UNIVERSITI TEKNOLOGI MARA

IDENTIFICATION AND CHARACTERIZATION OF ANTIMICROBIAL PEPTIDES FROM THE LARVAE OF BLACK SOLDIER FLY, *HERMETIA ILLUCENS* (DIPTERA: STRATIOMYIDAE) AND BLOWFLY, *CHRYSOMYA MEGACEPHALA* (DIPTERA: CALLIPHORIDAE) AS POTENTIAL SOLUTION FOR ANTIMICROBIAL RESISTANCE

NURUL AZMIERA BINTI ZAMRI

Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

February 2025

ACKNOWLEDGEMENT

Firstly, I wish to thank God for granting me the opportunity to pursue my PhD and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Assoc Prof Dr Heo Chong Chin, and my co-supervisors, Assoc Prof Dr Anna Krasilnikova, Assoc Prof Dr Hassanain Al-Talib, and Assoc Prof Dr Shariza Sahudin, for their countless support during the experiments and writing process of these thesis. My appreciation goes to as one of the grant holders of this project.

I extend my heartfelt gratitude to the staff at the Institute for Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM), especially and for their invaluable training and the experience I gained while working in the laboratory. I am also deeply appreciative of all the staff at the Analytical Unit and the Integrative Pharmacogenomics Institute (iPROMISE), Faculty of Pharmacy, UiTM.

This thesis is dedicated to my dear father, my mother, , and my brother, for their unwavering vision and support. This piece of victory is dedicated to all of you.

I would also like to appreciate my FUN ento lab members, especially , as well as the IMMB research students and intern students who have aided me throughout this journey. Your kind words and support are etched in my heart. To all others who may have contributed, whether directly or indirectly, your efforts are sincerely appreciated.

Lastly, to the flies and their larvae that were sacrificed for scientific research, your contributions have not been in vain. Your sacrifices have greatly advanced our knowledge and led to important scientific discoveries.

ABSTRACT

Antimicrobial resistance (AMR) has caused million fatalities on a worldwide scale, originating from the emergence of resistance in bacteria and fungi against commonly employed antibiotics. The larvae of *Hermetia illucens* (Diptera: Stratiomyidae) and *Chrysomya megacephala* (Diptera: Calliphoridae) thrive in environments contaminated with bacteria, such as decaying waste and carrion. Therefore, the objective of our research was to identify potential antimicrobial compounds that can protect the larvae from bacterial infections. Haemolymph from approximately 200 larvae of *C. megacephala* and *H. illucens*, previously exposed to Methicillin Resistant *Staphylococcus aureus* (patient's isolate) or *Escherichia coli*, was acquired, purified, and fractionated utilizing Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Fractions exhibiting antimicrobial properties were further examined through Quadrupole Time-of-Flight Liquid Chromatography Mass Spectrometry (QTOF-LCMS). The resultant mass spectra were converted into amino acid sequences through de novo algorithms. These sequences were subsequently analysed through bioinformatics using diverse protein databases, including the Antimicrobial Peptide Database version 3 (APD3), UniProt, and the National Centre for Biotechnology Information (NCBI), for comparative evaluation with previously reported peptides. Furthermore, antimicrobial prediction tools from databases such as APD3, Database of Antimicrobial Activity and Structure of Peptides (DBSAAP), Database of Antimicrobial Peptide (DBAMP), and Collection of Antimicrobial Peptide version 4 (CAMPR4) were utilized to anticipate the potential antimicrobial characteristics of the peptide sequences. The results indicated that a specific peptide (HGCGRLSKWFRQPGLLLSVKR), renamed as Chryso-AMP1 demonstrated significant similarity to *C. megacephala*'s heat shock protein 70 (hsp70), Meanwhile, for *H. illucens*, the peptide DPAVKPGRLPSAGLLAVLK (renamed as Hil-AMP1) displayed 100% identical identity to a beclin-1-like protein These peptides were synthesized and subjected to antimicrobial assessments against various pathogenic bacteria, including MRSA, *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 12228), and *Bacillus subtilis* (ATCC 6633). The examined peptide Chryso-AMP1 exhibited efficacy against these bacteria, with a minimum inhibitory concentration (MIC) of 0.06 mg/ml and an inhibition zone ranging from 8 to 11 mm at a concentration of 1 mg/ml. Unfortunately, no antibacterial activity was observed for the peptide Hil-AMP1 from *H. illucens*. This highlights the significance of supporting the utilization of antimicrobial databases for predictive intents with laboratory experiments. The cytotoxicity of the peptide was gauged using immortalized keratinocyte (HaCat) and human corneal epithelial cell lines (HCEC), revealing an average cell viability exceeding 80% at concentration of 0.06 mg/ml. In conclusion, the peptide Chryso-AMP1 could serve as a promising candidate for future antimicrobial drug development as potential solution to antimicrobial resistance.

TABLE OF CONTENTS

		Page
CONFIRMATION BY PANEL OF EXAMINERS		**ii**
AUTHOR'S DECLARATION		**iii**
ABSTRACT		**iv**
ACKNOWLEDGEMENT		**v**
TABLE OF CONTENTS		**vi**
LIST OF TABLES		**x**
LIST OF FIGURES		**xiii**
LIST OF PLATES		**xvii**
LIST OF SYMBOLS		**xviii**
LIST OF ABBREVIATIONS		**xx**
LIST OF NOMENCLATURE		**xxiv**
CHAPTER 1 INTRODUCTION		**25**
1.1	Background of Study	25
1.2	Problem Statement	28
1.3	Research Questions	29
1.4	Research Objectives	29
1.5	Hypotheses	30
1.6	Scope of Study	30
1.7	Significant of Study	31
1.8	Limitations of Study	31
CHAPTER 2 LITERATURE REVIEW		**33**
2.1	Antimicrobial Resistance (AMR)	33
2.1.1	Definition and History of AMR	33
2.1.2	Antimicrobial Resistance as Public Health Threats	35
2.1.3	Factors Accelerating the Emergence of AMR	37
2.1.4	The Mechanisms of Acquired Antibiotic Resistance	38
2.1.5	Strategies to Combat Antimicrobial Resistance	40

CHAPTER 1
INTRODUCTION

1.1 Background of Study

Recently, the increasing number of antimicrobial resistance (AMR) towards the commonly used antibiotics is alarming. AMR occurs when microorganisms such as bacteria, fungi, or viruses develop mechanisms to bypass the action of antimicrobial agents and survive. According to the World Health Organization (WHO) (2023), the overuse and misuse of antimicrobial drugs, insufficient treatment regimens, and poor infection control measures are key factors driving increase in AMR cases. Furthermore, globalization and travel contribute to the spread of resistant microorganisms, while a limited pipeline for new antibiotic development exacerbates the situation (WHO, 2023). Aside from that, environmental contamination, horizontal gene transfer, and selective pressure also contribute significantly to the emergence and spread of AMR (WHO, 2023). AMR has been associated with several health care problems such as increase in mortality rate, prolonged hospitalization, and increase in treatment costs (WHO, 2021). To date, AMR has been found to have led to 4.95 billion deaths reported from 204 countries in 2019 (Murray et al., 2022). Addressing this issue requires comprehensive methods that tackles on both antimicrobial use and the underlying reasons of resistance development and transmission.

AMR also poses a significant threat to the achievement of several United Nations Sustainable Development Goals (SDGs), including those related to health (SDG 3), poverty reduction (SDG 1), food security (SDG 2), and economic growth (SDG 8) (Greco et al., 2022; Bhattacharya et al., 2024). Addressing AMR is essential for advancing progress on the SDGs and promoting sustainable development globally.

To combat AMR through discovery and development of new antimicrobial drugs, various strategies can be pursued. These include targeted research to understand mechanisms of microbial resistance, novel drug discovery methods, exploration of combination therapies, and investigating alternative antimicrobial treatments such as bacteriophages and immune-based therapies (Reygaert, 2018; Berdigaliyev & Aljofan, 2020; Moo et al., 2020; Watson et al., 2020; Ling et al., 2022). Our interest lies in the strategies highlighted by Ye and Chen (2023) which include discovering new