Food, Drug, and Cosmetic Act (FD&C Act), the word "cosmeceutical" is being actively used in the personal care sector to describe beauty products whose natural ingredients provide a plethora of skin benefits such as promoting healthy and youthful skin as well as relieving the effects of photodamage by ultraviolet (UV) radiation.[5,6]

Due to the rising global concern about antibiotic resistance and side effects during antibiotic therapies, as well as the demand for the use of more "natural" raw materials to develop safer and more sustainable cosmeceutical products, it is no wonder that the world is turning to the Earth's most varied group of organisms as a natural resource: insects.[7–11] With more than two times the number of species compared to plants, insects are a reservoir of important bioactive compounds with various applications in the health sector.[12] One such promising insect species is *Hermetia illucens* Linnaeus, or the black soldier fly (BSF). Belonging to the order Dipterans and the *Stratiomyidae* family, the 20-mm-long insect is widely distributed in the tropics, subtropics, and temperate regions.[13,14] Its 45-day life cycle is comprised of four stages–egg, larva, pupa, and adult–whereby at the larval stage, they feed on a wide variety of decaying organic matter such as animal manure and vegetable waste.[9,15,16] Furthermore, BSF larvae have earned their place as an animal feed alternative to fish meal and fish oil due to their lipid- and protein-rich biomass.[17,18] The fatty acid profile of the BSF bears great resemblance to that of palm kernel oil and coconut oil, both of which are popular plant oils in skincare, with the most abundant shared component being lauric acid.[9,19,20] Lauric acid is a potent antimicrobial and is commonly used in cosmetic formulations as stabilizers and emulsifiers.[9] The insect also possesses a host of amino acids which can be incorporated into various cosmetic products, namely arginine, glycine, and proline, all of which play a role in moisturizing the skin.[9] To fully utilize the BSF as an active cosmeceutical ingredient, this insect's diverse biological properties and bioactive compounds should be explored further in the hopes of developing a multifaceted product that would tackle the various factors contributing to common skin pathologies. Hence, this review will discuss the antimicrobial, antioxidant, and anti-inflammatory properties of the various extracts derived from the BSF in past literature, identify the most appropriate extraction method used to process BSF biomass, as well as present the bioactive compounds that contribute to the aforementioned biological effects. Furthermore, the possible impact of nutrition on the bioactive composition of the BSF will also be addressed. Ultimately, the aim of this review is to establish a link between the biological properties of the BSF and potential cosmeceutical applications based on the existing literature. An overview depicting these applications of the BSF is shown in Figure 1.

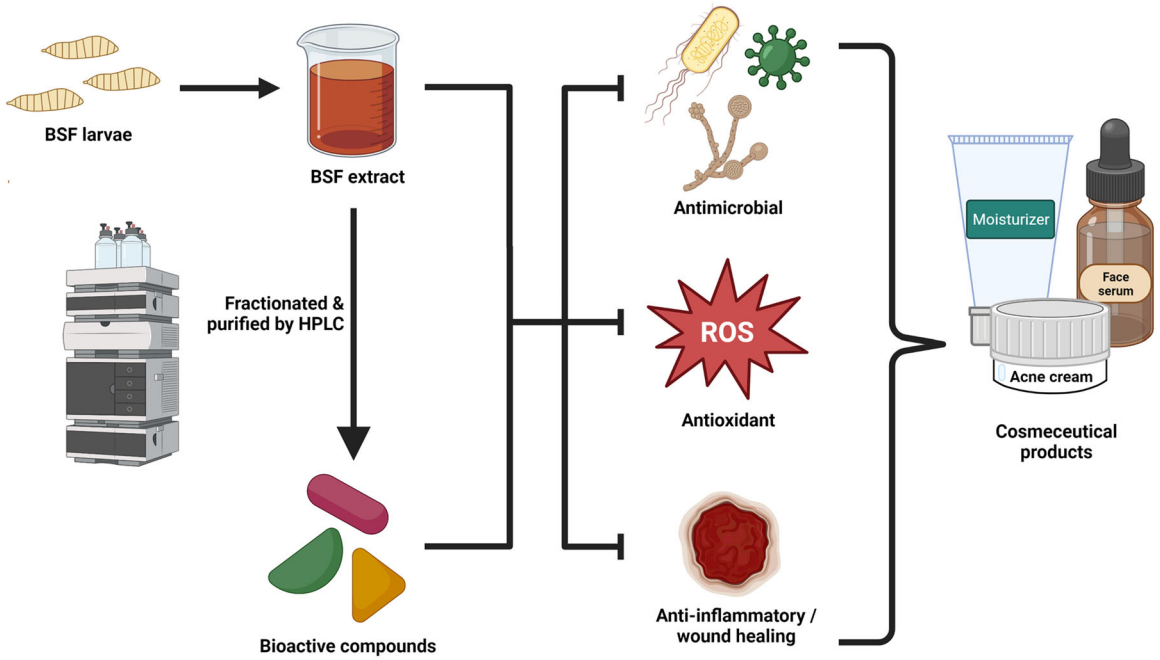

FIGURE 1 An overview summarizing the potential applications of black soldier fly (BSF) in cosmeceutical products. First, extracts and the desired bioactive compounds are derived from the insect using the appropriate solvents as well as fractionation and purification methods. The extract and/or bioactive compounds with promising therapeutic effects, such as antimicrobial, antioxidant, anti-inflammatory, and wound healing properties, may then be used as novel active ingredients in cosmeceutical products. (Created with BioRender. com).

2 | BSF AS A POTENT ANTIMICROBIAL

To date, there is more than one million described species of insects, making them the most diverse group of organisms in the world.[21] Their pervasiveness is owed to their high level of adaptability and broad-spectrum resistance against pathogens.[21] Due to the absence of an adaptive immune system that allows some organisms such as mammals to produce antibodies, insects have instead an innate immune system which comprises cellular and humoral immunity.[10,22] Cellular immunity encompasses phagocytosis, nodule formation, and encapsulation, while humoral immunity involves the production of proteins and peptides in the fat body that act against infection, and their secretion to the hemolymph.[10,22] The larvae of BSF are synonymous with scavengers, as they feed on animal waste, plant waste, and even carrion, and thus can survive in incredibly harsh environments rife with pathogenic microorganisms.[23] This unique behavior indicates that BSF larvae may be able to produce antimicrobial substances to combat infections by microorganisms present in manure and compost.[23] Previous studies have shown that BSF larvae managed to reduce the pathogen load in animal manure, for example in chicken manure, whereby *Escherichia coli* O157:H7 was reduced by 6 log CFU/g after 3 days and *Salmonella enterica* serotype Enteritidis was reduced by 4 log CFU/g after 2 days.[24] In addition, BSF larvae were also able to diminish the concentrations of *Salmonella* spp., thermotolerant coliforms, bacteriophage, and viruses in a mixture of pig manure, human feces, and dog food.[25]

2.1 | The antimicrobial capacity of whole BSF extracts

In the past decade, many solvents have been used to prepare BSF larvae extracts to study the insect's antimicrobial effects, such as methanol, chloroform, ethyl acetate, and even acetic acid.[10,23,26–30] Out of the various conventional extraction methods for natural products, only maceration and reflux extraction have been applied for the BSF. Maceration involved soaking the BSF larvae in a solvent at room temperature for a period of time, after which the resulting extract was filtered and concentrated.[26,28] Reflux extraction requires the introduction of heat by boiling the larvae.[10,30] Comparing the yields from different extraction methods, 306 g of extract was obtained from 1.5 kg of larvae after refluxing, bringing the yield to 20.4%; on the other hand, the yield of product from maceration was only 2% (12 g of extract from 600 g of larvae).[26,30] The higher yield achieved by refluxing compared to maceration was also observed in other publications.[31,32] This difference in yield may be due to the high temperatures during refluxing that increases the kinetic energy of solute molecules, thereby improving diffusion of the solute from the insect material to the solvent.[31,33] The presence of acetic acid in the aqueous solution used to reflux the BSF larvae may have also contributed to the greater yield.[10,30] A possible explanation for this is that acids may facilitate cell membrane disruption, thus smoothening the process of bioactive compound isolation.[34]

Upon reviewing the past literature on BSF extraction, it is evident that there is an association linking the extraction process with the antimicrobial activity of the resulting BSF extract. Extracts from

refluxing appear to be the stronger microbial inhibitors when compared to extracts produced from maceration. For example, the extract of *Lactobacillus casei*-immunized BSF larvae, prepared by refluxing the powdered larvae in 20% acetic acid solution, managed to inhibit the growth of both Gram-positive and -negative bacteria, that is *S. aureus* KCCM 40881, *E. coli* KCCM 11234, and *Salmonella* spp. at 1–2 mg/mL, unlike the methanol extract produced by maceration that could only inhibit Gram-negative bacteria.[10,26] Furthermore, at approximately 3 mg/mL, the refluxed extract has been shown to restrict the growth of the drug-resistant strains methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *P. aeruginosa* (MDRPA), as well as the AMP-resistant strains *Serratia marcescens* and *Pseudomonas tolaasii*.[30] Aside from antibacterial activity, this extract also possessed antifungal activity as it inhibited the growth of *Candida albicans* at an MIC value of 10 mg/mL.[30] These results are in contrast to those obtained with a BSF larvae extract prepared by maceration using acidified methanol (methanol/water/acetic acid; 90/9/1; v/v/v).[23] Despite being active against both Gram-positive and -negative bacteria, as well as against *C. albicans*, the activity of the extract was less intense, with the MIC against *E. coli* KCCM 11234 being 12.5 mg/mL and the MIC against both MRSA and *C. albicans* being 25 mg/mL.[23]

Based on the aforementioned advantages of refluxing over maceration (higher yields with higher antimicrobial activities) along with other benefits such as requiring a shorter extraction time and less solvent, refluxing should be considered for its potential application in the development of BSF-based cosmeceutical products that tackle pathogen-related skin disorders, especially if the practice were to be upscaled for commercialization.[35]

2.2 | Antimicrobial peptides (AMPs)

As mentioned before, the humoral immune response involves the production of AMPs, and the BSF possesses the largest AMP gene family among insects.[10,36] AMPs are small, cationic, evolutionarily conserved molecules of 10–100 amino acids that are found in all living organisms, including plants, bacteria, fungi, and insects.[21,37] Their most notable property is inhibitory activities against microorganisms such as bacteria, viruses, fungi, and even parasites, though they have also been found to have anticancer effects.[21,38] In response to the epidemic of antibiotic-resistant strains as a result of conventional antibiotic overuse, AMPs are gaining attention as potential alternatives due to their low production costs, antimicrobial activity against a wide variety of microorganisms, high heat tolerance, and low toxicity to eukaryotic cells.[7,10,39] In general, the mechanisms of action of AMPs can be categorized into two groups: firstly, the membranolytic mechanism, whereby pores are formed in the lipid membranes of the target microorganism, leading to membrane disruption and ultimately cell lysis, and secondly, the non-membranolytic mechanism, which involves the direct interaction between the AMP and the microbial proteins, DNA, and RNA.[21,40,41] Among insects, there are four families of AMPs, namely cysteine-rich peptides (e.g., defensins), α-helical peptides (e.g., cecropins), glycine-rich peptides, and proline-rich peptides.[21]

Transcriptomic analysis of larval and adult forms of the BSF revealed that the vast majority of identified putative AMPs belonged to the family of defensins.[21] These AMPs contain around 34–51 amino acids, are rich in cysteine, and inhibit the growth of mainly Gram-positive bacteria, but they have also shown activity against Gram-negative bacteria.[21] One such defensin successfully isolated from the BSF is defensin-like peptide 4 (DLP4).[42] This peptide was shown to exhibit inhibitory actions against Gram-positive bacteria *Bacillus subtilis* KCCM 11316, *S. aureus* KCCM 40881, *S. aureus* KCCM 12256, and *Staphylococcus epidermidis* KCCM 35494, with minimum inhibitory concentration (MIC) values of 0.1–0.2 μg/mL, 2.5–5 μg/mL, 5–10 μg/mL, and 2.5–5 μg/mL respectively.[42] However, when tested against Gram-negative bacteria, DLP4 did not show any antimicrobial effects.[42] On the other hand, another defensin-like peptide, DLP3, was reported to be a potent inhibitor with broad-spectrum activity, demonstrating antimicrobial effects against both Gram-positive and -negative bacteria.[37] Nevertheless, when comparing the strengths of both peptides, DLP4 appears to be the stronger inhibitor, as the MIC values of DLP3 against the same bacterial strains were relatively higher (5 μg/mL for *S. aureus* KCCM 40881, 10 μg/mL for *S. aureus* KCCM 12256, and 10 μg/mL for *S. epidermidis* KCCM 35494).[37,42]

Cecropins are α-helical AMPs that act against both Gram-positive and Gram-negative bacteria.[21,41,43] Cecropin-like peptide 1 (CLP1), isolated from BSF larvae, restricted the growth of Gram-negative bacteria *E. coli* KCCM 11234, *Pseudomonas aeruginosa* KCCM 11328, and *Enterobacter aerogenes* KCCM 12177 with MIC values of 2.5–5 μg/mL, 5–10 μg/mL, and 5–10 μg/mL respectively.[43] The same minimum bactericidal concentration (MBC) values were also achieved. Despite cecropins typically being active against both Gram-positive and -negative bacteria, CLP1 did not show any effect against Gram-positive bacteria.[43] Interestingly, when comparing CLP1 with DLP3 against the same panel of Gram-negative bacteria, CLP1 seems to be more potent, as DLP3 only managed to inhibit *E. coli* KCCM 11234 and *P. aeruginosa* KCCM 11328 at 10 μg/mL and 40 μg/mL respectively.[37,43]

2.3 | The antimicrobial capacity of the BSF against multidrug-resistant pathogens

The rapid emergence of various multidrug-resistant bacterial strains have made the search for novel drug candidates ever more dire.[26] MRSA is a significant contributor to drug resistance in dermatologic diseases, often presenting as painful red bumps in infected skin or, in more serious cases, causing diseases such as staphylococcal scalded skin syndrome in infants.[44–46] Due to acquiring the gene *mecA* which codes for penicillin-binding protein 2a (PBP2a), MRSA is resistant to β-lactam antibiotics such as methicillin and ampicillin, since PBP2a reduces the organism's affinity to bind to this class of antibiotics.[47–49] For decades, vancomycin has been the antibiotic of choice for MRSA;

however, the discovery of vancomycin-resistant *S. aureus* has urged researchers to turn to alternative methods for the treatment of MRSA infections.[50]

It is worth noting that DLP4 and DLP3 were both capable of inhibiting the growth of MRSA, with an MIC value of 2.5 to 5 μg/mL for DLP4 and 5 μg/mL for DLP3, while the MICs of positive controls methicillin and ampicillin were undetected.[37,42] So far, only DLP4 has been extensively studied for its mechanism of action against MRSA, which was reported to involve interference with the bacterial plasma membrane, cell cycle, and macromolecular synthesis.[51] It was speculated that DLP4 disrupts the plasma membrane through pore formation due to the presence of the charged residues Asp4 and Arg23.[51] Similar to vancomycin and ciprofloxacin, DLP4 may act as an inhibitor of cell wall, DNA, and RNA synthesis in MRSA.[51] When comparing the postantibiotic effects (PAEs) of this peptide with that of standard antibiotics, DLP4 displayed a greater PAE than vancomycin.[51] No resistance was observed in MRSA against DLP4 even after 30 serial passages, as opposed to ciprofloxacin whose MIC increased four-fold.[51] In light of these results, besides its antimicrobial actions against conventional bacterial species as detailed in Table 1, it is clear that the BSF possesses compounds such as DLP4 with antagonistic effects against multidrug-resistant bacteria, making this insect an invaluable resource of potential drug candidates capable of treating recalcitrant skin diseases.

2.4 | The impact of nutrition on the BSF immune system and subsequent antimicrobial properties

Nutritional immunology is a multidisciplinary field that investigates the relationship between dietary components and the intestinal immune system.[55] A well-nourished body would possess a robust immune system while malnutrition or unhealthy diets would render the immune system less capable of effectively combatting infections, as a result of the influence of nutrition on constitutive and inducible immune responses.[27,56] In the context of the BSF, this association between nutrition and immunity may be relevant in developing a better understanding of the mechanisms underlying the insect's antimicrobial capacity. As mentioned before, BSF larvae derive their dietary needs from pathogen-rich organic waste, and this ability to survive on harmful feedstuff is possibly owed to the AMPs and other antimicrobial substances within their biomass.[23] It is also likely that the larvae retained certain secondary metabolites, especially from plants, through sequestration, which is the accumulation of plant defense compounds simply by ingesting the plant material to increase fitness.[57] Sequestration among herbivorous insects is common, and the phytocompounds usually taken up by these insects include glucosinolates, salicinoids, and cyanogenic glycosides.[57,58] Although there has not been any research conducted on the sequestration of such metabolites by BSF larvae, it is indeed an exciting area to explore when considering the influence of nutrition on the antimicrobial capacity of the BSF.

Given that nutrition impacts the expression of immune-related genes, and therefore potentially affects AMP expression and subsequent antimicrobial properties, it was hypothesized that the diet-dependent expression of AMPs in the BSF adapts both the BSF gut microbiota and environmental microbiota (e.g., found in their diets), to enable the larvae to digest and survive on pathogen-rich substrates.[27] In a study by Vogel et al. investigating the effect of different diets on the antimicrobial potential of BSF larvae, the larvae extract obtained using chloroform was found to be active against both Gram-positive and -negative bacteria, namely *B. subtilis*, *Micrococcus luteus*, *E. coli* BL21 (DE3), and *Pseudomonas fluorescens* BL915.[27] In contrast, the chloroform extract prepared and tested by Park et al. showed no activity against a variety of Gram-positive and -negative bacteria, including *B. subtilis*, *M. luteus*, and *E. coli*.[23] Based on the aforementioned hypothesis regarding nutritional immunology, the diets of the BSF larvae may have been responsible for the inconsistent antimicrobial effects exerted by the chloroform extracts observed by Park et al. and Vogel et al., given that different immune-related genes may have been expressed in each case based on diet.[23,27] However, to declare that the two studies each fed their BSF larvae different diets would be considered speculation as Park et al. did not reveal the type of substrates used to feed their BSF larvae.[23] With regard to the bioactive compounds within certain diets that may have immunomodulatory effects on the BSF larvae, using the study by Vogel et al. as an example, it was found that brewer's grains and sunflower oil both generated strong immune responses in the larvae.[27] The protein-rich brewer's grains may have contributed to the production of AMPs in the BSF larvae, though the level of AMP expression did not correlate with the antimicrobial activity, most likely due to the reduced accuracy of transcriptome de novo analysis used by Vogel et al. compared to reference genome-based analysis.[27,59] The immune-regulating properties of sunflower oil may be owed to its rich sitosterol content, as sitosterol has previously shown antibacterial effects in several studies.[60–62] Overall, nutrition plays a significant role in BSF immunomodulation, which is reflected in the diet-dependent antimicrobial capacities of its larvae extracts that are composed of an array of high-value compounds. Understanding the nutritional immunology of the BSF would help researchers make informed decisions on the organism's dietary needs to express the desired bioactive compounds.

3 | A PROTECTIVE AGENT AGAINST OXIDATIVE STRESS AND INFLAMMATION

Aside from its antimicrobial effects, the BSF has also been reported to possess scavenging abilities against reactive oxygen species (ROS), as seen in Table 2. An extract of BSF larvae was tested for its antioxidant capacity following induction by UV irradiation (257.3 nm, 8 W) at a distance of 40 cm for varying durations, that is, 10 min, 20 min, and 30 min.[29] Compared to the Nonirradiated BSF larvae, the extract derived from UV-treated larvae displayed enhanced DPPH, ABTS, and hydroxyl radical scavenging activities.[29] These data

TABLE 1 Antimicrobial activity of the different extracts and bioactive compounds from the BSF against various microbial strains.

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
Hexane extract; chloroform extract; ethanol extract; methanol extract; aqueous extract	—	(A) 600 g of larvae were extracted with 6 L of n-hexane, chloroform, ethanol, methanol and distilled water for 24 h at room temperature. (B) The extracts were filtered using filter paper and vacuum pump, then evaporated under reduced pressure at 40°C with a rotary evaporator.	(A) Agar disk diffusion assay. (B) Turbidometric assay (TB).	Gram-positive: *B. subtilis* KCTC1325, *S. mutans* KCTC3289, *Sarcina lutea* ATCC9341 Gram-negative: *K. pneumoniae* KCTC2253, *S. sonnei* KCTC2581, *N. gonorrhoeae*	(A) Methanol extract showed highest antibacterial activity; inhibited Gram-negative bacteria only. (B) Agar disk diffusion assay (inhibition zones; methanol extract): (i) 20 mg/mL: 8.51 ± 0.48 mm (*K. pneumoniae*, *N. gonorrhoeae*, *S. sonnei*); (ii) 40 mg/mL: 10.46 ± 0.52 mm (*K. pneumoniae*, *N. gonorrhoeae*, *S. sonnei*); (iii) 80 mg/mL: 12.35 ± 0.48 mm (*K. pneumoniae*, *N. gonorrhoeae*, *S. sonnei*); (iv) Antibacterial activity decreased after 24 h; (v) Overall, higher antibacterial activity against *S. sonnei* and *N. gonorrhoeae* than against *K. pneumoniae*. (C) TB (IC_{50}; methanol extract): (i) Methanol extract showed strong inhibition from 20 mg/mL; (ii) Antibacterial activity was highest at 12 h and decreased after 24 h; (iii) At 12 h: • *K. pneumoniae*: 22.37 mg/mL; • *N. gonorrhoeae*: 21.99 mg/mL; • *S. sonnei*: 21.98 mg/mL.	[26]
Hexanedioic acid	–	(A) Larvae were extracted (solvent not mentioned) for 24 h at room temperature. (B) The extracts were filtered using filter paper and vacuum pump, then evaporated under reduced pressure at 40°C with a rotary evaporator. (C) Extracts were purified to obtain pure hexanedioic acid.	(A) In vitro: Spread plate method. (B) In vivo: Female BALB/c mice.	*K. pneumoniae* KCTC2253	(A) In vitro: (i) 1.25 or 12.5 mg/50 µL: Induced *K. pneumoniae* death. (ii) 0.75 mg/50 µL: Significant reduction. (B) In vivo: (i) 1.25 mg of hexanedioic acid provided protection to mice against *K. pneumoniae* infection.	[52]

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
					(ii) Body weight changes: • Significantly reduced weight loss in mice at 2 days p.i. (iii) Lung bacterial load: • 1.25 mg of hexanedioic acid completely inhibited *K. pneumoniae* in mice lungs (iv) Hexanedioic acid administered orally produced greater effect than control (ampicillin and streptomycin).	
(A) Extracts*: Acidified methanol extract; chloroform extract; ethyl acetate extract. (B) Antimicrobial peptide (AMP)*: Anti-MRSA substances. *Derived from larvae immunized with *S. aureus* at OD_{600} = 2.4.	(A) Temperature: 32°C. (B) Humidity: 62%. (C) Light conditions: 24 h dark cycle.	(A) Immunized larvae were lyophylized, ground, and extracted with methanol/water/acetic acid (90/9/1 v/v/v). (B) The acidified methanol extract was further fractionated and purified to give seven fractions and anti-MRSA substances. (C) Proteins and lipids were removed through sequential extraction using chloroform and ethyl acetate.	(A) MIC. (B) Inhibition zone assay.	Gram-positive: MRSA, *S. aureus* KCCM40881, *S. aureus* KCCM12256, *B. subtilis* KCCM11316, *Kocuria rhizophila* KCCM11236, *M. luteus* KCCM11326, *S. epidermidis* KCCM35494 Gram-negative: *E. coli* KCCM11234, *E. aerogenes* KCCM12177, *P. aeruginosa* KCCM11328 Fungi: *C. albicans* KCCM11282	(A) MIC (whole extracts): (i) Acidified methanol extract. • MRSA: 25 mg/mL; • *S. aureus* KCCM40881: >100 mg/mL; • *S. aureus* KCCM12256: 100 mg/mL; • *S. epidermidis*: 50 mg/mL; • *K. rhizophila*: 25 mg/mL; • *M. luteus*: 25 mg/mL; • *B. subtilis*: 12.5 mg/mL; • *E. coli*: 12.5 mg/mL; • *E. aerogenes*: 25 mg/mL; • *P. aeruginosa*: 12.5 mg/mL; • *C. albicans*: 25 mg/mL. (ii) Ethyl acetate extract: • MRSA: Not tested (NT); • *S. aureus* KCCM40881: >100 mg/mL; • *S. aureus* KCCM12256: NT; • *S. epidermidis*: 25 mg/mL; • *K. rhizophila*: NT; • *M. luteus*: NT; • *B. subtilis*: 25 mg/mL; • *E. coli*: >100 mg/mL; • *E. aerogenes*: NT; • *P. aeruginosa*: 25 mg/mL; • *C. albicans*: 50 mg/mL. (iii) Chloroform extract showed no antimicrobial activity.	[23]

(Continues)

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
					(B) Inhibition zone assay (acidified methanol fractions and anti-MRSA substances): (i) Fractions did not display any activity against *E. coli*, but displayed strong activity against *B. subtilis*, with the largest inhibitory zone for 30% acetonitrile fraction. (ii) Fractions 48, 61, and 78 gave large inhibitory zones against MRSA, while fractions 50 – 74 gave smaller zones.	
AMP*: DLP4 *Derived from larvae immunized with *S. aureus* at OD_{600} = 2.4.	(A) Temperature: 32°C. (B) Humidity: 62%.	(A) Immunized hemolymph was collected in ice-cold tubes containing phenylthiourea crystals, then centrifuged at 12,000 × *g* for 10 min for cell debris and hemocyte removal. (B) Hemolymph was fractionated and purified to obtain pure DLP4.	MIC.	Gram-positive: MRSA, *S. aureus* (KCCM40881, KCCM12256), *B. subtilis* KCCM11316, *S. epidermidis* KCCM35494 Gram-negative: *E. coli* KCCM11234, *E. aerogenes* KCCM12177, *P. aeruginosa* KCCM11328	(A) DLP4 showed high inhibitory activity against Gram-positive bacteria, but not Gram-negative bacteria. (B) DLP4 displayed lower MIC values against MRSA than the controls methicillin and ampicillin. (C) MIC: (i) MRSA: 0.59–1.17 μM; (ii) *S. aureus* KCCM40881: 0.59–1.17 μM; (iii) *S. aureus* KCCM12256: 1.17–2.34 μM; (iv) *S. epidermidis*: 0.59–1.17 μM; (v) *B. subtilis*: 0.02–0.04 μM.	[42]
AMP*: Recombinant Trx-stomoxynZH1 *Derived from larvae immunized with *S. aureus* and *E. coli* at OD_{600} = 1.5.	(A) Temperature: 28°C. (B) Humidity: 65%. (C) Diet: Artificial diet.	(A) Plasmid coding Trx-stomoxynZH1was transformed into *E. coli* BL21 (DE3) cells. (B) At exponential phase (OD600 = 0.6), cells were induced with IPTG, harvested by centrifugation, and lysed by ultrasonication on ice. (C) Supernatant was collected by centrifugation and dialyzed against phosphate-buffered saline (PBS).	(A) Inhibition zone assay. (B) MIC.	Bacteria: *E. coli*, *S. aureus* Fungi: *Rhizoctonia solani* Khün (rice)-10, *Sclerotinia sclerotiorum* (Lib.) de Bary-14.	(A) Inhibition zone assay: Trx-stomoxynZH1 exhibited strong activity against all bacterial and fungal strains. (B) MIC: (i) *E. coli*: 15–30 μg/mL; (ii) *S. aureus*: 27–54 μg/mL; (iii) *R. solani* Khün (rice)-10: >98 μg/mL; (iv) *S. sclerotiorum* (Lib.) de Bary-14: >98 μg/mL.	[39]

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
		(D) Target protein was eluted using nickel ion metal chelating affinity chromatography with gradient concentration of imidazole.				
AMP*: DLP3 *Derived from larvae immunized with *S. aureus* at OD_{600} = 2.4.	(A) Temperature: 32°C. (B) Humidity: 62%.	(A) Immunized hemolymph was collected in ice-cold tubes containing phenylthiourea crystals, then centrifuged at 12,000 × g for 10 min for cell debris and hemocyte removal. (B) Hemolymph was fractionated and purified to obtain pure DLP3.	MIC.	Gram-positive: MRSA, *S. aureus* (KCCM40881, KCCM12256), *S. epidermidis* KCCM35494 Gram-negative: *E. coli* KCCM11234, *P. aeruginosa* KCCM11328	(A) DLP3 showed potent inhibitory activity against both Gram-positive and Gram-negative bacteria. (B) MIC: (i) MRSA: 5 μg/mL; (ii) *S. aureus* KCCM40881: 5 μg/mL; (iii) *S. aureus* KCCM12256: 10 μg/mL; (iv) *S. epidermidis*: 10 μg/mL; (v) *E. coli*: 10 μg/mL; (vi) *P. aeruginosa*: 40 μg/mL.	[37]
AMP*: CLP1 *Derived from larvae immunized with *S. aureus* at OD_{600} = 2.4.	(A) Temperature: 32°C. (B) Humidity: 62%.	(A) Immunized hemolymph was collected in ice-cold tubes, then centrifuged at 10,000 rpm for 5 min at 4°C. (B) Hemolymph was fractionated and purified to obtain pure CLP1.	(A) MIC. (B) MBC.	Gram-positive: MRSA, *S. aureus* (KCCM40881, KCCM12256), *S. epidermidis* KCCM35494 Gram-negative: *E. coli* KCCM11234, *E. aerogenes* KCCM12177, *P. aeruginosa* KCCM11328	(A) CLP1 showed antimicrobial activity against Gram-negative bacteria but not against Gram-positive bacteria. (B) Antibacterial activity of CLP1: (i) *E. coli*: 0.52–1.03 μM (MIC); 0.52–1.03 μM (MBC); (ii) *E. aerogenes*: 1.03–2.07 μM (MIC); 1.03–2.07 μM (MBC); (iii) *P. aeruginosa*: 1.03–2.07 μM (MIC); 1.03–2.07 μM (MBC). (C) CLP1 showed activity against *E. coli* 50-fold greater than the control antibiotic ampicillin.	[43]
Acidified methanol extract; chloroform extract; ethyl acetate extract	(A) Temperature: 24°C. (B) Diet: 8 different diets to assess the impact of nutrition on the antibacterial activity of different BSF larvae extracts.	(A) Larvae were freeze-thawed at −20°C, then thoroughly ground in methanol/water/acetic acid (90/9/1 v/v/v). (B) The extract was centrifuged at 1600 × *g* for 20 min at 4°C and concentrated under reduced pressure using a rotary evaporator.	Inhibition zone assay.	Gram-positive: *M. luteus*, *B. subtilis* Gram-negative: *E. coli* BL21 (DE3), *P. fluorescens* BL915	(A) Antibacterial activity of extracts was diet-dependent. (B) Acidified methanol extract of larvae reared on brewer's grains and cellulose: Strongly inhibited Gram-negative bacteria. (C) Acidified methanol extract of larvae reared on chitin, cellulose, bacteria, and plant oil: Strongly inhibited Gram-positive bacteria.	[27]

(Continues)

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
		(C) Proteins and lipids were extracted using sequential extraction with chloroform and ethyl acetate. (D) All three extracts were then concentrated under reduced pressure using a rotary evaporator.			(D) Chloroform extract of larvae reared on lignin, bacteria, and plant oil: Inhibited all tested bacterial species. (E) Overall, acidified methanol extract displayed higher inhibition compared to chloroform extract. (F) Data on ethyl acetate extract not reported.	
DMSO-treated methanol extract (10, 20, 40, 80, 160, 320 mg/mL)	–	(A) 15-day-old larvae were harvested, oven-dried at 65°C for 24 h, then ground. (B) Ground larvae were extracted with methanol (1:10 b/v) at room temperature for 24 h. (C) The extracts were filtered using filter paper and a vacuum pump, then concentrated under reduced pressure with a rotary evaporator at 40°C.	Inhibition zone assay.	*E. coli*, *Salmonella* spp.	(A) Methanol extract showed dose-dependent antibacterial activity. (B) Anti-*E. coli* activity (inhibition zones): (i) 10 mg/mL = 0 mm; (ii) 20 mg/mL = 0 mm; (iii) 40 mg/mL = 0 mm; (iv) 80 mg/mL = 0 mm; (v) 160 mg/mL = 4.67 mm; (vi) 320 mg/mL = 6.00 mm. (C) Anti-*Salmonella* spp. activity (inhibition zones): (i) 10 mg/mL = 0 mm; (ii) 20 mg/mL = 0 mm; (iii) 40 mg/mL = 0 mm; (iv) 80 mg/mL = 0 mm; (v) 160 mg/mL = 4.33 mm; (vi) 320 mg/mL = 6.33 mm.	[28]
AMP*: rHI-attacin *Derived from larvae immunized with *E. coli* at OD_{600} = 2.4.	(A) Temperature: 32°C. (B) Humidity: 62%.	—	Inhibition zone assay.	*E. coli* KCCM11234, MRSA KCCM40881	(A) Cells transformed with recombinant protein rHI-attacin showed inhibitory activity against *E. coli* and MRSA. (B) Stronger activity was observed against *E. coli* compared to MRSA.	[22]
AMP*: Peptide HP/F9 *Derived from larvae immunized with *L. casei*.	–	(A) Small scissors were used to cut open the rear part of 600 g of immunized larvae to collect 10 µL of hemolymph from each larva. (B) Hemolymph was fractionated and purified to obtain pure peptide HP/F9.	(A) In vitro: Spread plate method. (B) In vivo: Female BALB/c mice.	*K. pneumoniae* ATCC13883	(A) In vitro: (i) HP/F9 inhibited the growth of *K. pneumoniae* (dose-dependent). (ii) 1.2 µg/50 µL: Significant reduction. (iii) 6 or 12 µg/50 µL: Complete inhibition.	[53]

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
					(B) In vivo: (i) Body weight changes: • 1.2, 6, and 15 μg of HP/F9 reduced weight loss in mice at 2 days p.i. (ii) Mice lung *K. pneumoniae* load: • 6 and 15 μg: Complete inhibition. (iii) Histopathology: • Both lungs and kidneys of mice treated with HP/F9 showed no signs of inflammation induced by *K. pneumoniae*.	
Extract of *Lactobacillus*-infected *H. illucens* larvae* *Derived from larvae immunized with various *Lactobacillus* species at various concentrations.	(A) Temperature: 26 ± 1°C. (B) Humidity: 60%.	(A) Immunized larvae were dried for 45 min using a microwave, then ground to a powder. (B) Powder was suspended in 20% acetic acid solution and boiled for 30 min. (C) The mixture was centrifuged at 4500 rpm for 30 min at 4°C, and the supernatant dried in a vacuum-spin drier for 9 h before being resuspended in sterilized distilled water as an extract.	MIC.	Gram-positive: *S. aureus* KCCM40881 Gram-negative: *E. coli* KCCM11234, *Salmonella pullorum* KVCC-BA0702509, *Salmonella typhimurium* KCCM40406, *S. enteritidis* KCCM12021	MIC: • *E. coli*: 100–200 μg/100 μL; • *S. aureus*: 100–200 μg/100 μL; • *Salmonella* spp.: 100–200 μg/100 μL.	[10]
Extract of *L. casei*-infected *H. illucens* larvae	(A) Temperature: 26 ± 1°C. (B) Humidity: 60%.	(A) 4.5 kg of larvae were dried for 45 min using a microwave. (B) 1.5 kg of dried larvae was ground to a powder, then suspended in 20% acetic acid solution, boiled for 30 min, and centrifuged at 4500 rpm for 30 min at 4°C. (C) Supernatant was dried in a vacuum-spin drier for 9 h, then dissolved in sterilized distilled water to produce 50 mg/mL extract.	(A) RDA. (B) MIC.	Gram-positive: *E. faecalis* KACC11859, *S. mutans* KACC16833, MRSA Gram-negative: *E. coli* KCCM11234, *S. pullorum* KVCC-BA0702509, *S. typhimurium* KCCM40406, *S. enteritidis* KCCM12021, *S. marcescens* KACC11961, *P. tolaasii* KACC15293, multidrug-resistant *P. aeruginosa* (MDRPA) Fungi: *C. albicans* KACC30071	(A) Antibacterial activity (MIC): (i) *E. faecalis*: 200 μg/100 μL; (ii) *S. mutans*: 500 μg/100 μL; (iii) *E. coli*: ~ 200 μg/100 μL; (iv) *S. aureus*: ~ 200 μg/100 μL; (v) *Salmonella* spp.: ~ 200 μg/100 μL; (vi) MRSA: ~ 300 μg/100 μL; (vii) MDRPA: ~ 300 μg/100 μL; (viii) *S. marcescens* (AMP-resistant): ~ 300 μg/100 μL; (ix) *P. tolaasii* (AMP-resistant): ~ 300 μg/100 μL. (B) Antifungal activity (MIC): (i) *C. albicans*: 1000 μg/100 μL.	[30]

(Continues)

TABLE 1 (Continued)

Extract/bioactive compound	Rearing method	Extraction method	Antimicrobial assay	Microbial strains	Antimicrobial activity	References
Fusion protein: TRX-Hidefensin1-1	(A) Temperature: 28–30°C. (B) Humidity: 60%–70%. (C) Diet: Artificial diet.	–	(A) Oxford cup method. (B) MIC.	Gram-positive: *S. aureus* Gram-negative: *E. coli*, *Salmonella* spp.	(A) Significant antimicrobial effects against *S. aureus* and *E. coli*, but less active against *Salmonella* spp. (B) Oxford cup method (inhibition zone; detection limit = 8 mm): (i) *S. aureus*: 15.33 ± 0.06 mm; (ii) *E. coli*: 14.56 ± 0.04 mm; (iii) *Salmonella* spp.: 8.03 ± 0.01 mm. (C) MIC (μg/mL): (i) *S. aureus*: 15–30 μg/mL; (ii) *E. coli*: 30–60 μg/mL; (iii) *Salmonella* spp.: >100 μg/mL.	[54]

Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; RDA, radial diffusion assay.

suggest that the insect would require stimulation to produce extracts with better results, given that UV irradiation can activate the immune system of the insect and trigger the generation of certain compounds involved in hormonal and cellular defense responses.[65] This is evident when comparing these data with that of protein hydrolysates obtained from BSF larvae without any prior induction, which demonstrated lower DPPH and ABTS scavenging activities.[63] However, when the same protein hydrolysate was ultrafiltered into fractions based on their molecular weights (< 3 kDa, 3–10 kDa, and >10 kDa), the fractions showed improvements in DPPH, ABTS, and hydroxyl radical scavenging abilities compared to the whole protein hydrolysate.[63] Protein hydrolysates are comprised of small peptides and free amino acids that would possess several valuable bioactivities such as antimicrobial, antihypertensive, anticoagulant, and antioxidant properties.[66–68] As such, fractionation is typically employed to these complex mixtures to concentrate or purify specific target peptides.[69,70] The difference in the scavenging capacity between the whole hydrolysate and the fractions may be due to the difference in molecular weight.[71] Several studies have reported the inverse relationship between the molecular weight of peptides and their antioxidant activities, with some suggesting that steric hindrance may restrict the access of fractions with a higher molecular weight to the radical portion of the target molecule, thus lowering the antioxidant capacity of the fraction.[72–75]

Apart from molecular weight, the antioxidant capacity of protein hydrolysates are also influenced by the amino acid composition of their peptides.[76] Hydrophobicity may contribute to the antioxidant activity of the overall peptide, as peptides with high antioxidant activity have been reported to contain a higher proportion of hydrophobic amino acids than hydrophilic amino acids.[71,77] This is reflected in the antioxidant fragments identified in protein hydrolysates from BSF larvae that contained predominantly hydrophobic amino acids (glycine, proline, phenylalanine, lysine, tyrosine, valine, and leucine).[63] It was suggested that hydrophobicity allows the antioxidative peptides to easily pass through the phospholipid bilayer and enter target cells to scavenge free radicals.[71,77] Another factor that may enhance the antioxidant capacity of protein hydrolysates is the presence of aromatic rings, which are rich in electrons and therefore easily attacked by oxidants.[78] This would explain the contribution of tyrosine to the antioxidant capacity of BSF larvae protein hydrolysates, as tyrosine contains an electron-donating hydroxyl group that activates a benzene ring in its side chain, thereby making it susceptible to oxidation.[63,78] Lastly, the amino acids arginine, histidine, as well as lysine have nitrogen atoms in their side chains, and the single lone electron pair within these atoms allows them to be easily oxidized, conferring antioxidant capacity to the BSF larvae protein hydrolysate.[63,78,79]

When reviewing literature focusing on the amino acids identified in the protein hydrolysate, it appears that tyrosine may have the largest effect on the overall antioxidant activity of the hydrolysate. Free tyrosine has been shown to be an effective antioxidant against DPPH, ABTS, and superoxide anion radicals.[80,81] Moreover, it was discovered that dipeptides and tripeptides with tyrosine residues,

TABLE 2 Antioxidant activity of different products from the BSF.

BSF product	Rearing method	Extraction method	Antioxidant assay	Antioxidant activity	References
Extract of UV-induced *H. illucens* larvae	(A) Temperature: 32°C. (B) Humidity: 50%.	—	(A) DPPH radical scavenging assay. (B) ABTS radical scavenging assay. (C) Hydroxyl radical scavenging assay.	(A) DPPH radical scavenging activity: (i) Nonirradiated control: 72.20%; (ii) UV-10: 92.60%; (iii) UV-20: ~85.00%; (iv) UV-30: ~70.00%. (B) ABTS radical scavenging activity: (i) Nonirradiated control: 29.10%; (ii) UV-10: ~35.00%; (iii) UV-20: 46.08%; (iv) UV-30: ~40.00%. (C) Hydroxyl radical scavenging assay: (i) Nonirradiated control: 29.80%; (ii) UV-10: ~65.00%; (iii) UV-20: 68.31%; (iv) UV-30: ~62.50%.	[29]
Protein hydrolysate and its ultrafiltered fractions (<3, 3–10, >10 kDa)	Temperature: 30–32°C.	(A) Starved larvae were freeze-dried and crushed. (B) To defat the larvae, the powdered larvae was combined with chloroform and methanol (4:1 v/v) in a 5:1 ratio, sonicated for 15 min at 100 W, then centrifuged at 5000 rpm at 4°C for 5 min. (C) The defatted residue was dried at 50°C to remove the solvent. (D) Alkaline protease was used to hydrolyze the proteins at pH 8.0°C and 55°C. The enzyme-defatted larvae mixture was incubated for 2 h in a 1.5:100 (w/v) ratio before heating for 10 min at 100°C. (E) After centrifugation for 10 min, the supernatant (protein hydrolysate; BLPH) was freeze-dried and kept at − 20°C. (F) The hydrolysate was ultrafiltered using filters with cut-off molecular weights of 3 kDa and 10 kDa to obtain three fractions: < 3 kDa (B1), 3–10 kDa (B2), and > 10 kDa (B3).	(A) DPPH radical scavenging assay. (B) ABTS radical scavenging assay. (C) Hydroxyl radical scavenging assay.	(A) DPPH radical scavenging activity: (i) BLPH: 31.06%; (ii) B1: 42.29%; (iii) B2: 72.23%; (iv) B3: 0%. (B) ABTS radical scavenging activity: (i) BLPH: 17.34%; (ii) B1: 89.99%; (iii) B2: 29.00%; (iv) B3: 0%. (C) Hydroxyl radical scavenging assay: (i) BLPH: 29.85%; (ii) B1: 77.11%; (iii) B2: 91.67%; (iv) B3: 83.13%.	[63]
Protein hydrolysate	—	(A) Defatting of larvae was carried out via Soxhlet extraction. The larvae was soaked in N-hexane in a ratio of 1:4 (biomass: solvent) for 6 h at 70°C. (B) The solvent was removed using a rotary evaporator for 2 h at 60°C. (C) 10 g of defatted larvae was dissolved in 50 mL of PBS before addition of bromelain enzyme. The	DPPH radical scavenging assay.	(A) Protein hydrolysate showed DPPH inhibition in a dose-dependent manner. (B) Percentage DPPH inhibition at 1.25% v/v: (i) Protein hydrolysate: 72.60 ± 0.41% v/v;	[64]

(Continues)

TABLE 2 (Continued)

BSF product	Rearing method	Extraction method	Antioxidant assay	Antioxidant activity	References
		hydrolysis reaction was carried out at 50°C and 150 rpm. The reaction was terminated by heating at 90°C for 10 min. (D) The protein hydrolysate was centrifuged at 4000 rpm, 25°C for 30 min. The supernatant was then freeze-dried and stored at 4°C.		(ii) Control: 66.30 ± 0.40% v/v. (C) IC_{50}: (i) Protein hydrolysate: 0.840 ± 0.001% v/v; (ii) Control: 0.920 ± 0.015% v/v.	

Note: UV-10: 10-min UV induction; UV-20: 20-min UV induction; UV-30: 30-min UV induction.

especially if they are found at the C-terminal, displayed potent radical scavenging abilities.[79,82,83] However, formulating a mixture of antioxidant amino acids at the same proportions found in the protein hydrolysate may not exert the same level of antioxidant effects, indicating that the relative spatial structure of the amino acids within the peptide should also be taken into consideration when it comes to the overall protective effect of the hydrolysate.[71]

Unlike that with the antimicrobial abilities of BSF, there are relatively more studies exploring the antioxidative and anti-inflammatory properties of this insect in vivo. The inclusion of BSF larvae as either insect meals or oils in the diets of animals (e.g., dogs, broiler chickens, carps, and shrimp) enhanced their antioxidative capacities, as indicated by the increase in their serum total antioxidant capacity (TAC) and antioxidant enzymes.[84–88] For example, during a 30-day trial on male broiler chickens fed with a corn and soybean meal-based diet supplemented with BSF larvae oil, the serum TAC of the chickens were increased from 0.77 mmol/L to 1.32 mmol/L from Day 15 to Day 30.[85] Moreover, a feeding trial on Pacific white shrimp with insect meals from different species demonstrated that shrimp fed BSF larvae meal had a significantly higher glutathione peroxidase activity compared to shrimp fed with the control diet.[87] Shrimp fed BSF larvae meal also showed a higher nitro-blue tetrazolium activity than the control group, though the difference is insignificant, while superoxide dismutase activity did not differ between the BSF group and the control group.[87] Nevertheless, it is clear that the BSF positively influences the antioxidant capacity of livestock and aquatic organisms. Besides improving antioxidative properties in vivo, BSF has also been shown to reduce the expression of pro-inflammatory genes in animals, namely *TNF-α* and *IL-1β*.[84,86,88] Female beagle dogs fed a commercial basal diet with varying inclusions of BSF larvae meal for 6 weeks revealed a decrease in serum TNF-α concentration with increasing inclusions of BSF larvae meal, whereas in juvenile mirror carp, *TNF-α* and *IL-1β* were both significantly downregulated in the kidneys and hepatopancreas of fish given 12.5–25 g kg^{-1} of BSF larvae oil in their diets compared to those given 0–6.25 g kg^{-1} of BSF larvae oil.[84,88] On the other hand, BSF larvae oil significantly increased the expression of interleukin 10 (IL-10), the gene that encodes the anti-inflammatory cytokine IL-10, in juvenile mirror carp.[86,88,89] Probing further into the anti-inflammatory potential of BSF,a composite comprising chitosan from BSF and silver nanoparticles synthesized using propolis was tested on the skin wounds of young Wistar rats.[90] The results showed that rats treated with the composite healed much faster than the untreated rats, without any indications of microbial infection such as pus formation.[90] Therefore, in addition to the aforementioned bioproperties, the BSF also have wound healing abilities, which is testament to anti-inflammatory as well as antimicrobial actions.[90] Although there is significantly less published research on the anti-inflammatory properties of BSF, they serve as a guideline for other researchers in understanding the mechanisms by which different BSF preparations might counteract the effects of inflammation.

3.1 | Potential antioxidant and anti-Inflammatory bioactive compounds in the BSF

Well-known biomolecules, such as the pigments melanins and ommochromes, may also play an important role in the antioxidative capacity of the BSF. A comprehensive study into the antioxidant and antiradical activity of melanins and ommochromes from the BSF showed that BSF water-soluble melanin (prepared by extraction using 10% NaOH) had higher antioxidant activity than BSF ommochromes despite having lower antiradical activity, while BSF chitin-melanin complex (prepared by acid hydrolysis using 25% sulfuric acid) displayed relatively low antioxidant activity compared to the water-soluble melanin and ommochromes.[91] In another study, when comparing the DPPH radical scavenging activities between melanin, chitosan alone, and chitosan-melanin complex from BSF, the chitosan-melanin complex displayed the greatest scavenging capacity.[92]

Aside from these biomolecules, however, there is an apparent lack in studies assessing the antioxidative potential of individual compounds derived from the BSF and the optimal methods for isolating these compounds. An untargeted metabolomics analysis was conducted on the methanol extract of BSF prepupae whereby bioactive compounds with potential cosmeceutical applications were identified.[29] Most of these bioactive compounds, specifically alkaloids and phenolic compounds, were actually phytocompounds derived from traditional herbs.[29,93–97] This similarity in compounds with sometimes identical structures found in both insects and plants was first discovered over 50 years ago, such as *cis*-zingiberenol identified in both *Zingiber officinale* (or ginger plant) and *Oebalus poecilus* (or rice stink bug), actinidine (iridoid) identified in honeysuckle and valerian as well as in the stick insect, and the cyanogenic glycoside linamarin found in cassava and *Zygaena filipendulae* (or burnet moth).[58,98–104] There are several theories for how this phenomenon came about, for example through convergent evolution to adapt to similar needs such as for defense and pollination/mating purposes, or through the sequestration of phytocompounds by insects through ingestion.[58] As mentioned before with the antimicrobial properties of the BSF, sequestration may also play a major role in the insect's diet-dependent antioxidant or anti-inflammatory capacity, and should be explored by screening the metabolites produced under different diets. Although sequestration refers mostly to plant toxins as well as sex, aggregation, and alarm pheromones being taken up by insects, the mechanisms underlying sequestration may also be applied to other accumulated plant metabolites that pose therapeutic properties.[58] Factors such as substrate specificity of transport proteins to facilitate the absorption of certain phytocompounds from the insect gut lumen, along with the separate storage of enzymes from sequestered metabolites to prevent the possible breakdown of these compounds, may help to discern the phytocompounds retained within the insect biomass from those eventually excreted.[57,58] Overall, prospecting for phytocompounds accumulated by the BSF instead of isolating these compounds from the plants themselves reduces the need for large areas of agricultural land to cultivate the crops, and the larvae's bioconverting abilities make them a more sustainable resource for cosmeceutical-related ingredients.[17]

One of the compounds the BSF shares with plants is withaferin A, a well-studied steroidal ester originally isolated from *Withania somnifera* (or winter cherry) with antioxidant and anti-inflammatory effects.[29,95,105,106] Based on these bioproperties of withaferin A, it may be responsible for the antioxidant capacity of the BSF, and it can be hypothesized that the compound could act as a UV protective agent. Withaferin A may even double as a remedy for acne, due to its reported potent antibacterial activity after isolation from *W. somnifera*, against *P. aeruginosa* ATCC 27853 and drug-resistant clinical isolates of *E. coli* and *E. aerogenes*, but of course, tests must be done on the compound's inhibitory actions against the relevant strains.[106,107] To isolate withaferin A, previous studies have used solvents such as methanol and ethyl acetate to obtain the crude extracts that would contain this compound.[105,108] In the study using methanol for the crude extraction, 0.25 mg of withaferin A was purified from 100 g of dried *W. somnifera* roots, whereas the study involving ethyl acetate resulted in a yield of 1.25 mg of withaferin A per 100 g of dried *Withania adpressa* leaves.[105,108] This four-fold increase in yield would suggest that ethyl acetate is the more efficient solvent for the extraction of withaferin A. However, factors such as species (*W. somnifera* vs. *W. adpressa*) and plant parts (roots vs. leaves) may have played a role in the varying levels of the extracted compound, not to mention insects may possess a dissimilar bioactive composition compared to plants, and therefore the outcome may be different when isolating withaferin A from BSF using ethyl acetate.

Furthermore, the flavonoid epigallocatechin (EGC), a well-known polyphenolic antioxidant derived from green tea, was identified in the BSF.[29,93] EGC may be employed as a potential active ingredient against inflammatory and oxidative stress-related skin disorders, including skin photodamage and atopic dermatitis. In terms of the extraction of EGC, ethanol seems to be a suitable choice, possibly due to its status as a universal solvent for studies on phytocompounds, as shown in the preparation of EGC-enriched green tea extracts in a past study by brewing the tea in 75% ethanol at a temperature of 30°C for 10 min.[35,93] Apart from this study, however, there is little reported on the extraction and isolation methods of EGC compared to that of its ester, epigallocatechin gallate (EGCG), due to the low abundance of EGC in green tea.[109] Therefore, researchers should use their own discretion when choosing their solvent for the isolation of EGC from the BSF, based on factors such as the polarity and solubility of the compound. As for the purification of EGC, another previous study used Sephadex LH-20 chromatography to purify EGC obtained from the hydrolysis of crude EGCG extracted from green tea, achieving a purity of 93.7%.[109] Hence, this purification method may also be applied in future experiments involving the isolation of BSF-derived EGC.

Aside from EGC, other phytocompounds in the flavonoid family that were also found in the BSF were luteolin, apigenin, quercetin, rutin, and kaempferol.[110] Flavonoids are a fount of biological activities, showing anticarcinogenic, antimicrobial, and antioxidative

effects, and are known for their protective actions against UVB.[111,112] They exert their photoprotective effects using various mechanisms such as UV radiation absorption and attenuation of oxidative damage through ROS scavenging.[112,113] To isolate these "sunscreens", as mentioned in previous sections of this review, consideration must be given to the polarities of the flavonoids. Within the context of BSF, the luteolin, apigenin, and rutin identified in the larvae are classified as flavones, whereas quercetin and kaempferol are flavonols.[110] Since flavones and flavonols are nonpolar, solvents such as ethyl acetate, chloroform, dichloromethane, and diethyl ether may be used to attempt the extraction of these compounds from the BSF.[114]

From the existing literature, there is indeed evidence of antioxidative metabolites present in the BSF. However, more streamlined studies focusing on the specific extraction of these metabolites should be conducted, using the extraction methods suggested in this review. Seeing as different metabolites require a specific type of extracting solvent, perhaps a fractionation approach should be applied to maximize the isolation of valuable compounds from the BSF, starting with a crude extraction followed by fractionation using various solvents based on the polarity of the target compounds.

4 | POTENTIAL APPLICATIONS OF THE BSF IN COSMECEUTICALS

Given the vast biological properties of the BSF discussed thus far, this insect may become an alternative resource of natural cosmeceutical ingredients. This is clear based on the number of patents filed in recent years for the use of BSF-derived products in the cosmetic field, as shown in Table 3. As previously mentioned, the BSF has been explored as a feed alternative for livestock, farmed fish, and pets.[17,18,84,121,122] The BSF is also now gaining traction as a human food source, though special measures such as blanching would have to be implemented to reduce the microbial load and heavy metal content.[123,124] In terms of BSF-based products, extracts and peptides derived from the larvae have shown low cytoxicity in cell viability assays.[7,10] Overall, the noteworthy safety profile of the BSF would help ease its incorporation into cosmeceutical formulations.

The antimicrobial effects of the BSF can be exploited for a number of skin-based applications. Our skin is teeming with microorganisms such as bacteria, viruses, and fungi forming our skin microbiota, which plays vital roles in immunity through crosstalk between skin microorganisms and the immune system, as well as modulating inflammatory processes.[125,126] An imbalance in this microbiota, also termed dysbiosis, would bring about skin diseases like chronic wounds, acne, and atopic dermatitis.[125,127] For example, nonhealing diabetic foot ulcers are atttributed to hyperglycemia that which inhibits angiogenesis, an essential component of wound healing, and such poor blood glucose control was found to be associated with greater colonization by *Staphylococcus* spp. and *Streptococcus* spp.[128,129] *Propionibacterium acnes*, the most commonly found bacterium in the skin microbiota, is associated with the pathophysiology of acne vulgaris, a chronic inflammatory disease of the pilosebaceous unit which includes the hair follicle, hair, and sebaceous gland.[125,130] Furthermore, another chronic inflammatory skin condition, atopic dermatitis (or eczema, as it is more commonly known), is linked with colonization by *Staphylococcus aureus* and the yeast *Malassezia* spp.[125,131,132] Aside from skin diseases, there is evidence of a possible relationship between cutaneous microbial composition and skin aging, whether it be intrinsic aging (natural aging) or photoaging (skin aging due to overexposure to the sun).[126] For instance, cyanobacteria, a microorganism with natural protective properties against UV radiation, were found in higher abundance on the skin of children, suggesting that children would be less susceptible to UV-induced skin damage.[126] On the other hand, the higher levels of *Streptococcus* found in the elder group were positively correlated with the acceleration of certain clinical parameters of skin aging, such as wrinkles and hyperpigmentation.[126] Therefore, cosmeceuticals that contain BSF products as an active ingredient may be developed as a remedy for the aforementioned skin disorders, by making use of the natural mechanisms of the BSF that inhibit the growth of harmful cutaneous microorganisms.

Beyond its admirable range of antimicrobial properties, the BSF has also shown promise as an agent for skin repair. Both intrinsic aging and photoaging are characterized by wrinkles and diminished elasticity as a result of the reduction in the amount of collagen in the extracellular matrix (ECM) within the dermis.[133] Compared to young skin, aged skin contains less collagen, due to the simultaneous decrease in collagen synthesis and increase in collagen breakdown owing to various factors, such as elevated levels of matrix metalloproteinase (MMP) and disrupted signaling of transforming growth factor-β (TGF-β).[133] MMPs are endopeptidases that break down ECM proteins, including collagen.[134,135] The levels of these endopeptidases were reported to be increased in aged skin, most likely triggered by ROS generated through both internal and external means such as metabolic processes and UV radiation.[133] ROS induce oxidative damage in photoaged skin through an activation cascade involving the transcription factors activator protein 1 (AP-1) and nuclear factor-κB (NF-κB), both of which are implicated in the upregulation of MMPs.[133,136] AP-1 is also responsible for the disruption of the TGF-β signaling pathway in fibroblasts, cells found in the dermis that play an essential role in the production and breakdown of ECM proteins.[133] Given that TGF-β regulates both the synthesis and degradation of collagen through the Smad pathway, impairment of its signaling would result in reduced neocollagen synthesis, leading to a decreased amount of collagen in the dermis of aged skin.[133,137] Besides inducing the fragmentation of collagen in aged skin, ROS are also heavily involved in the pathogenesis of inflammatory diseases, with a number of chronic skin conditions being a result of pro-inflammatory responses, namely chronic wounds, acne vulgaris, and atopic dermatitis.[130,132,138,139] Thus, seeing as ROS have a hand in various skin pathologies and abnormalities, cosmetic ingredients that possess both antioxidative and anti-inflammatory properties, such as the BSF, would be the key to mitigating skin damage incited by oxidative stress.

TABLE 3 List of patents for the cosmeceutical applications of BSF larvae.

Patent no.	Patent title	BSF larvae product	Functions/Biological properties	Year of publication	References
CN105456155A	Method for preparing cosmetic raw materials from *Hermetia illucens* larvas and application of method	Oil	Moisturizing, antiwrinkle	2016	[115]
CN105878113A	External preparation capable of preventing and treating skin inflammations and protecting skins	Oil	Anti-inflammatory, skin protecting: (A) Reduction of cracked skin in patients after 5 days.	2016	[116]
US20180256483A1	Skincare product containing *Hermetia illucens* extract	Oil/fat	(A) Antimicrobial (MIC–MBC; % w/v): (i) *C. albicans*: 50–50; (ii) *E. coli*: 25–50; (iii) *P. aeruginosa*: 50–50; (iv) *S. aureus*: 25–50; (v) *Kytococcus sedentarius*: 25–50; (vi) *P. acnes*: 12.5–50. (B) Antioxidant (DPPH assay): (i) SC_{50}: 14.43 ± 0.14 (unit not given). (C) Skin whitening (tyrosinase inhibition): (i) IC_{50}: 0.13 ± 0.04 (unit not given).	2018	[117]
KR102114996B1	An antimicrobial composition comprising an extract of an *Hermetia illucens*	Acetic acid extract	Antimicrobial (MIC): (A) *Salmonella* spp.: 100–200 μg/100 μL; (B) *S. aureus* and *E. faecalis*: 100–200 μg/100 μL; (C) *S. mutans*: 300–400 μg/100 μL; (D) *Candida vaginitis*: 500–1000 μg/100 μL; (E) *S. marcescens* and *P. tolaasii*: Strong inhibition; (F) MRSA and MDRPA: Strong inhibition.	2020	[118]
KR102120475B1	Cosmetic composition comprising *Hermetia illucens* larva	Ethanol extract	(A) Antibacterial; (B) Antiwrinkle (elastase inhibition): (i) Inhibitory activity increases from 100 μg/mL; (C) Skin whitening (tyrosinase inhibition): (i) Inhibitory activity increases from 100 μg/mL; (D) Antioxidant (DPPH assay): (i) Inhibition increases as extract concentration increases.	2020	[119]
CN113398043A	Composition compounded with the *Hermetia illucens* oil and application thereof	Oil	Antioxidant, moisturizing, skin barrier protecting	2021	[120]

Abbreviations: MBC, minimum bactericidal concentration; MDRPA, multidrug-resistant *Pseudomonas aeruginosa*; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.

5 | CONCLUSIONS

Based on the evidence presented, the prospects of BSF as a natural ingredient in cosmeceutical products are bright, especially in this day and age when the demand for "greener" cosmetics is ever increasing. Nevertheless, there is still a lack of comprehensive published data on the potential use of BSF larvae extracts and derived compounds in the cosmeceutical context, and this research gap pertaining to the direct relationship between the BSF extract/components and our skin must be acknowledged. In an effort to fill this gap, more research on BSF products should focus on components related to the skin, such as by utilizing HaCaT keratinocytes as an in vitro model. It would also be helpful to extend this type of research beyond the single cell and use human skin as models to deepen our understanding of the pathogenesis of skin damage and microorganism-induced skin diseases, as well as of how BSF products may act to rectify these cutaneous abnormalities, before eventually progressing to clinical trials using BSF-based cosmeceutical formulations. Furthermore, explorations into the relationship between diet and bioactive composition would be an interesting avenue in the search for novel BSF metabolites, and would allow for the fine-tuning of the insect's dietary needs to obtain specific cosmeceutical compounds. Comparative phylogenetic analyzes would be useful when determining the biosynthetic origins of BSF compounds that share similarities with certain phytocompounds, whether these compounds were sequestered through ingestion of plant material or arose through convergent evolution.

AUTHOR CONTRIBUTIONS

Ashley Sean Lai-Foenander: Writing—original draft. **Giva Kuppusamy**: Writing—review and editing. **Janaranjani Manogoran**: Writing—review and editing. **Tengfei Xu**: Writing—review and editing. **Yong Chen**: Writing—review and editing. **Siah Ying Tang**: Writing—review and editing. **Hooi-Leng Ser**: Writing—review and editing. **Yoon-Yen Yow**: Writing—review and editing. **Khang Wen Goh**: Writing—review and editing. **Long Chiau Ming**: Writing—review and editing. **Lay-Hong Chuah**: Writing—review and editing. **Wei-Hsum Yap**: Writing—review and editing. **Bey-Hing Goh**: Conceptualization; supervision.

ACKNOWLEDGMENTS

We are grateful to all those who helped with our article. Open access publishing facilitated by Monash University, as part of the Wiley - Monash University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data used in this study are present within the study itself. No new data were created or analyzed in this study.

ETHICS STATEMENT

The authors have nothing to report.

TRANSPARENCY STATEMENT

The lead author Khang Wen Goh, Bey-Hing Goh affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID

Khang Wen Goh https://orcid.org/0000-0001-6686-7019
Bey-Hing Goh http://orcid.org/0000-0003-1006-3649

REFERENCES

1. Parish LC, Crissey JT. Cosmetics: a historical review. *Clin Dermatol*. 1988;6(3):1-4.
2. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. *Metabolites*. 2012;2(2):303-336.
3. Beutler JA. Natural products as a foundation for drug discovery. *Curr Protoc Pharmacol*. 2009;46:9.11.1-9.21. doi:10.1002/0471141755.ph0911s46
4. Kligman AM. Cosmetics. *Dermatol Clin*. 2000;18(4):699-709.
5. Zappelli C, Barbulova A, Apone F, Colucci G. Effective active ingredients obtained through biotechnology. *Cosmetics*. 2016;3(4):39.
6. Ahmad A, Ahsan H. Lipid-based formulations in cosmeceuticals and biopharmaceuticals. *Biomed Dermatol*. 2020;4(1):12.
7. Choi WH, Choi H-J, Goo TW, Quan F-S. Novel antibacterial peptides induced by probiotics in *Hermetia illucens* (Diptera: *Stratiomyidae*) larvae. *Entomol Res*. 2018;48(4):237-247.
8. Alvarez D, Wilkinson KA, Treilhou M, Téné N, Castillo D, Sauvain M. Prospecting peptides isolated from black soldier fly (Diptera: *Stratiomyidae*) with antimicrobial activity against *Helicobacter pylori* (Campylobacterales: *Helicobacteraceae*). *J Insect Sci*. 2019;19(6). doi:10.1093/jisesa/iez120
9. Almeida C, Rijo P, Rosado C. Bioactive compounds from *Hermetia illucens* larvae as natural ingredients for cosmetic application. *Biomolecules*. 2020;10(7):976.
10. Lee K-S, Yun E-Y, Goo T-W. Antimicrobial activity of an extract of *Hermetia illucens* larvae immunized with *Lactobacillus casei* against *Salmonella* species. *Insects*. 2020;11(10):704.
11. Franco A, Salvia R, Scieuzo C, Schmitt E, Russo A, Falabella P. Lipids from insects in cosmetics and for personal care products. *Insects*. 2022;13(1):41.
12. Seabrooks L, Hu L. Insects: an underrepresented resource for the discovery of biologically active natural products. *Acta Pharm Sin B*. 2017;7(4):409-426.
13. Müller A, Wolf D, Gutzeit HO. The black soldier fly, *Hermetia illucens* – A promising source for sustainable production of proteins, lipids and bioactive substances. *Zeitschrift für Naturforschung C*. 2017;72(9-10):351-363.
14. Kaya C, Generalovic TN, Ståhls G, et al. Global population genetic structure and demographic trajectories of the black soldier fly, *Hermetia illucens*. *BMC Biol*. 2021;19(1):94.
15. Li Q, Zheng L, Qiu N, Cai H, Tomberlin JK, Yu Z. Bioconversion of dairy manure by black soldier fly (Diptera: *Stratiomyidae*) for biodiesel and sugar production. *Waste Manage*. 2011;31(6):1316-1320.
16. Liu X, Chen X, Wang H, et al. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS One*. 2017;12(8):e0182601.

17. van Huis A, van Itterbeeck J, Klunder H, et al. *Edible Insects: Future Prospects for Food and Feed Security*. Food and Agriculture Organization of the United Nations; 2013:187.
18. Wang Y-S, Shelomi M. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. *Foods*. 2017;6(10):91.
19. Chiabi A, Kenmogne MH, Nguefack S, et al. The empiric use of palm kernel oil in neonatal skin care: justifiable or not? *Chin J Integr Med*. 2011;17(12):950-954.
20. Ng YJ, Tham PE, Khoo KS, Cheng CK, Chew KW, Show PL. A comprehensive review on the techniques for coconut oil extraction and its application. *Bioprocess Biosyst Eng*. 2021;44(9):1807-1818.
21. Moretta A, Salvia R, Scieuzo C, et al. A bioinformatic study of antimicrobial peptides identified in the black soldier fly (BSF) *Hermetia illucens* (Diptera: *Stratiomyidae*). *Sci Rep*. 2020;10(1):16875.
22. Shin HS, Park SI. Novel attacin from *Hermetia illucens*: cDNA cloning, characterization, and antibacterial properties. *Prep Biochem Biotechnol*. 2019;49(3):279-285.
23. Park S-I, Chang BS, Yoe SM. Detection of antimicrobial substances from larvae of the black soldier fly, *Hermetia illucens* (Diptera: *Stratiomyidae*). *Entomol Res*. 2014;44(2):58-64.
24. Erickson MC, Islam M, Sheppard C, Liao J, Doyle MP. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar enteritidis in chicken manure by larvae of the black soldier fly. *J Food Prot*. 2004;67(4):685-690.
25. Lalander CH, Fidjeland J, Diener S, Eriksson S, Vinnerås B. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier Fly for waste recycling. *Agron Sustain Dev*. 2015;35(1):261-271.
26. Choi W-H, Yun J-H, Chu J-P, Chu K-B. Antibacterial effect of extracts of *Hermetia illucens* (Diptera: *Stratiomyidae*) larvae against gram-negative bacteria. *Entomol Res*. 2012;42(5):219-226.
27. Vogel H, Müller A, Heckel DG, Gutzeit H, Vilcinskas A. Nutritional immunology: diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly *Hermetia illucens*. *Dev Comp Immunol*. 2018;78:141-148.
28. Harlystiarini H, Mutia R, Wibawan IWT, Astuti DA. *In vitro* antibacterial activity of black soldier fly (*Hermetia illucens*) larva extracts against Gram-negative bacteria. *Buletin Peternakan*. 2019;43(2):5.
29. Lu J, Guo Y, Muhmood A, et al. Food waste management employing UV-induced black soldier flies: metabolomic analysis of bioactive components, antioxidant properties, and antibacterial potential. *Int J Environ Res Public Health*. 2022;19(11):6614.
30. Lee K-S, Yun E-Y, Goo T-W. Evaluation of the antimicrobial activity of an extract of *Lactobacillus casei*-infected *Hermetia illucens* larvae produced using an automatic injection system. *Animals*. 2020;10(11):2121.
31. Saptarini NM, Wardati Y. Effect of extraction methods on antioxidant activity of papery skin extracts and fractions of maja cipanas onion (*Allium cepa* L. var. ascalonicum). *ScientificWorldJournal*. 2020;2020:1-6.
32. Kongkiatpaiboon S, Gritsanapan W. Optimized extraction for high yield of insecticidal didehydrostemofoline alkaloid in Stemona collinsiae root extracts. *Ind Crops Prod*. 2013;41:371-374.
33. Cheok CY, Salman HAK, Sulaiman R. Extraction and quantification of saponins: A review. *Food Res Int*. 2014;59:16-40.
34. Amr A, Al-Tamimi E. Stability of the crude extracts of *Ranunculus asiaticus* anthocyanins and their use as food colourants. *Int J Food Sci Technol*. 2007;42(8):985-991.
35. Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin Med*. 2018;13(1):20.
36. Zhan S, Fang G, Cai M, et al. Genomic landscape and genetic manipulation of the black soldier fly *Hermetia illucens*, a natural waste recycler. *Cell Res*. 2020;30(1):50-60.
37. Park S-I, Yoe SM. Defensin-like peptide3 from black solder Fly: identification, characterization, and key amino acids for anti-gram-negative bacteria. *Entomol Res*. 2017;47(1):41-47.
38. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. *Biochim et Biophys Acta Biomembranes*. 2008;1778(2):357-375.
39. Elhag O, Zhou D, Song Q, et al. Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from *Hermetia illucens* (L.). *PLoS One*. 2017;12(1):e0169582.
40. Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LLS, Silva-Pereira I, Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. *Front Microbiol*. 2013;4:353.
41. Van Moll L, De Smet J, Paas A, et al. *In vitro* evaluation of antimicrobial peptides from the black soldier fly (*Hermetia illucens*) against a selection of human pathogens. *Microbiol Spectr*. 2022;10(1):e01664-21.
42. Park S-I, Kim J-W, Yoe SM. Purification and characterization of a novel antibacterial peptide from black soldier fly (*Hermetia illucens*) larvae. *Dev Comp Immunol*. 2015;52(1):98-106.
43. Park S-I, Yoe SM. A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: isolation, structural and functional characterization. *Entomol Res*. 2017;47(2):115-124.
44. Shah H, Safi SZ. Drug Resistance in Skin Diseases. In: Ahmed S, Chandra Ojha S, Najam-ul-Haq M, Younus M, Hashmi MZ, editors. *Biochemistry of Drug Resistance*. Springer International Publishing; 2021:197-234.
45. Yao Z, Wu Y, Xu H, et al. Prevalence and clinical characteristics of methicillin-resistant *Staphylococcus aureus* infections among dermatology inpatients: A 7-year retrospective study at a tertiary care center in southwest China. *Front Public Health*. 2023;11:1124930.
46. CDC. Methicillin-resistant *Staphylococcus aureus* (MRSA): U.S. Department of Health & Human Services; 2019: Available from https://www.cdc.gov/mrsa/community/index.html.
47. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Sci Prog*. 2002;85(Pt 1):57-72.
48. Shahkarami F, Rashki A, Rashki Ghalehnoo Z. Microbial susceptibility and plasmid profiles of methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *S. aureus*. *Jundishapur J Microbiol*. 2014;7(7):e16984.
49. Yip DW, Gerriets V. *Penicillin Treasure Island (FL)*. StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK554560/
50. Cong Y, Yang S, Rao X. Vancomycin resistant *Staphylococcus aureus* infections: a review of case updating and clinical features. *J Adv Res*. 2020;21:169-176.
51. Li Z, Mao R, Teng D, et al. Antibacterial and immunomodulatory activities of insect defensins-DLP2 and DLP4 against multidrug-resistant *Staphylococcus aureus*. *Sci Rep*. 2017;7(1):12124.
52. Chu K-B, Jeon G-C, Quan F-S. Hexanedioic acid from *Hermetia illucens* larvae (Diptera: *Stratiomyidae*) protects mice against *Klebsiella pneumoniae* infection. *Entomol Res*. 2014;44(1):1-8.
53. Lee D-H, Chu K-B, Kang H-J, Lee S-H, Quan F-S. Peptides in the hemolymph of *Hermetia illucens* larvae completely inhibit the growth of *Klebsiella pneumonia in vitro* and *in vivo*. *J Asia-Pacific Entomol*. 2020;23(1):36-43.
54. Zhang J, Li J, Peng Y, et al. Structural and functional characterizations and heterogenous expression of the antimicrobial peptides, Hidefensins, from black soldier fly, *Hermetia illucens* (L.). *Protein Expr Purif*. 2022;192:106032.
55. Frontiers. Nutritional Immunology. Available from: https://www.frontiersin.org/journals/nutrition/sections/nutritional-immunology
56. Saeed F, Nadeem M, Ahmed RS, Tahir Nadeem M, Arshad MS, Ullah A. Studying the impact of nutritional immunology underlying

the modulation of immune responses by nutritional compounds – a review. *Food Agric Immunol*. 2016;27(2):205-229.
57. Beran F, Petschenka G. Sequestration of plant defense compounds by insects: from mechanisms to insect–plant coevolution. *Annu Rev Entomol*. 2022;67(1):163-180.
58. Beran F, Köllner TG, Gershenzon J, Tholl D. Chemical convergence between plants and insects: biosynthetic origins and functions of common secondary metabolites. *New Phytol*. 2019;223(1):52-67.
59. Jin N, Liu Y, Zhang S, et al. C/N-dependent element bioconversion efficiency and antimicrobial protein expression in food waste treatment by black soldier fly larvae. *Int J Mol Sci*. 2022;23(9):5036.
60. Sen A, Dhavan P, Shukla Kk, Singh S, Tejovathi G. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from *Momordica charantia*. science secure. *J Biotech*. 2013;1:9-13.
61. Ododo MM, Choudhury MK, Dekebo AH. Structure elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. *SpringerPlus*. 2016;5(1):1210.
62. Alawode TT, Lajide L, Olaleye M, Owolabi B. Stigmasterol and β-sitosterol: antimicrobial compounds in the leaves of *Icacina trichantha* identified by GC–MS. *Beni-Suef Univ J Basic Appl Sci*. 2021;10(1):80.
63. Lu J, Guo Y, Muhmood A, et al. Probing the antioxidant activity of functional proteins and bioactive peptides in *Hermetia illucens* larvae fed with food wastes. *Sci Rep*. 2022;12(1):2799.
64. Firmansyah M, Abduh MY. Production of protein hydrolysate containing antioxidant activity from *Hermetia illucens*. *Heliyon*. 2019;5(6):e02005.
65. Lavine MD, Strand MR. Insect hemocytes and their role in immunity. *Insect Biochem Mol Biol*. 2002;32(10):1295-1309.
66. Clare DA, Swaisgood HE. Bioactive milk peptides: A prospectus. *J Dairy Sci*. 2000;83(6):1187-1195.
67. de Castro RJS, Sato HH. A response surface approach on optimization of hydrolysis parameters for the production of egg white protein hydrolysates with antioxidant activities. *Biocatal Agric Biotechnol*. 2015;4(1):55-62.
68. Halim NRA, Azlan A, Yusof HM, Sarbon NM. Antioxidant and anticancer activities of enzymatic eel (*Monopterus* sp) protein hydrolysate as influenced by different molecular weight. *Biocatal Agric Biotechnol*. 2018;16:10-16.
69. Bourseau P, Vandanjon L, Jaouen P, et al. Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: impact on peptidic populations. *Desalination*. 2009;244(1):303-320.
70. Roslan J, Mustapa Kamal SM, Md. Yunos KF, Abdullah N. Fractionation of tilapia by-product protein hydrolysate using multilayer configuration of ultrafiltration membrane. *Processes*. 2021;9(3):446.
71. Zou TB, He TP, Li HB, Tang HW, Xia EQ. The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules*. 2016;21(1):72.
72. Ngoh Y-Y, Gan C-Y. Enzyme-assisted extraction and identification of antioxidative and α-amylase inhibitory peptides from Pinto beans (*Phaseolus vulgaris* cv. Pinto). *Food Chem*. 2016;190:331-337.
73. Ma J, Zeng X, Zhou M, Cheng L, Ren D. Inhibitory effect of low-molecular-weight peptides (0–3 kDa) from *Spirulina platensis* on H_2O_2-induced oxidative damage in L02 human liver cells. *Bioresour Bioprocess*. 2021;8(1):36.
74. Chen H-J, Dai F-J, Chen C-Y, et al. Effects of molecular weight fraction on antioxidation capacity of rice protein hydrolysates. *Sci Rep*. 2023;13(1):3464.
75. Hunsakul K, Laokuldilok T, Prinyawiwatkul W, Utama-ang N. Effects of thermal processing on antioxidant activities, amino acid composition and protein molecular weight distributions of jasmine rice bran protein hydrolysate. *Int J Food Sci Technol*. 2021;56(7):3289-3298.
76. Putra SNKM, Ishak NH, Sarbon NM. Preparation and characterization of physicochemical properties of golden apple snail (*Pomacea canaliculata*) protein hydrolysate as affected by different proteases. *Biocatal Agric Biotechnol*. 2018;13:123-128.
77. Girgih AT, He R, Malomo S, Offengenden M, Wu J, Aluko RE. Structural and functional characterization of hemp seed (*Cannabis sativa* L.) protein-derived antioxidant and antihypertensive peptides. *J Func Foods*. 2014;6:384-394.
78. Xu N, Chen G, Liu H. Antioxidative categorization of twenty amino acids based on experimental evaluation. *Molecules*. 2017;22(12):2066.
79. Guo H, Kouzuma Y, Yonekura M. Structures and properties of antioxidative peptides derived from royal jelly protein. *Food Chem*. 2009;113(1):238-245.
80. Gülçin İ. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. *Amino Acids*. 2007;32(3):431-438.
81. Torkova A, Koroleva O, Khrameeva E, Fedorova T, Tsentalovich M. Structure-functional study of tyrosine and methionine dipeptides: an approach to antioxidant activity prediction. *Int J Mol Sci*. 2015;16(10):25353-25376.
82. Chen N, Chen J, Yao B, Li Z. QSAR study on antioxidant tripeptides and the antioxidant activity of the designed tripeptides in free radical systems. *Molecules*. 2018;23(6):1407.
83. Saito K, Jin D-H, Ogawa T, et al. Antioxidative properties of tripeptide libraries prepared by the combinatorial chemistry. *J Agricult Food Chem*. 2003;51(12):3668-3674.
84. Lei XJ, Kim TH, Park JH, Kim IH. Evaluation of supplementation of defatted black soldier fly (*Hermetia illucens*) larvae meal in beagle dogs. *Ann Animal Science*. 2019;19(3):767-777.
85. Kim YB, Kim D-H, Jeong S-B, et al. Black soldier fly larvae oil as an alternative fat source in broiler nutrition. *Poult Sci*. 2020;99(6):3133-3143.
86. Xu X, Ji H, Belghit I, Sun J. Black soldier fly larvae as a better lipid source than yellow mealworm or silkworm oils for juvenile mirror carp (*Cyprinus carpio* var. *specularis*). *Aquaculture*. 2020;527:735453.
87. Shin J, Lee K-J. Digestibility of insect meals for pacific White shrimp (*Litopenaeus vannamei*) and their performance for growth, feed utilization and immune responses. *PLoS One*. 2021;16(11):e0260305.
88. Xu X, Ji H, Belghit I, Liland NS, Wu W, Li X. Effects of black soldier fly oil rich in n-3 HUFA on growth performance, metabolism and health response of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). *Aquaculture*. 2021;533:736144.
89. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critic Rev Immunol*. 2012;32(1):23-63.
90. Al-saggaf MS. Formulation of insect chitosan stabilized silver nanoparticles with propolis extract as potent antimicrobial and wound healing composites. *Int J Polym Sci*. 2021;2021:5578032.
91. Ushakova N, Dontsov A, Sakina N, Bastrakov A, Ostrovsky M. Antioxidative properties of melanins and ommochromes from black soldier fly *Hermetia illucens*. *Biomolecules*. 2019;9(9):408.
92. Khayrova A, Lopatin S, Varlamov V. Obtaining and study of physicochemical properties of chitin/chitosan-melanin complexes from *Hermetia illucens*. *J Phys Conf Ser*. 2021;1942(1):012003.
93. Hu J, Zhou D, Chen Y. Preparation and antioxidant activity of green tea extract enriched in epigallocatechin (EGC) and epigallocatechin gallate (EGCG). *J Agricult Food Chem*. 2009;57(4):1349-1353.
94. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their

molecular mechanisms and experimental models. *Phytother Res*. 2015;29(3):323-331.
95. Batumalaie K, Amin MA, Murugan DD, Sattar MZA, Abdullah NA. Withaferin A protects against palmitic acid-induced endothelial insulin resistance and dysfunction through suppression of oxidative stress and inflammation. *Sci Rep*. 2016;6(1):27236.
96. Bandatmakuru SR, Arava VR. Novel synthesis of graveoline and graveolinine. *Synth Commun*. 2018;48(20):2635-2641.
97. Llano S, Gómez S, Londoño J, Restrepo A. Antioxidant activity of curcuminoids. *Phys Chem Chem Phys*. 2019;21(7):3752-3760.
98. Seigler DS. Plants of the northeastern United States that produce cyanogenic compounds. *Econ Bot*. 1976;30(4):395-407.
99. Wray V, Davis RH, Nahrstedt A. Biosynthesis of cyanogenic glycosides in butterflies and moths: incorporation of valine and isoleucine into linamarin and lotaustralin by *Zygaena* and *Heliconius* species (Lepidoptera). *Zeitschrift für Naturforschung C*. 1983;38(7-8):583-588.
100. Eisner T, Meinwald J. Chemical ecology. *Proc Nat Acad Sci*. 1995;92(1):1.
101. Prescott TAK, Bramham J, Zompro O, Maciver SK. Actinidine and glucose from the defensive secretion of the stick insect *Megacrania nigrosulfurea*. *Biochem Sys Ecol*. 2009;37(6):759-760.
102. de Oliveira MWM, Borges M, Andrade CKZ, Laumann RA, Barrigossi JAF, Blassioli-Moraes MC. Zingiberenol, (1S,4R,1′ S)-4-(1′,5′-dimethylhex-4′-enyl)-1-methylcyclohex-2-en-1-ol, identified as the sex pheromone produced by males of the rice stink bug *Oebalus poecilus* (Heteroptera: *Pentatomidae*). *J Agricult Food Chem*. 2013;61(32):7777-7785.
103. Khrimian A, Shirali S, Guzman F. Absolute configurations of zingiberenols isolated from ginger (*Zingiber officinale*) rhizomes. *J Nat Prod*. 2015;78(12):3071-3074.
104. Bol S, Caspers J, Buckingham L, et al. Responsiveness of cats (*Felidae*) to silver vine (*Actinidia polygama*), tatarian honeysuckle (*Lonicera tatarica*), valerian (*Valeriana officinalis*) and catnip (*Nepeta cataria*). *BMC Vet Res*. 2017;13(1):70.
105. Ben Bakrim W, El Bouzidi L, Nuzillard J-M, et al. Bioactive metabolites from the leaves of *Withania adpressa*. *Pharm Biol*. 2018;56(1):505-510.
106. Murugan R, Rajesh R, Seenivasan B, et al. Withaferin A targets the membrane of *Pseudomonas aeruginosa* and mitigates the inflammation in zebrafish larvae; an *in vitro* and *in vivo* approach. *Microb Pathog*. 2022;172:105778.
107. Rossato Viana A, Godoy Noro B, Lenz JC, et al. Cytotoxic screening and antibacterial activity of withaferin A. *J Toxicol Environ Health, Part A*. 2022;85(16):685-698.
108. Keesara BR, Jat RK. Isolation and characterization of Withaferin-A from the *Withania somnifera* (Ashwagandha). *J Drug Delivery Ther*. 2017;7(1):65-69.
109. Li M, Kelly BT, Hagerman AE. Isolation of (−)-epigallocatechin from green tea extract by means of immobilized tannase. *Thieme*. 2016.
110. Shumo M, Osuga IM, Khamis FM, et al. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Sci Rep*. 2019;9(1):10110.
111. Saeed M, Naveed M, Arain MA, et al. Quercetin: nutritional and beneficial effects in poultry. *Worlds Poult Sci J*. 2017;73(2):355-364.
112. Agati G, Tattini M. Multiple functional roles of flavonoids in photoprotection. *New Phytol*. 2010;186(4):786-793.
113. Sisa M, Bonnet SL, Ferreira D, Van der Westhuizen JH. Photochemistry of flavonoids. *Molecules*. 2010;15(8):5196-5245.
114. Rodríguez De Luna SL, Ramírez-Garza RE, Serna Saldívar SO. Environmentally friendly methods for flavonoid extraction from plant material: impact of their operating conditions on yield and antioxidant properties. *ScientificWorldJournal*. 2020;2020:6792069.
115. Hu W, Li W, Yang S, Wang J, inventors; Taicang Anyou Biological Tech Co Ltd, assignee. Method for preparing cosmetic raw materials from *Hermetia illucens* larvas and application of method. CN patent CN 105456155 A. 2016 2015/11/23.
116. Yang D, Xue XUE, Zhao Z, et al, inventors; Zhongshan Unicare Natural Medicine Co Ltd UNIV SUN YAT SEN, assignee. External preparation capable of preventing and treating skin inflammations and protecting skins. CN patent CN 105878113 A. 2016 2016/05/18.
117. Sangduan C, inventor; Sangduan Chanarong, assignee. Skincare product containing *Hermetia illucens* extract. US patent US 2018/0256483 A1. 2018 2018/05/16.
118. 구태원, 이상훈, inventors; 동국대학교 경주캠퍼스 산학협력단 그린테코 주식회사, assignee. An Antimicrobial Composition comprising an Extract of an *Hermetia illucens*. KR patent KR 102114996 B1. 2020 2017/08/31.
119. 김태형, inventor; 김태형, assignee. Cosmetic composition comprising *Hermetia illucens* larva. KR patent KR 102120475 B1. 2020 2018/11/26.
120. Xu J, Hu W, Pang XU, inventors; Guangzhou Biofume Bio Tech Co Ltd, assignee. Composition compounded with the *Hermetia illucens* oil and application thereof. CN patent CN 113398043 A. 2021 2021/05/31.
121. Sealey WM, Gaylord TG, Barrows FT, et al. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. *J World Aquac Soc*. 2011;42(1):34-45.
122. Spranghers T, Michiels J, Vrancx J, et al. Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. *Anim Feed Sci Technol*. 2018;235:33-42.
123. DiGiacomo K. Black soldier fly larvae protein production in Australia. *Animal Frontiers*. 2023;13(4):8-15.
124. Bessa LW, Pieterse E, Marais J, Dhanani K, Hoffman LC. Food safety of consuming black soldier Fly (*Hermetia illucens*) larvae: microbial, heavy metal and Cross-Reactive allergen risks. *Foods*. 2021;10(8):1934.
125. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018;16(3):143-155.
126. Li Z, Bai X, Peng T, et al. New insights into the skin microbial communities and skin aging. *Front Microbiol*. 2020;11. doi:10.3389/fmicb.2020.565549
127. Mahendra CK, Goh KW, Ming LC, et al. The prospects of *Swietenia macrophylla* king in skin care. *Antioxidants*. 2022;11(5):913.
128. Marston WA, Dermagraft Diabetic Foot Ulcer Study G. Risk factors associated with healing chronic diabetic foot ulcers: the importance of hyperglycemia. *Ostomy Wound Manage*. 2006;52(3):26-28.
129. Gardner SE, Hillis SL, Heilmann K, Segre JA, Grice EA. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes*. 2013;62(3):923-930.
130. McLaughlin J, Watterson S, Layton AM, Bjourson AJ, Barnard E, McDowell A. *Propionibacterium acnes* and acne vulgaris: New insights from the integration of population genetic, multi-omic, biochemical and host-microbe studies. *Microorganisms*. 2019;7(5):128.
131. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol*. 1974;90(5):525.
132. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet*. 2020;396(10247):345-360.
133. Shin JW, Kwon SH, Choi JY, et al. Molecular mechanisms of dermal aging and antiaging approaches. *Int J Mol Sci*. 2019;20(9):2126.
134. Verma RP, Hansch C. Matrix metalloproteinases (MMPs): chemical–biological functions and (Q)SARs. *Bioorg Med Chem*. 2007;15(6):2223-2268.
135. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int J Mol Sci*. 2016;17(6):868.

136. Kim J, Lee C-W, Kim EK, et al. Inhibition effect of *Gynura procumbens* extract on UV-B-induced matrix-metalloproteinase expression in human dermal fibroblasts. *J Ethnopharmacol*. 2011; 137(1):427-433.
137. Quan T, Shao Y, He T, Voorhees JJ, Fisher GJ. Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. *J Invest Dermatol*. 2010;130(2):415-424.
138. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signaling*. 2014;20(7):1126-1167.
139. Holzer-Geissler JCJ, Schwingenschuh S, Zacharias M, et al. The impact of prolonged inflammation on wound healing. *Biomedicines*. 2022;10, 856(4).

How to cite this article: Lai-Foenander AS, Kuppusamy G, Manogoran J, et al. Black soldier fly (*Hermetia illucens* L.): a potential small mighty giant in the field of cosmeceuticals. *Health Sci Rep*. 2024;7:e2120. doi:10.1002/hsr2.2120