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Antibacterial Activity of Extracts from Seven Insect Species Against Mediterranean Marine Aquaculture Bacterial Pathogens

Gatien Debailleul [1,2], Hugo Gatouillat [1,2], Dimitrios Chatzimpalasis [2,3], Matteo Chatteleyn [2,4], Petros Chronopoulos [2], Chrisanthi Nikoloudaki [2], Antigoni Vasilaki [2], Dimitra Kogiannou [2] and Morgane Henry [2,*]

1 L'Institut Agro Rennes Angers, 65 Rue de St-Brieuc, FR-35000 Rennes, France; gatien.debailleul@inrae.fr (G.D.); gatouillathugo@gmail.com (H.G.)
2 Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), 46.7 km Athens-Sounio Ave., GR-19013 Anavyssos, Greece; chatzimpalasis@gmail.com (D.C.); matteo.chatteleyn@etu.utc.fr (M.C.); p.chronopoulos@hcmr.gr (P.C.); cnikol@hcmr.gr (C.N.); avasilaki@hcmr.gr (A.V.); dkogiannou@hcmr.gr (D.K.)
3 Department of Zoology, School of Biology, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
4 Department of Bio-Engineering, University of Technology of Compiegne, CS 60319, Cedex, FR-60203 Compiègne, France
* Correspondence: morgane@hcmr.gr; Tel.: +30-2291076493

Abstract

Beyond their role as fishmeal replacements in aquafeeds, insects may enhance fish resistance to microbial diseases and improve feed shelf life through the presence of antibacterial compounds. This study evaluated the antibacterial activity of extracts from silkworm pupae (*Bombyx mori*, BM), larval meals of black soldier fly (*Hermetia illucens*, HI), mealworm (*Tenebrio molitor*, TM), and superworm (*Zophobas morio*, ZM), and adults of house cricket (*Acheta domesticus*, AD), two-spotted cricket (*Gryllus bimaculatus*, GB) and weaver ant (*Oecophylla smaragdina*, OS). The extracts were tested against ten Gram-negative marine bacterial pathogens associated with aquaculture disease outbreaks, as well as the Gram-positive bacterium, *Bacillus thuringiensis*. The antibacterial activity of aqueous and solvent-soluble extracts was assessed using agar diffusion assays, turbidimetric growth kinetics, and MTT metabolic assays. Several insect extracts exhibited moderate to strong antibacterial activity. In particular, the aqueous extracts of BM and GB, the methanolic extract of HI, and the isopropanolic extracts of HI and OS were effective against multiple bacterial strains, including strains with reduced susceptibility to the reference antibiotics bacitracin, oxolinic acid, and streptomycin. These findings highlight the potential of HI, GB and especially OS as functional ingredients or feed additives for aquaculture.

Keywords: IC_{50}; MIC; growth inhibition zone; bacteria; aquaculture

Key Contribution: Water, methanol and isopropanol effectively extract antibacterial compounds. High concentrations of silkworm extracts are effective against many fish pathogens. GB (water), HI (methanol and isopropanol) and OS (isopropanol) yield moderate to strong extracts. GBa (water) and OSa (isopropanol) extracts active against *Vibrio harveyi* resistant to bacitracin. HI extracts as potent as streptomycin against *B. thuringiensis* and *V. splendidus.*

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1. Introduction

Aquaculture is one of the fastest-growing food production sectors and plays a crucial role in global food security by supplying high-quality animal protein to an increasing human population [1,2]. Mediterranean aquaculture contributes substantially to regional economy and food security, producing over 600,000 tons of finfish annually, with gilthead seabream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), and meagre (*Argyrosomus regius*) representing the principal cultured species [2]. However, the sustainable expansion of the sector is increasingly constrained by infectious diseases, which reduce productivity, compromise animal welfare, and cause substantial economic losses [3,4].

Bacterial pathogens are among the most significant infectious agents affecting Mediterranean aquaculture [5,6]. Species of the genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Pseudomonas* are responsible for recurrent outbreaks associated with hemorrhagic septicemia, ulcerative lesions, systemic infections, and high mortality rates in cultured fish [5–8]. These pathogens are particularly problematic under intensive farming conditions and elevated seawater temperatures, conditions commonly encountered in Mediterranean production systems [5]. Disease management has traditionally relied on antibiotics such as oxytetracycline, florfenicol, and quinolones [5,6]. However, their extensive use has contributed to the emergence and dissemination of antimicrobial resistance (AMR), reducing treatment efficacy and raising concerns regarding environmental contamination, food safety, and public health [9,10]. Multidrug resistant *Vibrio* and *Aeromonas* isolates have already been reported from Mediterranean aquaculture environments [9,10], while global efforts to reduce antimicrobial use have led to stricter regulations, including restrictions on prophylactic antibiotic administration in the European Union. Consequently, the identification of alternative antimicrobial agents capable of controlling fish pathogens has become a research priority.

Natural products represent an important source of novel antimicrobial compounds, and insects have recently attracted considerable attention in this regard. Living in microbially rich environments, insects have evolved sophisticated innate immune systems that produce a wide range of bioactive molecules to combat pathogenic microorganisms. These include antimicrobial peptides (AMPs), such as defensins, cecropins, attacins, and coleoptericins, as well as medium-chain fatty acids—particularly lauric acid—chitin, chitosan, phenolic compounds, and other metabolites with antimicrobial and immunomodulatory properties [11–17]. Numerous studies have demonstrated antimicrobial activity of insect-derived extracts or purified compounds against human, veterinary, and aquatic pathogens, highlighting insects as promising reservoirs of natural antimicrobial agents [11–18]. Beyond their direct antibacterial effects, several insect-derived compounds have also been reported to stimulate host immunity and improve resistance to infection [18], suggesting potential applications in both disease prevention and functional feed development.

In parallel, insects have gained considerable interest as sustainable feed ingredients for aquaculture because of their high nutritional value and a relatively low environmental footprint [19,20]. They efficiently convert low-value organic substrates into high-quality biomass while requiring less land and water than conventional protein sources [19,20]. The European Union has progressively recognized their importance by authorizing several insect species for use in aquafeeds, including the black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), house cricket (*Acheta domesticus*), and silkworm (*Bombyx mori*), among others. However, although the nutritional properties of these species have been extensively investigated, comparatively fewer studies have systematically evaluated and compared their antibacterial potential against Mediterranean fish pathogens. Furthermore, emerging edible insect species, such as the two-spotted cricket (*Gryllus bimaculatus*), the

superworm (*Zophobas morio*), and the weaver ant (*Oecophylla smaragdina*), are increasingly attracting scientific and commercial interest because of their nutritional and medicinal properties. In particular, *G. bimaculatus* and *O. smaragdina* have long histories of use in traditional foods and medicine in Asia and have been reported to contain bioactive compounds with antimicrobial and antioxidant activities, although their activity against fish pathogens remains poorly characterized [21–24].

Previous studies have largely focused on individual insect species, specific antimicrobial peptides, or antibacterial activity against human pathogens, making direct comparisons between different edible insects and extraction methods difficult. Moreover, information regarding the antibacterial potential of insect extracts against bacterial pathogens relevant to Mediterranean aquaculture remains limited. Comparative screening studies are therefore needed to identify promising insect species and extraction approaches for future characterization of active compounds and potential application in sustainable aquaculture.

The present study aimed to comparatively evaluate the antibacterial activity of crude extracts obtained from seven insect species, including four species authorized for use in European aquafeeds (*B. mori*, *H. illucens*, *T. molitor*, *A. domesticus*) and three additional edible insect species of emerging interest (*Z. mori*, *G. bimaculatus*, *O. smaragdina*). Aqueous, methanolic, and isopropanolic extracts were prepared and screened against a panel of Mediterranean fish bacterial pathogens, including *Aeromonas veronii*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *Photobacterium damselae* subsp. *damselae*, *P. damselae* subsp. *piscicida*, *Vibrio alginolyticus*, *V. anguillarum*, *V. harveyi*, *V. splendidus*). By providing a comparative assessment of the antibacterial activity of crude insect extracts, this study seeks to identify promising candidates for future isolation of bioactive compounds and to support the development of insect-derived functional ingredients for sustainable aquaculture.

2. Materials and Methods

2.1. Insects

Silkworm pupae (*B. mori*, BMp) were purchased from Reptilia Nostra (Athina, Greece), larval meals from yellow mealworm (*T. molitor*, TMl), black soldier fly (*H. illucens,* HIl), and superworm (*Z. morio*, ZMl), were kindly provided by Dr. I. Karapanagiotidis (Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, Faculty of Agricultural Sciences, University of Thessaly, Volos, Greece). Adults of weaver ant (*O. smaragdina*, OSa), house cricket (*A. domesticus*, ADa) and two-spotted cricket (*G. bimaculatus*, GBa), were purchased from Next-Food (Ras Al Khaima, United Arab Emirates).

The insects were milled using a food blender and subsequently freeze-dried. Their proximate composition was analyzed in triplicate according to standardized analytical methods [25]. Moisture content was determined by drying pre-weighed samples in porcelain cups at 104 °C for 24 h (Method number 950.46). Ash content was determined by combustion at 500 °C for 12 h (Method number 920.153). Crude protein (CP) was quantified using to the Kjeldahl method (Method number 988.05) and calculated using a nitrogen-to-protein conversion factor (Kp) of 4.76 rather than the conventional coefficient of 6.25 commonly used for animal proteins, in order to avoid overstimulation due to nitrogen associated with chitin [26]. Total lipid content (ether extract, EE) was determined using a Soxhlet system (HT, 1043 Extraction unit Foss Tecator, Foss Analytical, Hilleroed, Denmark) according to the ISO method 6492:1999. Nitrogen-free extract (NFE) and gross energy (GE) were calculated as follows:

$$\text{NFE} = 100 - (\text{Moisture} + \text{Ash} + \text{CP} + \text{EE})$$

$$\text{GE} = (\text{CP} \times 23.6 + \text{EE} \times 39.5 + \text{NFE} \times 17.3)/100$$

2.2. Extraction Protocol

A preliminary experiment was conducted to select the most effective solvents for extracting antibacterial compounds from BMp, following a standard maceration protocol [27]. Briefly, 3 g of the milled freeze-dried BMp were extracted with 30 mL of different solvents (1:10 w/v) of increasing polarities (n-hexane, ethyl acetate, dichloromethane, chloroform, chloroform/methanol (1:1), isopropanol, methanol or distilled water) in sealed glass tubes (Parafilm). Extractions were performed for 48 h at room temperature under continuous magnetic stirring in a fume hood.

The resulting extracts were clarified by sequential centrifugation at 1000× *g* for 15 min followed by 2700× *g* for 10 min. Supernatants were collected, and solvents were removed according to their physicochemical properties: aqueous extracts were freeze-dried, methanol and isopropanol extracts were evaporated at 40 °C under a nitrogen stream (TurboVap LV system, Caliper Life Sciences, Hopkinton, MA, USA); and other organic extracts were concentrated using rotary evaporation at 35 °C.

Based on these results, three solvents (distilled water, methanol and isopropanol) were selected for further investigation of seven insect species, including silkworm pupae (BMp), three insect larval meals (HMl, TMl and ZMl) and three whole adult insects (ADa, GBa and OSa). Freeze-dried insect powders were macerated in each of the selected solvents (1:10 w/v) following the same procedure described above. The dry weight of each extract was determined, and extraction yield was calculated using the following equation:

$$\text{Yield (\%)} = 100 \times \text{dried extract weight (g)/initial freeze-dried insect weight (g)}$$

Concerning BMp extracts in the preliminary experiment, extracts were diluted either in distilled water (aqueous extracts) or in acetone (all other extracts), and working solution were adjusted with distilled water to 100–150 mg/mL depending on extract availability: methanol yielded high amount of extracts that were tested at 150 mg/mL, whereas extracts obtained with all other solvents were tested at 100 mg/mL. The final acetone concentration in the working solutions was $\leq$20% to avoid a turbidity that could interfere with optical density (OD) measurements. This acetone concentration did not exhibit antibacterial activity, in contrast to other tested solvents such as dimethyl sulfoxide (DMSO), dimethyl sulfide (DMS), dimethylformamide (DMF), or polysorbate 20 (Tween 20), which showed antibacterial activity even at low concentrations that were insufficient to fully solubilize the extracts.

Based on the preliminary solvent screening results using BMp, further experiments compared the seven insect species in standardized conditions. Stock solutions were prepared at 250 mg/mL, using either distilled water (for aqueous extracts) or acetone (for methanolic and isopropanolic extracts). Sonication in a water bath at 35 °C for 10 min was applied to facilitate dissolution of the extracts. Working solutions of all insects were adjusted to 50 mg/mL with distilled water. As before, the acetone concentration in all extracts adjusted to 50 mg/mL was 20%, except for the aqueous extracts, which were dissolved in water. Finally, all extracts were sterilized by filtration through 0.2 µm Whatman syringe filters (Whatman, Maidstone, UK) under a laminar flow hood to prevent microbial contamination. Samples were aliquoted, protected from light, and stored at −20 °C for a maximum of 2 months before analyses.

2.3. Bacterial Strains and Culture Conditions

Eleven strains of pathogenic bacteria commonly associated with disease outbreaks in Mediterranean fish farms were selected from the microbiological collection of the Microbiology Laboratory of the Hellenic Centre for Marine Research (Gournes, Greece). These

strains were originally isolated from diseased fish or shellfish in Greek aquaculture systems and are maintained as glycerol stocks within the HCMR collection (Table 1).

Table 1. List of tested bacteria with their host origin and organ when known.

Code	Gram **	Bacterium Species	Origin
Av	−	*Aeromonas veronii*	European seabass, *Dicentrarchus labrax*
Bt	+	*Bacillus thuringiensis*	Gilthead seabream, *Sparus aurata*
Pa	−	*Pseudomonas aeruginosa*	unknown
Pdd	−	*Photobacterium damselae damselae*	Gilthead seabream, *S. aurata*
Pdp	−	*Photobacterium damselae piscicida*	European seabass, *D. labrax*
Val V1	−	*Vibrio alginolyticus*	Gilthead seabream, *S. aurata*
Val	−	*Vibrio alginolyticus*	Kidney European seabass, *D. labrax*
Va	−	*Vibrio anguillarum*	Spleen European seabass, *D. labrax*
Vh	−	*Vibrio harveyi*	Spleen European seabass, *D. labrax*
Vh2 *	−	*Vibrio harveyi*	Greater amberjack, *Seriola dumerilii*
Vs	−	*Vibrio splendidus*	Mussels

* *Vibrio harveyi* VH2 is extensively described in a recent publication [28]; ** Gram negative (−), Gram positive (+).

All experiments were conducted under aseptic conditions in a laminar-flow hood (BioAura 2000, BioAir, Siziano, Italy). Bacterial strains were cultured at 22 °C in tryptic soy broth supplemented with 2% NaCl (TSB-2%) for 24 h for most bacteria, or 48 h concerning *P. aeruginosa* and *P. damselae piscicida*, to reach the exponential growth phase. Cultures were then centrifuged at 2300× *g* for 10 min, washed in phosphate-buffered saline (PBS), and resuspended in PBS to 0.5 McFarland, corresponding to an optical density of 0.10 ± 0.02 at 625 nm and approximately 1.5×10^8 CFU/mL [29,30].

2.4. Antibacterial Assays

Negative controls consisted of the solvent-only treatments, which were included in each agar plate and microplate. Positive controls consisted of antibiotics from different classes, including bacitracin (50 mg/mL), streptomycin (25 mg/mL), gentamycin (10 mg/mL), and oxolinic acid, flumequine, florfenicol and oxytetracycline (50 µg/mL). The reference antibiotic concentrations were selected based on preliminary optimization experiments. Each assay was repeated at least three times on different bacterial culture batches giving 3 to 6 biological replicates to ensure reproducibility.

2.4.1. Agar Well Diffusion Method

A pour-plate technique was performed following standard microbiological procedures [31]. Briefly, 1 mL of each logarithmic-phase bacterial suspension adjusted to 0.5 McFarland was mixed with 20 mL of freshly autoclaved tryptic soy agar supplemented with 2% NaCl (TSA-2%), cooled to 48 °C in a water bath, and poured into sterile Petri dishes. After complete solidification of the agar, wells of 2 mm diameter were aseptically punched using a sterile Pasteur pipette. Subsequently, 2 µL of each extract at the highest tested concentration or antibiotic at concentrations stated in Section 2.4 was dispensed into the wells. Plates were incubated at 22 °C for 24 h, or 48 h for *P. aeruginosa* and *P. damselae* piscicida). The diameters of the growth inhibition zones (GIZs) were then measured in millimeters.

2.4.2. Kinetic Microdilution Assay

Antibacterial activity was evaluated using a kinetic microdilution assay adapted from Clinical Laboratory Standard Institute (CLSI) guidelines [32,33], as reviewed by Balouiri and colleagues [29] in 384 wells flat-bottom transparent microplates (Greiner, Kremsmunster, Austria). Outer wells were filled with sterility controls (medium only or medium with

solvent, i.e., water or 20% acetone), to both monitor the sterility of the assay and minimize evaporation. Growth controls (medium plus inoculum without extract) were also included on each plate. Serial two-fold dilutions of each insect extract were prepared in sterile distilled water and distributed 10 wells of the microplate. The 2-fold serial dilution of the positive controls (reference antibiotics) was done in 21 wells to cover the amplitude of antibacterial activities of these antibiotics against the various bacterial strains. The solvent control (medium plus inoculum plus 20% acetone) was also serially diluted in 10 wells of each microplate.

Each well contained 25 µL of extract solution, 50 µL of TSB-2%, and 25 µL of bacterial inoculum adjusted to 0.5 McFarland and diluted 1:150 in TSB-2% corresponding to $1{\cdot}10^6$ CFU/mL (final volume: 100 µL per well). Bacterial growth kinetics were monitored at 22 °C every 15 min for 16 h for *Aeromonas veronii* (Av), *Bacillus thuringiensis* (Bt), *Photobacterium damselae* subsp. *damselae* (Pdd), *Vibrio anguillarum* (Va), *V. splendidus* (Vs), *V. alginolyticus* (Valg and Valg V1), 24 h for *V. harveyi* (Vh and VH2) or 48 h for *Pseudomonas aeruginosa* (Pa) and *P. damselae* subsp. *piscicida* (Pdp), by measuring the optical density at 600 nm (OD_{600}) using a microplate spectrophotometer (Genios Pro, Tecan, Mannedorf, Switzerland).

Antibacterial activity was expressed as the IC_{50}, defined as the lowest concentration of the serial dilution resulting in a ≥50% reduction in bacterial growth relative to the untreated solvent control. Subsequently, 10 µL of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL) were added in each well, incubated for 15 min, and absorbance was measured at 540 nm. Metabolically active bacteria reduce the yellow MTT to blue formazan crystals. The minimal metabolic inhibitory concentration (MIC) corresponded to the lowest concentration of the serial dilution of the extract or antibiotic that completely inhibited bacterial metabolic activity (i.e., absence of color change in the MTT assay). Antibacterial activity was interpreted using a semi-quantitative activity classification scale where MIC or IC_{50} values ≤ 1 mg/mL indicate very strong activity, 1–5 mg/mL strong activity, 5–25 mg/mL moderate activity, and >25 mg/mL weak activity.

3. Results

3.1. Proximate Composition of the Seven Insects

The proximate composition of the seven insect species is presented in Table 2. Among the insects analyzed, superworm larval meal (ZMl) exhibited the highest lipid content (39.7% crude lipids), whereas adult house cricket (ADa) showed the highest protein content, reaching 44.7% crude protein (N × 4.76), equivalent to 58.7% when calculated using the conventional nitrogen-to-protein conversion factor of 6.25.

Table 2. Proximate composition (%) of the 7 insect species tested in the present study.

	BMp	HIl	TMl	ZMl	ADa	GBa	OSa
Moisture (%)	7.1 ± 0.1	8.0 ± 0.3	12.5 ± 0.2	8.7 ± 0.2	5.3 ± 0.1	5.4 ± 0.1	6.1 ± 0.2
Ash (%)	4.8 ± 0.1	10.0 ± 0.1	3.7 ± 0.2	2.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	2.5 ± 0.1
CP (%) (Kp = 4.76)	40.4 ± 0.6	31.1 ± 0.7	39.4 ± 0.3	32.9 ± 0.4	44.7 ± 0.4	39.7 ± 0.2	37.5 ± 0.3
CP (%) (Kp = 6.25)	53.0 ± 0.7	40.9 ± 0.9	51.8 ± 0.4	43.2 ± 0.5	58.7 ± 0.6	52.1 ± 0.2	49.2 ± 0.3
Crude Lipids (%)	23.2 ± 0.6	29.6 ± 1.0	22.1 ± 0.5	39.7 ± 0.4	22.1 ± 0.1	31.6 ± 0.2	32.6 ± 0.1
NFE	24.6	21.3	22.2	16.1	24.3	19.8	21.5
GE (kJ/g)	22.9	22.7	21.9	26.3	23.5	25.3	25.4

Values represent mean ± standard deviation (n = 3). Abbreviations: CP: crude protein; NFE: nitrogen-free extract, GE: gross energy.

3.2. Preliminary Experiment on BMp

3.2.1. Extraction Yield of Silkworm Pupae

The extraction yields of BMp using eight different solvents are given in Table 3. The highest extraction yield of BMp was obtained with methanol (33.5%).

Table 3. Solvents used for the extraction of silkworm pupae; their polarity and extraction yields.

Solvent	Polarity	BMP
n-Hexane	0.009	24.0%
Ethyl-acetate	0.228	25.5%
Dichloromethane	0.234	24.2%
Chloroform	0.259	28.0%
Chloroform/Methanol	0.259/0.762	31.8%
Isopropanol	0.546	23.6%
Methanol	0.762	33.5%
DH_2O	1	7.3%

3.2.2. Antibacterial Activity of Silkworm Pupae Extracts

The results of the optimization experiment to identify the most effective extraction solvents for silkworm pupae, based on the growth inhibition zone (GIZ) assay, the minimum BMp extract concentration required to inhibit 50% of bacterial growth (IC_{50}), and the minimum metabolic inhibitory concentration (MIC), are presented in Table 4, Table 5 and Table 6 respectively.

Table 4. Growth inhibition zone (in mm) of BMp extracts.

		Bacteria									
		Av	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh2**	**Vs**
Solvents	water	3.0 ± 0.0 *	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	methanol	4.5 ± 0.5 *	0.0	23.0 ± 5.9	0.0	20.0 ± 0.0	22.3 ± 1.5 *	22.5 ± 2.1 *	0.0	22.0 ± 2.8 *	0.0
	isopropanol	0.0	0.0	17.2 ± 5.5	0.0	20.0 ± 0.0	12.3 ± 2.6.0 *	14.0 ± 0.0 *	0.0	16.0 ± 1.0 *	0.0
	n-hexane	0.0	0.0	12.7 ± 2.9	4.0 ± 0.0	11.0 ± 1.4	10.7 ± 1.0.0 *	11.5 ± 0.7 *	0.0	10.7 ± 5.7 *	0.0
	chloroform	0.0	0.0	14.0 ± 4.9	0.0	10.0 ± 0.0	12.0 ± 2.5 *	12.0 ± 4.2 *	0.0	11.3 ± 2.3	0.0
	chloroform/methanol	0.0	0.0	13.3 ± 5.7	0.0	0.0	12.0 ± 2.5 *	16.0 ± 0.0 *	0.0	11.0 ± 1.4	0.0
	dichloromethane	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ethyl acetate	0.0	0.0	14.7 ± 4.6	0.0	6.0 ± 1.4	7.7 ± 2.1 *	6.5 ± 0.7 *	0.0	8.0 ± 4.0 *	0.0

Growth inhibition zone (in mm) produced by 2 µL of at 100 mg/mL using different solvents (water, isopropanol, n-hexane, chloroform, chloroform/methanol, dichloromethane or ethyl acetate) or by the methanolic extract at 150 mg/mL against tested bacterial pathogens. * Asterisks indicate inhibition zones that were not fully translucent (i.e., blurred). Solvent-alone negative controls were included in each agar plate and exhibited no GIZ. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

3.3. Comparison of Extracts from the 7 Insect Species

3.3.1. Extraction Yield of the Seven Insect Species

The extraction yields of the seven insect species using the three selected solvents (water, methanol, isopropanol) are presented in Table 7.

3.3.2. Antibacterial Activity of Extracts from the Seven Insects and Reference Antibiotics

Thereafter, insect extracts were adjusted to 50 mg/mL. The GIZ results are presented in Table 8. Photographs of two agar plates are given in the Supplementary Material S1. The IC_{50} values of the extracts (mg/mL) and antibiotics (µg/mL) are shown in Tables 9 and 10, respectively. The MIC values for the extracts (mg/mL) and reference antibiotics (µg/mL) are reported in Tables 11 and 12, respectively.

Table 5. Minimum BMp extract concentration inhibiting 50% of bacterial growth (IC_{50} in mg/mL).

		Bacteria									
		Av	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh2**	**Vs**
Solvents	water	35.4 ± 16.6	31.8 ± 18.0	50 ± 0.0	87.5 ± 25.0	50 ± 0.0	34.4 ± 18.7	31.3 ± 21.7	44.6 ± 14.2	>100	>100
	methanol	100 ± 43.3	48.4 ± 18.9	125.0 ± 43.3	>150	18.6 ± 14.0	29.2 ± 19.1	32.5 ± 20.4	>150	>150	>150
	isopropanol	66.7 ± 28.9	>100	100 ± 0.0	83.3 ± 28.9	>100	100 ± 0.0	75.0 ± 28.9	>100	>100	>100
	n-hexane	>100	>100	>100	>100	>100	100 ± 0.0	>100	>100	>100	>100
	chloroform	>100	66.7 ± 28.9	100 ± 0.0	>100	>100	100 ± 0.0	>100	100 ± 0.0	>100	50.0 ± 0.0
	chloroform/methanol	>100	nd	50.0 ± 0.0	>100	>100	16.7 ± 7.2	>100	91.7 ± 14.4	20.8 ± 7.2	>100
	dichloromethane	>100	>100	100 ± 0.0	>100	>100	100 ± 0.0	83.3 ± 28.9	>100	100 ± 0.0	83.3 ± 28.9
	ethyl acetate	>100	>100	83.3 ± 28.9	>100	>100	100 ± 0.0	83.3 ± 28.9	>100	100 ± 0.0	66.7 ± 28.9

Extracts were serially 2-fold diluted in 10 wells from 100 mg/mL to 0.2 mg/mL for all extracts except for methanolic extracts which were diluted from 150 mg/mL to 0.3 mg/mL in a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

Table 6. Minimum metabolic inhibitory concentration (MIC in mg/mL) of BMp extracts using various solvents against tested bacterial pathogens.

		Bacteria									
		Av	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh2**	**Vs**
Solvents	water	34.4 ± 18.7	44.8 ± 27.1	nd.	50.0 ± 0.0	nd.	50.0 ± 0.0	56.3 ± 12.5	59.7 ± 21.4	100.0 ± 0.0	83.3 ± 28.8
	methanol	23.4 ± 10.7	75.0 ± 0.0	nd.	46.8 ± 19.8	nd.	62.5 ± 21.7	62.5 ± 21.7	53.3 ± 5.8	75 ± 11.2	31.2 ± 10.8
	isopropanol	62.5 ± 15.6	100 ± 0.0	nd.	>100.0	nd.	100.0 ± 0.0	68.75 ± 23.9	75.0 ± 25.0	>100	41.6 ± 14.4
	n-hexane	>100	100 ± 0.0	nd.	>100	nd.	100.0 ± 0.0	>100	>100	>100	100.0 ± 0.0
	chloroform	100 ± 0.0	100 ± 0.0	nd.	>100	nd.	100.0 ± 0.0	100.0 ± 0.0	>100	>100	25.0 ± 0.0
	chloroform/methanol	100 ± 0.0	25.0 ± 0.0	nd.	100.0 ± 0.0	nd.	20.8 ± 7.2	25.0 ± 0.0	>100	20.8 ± 7.2	6.3 ± 0.0
	dichloromethane	100 ± 0.0	100 ± 0.0	nd.	>100	nd.	100.0 ± 0.0	100.0 ± 0.0	100 ± 0.0	>100	18.8 ± 8.8
	ethyl acetate	75.0 ± 35.4	100 ± 0.0	nd.	>100	nd.	100.0 ± 0.0	100.0 ± 0.0	100 ± 0.0	75.0 ± 35.4	50.0 ± 14.5

Extracts were serially 2-fold diluted in 10 wells from 100 mg/mL to 0.2 mg/mL for all extracts except for methanolic extracts which were diluted from 150 mg/mL to 0.3 mg/mL in a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Data are presented as mean ± standard deviation (S.D.), $n = 3$. nd = not determined.

Table 7. Solvents used for insect extraction; their polarity and extraction yields for each insect tested.

		Extraction Yield						
Solvent	**Polarity**	**BMp**	**HIl**	**TMl**	**ZMl**	**ADa**	**GBa**	**OSa**
DH_2O	1	7.3%	11.8%	42.9%	28.2%	9.3%	13.7%	18.7%
Methanol	0.762	33.5%	37.4%	41.9%	50.0%	24.9%	21.6%	19.2%
Isopropanol	0.546	23.6%	26.9%	24.9%	37.2%	38.1%	28.0%	27.3%

BMp = *Bombyx mori* pupae, HIl = *H. illuscens* larval meal, TMl = *T. molitor* larval meal, ZMl = *Z. mori* larval meal, ADa = *A. domesticus* adult, GBa = *G. bimaculatus* adult, OSa = *O. smaragdana* adult.

Table 8. Growth inhibition zone (in mm) of insect extracts or by different antibiotics against tested bacterial pathogens.

		Bacteria										
	Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Water	BMp	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	HIl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TMl	0.0	0.0	0.0	8.0 ± 4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ZMl	0.0	0.0	0.0	7.0 ± 2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ADa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	GBa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	OSa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Methanol	BMp	0.0	0.0	6.5 * ± 2.1	0.0	0.0	4.5 ± 0.7	0.0	0.0	0.0	0.0	0.0
	HIl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TMl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ZMl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ADa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	GBa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	OSa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isopropanol	BMp	0.0	0.0	5.5 * ± 4.9	0.0	0.0	6.0 ± 1.4	0.0	0.0	0.0	0.0	0.0
	HIl	0.0	0.0	7.5 * ± 4.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TMl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ZMl	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	ADa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	GBa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	OSa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 8. *Cont.*

	Bacteria										
Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Abiotics											
Bacitracin	0.0	15.5 * ± 6.5	0.0	0.0	52.0 ± 22.0	3.0 ± 0.9	0.0	18.7 ± 10.1	15.2 ± 2.6	13.5 ± 2.7	10.0 ± 3.7
Streptomycin	8.8 ± 1.9	12.2 ± 5.2	11.3 ± 2.1	10.5 ± 3.5	22.5 ± 0.7	21.3 ± 5.5	10.0 ± 6.5	14.7 ± 7.0	11.7 ± 6.7	16.7 ± 2.5	17.0 ± 7.0
Gentamicin	20.5 ± 2.1	12.7 ± 4.5	16.3 ± 2.1	0	0.0	19.5 ± 0.7	16.5 ± 0.7	25.0 ± 12.7	23.5	22.5 ± 6.4	24.5 ± 0.7
Oxolinic Acid	0.0	0.0	0.0	2.0 ± 1.4	0.0	1.5 ± 0.7	0.0	0.0	0.0	0.0	0.0
Flumequine	0.0	0	0.0	6.5 ± 2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Florfenicol	0.0	6.3 ± 2.1	0.0	7.0 ± 1.4	0.0	0.0	0.0	0.0	3.0 ± 2.8	5.5 ± 2.1	7.5 ± 4.9
Oxytetracycline	3.5 ± 0.7 *	2.0 ± 0.7	0.0	4.3 ± 0.6	14.0 ± 1.4	3.7 ± 2.1	3.5 ± 1.9	9.0 ± 0.0	6.3 ± 0.6	7.3 ± 1.5	8.0 ± 4.0

Growth inhibition zone (in mm) produced by 2 µL of insects extracts at 50 mg/mL using different solvents (water, methanol and isopropanol) or antibiotics (bacitracin at 50 mg/mL, streptomycin at 25 mg/mL, gentamycin at 10 mg/mL, and oxolinic acid, flumequine, florfenicol and oxytetracycline at 50 µg/mL) against tested bacterial pathogens. * Asterisks represent inhibition zones that were not totally translucid. Solvent-alone negative controls were included in each agar plate and exhibited no GIZ. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

Table 9. Minimum extract or bacitracin concentration inhibiting 50% of the growth of tested bacterial pathogens (IC50 in mg/mL).

		Bacteria										
	Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Water	BMp	35.4 ± 16.6	31.8 ± 18.0	>50	>50	50 ± 0.0	34.4 ± 18.7	31.3 ± 21.7	44.6 ± 14.2	>50	>50	>50
	HIl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	TMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	3.4 ± 2.7	>50	9.4 ± 4.4	37.8 ± 26.3	>50	9.4 ± 4.4	25.0 ± 0.0	41.7 ± 14.4	7.3 ± 4.77	33.3 ± 14.4	20.8 ± 7.2
	OSa	>50	32.5 ± 24.0	>50	>50	>50	>50	>50	>50	>50	>50	>50
Methanol	BMp	>50	48.4 ± 18.9	>50	>50	18.6 ± 14.0	29.2 ± 19.1	32.5 ± 20.44	>50	>50	>50	>50
	HIl	0.5 ± 0.3	33.5 ± 28.6	>50	>50	1.6 ± 0.0	0.9 ± 0.7	0.7 ± 0.5	>50	>50	0.3 ± 0.1	1.3 ± 1.1
	TMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	8.3 ± 3.6
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	OSa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

Table 9. *Cont.*

		Bacteria										
	Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Isopropanol	BMp	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HIl	27.1 ± 21.9	1.7 ± 1.4	>50	>50	1.9 ± 0.8	1.0 ± 0.4	1.1 ± 1.0	>50	>50	1.8 ± 1.2	0.8 ± 0.7
	TMl	47.5 ± 33.5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	OSa	3.4 ± 2.7	37.5 ± 14.4	2.6 ± 0.9	8.3 ± 3.6	>50	6.8 ± 5.5	5.2 ± 1.8	29.7 ± 23.6	6.8 ± 5.5	16.7 ± 7.2	8.3 ± 3.6
Bacitracin		11.3 ± 2.8	40.0 ± 13.7	35.4 ± 25.3	10.9 ± 8.5	30.8 ± 25.5	11.7 ± 9.7	3.94 ± 3.86	43.8 ± 12.5	>50	5.9 ± 4.8	2.8 ± 2.2

Extracts and bacitracin were serially 2-fold diluted in 10 wells from 50 mg/mL to 0.1 mg/mL in a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Solvent-alone negative controls were included. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

Table 10. Minimum antibiotic concentration inhibiting 50% of the growth of tested bacterial pathogens (IC_{50} in µg/mL).

	Bacteria										
Antibiotics	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Streptomycin	91.5 ± 75.7	439.5 ± 245.8	162.8 ± 56.4	107.4 ± 64.8	81.4 ± 28.1	130.2 ± 56.4	162.8 ± 56.4	336.5 ± 279.9	146.5 ± 84.6	146.5 ± 69.1	3125.0 ± 0.0
Gentamicin	19.6 ± 13.7	58.6 ± 27.6	9.8 ± 6.9	29.3 ± 13.8	17.1 ± 4.8	17.6 ± 4.4	26.0 ± 10.1	97.7 ± 39.1	17.6 ± 4.4	86.1 ± 33.7	43.0 ± 21.4
Oxolinic Acid	9.5 ±8.2	14.1 ± 12.6	>50	15.6 ± 10.8	>50	29.2 ± 19.1	38.0 ± 19.5	30.6 ± 28.3	41.7 ± 14.4	>50	20.8 ± 7.2
Flumequine	5.4 ± 4.5	1.0 ± 0.4	10.0 ± 3.4	3.8 ± 4.3	11.2 ± 2.8	9.9 ± 4.2	8.2 ± 4.5	1.5 ± 1.0	11.7 ± 8.6	13.3 ± 9.0	10.4 ± 8.3
Florfenicol	0.7 ±0.5	0.3 ± 0.2	18.7 ± 7.2	0.5 ± 0.2	1.4 ± 0.4	0.6 ± 0.3	0.8 ± 0.4	0.4 ± 0.2	1.3 ± 0.4	1.6 ± 1.0	0.9 ± 0.5
Oxytetracycline	0.5 ± 0.3	0.2 ± 0.1	5.6 ± 1.4	0.3 ± 0.1	0.7 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.2 ± 0.1

Antibiotics (streptomycin at 25 mg/mL, gentamycin at 10 mg/mL, and oxolinic acid, flumequine, florfenicol and oxytetracycline at 50 µg/mL) were serially 2-fold diluted in 21 wells of a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Solvent-alone negative controls were included. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

Table 11. Minimum metabolic inhibitory concentration (MIC in mg/mL) of each insect extracts and bacitracin against tested bacterial pathogens.

		Bacteria										
	Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Water	BMp	34.4 ± 18.7	44.8 ± 27.1	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HIl	50.0 ± 0.0	>50	>50	48.3 ± 18.1	>50	>50	>50	>50	>50	>50	>50
	TMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	50.0 ± 0.0	>50	>50	>50	>50	>50	>50	44.0 ± 17.82	>50	>50	>50
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	3.4 ± 2.7	>50	8.3 ± 3.6	9.3 ± 4.4	12.5 ± 0.0	9.4 ± 4.4	25.0 ± 0.0	46.0 ± 19.2	14.6 ± 9.5	>50	20.8 ± 7.2
	OSa	>50	8.3 ± 3.6	>50	>50	>50	>50	>50	>50	>50	>50	>50

Table 11. *Cont.*

		Bacteria										
	Insect	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Methanol	BMp	23.4 ± 10.7	>50	>50	46.8 ± 19.8	>50	>50	>50	>50	>50	>50	31.2 ± 10.8
	HIl	8.3 ± 3.6	0.3 ± 0.1	>50	>50	>50	1.2 ± 0.7	1.5 ± 1.2	0.6 ± 0.5	>50	0.5 ± 0.4	2.2 ± 1.7
	TMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	35.7 ± 24.5
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	OSa	50.0 ± 0.0	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Isopropanol	BMp	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	41.6 ± 14.4
	HIl	12.5 ± 10.8	0.5 ± 0.2	43.4 ± 23.3	40.8 ± 20.8	34.5 ± 19.7	7.0 ± 4.6	1.3 ± 0.6	0.9 ± 0.5	>50	1.0 ± 0.9	1.2 ± 0.6
	TMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ZMl	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	ADa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	GBa	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	OSa	4.2 ± 1.8	32.5 ± 16.8	2.6 ± 0.9	5.7 ± 4.5	3.1 ± 0.0	37.5 ± 17.7	13.5 ± 11.0	42.0 ± 16.0	6.8 ± 5.5	16.7 ± 7.2	10.4 ± 3.6
Antibiotics												
Bacitracin		10.9 ± 3.1	33.3 ± 16.5	32.9 ± 26.8	31.0 ± 21.2	7.0 ± 3.9	15.0 ± 5.6	11.2 ± 2.8	37.5 ± 17.7	>50	40.9 ± 26.1	29.2 ± 19.1

Extracts were serially 2-fold diluted in 10 wells from 50 mg/mL to 0.1 mg/mL for all extracts in a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

Table 12. Metabolic minimum inhibitory concentration (MIC in µg/mL) of antibiotics against tested bacterial pathogens.

	Bacteria										
Antibiotics	**Av**	**Bt**	**Pa**	**Pdd**	**Pdp**	**Val V1**	**Val**	**Va**	**Vh**	**Vh2**	**Vs**
Streptomycin	48.8 ± 42.3	455.7 ± 298.3	130.2 ± 56.4	115.4 ± 51.8	390.3 ± 0.0	195.3 ± 0.0	227.9 ± 149.2	415.0 ± 280.5	211.6 ± 171.5	97.7 ± 69.1	4687.5 ± 2209.7
Gentamicin	18.3 ± 15.1	55.8 ± 20.9	15.5 ± 12.5	29.1 ± 13.8	19.5 ± 0.0	52.7 ± 34.9	32.6 ± 10.1	82.0 ± 48.6	29.3 ± 10.7	39.1 ± 27.6	25.6 ± 16.6
Oxolinic Acid	17.7 ± 12.6	28.8 ± 20.5	44.7 ± 19.6	39.7 ± 22.7	>50	37.5 ± 21.6	45.4 ± 19.1	39.0 ± 19.2	>50	>50	>50
Flumequine	8.6 ± 6.8	7.0 ± 6.0	32.0 ± 26.3	1.8 ± 1.2	12.5 ± 0.0	20.0 ± 6.0	16.7 ± 6.5	5.0 ± 4.6	17.5 ± 6.8	11.3 ± 9.0	6.9 ± 3.4
Florfenicol	1.1 ± 0.7	0.7 ± 0.7	12.8 ± 9.0	0.4 ± 0.2	1.4 ± 0.3	1.6 ± 0.0	1.4 ± 0.4	0.8 ± 0.6	1.7 ± 0.8	2.3 ± 0.9	1.4 ± 1.0
Oxytetracycline	0.7 ± 0.6	0.3 ± 0.3	4.4 ± 2.6	0.3 ± 0.1	0.8 ± 0.0	1.1 ± 0.4	0.6 ± 0.4	0.5 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.3 ± 0.2

Antibiotics (streptomycin at 25 mg/mL, gentamycin at 10 mg/mL, and oxolinic acid, flumequine, florfenicol and oxytetracycline at 50 µg/mL) were serially 2-fold diluted in 21 wells of a 384 wells-microplate where edges were filled with sterility controls to avoid evaporation. Solvent-alone negative controls were included. Data are presented as mean ± standard deviation (S.D.), $n = 3$.

4. Discussion

4.1. Proximate Composition

This study evaluated the proximate composition of seven insect species for their suitability as alternative ingredients in aquafeeds. Lipid and ash contents were consistent with previously reported ranges, although lipid levels varied considerably, likely due to differences in insect diets, except for the more specialized silkworm. Protein contents were generally at the lower end of published ranges, because a revised nitrogen-to-protein conversion factor (Kp = 4.76) [26] was used accounting non-protein nitrogen sources, such as chitin, chitosan, and other nitrogenous compounds commonly present in insects [34,35]. This adjustment reduces the overestimation of protein and the corresponding underestimation of nitrogen-free extract (NFE) common with the traditional Kp of 6.25 used for animal protein. After correction, protein values largely aligned with published ranges of 35.1–55.1% for BMp [35–38], 24.1–45.7% for HIl [34,39–43], 27.6–56.4% for TMl [44–46], 27.7–40.6% for ZMl [47–50] and 31.8.5–54.6% for ADa [21,36,46,51,52]. Values for OSa (39.2–42.1%) [22,23] and GBa (42.0–52.2%) [21,24,53] showed slightly lower protein and higher lipid contents, possibly reflecting limited available data. Variability in nutrient composition across studies can largely be attributed to differences in insect diet and processing methods [35,45,51,54].

Among the species analyzed, ADa combined high protein content and low moisture, indicating good potential for feed stability and fish growth performance. However, elevated NFE levels in species ADa and BMp may reduce their nutritional value due to the generally poor digestibility of carbohydrates in fish. The larvae of ZM exhibited the highest lipid content and are known to be rich in mono-unsaturated and poor in poly-unsaturated fatty acids, suggesting potential advantages of using defatted forms [47]. The larvae of HI were notably rich in mineral (ash content) (Table 2), likely related to elevated potassium, calcium, and phosphorus levels reported in the literature [55]. Overall, the findings confirm the favorable nutritional profile of the commonly studied HIl and highlight the potential less-explored, protein-rich species such as ADa and GBa for aquaculture feeds.

4.2. Efficacy of the Extraction Process and Selection of Optimal Solvents for Antibacterial Compound Extraction

Eight solvents of increasing polarity were tested on BMp, with extraction yields ranging from 7.3% (water) to 33.5% (methanol) (Table 3). Lipid-targeting solvents (ethyl acetate and n-hexane) produced yields (25.5 and 24% respectively, Table 3) comparable to BMp lipid content (23.17%; Table 2), whereas methanol achieved the highest recovery due to its ability to extract both hydrophilic and lipophilic compounds [56]. Growth inhibition zone assays revealed that most BMp extracts—except those obtained with dichloromethane—exhibited inhibitory activity against the majority of tested bacteria, with the exception of *V. anguillarum*, *V. splendidus* and *B. thuringiensis* (Table 4). Regarding the minimal extract concentration required to inhibit 50% of bacterial growth (IC_{50}) and the minimum inhibitory concentration (MIC), BMp extracts obtained with methanol, a chloroform/methanol mixture, and to a lesser extent water, exhibited mostly weak antibacterial activity against six fish pathogens, whereas isopropanol extracts were active against strains, but with relatively lower potency (Table 6). In contrast, chloroform and dichloromethane extracts showed relatively higher metabolic inhibition against *V. splendidus* but limited effect on other bacteria. These results indicate that both polar and nonpolar compounds contribute to the observed antimicrobial activity. Despite the higher efficacy of the chloroform/methanol mixture, subsequent analyses focused on methanol, isopropanol, and water due to chloroform's toxicity and environmental concerns [57]. Methanol and isopropanol were selected for their efficiency in extracting antibacterial compounds and their relatively lower environmental persistence, despite known toxicity at high exposure levels [56–58]. Water, although less

effective, was included as a green solvent for recovering hydrophilic bioactive compounds. Across the seven insect species, extraction yields varied widely, ranging from 7.3–42.9% for water, 19.2–50.0% for methanol, and 23.6–38.1% for isopropanol (Table 7). The 48 h extraction period substantially improved yields compared with previous 24 h extractions of HI, which achieved ≤2% yields [14].

4.3. Antibacterial Activity of Common Antibiotics Against Marine Aquaculture Pathogens

In the context of global rise of antimicrobial resistance and public health risks associated with the wide use of antibiotics [16,59], insect-derived feed ingredients enriched in antibacterial bioactive compounds may represent a more sustainable disease management strategy. The present study represents a preliminary screening of antibacterial potential of seven insect species. Although the antibacterial assays used in the present study do not distinguish between antibiotic-sensitive and resistant bacterial strains, they enable a comparative evaluation of the efficacy of insect extracts and conventional antibiotics. Most antibiotics tested showed very strong antibacterial activity (IC_{50} and MIC < 1 mg/mL) against all fish pathogens. An exception was the case of streptomycin which exhibited a lower but still strong activity against *V. splendidus* (1 mg/mL < IC_{50} and MIC < 5 mg/mL) (Tables 10 and 12). This aligns with widespread reports of streptomycin resistance in aquaculture pathogens, including *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Vibrio* species [10,59–64]. Similarly, oxolinic acid showed weak activity (IC_{50} and/or MIC > 25 mg/mL) against several pathogens, including *P. aeruginosa*, *P. damselae* subsp. *piscicida*, and *Vibrio* spp., in agreement with previously documented resistance in aquaculture environments [10,59,65,66]. In contrast, florfenicol and oxytetracycline demonstrated the highest efficacy (IC_{50} and MIC < 3 µg/mL) against most tested bacteria, except *P. aeruginosa* (Tables 10 and 12), although resistance to these two antibiotics has been widely reported in the Mediterranean region [10]. Notably, some insect-derived compounds investigated in the present study showed antibacterial activity comparable to that of bacitracin and, in certain cases, streptomycin, as discussed below.

4.4. Antibacterial Activity of the Extracts of Each Insect Species Tested

Insect extracts—particularly GB aqueous extracts, HI methanolic extract and isopropanolic extracts of HI and OS exhibited notable antibacterial activity against a broad range of bacteria, including strains with relatively reduced sensitivity to reference antibiotics such as *B. thuringiensis*, *P. damsela piscida*, *V. harveyi* and *V. splendidus,* which showed relatively low susceptibility to streptomycin and/or oxolinic acid. Compared to the polypeptide antibiotic bacitracin produced by *Bacillus licheniformis*, aqueous extracts of OS and GB, as well as methanolic extracts of HI and isopropanolic extractsof HI and OS, showed lower IC_{50} and MIC values against most tested bacteria. Methanolic and isopropanolic extracts of BM also produced larger growth inhibition zones (GIZs) against Pa and ValV1. In addition, aqueous extracts of TM and ZM generated a larger GIZ than bacitracin against Pdd (Table 8).

Insect-derived bioactive compounds with known antibacterial activity include antimicrobial peptides (AMPs), which often outperform conventional antibiotics against multidrug-resistant strains [67,68], medium-chain saturated fatty acids, including lauric (C12:0), capric (C10:0), and myristic (C14:0) acids [13], and chitosan, a derivative of chitin [69,70]. Details for each insect extracted with the three selected solvents are further discussed below.

The antibacterial activity of silkworm pupae (BMp) extracts obtained using solvents of varying polarity, with the exception of dichloromethane, was evidenced by growth inhibition zones against multiple fish pathogens (*A. veronii*, *P. aeruginosa*, *P. damselae subspecies*,

and *V.* spp.), as well as measurable IC_{50} and MIC values, albeit generally at concentrations above 6.3 mg/mL. This bioactivity is likely attributable to AMPs, such as moricin, BM-ponericin-L1, cecropins, defensins, enbocins, gloverins, and lebocins, which are well-documented in *B. mori*. [71–73], polysaccharide silkrose [11,74]. Previous studies have also reported moderate antibacterial activity of BM extracts rich in fatty acids against resistant bacteria [75], although results were variable depending on extraction methods [76]. Overall, these findings support the potential use of silkworm pupae as a functional ingredient or additive in aquafeeds, particularly in Mediterranean regions where sericulture by-products are readily available.

The antibacterial activity of HIl extracts obtained using the three selected solvents was evaluated against a range of fish pathogenic bacteria. In the growth inhibition zone assay, only the isopropanolic extract exhibited weak activity against *P. aeruginosa* (7.5 mm), although the diffuse inhibition zone suggested a primarily bacteriostatic rather than bactericidal effect. This observation was supported by MTT assays, which showed a weak inhibitory activity against *P. aeruginosa*. The aqueous HIl extract displayed little or no antibacterial activity, contrasting with previous reports of broader efficacy [42]. In contrast, the methanolic and isopropanolic HIl extracts exhibited strong antibacterial activity, against several fish pathogens, including *A. veronii*, *B. thuringiensis*, *P. damselae* subsp. *piscida*, and multiple *Vibrio* species (*V. alginolyticus* and *V. harveyi*). Their activity generally exceeded that of bacitracin and, in the case of *V. splendidus*, those of both bacitracin and streptomycin. These findings agree with earlier reports of antibacterial activity of HI methanolic extracts against both Gram-positive and Gram-negative bacteria, in contrast to extracts using other solvents, including n-hexane, chloroform, ethanol and water [14,30,77–79]. The broad antimicrobial activity of HIl is likely due to its diverse array of bioactive compounds, including antimicrobial peptides (defensins, cecropins, attacins, diptericins), polysaccharides (dipterose), fatty acids (palmitic acid, lauric acid), sterols, and chitin/chitosan [13,42,75,78,80–87], which help protect larvae from microorganisms developing in the moist organic substrates. Moreover, antimicrobial compound production can be enhanced through immune stimulation [18,87–90], and dietary HIl has consistently improved disease resistance in aquaculture species [28,91–94]. Overall, these results highlight HIl as a highly promising insect species for aquaculture applications owing to its broad-spectrum antimicrobial properties.

The larval extracts of yellow mealworm (TMl) and superworm (ZMl), generally exhibited weak to no antibacterial activity. Unlike HIl, these species develop on relatively dry substrates, such as flour, wheat bran or cereal grains, which may reduce the need for antimicrobial defenses. Nevertheless, the aqueous extracts produced notable GIZ against *P. damselae* subsp. *damselae*, a pathogen resistant to the other insect extracts and to bacitracin and gentamycin, although this activity was not confirmed by microdilution assays. TMl isopropanolic extract showed weak activity against *A. veronii*, while ZMl methanolic extract moderately inhibited *V. splendidus*, a strain with low sensitivity to bacitracin and streptomycin. Previous studies have reported broader antibacterial activity of ZMl extracts, particularly using acidified methanol [95]. The antibacterial properties of TMl are attributed to bioactive compounds, including antimicrobial peptides (e.g., tenecin, coleoptericin, attacin, and defensins; reviewed by Errico [96]), chitosan and lipid-derived molecules. TMl-derived chitosan has demonstrated greater antibacterial activity than commercial preparations [97,98], while its weaker lipid-associated activity likely arises from compounds other than lauric acid, which is present at much lower levels than in HIl [13,80]. As with HIl, immune stimulation can enhance the production of bioactive compounds in both TMl and ZMl [95,99]. Moreover, the stability of TMl bioactive compounds under a wide range of processing conditions (temperature, pH, and salinity) [99] and evidence that

dietary TMl enhances the resistance of red seabream (*Pagrus major*) to *Edwardsiella tarda* infection [100] further support its potential for aquaculture applications.

Concerning ADa, none of the aqueous, methanolic or isopropanolic extracts exhibited antibacterial activity under the tested conditions, as determined by the three assays (GIZ, IC_{50}, MIC). Although ADa is authorized for use in animal feed within the European Union, available literature on the antimicrobial properties of ADa remains scarce. Aqueous extracts have previously demonstrated antibacterial activity against several Gram-negative bacteria, although the concentrations tested were not specified [101]. Additionally, chitin derived from ADa has shown antibacterial effects against both Gram-positive and Gram-negative bacteria [102].

Another cricket species, GBa, is commonly used in animal nutrition (pets, reptiles, birds, and livestock) and in traditional medicine due to its high protein content and richness in functional bioactive compounds [103]. However, it has not yet been authorized by the European Commission for use in aquafeeds. Previous studies have demonstrated that dichloromethane extracts enriched in sterols exhibit antibacterial activity against *Staphylococcus aureus* [75], while chitosan derived from this species shares key physicochemical and potentially antimicrobial properties with shrimp-derived chitosan [104]. In the present study, the aqueous extract of GBa showed antibacterial activity against 9 of the 11 bacterial strains tested, with IC_{50} and MIC values as low as 3.4 mg/mL against *Av* (Tables 9 and 11). In contrast, the methanolic and isopropanolic extracts exhibited no detectable antibacterial activity. The pronounced activity of the aqueous extract is likely associated with hydrophilic bioactive compounds, such as chitin/chitosan derived molecules or antimicrobial peptides [104]. Collectively, these findings highlight GBa as a promising functional ingredient for aquafeeds, combining nutritional value with a diverse range of bioactive properties.

Weaver ants (OSa) are known for their nest-building behavior and production of antiseptic formic acid, and have long been used in traditional Southeast Asian medicine to treat wounds and infections [105]. Previous studies have reported variable antimicrobial activity, with methanolic extracts active against *S. aureus* and *Escherichia coli* and aqueous extracts attributed to benzoic acid derivatives [22,28,106]. In the present study, OSa extracts did not produce growth inhibition zones (GIZs) at 50 mg/mL against the tested bacteria. However, the aqueous extract exhibited weak inhibitory activity against *B. thuringiensis*, the methanolic extract showed only marginal effect, and the isopropanolic extract exhibited moderate to strong antibacterial activity against 8 out of the 11 bacterial strains, with IC_{50} and MIC values usually lower than those of bacitracin. Given the growing interest in commercially available ant-derived compounds [107], OSa represents a very promising natural source of novel antibacterial agents in the fight against bacterial pathogens.

4.5. Feasibility of Insect-Derived Antibacterial Compounds in Aquafeeds

Extracts from three of the seven insect species evaluated in the present study (GBa, HIl and OSa) demonstrated moderate to strong antibacterial activity against fish pathogens. The extraction of antibacterial compounds from GBa using the most environmentally friendly solvent, i.e., water, was particularly interesting, but its relatively low extraction yield (13.7%) may limit practical application. In contrast, the methanolic and isopropanolic extracts of HIl, as well as the isopropanolic extract of OSa, combined higher extraction yields (>26.9%) with strong antibacterial activity across a broad range of pathogens, in some cases equivalent to antibiotics such as streptomycin and bacitracin.

The use of antibiotics in fish feeds for the treatment of bacterial infections typically requires concentrations ranging from 20 to 250 mg/kg body weight (BW) [59], corresponding to approximately 1–12.5 g/kg of feed when fish are fed at 2% of BW per day. Considering that bioactive compounds extracted from insects in the present study were, at best,

approximately 500-fold less potent than oxytetracycline (as obtained for the methanolic extract of HIl and the isopropanolic extract of OSa), which is commonly used in aquaculture at 1–2 g/kg feed, the use of crude insects for therapeutic purposes is neither feasible nor practical. On the contrary, given the extraction yields calculated for each extract, the use of whole insects (adults or larvae) in functional feeds at 0.1 to 2% inclusion, or as a sustainable alternative to fishmeal at inclusion levels of up to 30%, would correspond to an estimated incorporation of approximately 0.0137–4.1% of the aqueous GBa extract, 0.037–11.2% of the methanolic HIl extract, and 0.027–8.2% of the isopropanol HIl or OSa extracts, equivalent to 137 mg to 112 g of insect-derived extract per kg of feed. Notably, all four extract types exhibited relatively higher activity than bacitracin against most of the tested bacterial pathogens and the recommended inclusion level of bacitracin as feed additive ranges from 50 to 275 mg/kg animal feed. However, the European Union has banned the use of bacitracin in 1998 (Council Regulation No 2820/98). Alternative antibacterial additives, such as medium-chain fatty acids, are usually administered at inclusion levels of 1–2 g/kg of aquafeed [107]. At realistic inclusion levels, namely 0.1–5.3 g HIl/kg feed, 0.4–14.6 g GBa/kg feed or 0.2–7.3 g OSa/kg feed, insect-derived ingredients could deliver biologically relevant concentrations of antibacterial compounds (approximately 50–2000 mg/kg feed), while aligning with current regulatory restrictions in aquaculture. This approach therefore represents a feasible nutritional strategy for antimicrobial intervention [108], either through direct inhibition of bacterial pathogens or indirectly via immunostimulation and increase of the fish resistance to bacterial diseases. From an economic and environmental perspective, insect meals can become competitive with fishmeal when considering not only ingredient costs, but also benefits such as improved growth performance, reduced disease-related losses, lower antibiotic usage, potential price premiums for sustainably produced seafood, and a reduced environmental footprint through decreased pressure on wild fish stocks, lower greenhouse gas emissions, and valorizing organic waste streams. However, the generation of precise mechanistic and applied knowledge remains essential to fully establish insects as viable and resilient innovative ingredients for aquafeeds.

5. Conclusions

The present study highlights the antibacterial activity of GBa and HIl and the untapped potential of the less-studied species OSa, as well as the potential for these three insect species to be sustainable ingredients for the development of next-generation aquafeeds reducing reliance on conventional antibiotics. Future research should include the purification and identification of active compounds, investigation of mechanisms of antibacterial action, assessment of toxicity and safety profiles, and validation of efficacy of dietary HIl, GBa and OSa in Mediterranean fish, focusing on growth performance, gut microbiota modulation, immune responses, and disease resistance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes11070389/s1, Suppementary Material S1: Photographs of two agar plates.

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Abbreviations

The following abbreviations are used in this manuscript:

ADa	House cricket (*Acheta domesticus*) adult
AMPs	Antimicrobial peptides
BMp	silkworm (*Bombyx mori*) pupae
CP	Crude protein
EE	Ether extract
GBa	two-spotted cricket (*Gryllus bimaculatus*) adult
GE	Gross energy
GIZ	Growth Inhibition Zone
HIl	Black soldier fly (*Hermetia illuscens*) larvae
IC_{50}	minimal concentration inhibiting 50% of bacterial growth
MIC	minimal metabolic inhibitory concentration
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NFE	Nitrogen-free extract
OSa	weaver ant (*Oecophylla smaragdina*) adult
TSA	Tryptic soy agar
TSB	Tryptic soy broth
TMl	Yellow mealworm (*Tenebrio molitor)* larvae
ZMl	Superworm (*Zophobas morio)* larvae

References

1. Boyd, C.E.; McNevin, A.A.; Davis, R.P. The contribution of fisheries and aquaculture to the global protein supply. *Food Secur.* **2022**, *14*, 805–827. [CrossRef]
2. FAO. *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*; FAO: Rome, Italy, 2022. [CrossRef]
3. Defoirdt, T.; Sorgeloos, P.; Bossier, P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.* **2011**, *14*, 251–258. [CrossRef]
4. Austin, B.; Austin, D.A. *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish*, 4th ed.; Springer: Berlin/Heidelberg, Germany, 2007; pp. 552.
5. Rigos, G.; Kogiannou, D.; Padrós, F.; Cristòfol, C.; Florio, D.; Fioravanti, M.; Zarza, C. Best therapeutic practices for the use of antibacterial agents in finfish aquaculture: A particular view on European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) in Mediterranean aquaculture. *Rev. Aquacult.* **2021**, *13*, 1285–1323. [CrossRef]
6. Zrnčić, S.; Katharios, P.; Padrós, F.; Fioravanti, M.; Gustinelli, A.; Le Breton, A.; Toffan, A. New challenges and achievements in Mediterranean fish health management. *Bull. Eur. Assoc. Fish. Pathol.* **2024**, *45*. [CrossRef]
7. Rivas, A.J.; Lemos, M.L.; Osorio, C.R. *Photobacterium damselae* subsp. *damselae*, an emerging pathogen of marine fish. *Front. Microbiol.* **2013**, *4*, 283. [CrossRef]
8. Janda, J.M.; Abbott, S.L. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.* **2010**, *23*, 35–73.
9. Deekshit, V.K.; Maiti, B.; Krishna Kumar, B.; Kotian, A.; Pinto, G.; Bondad-Reantaso, M.G.; Karunasagar, I. Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. *Rev. Aquacult.* **2023**, *15*, 261–273. [CrossRef]
10. Massaccesi, N.; Coci, M.; Basili, M.; Amalfitano, S.; Melita, M.; Di Cesare, A.; Sabatino, R.; Manini, E.; Luna, G.M.; Quero, G.M. A Mediterranean perspective on antimicrobial resistance in aquaculture. *Aquaculture* **2026**, *617*, 743773. [CrossRef]

11. Ali, M.F.Z.; Yasin, I.A.; Ohta, T.; Hashizume, A.; Ido, A.; Takahashi, T.; Miura, C.; Miura, T. The silkrose of *Bombyx mori* effectively prevents vibriosis in penaeid prawns via the activation of innate immunity. *Sci. Rep.* **2018**, *8*, 8836. [CrossRef]
12. Alvarez, D.; Wilkinson, K.A.; 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*, 17. [CrossRef]
13. Borrelli, L.; Varriale, L.; Dipineto, L.; Pace, A.; Menna, L.F.; Fioretti, A. Insect-derived lauric acid as a promising alternative strategy to antibiotics in the antimicrobial resistance scenario. *Front. Microbiol.* **2021**, *12*, 620798. [CrossRef]
14. 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*, 219–226. [CrossRef]
15. Meylaers, K.; Clynen, E.; Daloze, D.; DeLoof, A.; Schoofs, L. Identification of 1-lysophosphatidylethanolamine (C16:1) as an antimicrobial compound in the housefly, *Musca domestica*. *Insect Biochem. Mol. Biol.* **2004**, *34*, 43–49. [CrossRef]
16. Ohta, T.; Kusano, K.; Ido, A.; Miura, C.; Miura, T. Silkrose: A novel acidic polysaccharide from the silkmoth that can stimulate the innate immune response. *Carbohydr. Polym.* **2015**, *136*, 995–1001. [CrossRef]
17. Vogel, H.; Müller, A.; Heckel, D.G.; 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. [CrossRef]
18. Gasco, L.; Józefiak, A.; Henry, M. Beyond the protein concept: Health aspects of using edible insects on animals. *J. Insects Food Feed* **2021**, *7*, 715–741. [CrossRef]
19. Henry, M.; Gasco, L.; Piccolo, G.; Fountoulaki, E. Review on the use of insects in the diet of farmed fish: Past and future. *Anim. Feed Sci. Technol.* **2015**, *203*, 1–22. [CrossRef]
20. Lisboa, H.M.; Nascimento, A.; Arruda, A.; Sarinho, A.; Lima, J.; Batista, L.; Dantas, M.F.; Andrade, R. Unlocking the Potential of Insect-Based Proteins: Sustainable Solutions for Global Food Security and Nutrition. *Foods* **2024**, *13*, 1846. [CrossRef]
21. Udomsil, N.; Imsoonthornruksa, S.; Gosalawit, C.; Ketudat-Cairns, M. Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). *Food Sci. Technol. Res.* **2019**, *25*, 597–605. [CrossRef]
22. Alagappan, S.; Chaliha, M.; Sultanbawa, Y.; Fuller, S.; Hoffman, L.C.; Netzel, G.; Weber, N.; Rychlik, M.; Cozzolino, D.; Smyth, H.E.; et al. Nutritional analysis, volatile composition, antimicrobial and antioxidant properties of Australian green ants (*Oecophylla smaragdina*). *Future Foods* **2021**, *3*, 100007. [CrossRef]
23. Chakravorty, J.; Ghosh, S.; Megu, K.; Jung, C.; Meyer-Rochow, V.B. Nutritional and anti-nutritional composition of *Oecophylla smaragdina* (Hymenoptera: Formicidae) and *Odontotermes* sp. (Isoptera: Termitidae): Two preferred edible insects of Arunachal Pradesh, India. *J. Asia-Pac. Entomol.* **2016**, *19*, 711–720. [CrossRef]
24. Phesatcha, B.; Phesatcha, K.; Viennaxay, B.; Matra, M.; Totakul, P.; Wanapat, M. Cricket meal (*Gryllus bimaculatus*) as a protein supplement on in vitro fermentation characteristics and methane mitigation. *Insects* **2022**, *13*, 129. [CrossRef]
25. AOAC. *Official Methods of Analysis of Association of Official Agricultural Chemists International*, 18th ed.; AOAC International: Gaithersburg, MD, USA, 2005.
26. Janssen, R.H.; Vincken, J.-P.; Van den Broek, L.A.M.; Fogliano, V.; Lakemond, C.M.M. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J. Agric. Food Chem.* **2017**, *65*, 2275–2278. [CrossRef]
27. Pan, J.; Xu, H.; Cheng, Y.; Mintah, B.K.; Dabbour, M.; Yang, F.; Chen, W.; Zhang, Z.; Dai, C.; He, R.; et al. Recent insight on edible insect protein: Extraction, functional properties, allergenicity, bioactivity, and applications. *Foods* **2022**, *11*, 2931. [CrossRef]
28. Droubogiannis, S.; Triga, A.; Pavlidis, M.; Katharios, P. Genome sequences of *Vibrio harveyi* VH2 and *Vibrio harveyi* Vhp1-sp. *Microbiol. Resour. Announc.* **2025**, *14*, e00087-25. [CrossRef]
29. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef]
30. Mohamed, H.; Marusich, E.; Afanasev, Y.; Leonov, S. Fatty acids-enriched fractions of *Hermetia illucens* (black soldier fly) larvae fat can combat MDR pathogenic fish bacteria *Aeromonas* spp. *Int. J. Mol. Sci.* **2021**, *22*, 8829. [CrossRef]
31. Sanders, E.R. Aseptic laboratory techniques: Plating methods. *J. Vis. Exp.* **2012**, *11*, e3064. [CrossRef]
32. Clinical and Laboratory Standards Institute (CLSI). *M07-A10, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 10th ed.; CLSI: Wayne, PA, USA, 2015.
33. Clinical and Laboratory Standards Institute (CLSI). *M100 Performance Standards for Antimicrobial Susceptibility Testing*, 29th ed.; CLSI: Wayne, PA, USA, 2019.
34. Caligiani, A.; Marseglia, A.; Leni, G.; Baldassarre, S.; Maistrello, L.; Dossena, A.; Sforza, S. Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids, and chitin. *Food Res. Int.* **2018**, *105*, 812–820. [CrossRef]
35. Tassoni, L.; Cappellozza, S.; Dalle Zotte, A.; Belluco, S.; Antonelli, P.; Marzoli, F.; Saviane, A. Nutritional composition of *Bombyx mori* pupae: A systematic review. *Insects* **2022**, *13*, 644. [CrossRef]

36. Brogan, E.N.; Park, Y.L.; Shen, C.; Matak, K.E.; Jaczynski, J. Characterization of lipids in insect powders. *LWT* **2023**, *184*, 115040. [CrossRef]
37. Kashyap, S.; Priyadharshini, P.; Murugesh, K.A.; Anand, S.; Tilak, M.; Radha, P. Proximate and amino acid analysis of mulberry and non-mulberry silkworm pupae. *Pharma Innov. J.* **2023**, *12*, 1140–1142. [CrossRef]
38. Yuet Ying, L.; Ying, L.H.; Sofian-Seng, N.S.; Mustapha, W.A.W.; Razali, N.S.M. Physicochemical characteristics and microbiological quality of silkworm (*Bombyx mori*) larval and pupae powder: Comparative study. *Sains Malays.* **2022**, *51*, 547–558. [CrossRef]
39. Chia, S.Y.; Tanga, C.M.; Osuga, I.M.; Cheseto, X.; Ekesi, S.; Dicke, M.; van Loon, J.J.A. Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products. *Entomol. Exp. Appl.* **2020**, *168*, 472–481. [CrossRef]
40. Holeh, G.M.; Opiyo, M.A.; Brown, C.L.; Sumbule, E.; Gatagwu, J.; Oje, E.O.; Munyi, F. Effect of different waste substrates on the growth, development and proximate composition of black soldier fly (*Hermetia illucens*) larvae. *Livest. Res. Rural Dev.* **2022**, *34*, 57.
41. Loho, L.; Lo, D. Proximate and fatty acid analysis of black soldier fly larvae (*Hermetia illucens*). *IOP Conf. Ser. Earth Environ. Sci.* **2023**, *1169*, 012082. [CrossRef]
42. Müller, A.; Wolf, D.; Gutzeit, H.O. The black soldier fly, *Hermetia illucens*—A promising source for sustainable production of proteins, lipids and bioactive substances. *Z. Naturforsch. C* **2017**, *72*, 351–363. [CrossRef]
43. Yusoff, N.A.M.; Hai, C.T.; Komilua, C.F. Proximate composition of larvae, prepupae and adult in black soldier fly (*Hermetia illucens*). *J. Agrobiotechnol.* **2022**, *13*, 109–117. [CrossRef]
44. Iyapo, K.; Adewole, M.; Okanlawon, S.; Sule, O.; Olofintoye, B.O. Nutritional composition profile of mealworm larvae (*Tenebrio molitor*) reared on different substrate: A potential protein substitute for fishmeal. *Niger. J. Entomol.* **2024**, *40*, 92–100. [CrossRef]
45. Kröncke, N.; Benning, R. Influence of dietary protein content on the nutritional composition of mealworm larvae (*Tenebrio molitor* L.). *Insects* **2023**, *14*, 261. [CrossRef]
46. Laroche, M.; Perreault, V.; Marciniak, A.; Gravel, A.; Chamberland, J.; Doyen, A. Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods* **2019**, *8*, 572. [CrossRef]
47. Asimaki, A.; Psofakis, P.; Gkalogianni, E.-Z.; Katouni, A.-M.; Berillis, P.; Kormas, K.A.; Rumbos, C.I.; Athanassiou, C.G.; Vasilaki, A.; Fountoulaki, E.; et al. The effects of dietary fishmeal replacement by full-fat and defatted *Zophobas morio* larvae meals on juvenile gilthead seabream (*Sparus aurata*): An integrative approach. *Aquacult. Nutr.* **2025**, *21*, 8885509. [CrossRef]
48. Bogusz, R.; Onopiuk, A.; Żbik, K.; Pobiega, K.; Piasecka, I.; Nowacka, M. Chemical and microbiological characterization of freeze-dried superworm (*Zophobas morio* F.) larvae pretreated by blanching and ultrasound treatment. *Molecules* **2024**, *29*, 5447. [CrossRef]
49. Prachom, N.; Boonyoung, S.; Hassaan, M.S.; El-Haroun, E.; Davies, S.J. Preliminary evaluation of superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*. *Aquacult. Nutr.* **2021**, *27*, 1304–1314. [CrossRef]
50. Wadje, P.; Akshay, R.; Meenatchi, R. Effect of pre-treatments on proximate composition, protein extraction yields and extraction rate of superworm protein. *Biol. Forum* **2022**, *14*, 288–294.
51. Ndiritu, A.K.; Kinyuru, J.N.; Kenji, G.M.; Gichuhi, P.N. Extraction technique influences the physico-chemical characteristics and functional properties of edible crickets (*Acheta domesticus*) protein concentrate. *Food Meas.* **2017**, *11*, 2013–2021. [CrossRef]
52. Siddiqui, S.A.; Zhao, T.; Fitriani, A.; Rahmadhia, S.N.; Alirezalu, K.; Fernando, I. *Acheta domesticus* (house cricket) as human foods—An approval of the European Commission—A systematic review. *Food Front.* **2024**, *5*, 435–473. [CrossRef]
53. Wongsorn, D.; Todklang, W.; Pitiwittayakul, N.; Sanmahayak, B. Growth performance and nutritional composition of field crickets (*Gryllus bimaculatus* De Geer) fed with diets containing dried cassava leaves. *Int. J. Agric. Technol.* **2025**, *21*, 2621–2634. [CrossRef]
54. Mishyna, M.; Chen, J.; Benjamin, O. Sensory attributes of edible insects and insect-based foods–Future outlooks for enhancing consumer appeal. *Trends Food Sci. Technol.* **2020**, *95*, 141–148. [CrossRef]
55. Eke, M.; Tougeron, K.; Ngamo, L.; Hance, T. Effect of food waste composition on growth and nutritional value of black soldier fly larvae. *J. Insects Food Feed* **2025**, *12*, 631–655. [CrossRef]
56. Navarro del Hierro, J.; Cantero-Bahillo, E.; Fornari, T.; Martin, D. Effect of defatting and extraction solvent on the antioxidant and pancreatic lipase inhibitory activities of extracts from *Hermetia illucens* and *Tenebrio molitor*. *Insects* **2021**, *12*, 789. [CrossRef]
57. Richardson, P.; Bryan, M.C.; Diorazio, L.; Gallou, F.; Keily, S.; Martinez, I.; Plummer, S.; Reed, A.B.; Shields, J.; Wrigley, G.; et al. Solvent sustainability in drug discovery: Where are we now, and how can we improve? *J. Med. Chem.* **2025**, *68*, 25625–25664. [CrossRef]
58. Church, A.S.; Witting, M.D. Laboratory testing in ethanol, methanol, ethylene glycol, and isopropanol toxicities. *J. Emerg. Med.* **1997**, *15*, 687–692. [CrossRef]
59. Afroze, S.; Faisal, M.; Khan, M.N.A.; Barua, H.A. Comprehensive review of antibiotics and antimicrobial resistance in the aquaculture sector of the world and Bangladesh. *Int. J. Microbiol.* **2025**, *2025*, 8818516. [CrossRef]

60. Ibrahim, M.; Ahmad, F.; Yaqub, B.; Ramzan, A.; Imran, A.; Afzaal, M.; Mirza, S.A.; Mazhar, I.; Younus, M.; Akram, Q.; et al. Current trends of antimicrobials used in food animals and aquaculture. In *Antibiotics and Antimicrobial Resistance Genes in the Environment*; Hasmi, M.Z., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; Volume 1, pp. 39–69. [CrossRef]
61. Kathleen, M.M.; Samuel, L.; Felecia, C.; Reagan, E.L.; Kasing, A.; Lesley, M.; Toh, S.C. Antibiotic resistance of diverse bacteria from aquaculture in Borneo. *Int. J. Microbiol.* **2016**, *2016*, 2164761. [CrossRef]
62. Nguyen, H.N.K.; Van, T.T.H.; Nguyen, H.T.; Smooker, P.M.; Shimeta, J.; Coloe, P.J. Molecular characterization of antibiotic resistance in *Pseudomonas* and *Aeromonas* isolates from catfish of the Mekong Delta, Vietnam. *Vet. Microbiol.* **2014**, *171*, 397–405. [CrossRef]
63. Sudha, S.; Divya, P.S.; Francis, B.; Hatha, A.A. Prevalence and distribution of *Vibrio parahaemolyticus* in finfish from Cochin (South India). *Vet. Ital.* **2012**, *48*, e81.
64. Yang, Y.; Miao, P.; Li, H.; Tan, S.; Yu, H.; Yu, H. Antibiotic susceptibility and molecular characterization of *Aeromonas hydrophila* from grass carp. *J. Food Saf.* **2018**, *38*, e12393. [CrossRef]
65. Akinbowale, O.L.; Peng, H.; Barton, M.D. Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *J. Appl. Microbiol.* **2006**, *100*, 1103–1113. [CrossRef]
66. Akinbowale, O.L.; Peng, H.; Grant, P.; Barton, M.D. Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *Int. J. Antimicrob. Agents* **2007**, *30*, 177–182. [CrossRef]
67. Sahoo, A.; Swain, S.S.; Behera, A.; Sahoo, G.; Mahapatra, P.K.; Panda, S.K. Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm: A review. *Front. Microbiol.* **2021**, *12*, 661195. [CrossRef]
68. Wu, Q.; Patočka, J.; Kuča, K. Insect antimicrobial peptides, a mini review. *Toxins* **2018**, *10*, 461. [CrossRef]
69. Cheraghi, E.; Kababian, M.; Moradi-Asl, E.; Mousavi Bafrouyi, S.M.; Saghafipour, A. Structure and antibacterial activity of chitosan from the American cockroach, the German cockroach, and the mealworm beetle. *J. Arthropod Borne Dis.* **2022**, *16*, 325–339. [CrossRef]
70. Gul, T.; Khan, N.; Latif, R.; Faqir, Y. A review on the utilization of insect chitosan in the food industry: Challenges and opportunities. *Int. J. Biol. Macromol.* **2025**, *328*, 147587. [CrossRef]
71. Hara, S.; Yamakawa, M. Moricin, a novel type of antibacterial peptide isolated from the silkworm, *Bombyx mori*. *J. Biol. Chem.* **1995**, *270*, 29923–29927. [CrossRef]
72. Nesa, J.; Jana, S.K.; Sadat, A.; Biswas, K.; Kati, A.; Kaya, O.; Mondal, R.; Dam, P.; Thakur, M.; Kumar, A.; et al. Antimicrobial potential of a ponericin-like peptide isolated from *Bombyx mori* L. hemolymph in response to *Pseudomonas aeruginosa* infection. *Sci. Rep.* **2022**, *12*, 15493. [CrossRef]
73. Makwana, P.; Rahul, K.; Ito, K.; Subhadra, B. Diversity of antimicrobial peptides in silkworm. *Life* **2023**, *13*, 1161. [CrossRef]
74. Miura, T.; Nishikawa, M.; Otsu, Y.; Ali, M.F.Z.; Hashizume, A.; Miura, C. The effects of silkworm-derived polysaccharide (Silkrose) on ectoparasitic infestations in yellowtail (*Seriola quinqueradiata*) and white trevally (*Pseudocaranx dentex*). *Fishes* **2022**, *7*, 14. [CrossRef]
75. Mudalungu, C.M.; Mokaya, H.O.; Tanga, C.M. Beneficial sterols in selected edible insects and their associated antibacterial activities. *Sci. Rep.* **2023**, *13*, 10786. [CrossRef]
76. Ma, G.; Wu, L.; Shao, F.; Zhang, C.; Wan, H. Antimicrobial activity of 11 insects extracts against multi-drug resistant (MDR) strains of bacteria and fungus. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *252*, 022132. [CrossRef]
77. Bonomini, M.G.; Verstringe, S.; Bruggeman, G.; Vandercruyssen, R.; Carmans, H.; Caligiani, A. Characterisation, antibacterial activity and prebiotic potential of dried *Hermetia illucens* L. larvae and their fractions. *J. Insects Food Feed* **2025**, *11*, 273–288. [CrossRef]
78. Choi, W.H.; Jiang, M. Evaluation of antibacterial activity of hexanedioic acid isolated from *Hermetia illucens* larvae. *J. Appl. Biomed.* **2014**, *12*, 179–189. [CrossRef]
79. Harlystiarini, H.; Mutia, R.; Wibawan, I.W.T.; Astuti, D.A. In vitro antibacterial activity of black soldier fly (*Hermetia illucens*) larva extracts against gram-negative bacteria. *Bul. Peternak.* **2019**, *43*, 125–129. [CrossRef]
80. Dabbou, S.; Ferrocino, I.; Gasco, L.; Schiavone, A.; Trocino, A.; Xiccato, G.; Barroeta, A.C.; Maione, S.; Soglia, D.; Biasato, I.; et al. Antimicrobial effects of black soldier fly and yellow mealworm fats and their impact on gut microbiota of growing rabbits. *Animals* **2020**, *10*, 1292. [CrossRef]
81. Elhag, O.; Zhou, D.; Song, Q.; Soomro, A.A.; Cai, M.; Zheng, L.; Yu, Z.; Zhang, J. Screening, expression, purification, and functional characterization of novel antimicrobial peptide genes from *Hermetia illucens* (L.). *PLoS ONE* **2017**, *12*, e0169582. [CrossRef]
82. Guarnieri, A.; Triunfo, M.; Scieuzo, C.; Ianniciello, D.; Tafi, E.; Hahn, T.; Zibek, S.; Salvia, R.; DeBonis, A.; Falabella, P. Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*. *Sci. Rep.* **2022**, *12*, 8084. [CrossRef]
83. Lin, Y.-S.; Liang, S.-H.; Lai, W.-L.; Lee, J.-X.; Wang, Y.-P.; Liu, Y.-T.; Wang, S.-H.; Lee, M.-H. Sustainable extraction of chitin from spent pupal shell of black soldier fly. *Processes* **2021**, *9*, 976. [CrossRef]

84. Mudalungu, C.M.; Tanga, C.M.; Kelemu, S.; Torto, B. An overview of antimicrobial compounds from African edible insects and their associated microbiota. *Antibiotics* **2021**, *10*, 621. [CrossRef]
85. Park, S.I.; Chang, B.S.; Yoe, S.M. Detection of antimicrobial substances from larvae of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Entomol. Res.* **2014**, *44*, 58–64. [CrossRef]
86. Park, S.-I.; Yoe, S.M. A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: Isolation, structural and functional characterization. *Entomol. Res.* **2017**, *47*, 115–124. [CrossRef]
87. Giglio, F.; De Stefano, F.; Fusco, A.; Salvia, R.; Scieuzo, C.; Cos, P.; Donnarumma, G.; Falabella, P. Antibacterial and antifungal potential of *Hermetia illucens* hemolymph contained-peptides. *Probiotics Antimicrob. Prot.* **2026**, *18*, 2446–2461. [CrossRef]
88. 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*, 704. [CrossRef]
89. Park, S.I.; Kim, J.W.; Yoe, S.M. Purification and characterization of a novel antibacterial peptide from black soldier fly (*Hermetia illucens*) larvae. *Dev. Comp. Immunol.* **2015**, *52*, 98–106. [CrossRef]
90. Xiao, Y.; Zhu, L.; Liang, R.; Su, J.; Yang, J.; Cao, X.; Lu, Y.; Yu, Y.; Hu, J. A meta-analysis of the effects of black soldier fly meal on fish immune response and antioxidant capacity. *Comp. Immunol. Rep.* **2024**, *7*, 200162. [CrossRef]
91. Abdel-Latif, H.M.R.; Abdel-Tawwab, M.; Khalil, R.H.; Metwally, A.A.; Shakweer, M.S.; Ghetas, H.A.; Khallaf, M.A. Black soldier fly (*Hermetia illucens*) larvae meal in diets of European seabass: Effects on antioxidative capacity, non-specific immunity, transcriptomic responses, and resistance to the challenge with *Vibrio alginolyticus*. *Fish. Shellfish Immunol.* **2021**, *114*, 207–217. [CrossRef]
92. Nishiguchi, H.; Suryadi, I.B.B.; Ali, M.F.Z.; Miura, C.; Miura, T. Dietary black soldier fly (*Hermetia illucens*)—Dipterose-BSF—Enhanced zebrafish innate immunity gene expression and resistance to *Edwardsiella tarda* infection. *Insects* **2024**, *15*, 326. [CrossRef]
93. Sayramoglu, H.; Ozturk, R.C.; Ustaogly, D.; Terzi, Y.; Yandi, I.; Kayis, S.; Capkin, E.; Altinok, I. Effects of black soldier fly meal feeding on rainbow trout gut microbiota, immune-related gene expression, and *Lactococcus petauri* resistance. *J. Insects Food Feed* **2023**, *10*, 141–157. [CrossRef]
94. Yildirim Aksoy, M.; Eljack, R.; Schrimsher, C.; Beck, B. Use of dietary frass from black soldier fly larvae, *Hermetia illucens*, in hybrid tilapia (Nile × Mozambique, *Oreochromis niloticus* × *O. mozambique*) diets improves growth and resistance to bacterial diseases. *Aquacult. Rep.* **2020**, *17*, 100373. [CrossRef]
95. Du, M.; Liu, X.; Xu, J.; Li, S.; Wang, S.; Zhu, Y.; Wang, J. Antimicrobial effect of *Zophobas morio* hemolymph against bovine mastitis pathogens. *Microorganisms* **2020**, *8*, 1488. [CrossRef]
96. Errico, S.; Spagnoletta, A.; Verardi, A.; Moliterni, S.; Dimatteo, S.; Sangiorgio, P. *Tenebrio molitor* as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 148–197. [CrossRef]
97. Nafary, A.; Mousavi Nezhad, S.A.; Jalili, S. Extraction and characterization of chitin and chitosan from *Tenebrio molitor* beetles and investigation of its antibacterial effect against *Pseudomonas aeruginosa*. *Adv. Biomed. Res.* **2023**, *12*, 96. [CrossRef]
98. Chae-Shim, S.; Do-Yeong, K.; Weon-Sun, S. Characterization of chitosan extracted from mealworm beetle (*Tenebrio molitor*, *Zophobas morio*) and rhinoceros beetle (*Allomyrina dichotoma*) and their antibacterial activities. *Int. J. Biol. Macromol.* **2019**, *125*, 72–77. [CrossRef]
99. Hwang, D.; Lee, S.H.; Goo, T.W.; Yun, E.Y. Potential of antimicrobial peptide-overexpressed *Tenebrio molitor* larvae extract as a natural preservative for Korean traditional sauces. *Insects* **2022**, *13*, 381. [CrossRef]
100. Ido, A.; Hashizume, A.; Ohta, T.; Takahashi, T.; Miura, C.; Miura, T. Replacement of fish meal by defatted yellow mealworm (*Tenebrio molitor*) larvae in diet improves growth performance and disease resistance in red seabream (*Pagrus major*). *Animals* **2019**, *9*, 100. [CrossRef]
101. Ouango, M.; Cissé, H.; Romba, R.; Drabo, S.M.; Semde, R.; Savadogo, A.; Gnankine, O. Entomotherapy as an alternative treatment for diseases due to Gram-negative bacteria in Burkina Faso. *Sci. Rep.* **2024**, *14*, 7. [CrossRef]
102. Malm, M.; Liceaga, A.M. Physicochemical properties of chitosan from two commonly reared edible cricket species, and its application as a hypolipidemic and antimicrobial agent. *Polysaccharides* **2021**, *2*, 339–353. [CrossRef]
103. Yu, S.H.; Yu, S.-Y.; Lee, B.-S.; Kim, H.-J.; Kim, M.-R.; Lee, Y.-C. 28-day repeated oral dose toxicity study of an aqueous extract of *Gryllus bimaculatus* in Sprague-Dawley rat. *Toxicol. Rep.* **2020**, *7*, 577–582. [CrossRef]
104. Chae, K.S.; Shin, C.S.; Shin, W.S. Characteristics of cricket (*Gryllus bimaculatus*) chitosan and chitosan-based nanoparticles. *Food Sci. Biotechnol.* **2018**, *27*, 631–639. [CrossRef]
105. Offenberg, J. Weaver ants (*Oecophylla*). In *Encyclopedia of Social Insects*; Starr, C.K., Ed.; Springer: Cham, Switzerland, 2021; pp. 1009–1021. [CrossRef]
106. Offenberg, J.; Jensen, I.C.; Hansen, R.R. Combatting plant diseases with ant chemicals: A review and meta-analysis. *J. Appl. Ecol.* **2022**, *59*, 25–38. [CrossRef]

107. Liu, H.; Zhou, W.; Cai, C.; Feng, F.; Cai, H.; Yang, H. Biological function of medium-chain fatty acids and their application in aquatic animals: A review. *Animals* **2025**, *15*, 2294. [CrossRef]
108. Ibeagha-Awemu, E.M.; Omonijo, F.A.; Piché, L.C.; Vincent, A.T. Alternatives to antibiotics for sustainable livestock production in the context of the One Health approach: Tackling a common foe. *Front. Vet. Sci.* **2025**, *12*, 1605215. [CrossRef]