antibiotics

MDPI

Article

Antimicrobial Activity of *Apis mellifera* Bee Venom Collected in Northern Peru

Orlando Pérez-Delgado [1,*], Abraham Omar Espinoza-Culupú [1] and Elmer López-López [2]

[1] Health Science Research Laboratory, Universidad Señor de Sipán, Chiclayo 14001, Peru
[2] Faculty of Health Sciences, Universidad Señor de Sipán, Chiclayo 14001, Peru
* Correspondence: perezdelgado@crece.uss.edu.pe

Abstract: Due to the emergence of microorganisms resistant to antibiotics and the failure of antibiotic therapies, there is an urgent need to search for new therapeutic options, as well as new molecules with antimicrobial potential. The objective of the present study was to evaluate the in vitro antibacterial activity of *Apis mellifera* venom collected in the beekeeping areas of the city of Lambayeque in northern Peru against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Bee venom extraction was performed by electrical impulses and separated using the Amicon ultra centrifugal filter. Subsequently, the fractions were quantified by spectrometric 280 nm and evaluated under denaturant conditions in SDS-PAGE. The fractions were pitted against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. A purified fraction (PF) of the venom of *A. mellifera* and three low molecular weight bands of 7 KDa, 6 KDa, and 5 KDa were identified that showed activity against *E. coli* with a MIC of 6.88 µg/mL, while for *P. aeruginosa* and *S. aureus*, it did not present a MIC. No hemolytic activity at a concentration lower than 15.6 µg/mL and no antioxidant activity. The venom of A. mellifera contains a potential presence of peptides and a predilection of antibacterial activity against *E. coli*.

Keywords: venom; antimicrobial activity; fraction; bee; protein

Citation: Pérez-Delgado, O.; Espinoza-Culupú, A.O.; López-López, E. Antimicrobial Activity of *Apis mellifera* Bee Venom Collected in Northern Peru. *Antibiotics* **2023**, *12*, 779. https://doi.org/10.3390/antibiotics12040779

Academic Editors: Valério Monteiro-Neto and Elizabeth S. Fernandes

Received: 8 February 2023
Revised: 10 April 2023
Accepted: 16 April 2023
Published: 19 April 2023

1. Introduction

World Health Organization (WHO) published the first global surveillance report on antibiotic resistance, showing that five out of six regions had more than 50% resistance to third-generation cephalosporins and fluoroquinolones in *Escherichia coli* and methicillin resistance in *Staphylococcus aureus* in hospital settings [1].

S. aureus is a human commensal that can cause systemic infections in the host; this requires evading the immune response and the ability to proliferate in different niches in the host; currently, the infection by staphylococci in the face of immune mediators and the disease is not well known [2]. However, the main agent of bacteremia and infective endocarditis (IE), as well as osteoarticular, skin, and soft tissue infections, pleuropulmonary infections [3], and even the appearance of methicillin-resistant *S. aureus* (MRSA), which is a therapeutic problem in patients [4]. Staphylococcal infection has also been reported from hosts or carriers of asymptomatic nasopharyngeal bacteria, even with certain risk factors such as passive smoking and a large family [5]. The results of certain studies have determined that *S. aureus* has generated resistance against ampicillin, penicillin, rifampicin, clindamycin, oxacillin, and erythromycin [6]. Variable susceptibility to levofloxacin, ciprofloxacin, gentamicin, tetracycline, and sulfamethoxazole-trimethoprim has also been shown [7], and patterns have also been shown regarding the mecA, rpoB, blaZ, ermB, tetM, and nuc genes [6,8]. This resistance has been acquired through different mechanisms, the most frequent being reduced membrane permeability, excessive production of β-lactamase, and acquiring resistance genes or gene mutations [9].

Likewise, *Pseudomonas aeruginosa* can cause nosocomial outbreaks related to its resistance and virulence properties [10], being a producer of β-lactamases and multiresistant

to a wide range of antimicrobials such as penicillin, cephalosporin, cephamycin, and carbapenem [11]. In addition, 35 resistomes (antimicrobial resistance genes) have been identified that confer resistance to 18 different antibiotics (including four classes of beta-lactams) and 214 virulence factor genes [12], in addition to the susceptibility of *P. aeruginosa* to carbapenems, piperacillin-tazobactam, and amikacin has undergone alterations before and during COVID-19 [13]. There are phenotypic studies that *P. aeruginosa*, as producers of extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamases (MBL), also present genes associated with biofilm formation and virulence, such as toxA and lasB [14]. Even using polymerase chain reaction (PCR) techniques and molecular markers such as Random Amplified Polymorphic DNA (RAPD), they have identified strains resistant to imipenem, Ticarcillin + Clavulanate, Piperacillin, and Ticarcillin + Clavulanate, these strains being isolated from swimming pools [15].

E. coli is responsible for a large number of virulent variants associated with human diseases, such as urinary tract infection (UTI) with a resistance rate of >55% to first to fourth-generation cephalosporins [16], neonatal and traveler's diarrhea [17], and multiresistant isolates (MDRs) the most prevalent genes being CTX-M-1, followed by NDM-1 for Betalactamases and the genes ermB and aac(6′)-Ib for resistance to macrolides and aminoglycosides [18]. *E. coli* is frequently discharged into the environment through feces, including wastewater, and is considered an indicator of fecal contamination. Many strains can carry resistance genes [19]. According to isolates, they have usually been reported to be sensitive to netilmicin, gentamicin, chloramphenicol, pipemidic acid, nalidixic acid, ciprofloxacin, amoxicillin/clavulanic acid, and nitrofurantoin, as well as increased susceptibility to cefotaxime, ceftriaxone, and aztreonam [20]. Uncomplicated UTI isolates have been found to have a higher susceptibility than complicated UTI isolates to amoxicillin, amoxicillin/clavulanic acid, and ciprofloxacin [21].

Antimicrobial resistance has been reported in gram-negative and gram-positive bacteria, with reports of up to 96.2% for *Pseudomonas* spp. and 66.7% for *E. coli* [22], with isolates reported in heart disease intensive care units [23] and in bloodstream infections [24] antimicrobial-resistant bacteria are also considered the most frequent uropathogens [25]. There are reports of MRSA in up to 50% of patients [26], with resistance profiles for cefoxitin, chloramphenicol, clindamycin, and gentamicin [27].

The failure of antibiotics, including the latest generation, to counteract superbugs highlights the need to search for new molecules with antimicrobial potential to control the global problem of antimicrobial resistance. One group of these new molecules are peptides that are antimicrobial (AMP) and are promising molecules for combating antimicrobial resistance (AMR) [28]. AMP has been found the most in the venoms of different organisms, such as scorpions [29–31], snakes [32], spiders [33], and bees [34], among other venoms.

The composition of bee venom is very variable, having Peptides: Melitin (the main component of the venom), Apamin, Mast Cell-Degranulating Peptide (MCD), Tertiapin, Secapin, and Its Isoforms, Adolapin, Procamine, and Minimine; Polypeptides: Api m 6, Cardiopep, Icarapin, and Major Royal Jelly Proteins; Enzymes: Phospholipase A2 (PLA2), Hyaluronidase, Acid Phosphatase, and Dipeptidylpeptidase IV; Serine Proteases [35].

Bees are insects found on all continents, many of these species have yet to be described and are an exciting source for the study and search for new molecules with antimicrobial properties. There are experimental and clinical reports on *Apis mellifera* venom and its anti-inflammatory, antimicrobial, and anticancer effects; the components present in the venom, such as proteins, vary from a summer season compared to a winter season [36–38], in addition, have shown different therapeutic properties against oxidative stress induced by beta-amyloid [39–41]. For Parkinson's disease, the neuroprotective potential of bee venom against oxidative stress induced by rotenone (pesticide) has been demonstrated in a mouse model, including preventing the decrease in dopamine and also restoring locomotor activity in mice [42,43]. For Lyme disease, the melittin present in the venom showed in vitro antibacterial effects against the causative agent *Borrelia burgdorferi* [44] and even had significant antibacterial effects against *E. coli*, *S. aureus*, and *Salmonella*

typhyimurium [45]. Melittin also exhibited antibacterial activity against MRSA strains [46], with antimicrobial potential against agents that cause dental caries, with antifungal capacity including suppression of biofilm formation [47,48]. Its significant antiviral potential has also been demonstrated in in vitro and in vivo assays on different enveloped (Influenza A) and non-enveloped (enterovirus-71) viruses [49]. In addition, phospholipase A2 (PLA2) can also block the replication of the virus, being shown to be responsible for the inhibition of HIV replication [50]. The present study aimed to evaluate the in vitro antibacterial activity of *Apis mellifera* venom collected in the beekeeping areas of the city of Lambayeque in northern Peru against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

2. Results

As seen in Figure 1, 15% SDS-PAGE-Tricine of the purified fraction (PF) of crude venom from *A. mellifera* yielded 3 low molecular weight bands, i.e., 7 kDa, 6 kDa, and 5 kDa.

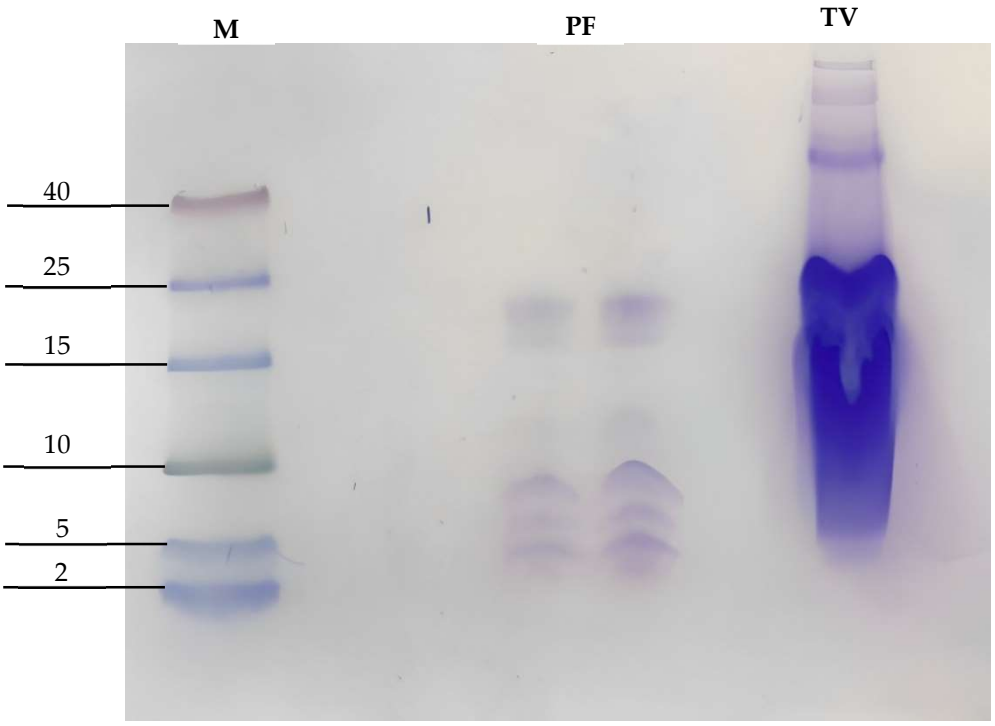

Figure 1. SDS polyacrylamide gel electrophoresis with Tricine (SDS-PAGE-Tricine) of the purified fraction; M denoted the marker lane (molecular weight 2–40 kDa) (molecular weight marker), PF denotes the protein fractions, and TV denotes total venom.

The PF of *A. mellifera* venom had a minimum inhibitory concentration (MIC) of 6.88 µg/mL ($p < 0.05$) for *E. coli.* For the *S. aureus* strain, at higher concentrations, the venom exhibited antibacterial activity. For *P. aeruginosa,* no antibacterial activity was observed (Figure 2).

Concentrations of the PF of *A. mellifera* venom above 125 µg/mL in erythrocyte suspension produced more than 50% hemolysis. At a concentration of 31.25 µg /mL, less than 10% hemolysis occurred, but at 7.8 µg /mL PF, no erythrocyte lysis was evidenced (Figure 3).

The PF of the *A. mellifera* venom showed no antioxidant activity was observed at the concentrations evaluated.

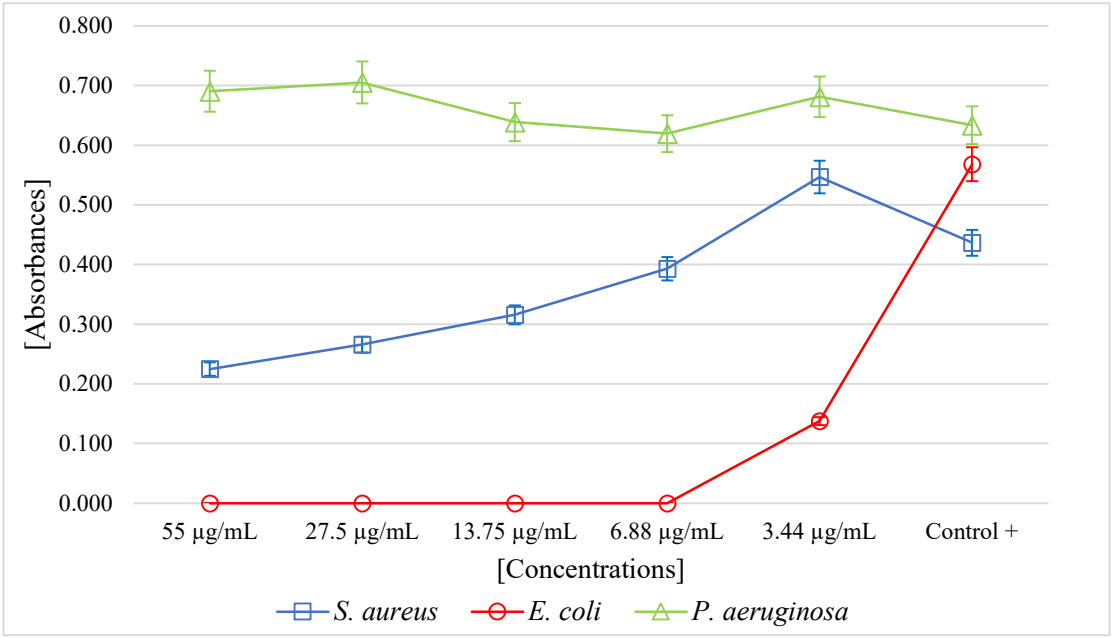

Figure 2. Microbial growth of *S. aureus*, *E. coli*, and *P. aeruginosa* incubated with the purified fraction of *Apis mellifera* venom.

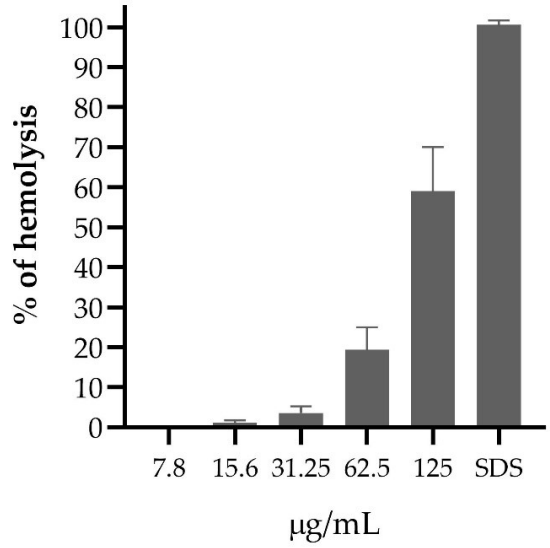

Figure 3. Hemolytic activity of the purified fraction of *Apis mellifera* venom.

3. Discussion

The purified fraction (PF) of the *A. mellifera* venom revealed the presence of peptides using the SDS-PAGE-Tricine technique and found three peptides of 7 kDa, 6 kDa, and 5 kDa. The chemical composition of the venom of *A. mellifera* is highly variable; such as melittin (3 kDa), apamin (2 kDa), and cecropin (4 kDa), enzymes, such as phospholipase A2 (19 kDa) and hyaluronidase (38 kDa), biologically active amines, such as histamine and epinephrine, as well as peptides not reported [39,51,52] and this suggests that many of these components may contribute their anti-inflammatory, antifungal, antiviral, healing and analgesic properties [36,53–55].

Our results were coherent with other studies; the PF of *A. mellifera* venom collected from Íllimo showed antibacterial activity against *E. coli*, but the same was not observed for *P. aeruginosa* despite being a gram-negative bacterium. For *S. aureus*, as the concentration of the venom fraction increased, bacterial growth was affected. Interestingly, the results

of other studies of the antibacterial activity of the crude venom showed highly variable MICs against *E. coli* and for *S. aureus* [56], including also demonstrating the inhibitory effect through a viability assay at a temperature of 25 °C against *E. coli* and *P. putida*, causing membrane permeability and loss of ATP, showing no effect against *P. fluorescens* [57], as well as the crude bee venom extract in a region of Egypt significantly inhibited the growth of *E. coli* ATCC8739 and *S. aureus* ATCC 6538P [58], in addition, the action of crude bee venom from Iran demonstrated inhibition through the Kirby–Bauer method against *E. coli* and *S. aureus*, but not against *P. aeruginosa* [59].

Other studies have shown the existence of antimicrobial peptides in the venom; it follows that the interaction against the cell envelope of the bacteria is due to the attraction between the positively charged venom peptides and the phospholipids, causing a rupture or instability of the venom membrane, in addition to forming pores; however, this mechanism requires a certain concentration threshold [60]. Direct insertion of melittin leads to pore formation, whereas the parallel conformation is inactive and prevents other melittin molecules from being inserted, thus, preventing pore formation [61]. However, melittin has a molecular weight of 3 KDa; in our study, we found three peptides in the range of 5 KDa to 7 KDa; this finding demonstrates that melittin is not the only peptide present in bee venom with antibacterial activity.

The PF of *A. mellifera* venom at a concentration lower than 15.6 µg/mL demonstrated low hemolytic activity. Few studies have revealed the hemolytic activity of bee venom in Peru; on the contrary, in other latitudes, they have revealed that melittin has not presented significant hemolytic activity below a concentration of 0.25 µg/mL [62], the hemolytic action was also demonstrated against erythrocytes of different species, with variable sensitivity to bee venom pools, with sheep erythrocytes being the most resistant to hemolytic action compared to equine erythrocytes, including humans erythrocytes showed good resistance to hemolytic action, it follows that hemolysis can be increased by the action of phospholipase 2 (PLA2) after the action of melittin [63].

The PF of the venom of *A. mellifera* has not shown antioxidant capacity, but in other studies, they demonstrated antioxidant capacity. Curiously, they worked with the total venom or apitoxin, having the capacity to inhibit the free radical DPPH (2,2-diphenyl-1-picrylhydrazine) between 60% and 75% of antioxidant activity [64]. In the same sense, demonstrated with the venom of *A. mellifera syriaca* eliminating DPPH radicals between 50 to 65% [65]. When analyzing the venom of four bee species, *A. dorsata*, *A. mellifera*, *A. florea*, and *A. cerena*, they showed that *A. dorsata* contained the highest amount of melittin; they also revealed that the extract of *A. dorsata* had the highest antioxidant activity from the DPPH and ABTS (3-ethylbenzothiazoline-6-sulfonic acid) assays, including melittin alone, revealed very poor antioxidant activity among all bee venom extracts [66], this suggests that in our study, of the peptides present in the venom PF, melittin was not present.

The bioactive components present in the venom have generated much interest in medicine through the different species of the *Apis* genus, and their application in in vitro antimicrobial activity [67], their cytotoxic action against cancer cells [68], even the synergistic effect of the venom with some antibiotics such as Cephotax, Cefepime, and Tavanic has been revealed [69]. Through Transmission Electron Microscopy, the deformation of the cell wall was appreciated, resulting in the destruction of the cell wall, changes in the permeability of the membrane, leakage of cell content, inactivation of metabolic activity, and finally, cell death [57], as the inhibitory effect on F1-F0—ATPase has also been demonstrated [70].

4. Materials and Methods

4.1. Bee Venom Samples

The venom was obtained from Africanized bees *A. mellifera* (Linnaeus, 1758) in hives from the Cruz Verde town center of the Ilimo District located at latitude 6°28′26″ and longitude 79°50′34″ (Lambayeque); an electrical impulse of 3 volts with an electrical intensity of 0.004 A was passed through a collecting box (beeWhisper 6.0; Model 2020), without harming the specimens (Figure 4a,b) [71].

Figure 4. (**a**) beeWhisper 6.0. collector box; (**b**) electrostimulation of the collector box; (**c**) recovery of *A. mellifera* dry venom; (**d**) storage of the venom.

The venom was collected on a glass plate and allowed to dry; it was then transferred into a 50-mL Falcon tube (Figure 4c,d). Then, the bee venom was resuspended in sterile deionized water and centrifuged at 10,000× *g* at 4 °C for 10 min to remove insoluble materials. The supernatant was collected and stored in 2 mL microtubes at −20 °C.

4.2. Fraction Concentration and Electrophoresis

The fractions were collected and quantified via absorbance at 280 nm (Navi UV/vis Nano spectrophotometer, Seongnam-si, Republic of Korea) using the formula [mg/mL] = (1.56 × Abs 280 nm) − (0.76 × Abs 260 nm) [72].

Crude fractions of bee venom were collected and concentrated using the Amicon ultra centrifugal filter (Merck Millipore, Cork, Ireland) with Cutoff from 3 kDa to 100 kDa [73], quantified via absorbance, and 50 µg protein from the FP were evaluated on a gel Tricine-SDS-PAGE (15%) under denaturing conditions [74] with a voltage of 100 volts and stained with Coomassie blue [75].

4.3. Antimicrobial Activity Test

The MIC values of the fraction were determined using the broth microdilution method in 96-well plates [76] against the strains of *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853; 50 µL of bacterial solution containing 5 × 10^4 CFU/mL was placed in each well, then 50 µL of different concentrations of the fraction (55 µg/mL to 3.44 µg/mL) were added and incubated at 37 °C for 24 h. The positive control was broth plus inoculum, and the negative control was only broth. Growth of the positive control was determined by a growth button of ≥2 mm or defined turbidity. Finally, the plates were read

by absorbance at 630 nm (SmartReader 96—Accuris) to determine the minimum inhibitory concentration (MIC). All assays were performed in triplicate.

4.4. Evaluation of Hemolytic Activity

The hemolytic test was evaluated following the protocol described by Oddo [77], red blood cells washed with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) and resuspended at a concentration of 0.5% and incubated for 1 h with different concentrations of the fractions and then centrifuged at 10,000× *g* for 10 min, 60 µL of supernatant was transferred to a 96-well polypropylene plate, and the absorbance was read at 405 nm. The results were normalized with the positive controls of hemolysis (0.25% SDS) and negative controls (PBS). Assays were performed in triplicate.

4.5. Evaluation of Antioxidant Activity

20 µL of different concentrations of the fraction (55 µg/mL to 3.44 µg/mL) were added with 380 µL of ABTS radical in ethanol, incubated at room temperature protected from light for 30 min, then the absorbance of the mixture was measured at 734 nm [66]. To calculate the % decoloration, the following equation was used: % decoloration = $[(C - S)/C] \times 100$, where C is the absorbance of the control, and S is the absorbance of the problem sample. Trolox was used as a positive control. The experiments were done in triplicate.

4.6. Statistic Analysis

The MegaStat add-in for Excel was used to determine the antibacterial activity of the purified fraction of *Apis mellifera* venom. Analysis of variance (ANOVA) was performed at a significance level of 5%.

5. Conclusions

In summary, *A. mellifera* bee venom contained peptides with weights of 7 kDa, 6 kDa, and 5 kDa and exhibited antibacterial activity against *E. coli* ATCC 25922 at a concentration of 6.88 µg/mL.

Author Contributions: Writing—preparation of the original draft, methodology, O.P.-D.; formal analysis, A.O.E.-C.; data curation, E.L.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Universidad Señor de Sipán with RD No. 074-2020/PD-USS. The funders had no role in the collection, analysis, and interpretation of the data.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data related to the manuscript were available in the main manuscript.

Acknowledgments: We are grateful for the assistance of Señor de Sipán University for the technical, translation, and logistical support provided for this research. We thank Luis Orlando Sánchez Suclupe, for his support with the hives for the extraction of bee venom, Karl Weiss Cruz Verde—Íllimo Bee-keeping Association. We thank the Thematic Network for the development of antiviral and antimicrobial peptides for multiresistant strains (RED BUDEPAV-AM) and for participating as an exhibitor at the international symposium on antimicrobial and antiviral peptides.

Conflicts of Interest: The authors declare that there are no conflict of interest with respect to the publication of this article.

References

1. World Health Organization. *Antimicrobial Resistance: Global Report on Surveillance*; World Health Organization: Geneva, Switzerland, 2014; ISBN 978-92-4-156474-8.
2. Pollitt, E.J.G.; Szkuta, P.T.; Burns, N.; Foster, S.J. Staphylococcus Aureus Infection Dynamics. *PLoS Pathog.* **2018**, *14*, e1007112. [CrossRef]

3. Tong, S.Y.C.; Davis, J.S.; Eichenberger, E.; Holland, T.L.; Fowler, V.G. Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clin. Microbiol. Rev.* **2015**, *28*, 603–661. [CrossRef]
4. Safdari, H.; Aryan, E.; Sadeghian, H.; Shams, S.F.; Aganj, M. Frequency of Methicillin-Resistant Staphylococcus Aureus (MRSA) in Nose and Cellular Phone of Medical and Non-Medical Personnel of Emergency Departments of Ghaem Hospital in Mashhad City. *Clin. Epidemiol. Glob. Health* **2020**, *8*, 1043–1046. [CrossRef]
5. Belayhun, C.; Tilahun, M.; Seid, A.; Shibabaw, A.; Sharew, B.; Belete, M.A.; Demsiss, W. Asymptomatic Nasopharyngeal Bacterial Carriage, Multi-Drug Resistance Pattern and Associated Factors among Primary School Children at Debre Berhan Town, North Shewa, Ethiopia. *Ann. Clin. Microbiol. Antimicrob.* **2023**, *22*, 9. [CrossRef]
6. Akanbi, O.E.; Njom, H.A.; Fri, J.; Otigbu, A.C.; Clarke, A.M. Antimicrobial Susceptibility of Staphylococcus Aureus Isolated from Recreational Waters and Beach Sand in Eastern Cape Province of South Africa. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1001. [CrossRef]
7. Qodrati, M.; SeyedAlinaghi, S.; Dehghan Manshadi, S.A.; Abdollahi, A.; Dadras, O. Antimicrobial Susceptibility Testing of Staphylococcus Aureus Isolates from Patients at a Tertiary Hospital in Tehran, Iran, 2018–2019. *Eur. J. Med. Res.* **2022**, *27*, 152. [CrossRef]
8. Nikbakht, M.; Ahangarzadeh Rezaee, M.; Hasani, A.; Nahaei, M.R.; Sadeghi, J.; Jedari Seifi, S. Molecular Characterization and Antimicrobial Susceptibility Patterns of Methicillin-Resistant Staphylococcus Aureus Isolates in Tabriz, Northwest of Iran. *Arch. Pediatr. Infect. Dis.* 2017, *in press*. [CrossRef]
9. Guo, Y.; Song, G.; Sun, M.; Wang, J.; Wang, Y. Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 107. [CrossRef]
10. Hu, P.; Chen, J.; Chen, Y.; Zhou, T.; Xu, X.; Pei, X. Molecular Epidemiology, Resistance, and Virulence Properties of *Pseudomonas aeruginosa* Cross-Colonization Clonal Isolates in the Non-Outbreak Setting. *Infect. Genet. Evol.* **2017**, *55*, 288–296. [CrossRef]
11. Nasser, M.; Gayen, S.; Kharat, A.S. Prevalence of β-Lactamase and Antibiotic-Resistant *Pseudomonas aeruginosa* in the Arab Region. *J. Glob. Antimicrob. Resist.* **2020**, *22*, 152–160. [CrossRef]
12. Hoque, M.N.; Jahan, M.I.; Hossain, M.A.; Sultana, M. Genomic Diversity and Molecular Epidemiology of a Multidrug-Resistant *Pseudomonas aeruginosa* DMC30b Isolated from a Hospitalized Burn Patient in Bangladesh. *J. Glob. Antimicrob. Resist.* **2022**, *31*, 110–118. [CrossRef]
13. Coșeriu, R.L.; Vintilă, C.; Mare, A.D.; Ciurea, C.N.; Togănel, R.O.; Cighir, A.; Simion, A.; Man, A. Epidemiology, Evolution of Antimicrobial Profile and Genomic Fingerprints of *Pseudomonas aeruginosa* before and during COVID-19: Transition from Resistance to Susceptibility. *Life* **2022**, *12*, 2049. [CrossRef]
14. Asadpour, L. Antimicrobial Resistance, Biofilm-Forming Ability and Virulence Potential of *Pseudomonas aeruginosa* Isolated from Burn Patients in Northern Iran. *J. Glob. Antimicrob. Resist.* **2018**, *13*, 214–220. [CrossRef]
15. Schiavano, G.F.; Carloni, E.; Andreoni, F.; Magi, S.; Chironna, M.; Brandi, G.; Amagliani, G. Prevalence and Antibiotic Resistance of *Pseudomonas aeruginosa* in Water Samples in Central Italy and Molecular Characterization of OprD in Imipenem Resistant Isolates. *PLoS ONE* **2017**, *12*, e0189172. [CrossRef]
16. Alotaibi, B.S.; Tantry, B.A.; Farhana, A.; Alammar, M.A.; Shah, N.N.; Mohammed, A.H.; Wani, F.; Bandy, A. Resistance Pattern in Mostly Gram-Negative Bacteria Causing Urinary Tract Infections. *Infect. Disord. Drug Targets* **2023**, *23*, 56–64. [CrossRef]
17. Umpiérrez, A.; Ernst, D.; Fernández, M.; Oliver, M.; Casaux, M.L.; Caffarena, R.D.; Schild, C.; Giannitti, F.; Fraga, M.; Zunino, P. Virulence Genes of *Escherichia coli* in Diarrheic and Healthy Calves. *Rev. Argent. Microbiol.* **2021**, *53*, 34–38. [CrossRef]
18. Sarjana Safain, K.; Bhuyan, G.S.; Hassan Hasib, S.; Islam, M.S.; Mahmud-Un-Nabi, M.A.; Sultana, R.; Tasnim, S.; Noor, F.A.; Sarker, S.K.; Islam, M.T.; et al. Genotypic and Phenotypic Profiles of Antibiotic-resistant Bacteria Isolated from Hospitalised Patients in Bangladesh. *Trop. Med. Int. Health* **2021**, *26*, 720–729. [CrossRef]
19. Jang, J.; Hur, H.-G.; Sadowsky, M.J.; Byappanahalli, M.N.; Yan, T.; Ishii, S. Environmental *Escherichia coli*.: Ecology and Public Health Implications—A Review. *J. Appl. Microbiol.* **2017**, *123*, 570–581. [CrossRef]
20. Edmond, T.; Yehouenou, L.C.; Malick, Z.F.; Arsene, K.A.; Rene, K.K.; Diouara, A.A.M.; Tonde, I.; Bankole, H.S.; Wilfried, B.K.; Marius, E.A.; et al. Antimicrobial Susceptibility of Community Acquired *Escherichia coli* in Urinary Tract Infections (UTI) in Benin for Eleven Years (2005–2015). *Am. J. Infect. Dis.* **2017**, *13*, 21–27. [CrossRef]
21. Grados, M.C.; Thuissard, I.J.; Alós, J.-I. Stratification by Demographic and Clinical Data of the Antibiotic Susceptibility of Escherichia Coli from Urinary Tract Infections of the Community. *Atención Primaria* **2019**, *51*, 494–498. [CrossRef]
22. Amanati, A.; Sajedianfard, S.; Khajeh, S.; Ghasempour, S.; Mehrangiz, S.; Nematolahi, S.; Shahhosein, Z. Bloodstream Infections in Adult Patients with Malignancy, Epidemiology, Microbiology, and Risk Factors Associated with Mortality and Multi-Drug Resistance. *BMC Infect. Dis.* **2021**, *21*, 636. [CrossRef]
23. Mahmoudi, S.; Mahzari, M.; Banar, M.; Pourakbari, B.; Haghi Ashtiani, M.T.; Mohammadi, M.; Keshavarz Valian, S.; Mamishi, S. Antimicrobial Resistance Patterns of Gram-Negative Bacteria Isolated from Bloodstream Infections in an Iranian Referral Paediatric Hospital: A 5.5-Year Study. *J. Glob. Antimicrob. Resist.* **2017**, *11*, 17–22. [CrossRef]
24. Shi, N.; Kang, J.; Wang, S.; Song, Y.; Yin, D.; Li, X.; Guo, Q.; Duan, J.; Zhang, S. Bacteriological Profile and Antimicrobial Susceptibility Patterns of Gram-Negative Bloodstream Infection and Risk Factors Associated with Mortality and Drug Resistance: A Retrospective Study from Shanxi, China. *Infect. Drug Resist.* **2022**, *15*, 3561–3578. [CrossRef]

25. Esposito, S.; Maglietta, G.; Di Costanzo, M.; Ceccoli, M.; Vergine, G.; La Scola, C.; Malaventura, C.; Falcioni, A.; Iacono, A.; Crisafi, A.; et al. Retrospective 8-Year Study on the Antibiotic Resistance of Uropathogens in Children Hospitalised for Urinary Tract Infection in the Emilia-Romagna Region, Italy. *Antibiotics* **2021**, *10*, 1207. [CrossRef]
26. Boschetti, G.; Sgarabotto, D.; Meloni, M.; Bruseghin, M.; Whisstock, C.; Marin, M.; Ninkovic, S.; Pinfi, M.; Brocco, E. Antimicrobial Resistance Patterns in Diabetic Foot Infections, an Epidemiological Study in Northeastern Italy. *Antibiotics* **2021**, *10*, 1241. [CrossRef]
27. Obakiro, S.B.; Kiyimba, K.; Paasi, G.; Napyo, A.; Anthierens, S.; Waako, P.; Royen, P.V.; Iramiot, J.S.; Goossens, H.; Kostyanev, T. Prevalence of Antibiotic-Resistant Bacteria among Patients in Two Tertiary Hospitals in Eastern Uganda. *J. Glob. Antimicrob. Resist.* **2021**, *25*, 82–86. [CrossRef]
28. Mahlapuu, M.; Håkansson, J.; Ringstad, L.; Björn, C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Front. Cell. Infect. Microbiol.* **2016**, *6*, 194. [CrossRef]
29. Tawfik, M.M.; Bertelsen, M.; Abdel-Rahman, M.A.; Strong, P.N.; Miller, K. Scorpion Venom Antimicrobial Peptides Induce Siderophore Biosynthesis and Oxidative Stress Responses in *Escherichia coli*. *mSphere* **2021**, *6*, e00267-21. [CrossRef]
30. Pérez-Delgado, O.; Rincon-Cortés, C.A.; Vega-Castro, N.A.; Reyes-Montaño, E.A.; Gómez-Garzón, M. Purificación Parcial de Péptidos Del Veneno de Escorpión Hadruroides Charcasus (Karsch, 1879) Con Actividad Antimicrobiana. *Bionat. Lat. Am. J. Biotechnol. Life Sci.* **2021**, *6*, 1917–1923. [CrossRef]
31. Zhao, Z.; Ma, Y.; Dai, C.; Zhao, R.; Li, S.; Wu, Y.; Cao, Z.; Li, W. Imcroporin, a New Cationic Antimicrobial Peptide from the Venom of the Scorpion *Isometrus maculates*. *Antimicrob. Agents Chemother.* **2009**, *53*, 3472–3477. [CrossRef]
32. de Barros, E.; Gonçalves, R.M.; Cardoso, M.H.; Santos, N.C.; Franco, O.L.; Cândido, E.S. Snake Venom Cathelicidins as Natural Antimicrobial Peptides. *Front. Pharmacol.* **2019**, *10*, 1415. [CrossRef]
33. Lee, B.; Shin, M.K.; Yoo, J.S.; Jang, W.; Sung, J.-S. Identifying Novel Antimicrobial Peptides from Venom Gland of Spider Pardosa Astrigera by Deep Multi-Task Learning. *Front. Microbiol.* **2022**, *13*, 971503. [CrossRef]
34. Ko, S.J.; Park, E.; Asandei, A.; Choi, J.-Y.; Lee, S.-C.; Seo, C.H.; Luchian, T.; Park, Y. Bee Venom-Derived Antimicrobial Peptide Melectin Has Broad-Spectrum Potency, Cell Selectivity, and Salt-Resistant Properties. *Sci. Rep.* **2020**, *10*, 10145. [CrossRef]
35. Abd El-Wahed, A.A.; Khalifa, S.A.M.; Sheikh, B.Y.; Farag, M.A.; Saeed, A.; Larik, F.A.; Koca-Caliskan, U.; AlAjmi, M.F.; Hassan, M.; Wahabi, H.A.; et al. Bee Venom Composition: From Chemistry to Biological Activity. In *Studies in Natural Products Chemistry*; Elsevier: Amsterdam, The Netherlands, 2019; Volume 60, pp. 459–484, ISBN 978-0-444-64181-6.
36. Amin, M.A.; Abdel-Raheem, I.T. Accelerated Wound Healing and Anti-Inflammatory Effects of Physically Cross Linked Polyvinyl Alcohol–Chitosan Hydrogel Containing Honey Bee Venom in Diabetic Rats. *Arch. Pharm. Res.* **2014**, *37*, 1016–1031. [CrossRef]
37. Ward, R.; Coffey, M.; Kavanagh, K. Proteomic Analysis of Summer and Winter *Apis mellifera* Workers Shows Reduced Protein Abundance in Winter Samples. *J. Insect Physiol.* **2022**, *139*, 104397. [CrossRef]
38. Kwon, N.-Y.; Sung, S.-H.; Sung, H.-K.; Park, J.-K. Anticancer Activity of Bee Venom Components against Breast Cancer. *Toxins* **2022**, *14*, 460. [CrossRef]
39. Kim, H.; Park, S.-Y.; Lee, G. Potential Therapeutic Applications of Bee Venom on Skin Disease and Its Mechanisms: A Literature Review. *Toxins* **2019**, *11*, 374. [CrossRef]
40. Hegazi, A.; Abdou, A.M.; El-Moez, S.I.A.; Allah, F.A. Evaluation of the Antibacterial Activity of Bee Venom from Different Sources. *World Appl. Sci. J.* **2014**, *30*, 266–270.
41. Nguyen, C.D.; Yoo, J.; Hwang, S.-Y.; Cho, S.-Y.; Kim, M.; Jang, H.; No, K.O.; Shin, J.C.; Kim, J.-H.; Lee, G. Bee Venom Activates the Nrf2/HO-1 and TrkB/CREB/BDNF Pathways in Neuronal Cell Responses against Oxidative Stress Induced by Aβ1–42. *Int. J. Mol. Sci.* **2022**, *23*, 1193. [CrossRef]
42. Tanner, C.M.; Kamel, F.; Ross, G.W.; Hoppin, J.A.; Goldman, S.M.; Korell, M.; Marras, C.; Bhudhikanok, G.S.; Kasten, M.; Chade, A.R.; et al. Rotenone, Paraquat, and Parkinson's Disease. *Environ. Health Perspect.* **2011**, *119*, 866–872. [CrossRef]
43. Khalil, W.K.B.; Assaf, N.; ElShebiney, S.A.; Salem, N.A. Neuroprotective Effects of Bee Venom Acupuncture Therapy against Rotenone-Induced Oxidative Stress and Apoptosis. *Neurochem. Int.* **2015**, *80*, 79–86. [CrossRef] [PubMed]
44. Socarras, K.; Theophilus, P.; Torres, J.; Gupta, K.; Sapi, E. Antimicrobial Activity of Bee Venom and Melittin against Borrelia Burgdorferi. *Antibiotics* **2017**, *6*, 31. [CrossRef]
45. Zolfagharian, H.; Mohajeri, M.; Babaie, M. Bee Venom (*Apis mellifera*) an Effective Potential Alternative to Gentamicin for Specific Bacteria Strains: Bee Venom an Effective Potential for Bacteria. *J. Pharmacopunct.* **2016**, *19*, 225–230. [CrossRef]
46. Han, S.; Kim, J.; Hong, I.; Woo, S.; Kim, S.; Jang, H.; Pak, S. Antibacterial Activity and Antibiotic-Enhancing Effects of Honeybee Venom against Methicillin-Resistant Staphylococcus Aureus. *Molecules* **2016**, *21*, 79. [CrossRef]
47. Leandro, L.F.; Mendes, C.A.; Casemiro, L.A.; Vinholis, A.H.C.; Cunha, W.R.; de Almeida, R.; Martins, C.H.G. Antimicrobial Activity of Apitoxin, Melittin and Phospholipase A2 of Honey Bee (*Apis mellifera*) Venom against Oral Pathogens. *Ann. Acad. Bras. Ciênc.* **2015**, *87*, 147–155. [CrossRef] [PubMed]
48. El-Didamony, S.E.; Kalaba, M.H.; El-Fakharany, E.M.; Sultan, M.H.; Sharaf, M.H. Antifungal and Antibiofilm Activities of Bee Venom Loaded on Chitosan Nanoparticles: A Novel Approach for Combating Fungal Human Pathogens. *World J. Microbiol. Biotechnol.* **2022**, *38*, 244. [CrossRef]
49. Uddin, M.B.; Lee, B.-H.; Nikapitiya, C.; Kim, J.-H.; Kim, T.-H.; Lee, H.-C.; Kim, C.G.; Lee, J.-S.; Kim, C.-J. Inhibitory Effects of Bee Venom and Its Components against Viruses In Vitro and In Vivo. *J. Microbiol.* **2016**, *54*, 853–866. [CrossRef] [PubMed]

50. Fenard, D.; Lambeau, G.; Valentin, E.; Lefebvre, J.C.; Lazdunski, M.; Doglio, A. Secreted Phospholipases A(2), a New Class of HIV Inhibitors That Block Virus Entry into Host Cells. *J. Clin. Investig.* **1999**, *104*, 611–618. [CrossRef]
51. de Brito, J.C.M.; Bastos, E.M.A.F.; Heneine, L.G.D.; de Souza Figueiredo, K.C. Fractionation of *Apis mellifera* Venom by Means of Ultrafiltration: Removal of Phospholipase A_2. *Braz. J. Chem. Eng.* **2018**, *35*, 229–236. [CrossRef]
52. El-Seedi, H.; Abd El-Wahed, A.; Yosri, N.; Musharraf, S.G.; Chen, L.; Moustafa, M.; Zou, X.; Al-Mousawi, S.; Guo, Z.; Khatib, A.; et al. Antimicrobial Properties of *Apis mellifera*'s Bee Venom. *Toxins* **2020**, *12*, 451. [CrossRef] [PubMed]
53. Memariani, H.; Memariani, M.; Moravvej, H.; Shahidi-Dadras, M. Melittin: A Venom-Derived Peptide with Promising Anti-Viral Properties. *Eur. J. Clin. Microbiol. Infect. Dis.* **2020**, *39*, 5–17. [CrossRef] [PubMed]
54. Kurek-Górecka, A.; Komosinska-Vassev, K.; Rzepecka-Stojko, A.; Olczyk, P. Bee Venom in Wound Healing. *Molecules* **2020**, *26*, 148. [CrossRef] [PubMed]
55. Huh, J.-E.; Seo, B.-K.; Lee, J.-W.; Park, Y.-C.; Baek, Y.-H. Analgesic Effects of Diluted Bee Venom Acupuncture Mediated by δ-Opioid and A2-Adrenergic Receptors in Osteoarthritic Rats. *Altern. Ther. Health Med.* **2018**, *24*, 28–35. [PubMed]
56. Maitip, J.; Mookhploy, W.; Khorndork, S.; Chantawannakul, P. Comparative Study of Antimicrobial Properties of Bee Venom Extracts and Melittins of Honey Bees. *Antibiotics* **2021**, *10*, 1503. [CrossRef]
57. Haktanir, I.; Masoura, M.; Mantzouridou, F.T.; Gkatzionis, K. Mechanism of Antimicrobial Activity of Honeybee (*Apis mellifera*) Venom on Gram-Negative Bacteria: *Escherichia coli* and *Pseudomonas* spp. *AMB Expr.* **2021**, *11*, 54. [CrossRef]
58. Bakhiet, E.K.; Hussien, H.A.M.; Elshehaby, M. *Apis mellifera* Venom Inhibits Bacterial and Fungal Pathogens In Vitro. *Pak. J. Biol. Sci.* **2022**, *25*, 875–884. [CrossRef] [PubMed]
59. Babaie, M.; Ghaem panah, A.; Mehrabi, Z.; Mollaei, A.; Sima Khalilifard, B. Partial Purification and Characterization of Antimicrobial Effects from Snake (*Echis carinatus*), Scorpion (*Mesosobuthus epues*) and Bee (*Apis mellifera*) Venoms. *Iran. J. Med. Microbiol.* **2020**, *14*, 460–477. [CrossRef]
60. Pucca, M.B.; Cerni, F.A.; Oliveira, I.S.; Jenkins, T.P.; Argemí, L.; Sørensen, C.V.; Ahmadi, S.; Barbosa, J.E.; Laustsen, A.H. Bee Updated: Current Knowledge on Bee Venom and Bee Envenoming Therapy. *Front. Immunol.* **2019**, *10*, 2090. [CrossRef]
61. van den Bogaart, G.; Guzmán, J.V.; Mika, J.T.; Poolman, B. On the Mechanism of Pore Formation by Melittin. *J. Biol. Chem.* **2008**, *283*, 33854–33857. [CrossRef]
62. Zarrinnahad, H.; Mahmoodzadeh, A.; Hamidi, M.P.; Mahdavi, M.; Moradi, A.; Bagheri, K.P.; Shahbazzadeh, D. Apoptotic Effect of Melittin Purified from Iranian Honey Bee Venom on Human Cervical Cancer HeLa Cell Line. *Int. J. Pept. Res. Ther.* **2018**, *24*, 563–570. [CrossRef]
63. de Roodt, A.R.; Lanari, L.C.; Lago, N.R.; Bustillo, S.; Litwin, S.; Morón-Goñi, F.; Gould, E.G.; van Grootheest, J.H.; Dokmetjian, J.C.; Dolab, J.A.; et al. Toxicological Study of Bee Venom (*Apis mellifera* Mellifera) from Different Regions of the Province of Buenos Aires, Argentina. *Toxicon* **2020**, *188*, 27–38. [CrossRef] [PubMed]
64. Viana, G.A.; Freitas, C.I.A.; Almeida, J.G.L.d.; Medeiros, G.V.D.d.; Teófilo, T.d.S.; Rodrigues, V.H.V.; Coelho, W.A.C.; Batista, J.S. Antioxidant, Genotoxic, Antigenotoxic, and Antineoplastic Activities of Apitoxin Produced by *Apis mellifera* in Northeast, Brazil. *Cienc. Rural* **2021**, *51*, e20200545. [CrossRef]
65. Frangieh, J.; Salma, Y.; Haddad, K.; Mattei, C.; Legros, C.; Fajloun, Z.; El Obeid, D. First Characterization of the Venom from *Apis mellifera* Syriaca, a Honeybee from the Middle East Region. *Toxins* **2019**, *11*, 191. [CrossRef] [PubMed]
66. Somwongin, S.; Chantawannakul, P.; Chaiyana, W. Antioxidant Activity and Irritation Property of Venoms from Apis Species. *Toxicon* **2018**, *145*, 32–39. [CrossRef]
67. Tanuwidjaja, I.; Svečnjak, L.; Gugić, D.; Levanić, M.; Jurić, S.; Vinceković, M.; Mrkonjić Fuka, M. Chemical Profiling and Antimicrobial Properties of Honey Bee (*Apis mellifera* L.) Venom. *Molecules* **2021**, *26*, 3049. [CrossRef]
68. Yaacoub, C.; Rifi, M.; El-Obeid, D.; Mawlawi, H.; Sabatier, J.-M.; Coutard, B.; Fajloun, Z. The Cytotoxic Effect of *Apis mellifera* Venom with a Synergistic Potential of Its Two Main Components—Melittin and PLA2—On Colon Cancer HCT116 Cell Lines. *Molecules* **2021**, *26*, 2264. [CrossRef]
69. Kamel, A.; Suleiman, W.; Elfeky, A.; El-Sherbiny, G.; Elhaw, M. Characterization of Bee Venom and Its Synergistic Effect Combating Antibiotic Resistance of *Pseudomonas aeruginosa*. *Egypt. J. Chem.* **2021**, *65*, 1–2. [CrossRef]
70. Nehme, H.; Ayde, H.; El Obeid, D.; Sabatier, J.M.; Fajloun, Z. Potential Inhibitory Effect of *Apis mellifera*'s Venom and of Its Two Main Components—Melittin and PLA2—On *Escherichia coli* F1F0-ATPase. *Antibiotics* **2020**, *9*, 824. [CrossRef]
71. Sobral, F.; Sampaio, A.; Falcão, S.; Queiroz, M.J.R.P.; Calhelha, R.C.; Vilas-Boas, M.; Ferreira, I.C.F.R. Chemical Characterization, Antioxidant, Anti-Inflammatory and Cytotoxic Properties of Bee Venom Collected in Northeast Portugal. *Food Chem. Toxicol.* **2016**, *94*, 172–177. [CrossRef]
72. Noble, J.E. Quantification of Protein Concentration Using UV Absorbance and Coomassie Dyes. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 2014; Volume 536, pp. 17–26, ISBN 978-0-12-420070-8.
73. Pérez-Delgado, O.; Espinoza-Vergara, M.A.; Castro-Vega, N.A.; Reyes-Montaño, E.A. Evaluación Preliminar de Actividad Antibacteriana in Vitro Del Veneno de Escorpión Hadruroides Charcasus (Karsch, 1879) Contra *Pseudomonas aeruginosa* y *Staphylococcus aureus*. *Rev. Cuerpo Med. HNAAA* **2019**, *12*, 6–12. [CrossRef]
74. Schägger, H.; von Jagow, G. Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis for the Separation of Proteins in the Range from 1 to 100 KDa. *Anal. Biochem.* **1987**, *166*, 368–379. [CrossRef] [PubMed]
75. Brunelle, J.L.; Green, R. Coomassie Blue Staining. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 2014; Volume 541, pp. 161–167, ISBN 978-0-12-420119-4.

76. *Approved Standard M07*; Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 9th ed. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012; Volume 32, ISBN 1-56238-784-7.
77. Oddo, A.; Hansen, P.R. Hemolytic Activity of Antimicrobial Peptides. In *Antimicrobial Peptides*; Hansen, P.R., Ed.; Methods in Molecular Biology; Springer: New York, NY, USA, 2017; Volume 1548, pp. 427–435, ISBN 978-1-4939-6735-3.