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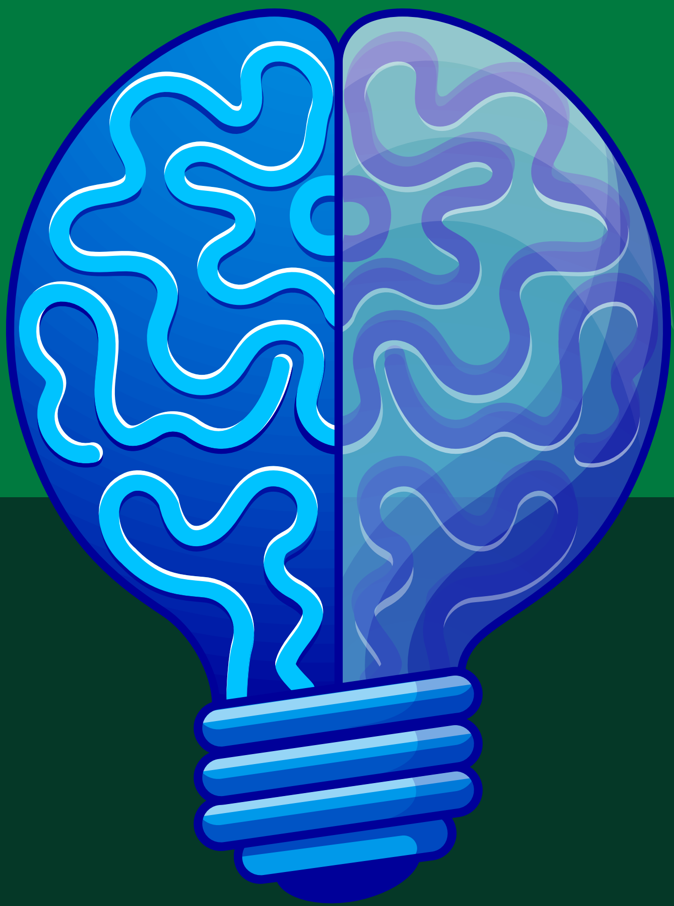

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Preface

The Scientific Project Colloquium offers a platform for publishing Diploma Science final year projects (FYP). The objective is to effectively distribute research findings throughout all scientific disciplines. The primary objective of including final year projects into the course curriculum is to encourage students to put their theoretical knowledge into practical applications.

We would like to express our gratitude to our primary establishment, the Faculty of Applied Sciences and Universiti Teknologi MARA, Perak Branch, for their invaluable assistance.

Lastly, we would like to express our gratitude to all of the authors for the tremendous help in preparing the articles, without which this undertaking would not have been completed.

Editors

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ANTIBACTERIAL ACTIVITY OF HAEMOLYMPH EXTRACTED FROM *Coccinella septempunctata* AGAINST *Escherichia coli*: A PRELIMINARY STUDY

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Abstract: This study investigated the antibacterial activity of haemolymph extracted from *Coccinella septempunctata* against bacteria *Escherichia coli*. The rising issue of antibiotic resistance has encouraged the search for alternative natural antibacterial sources, including from insect haemolymph, which contains antimicrobial peptides (AMPs). In this study, haemolymph was extracted and tested using the agar well diffusion method on nutrient agar inoculated with *E. coli.* Four (4) treatment groups were prepared by mixing haemolymph (H) with buffer A (BA) (pH 6), B (BB) (pH 6.5), C (BC) (pH 5) and distilled water (DW) respectively. The results showed that haemolymph combined with BB resulted in the largest inhibition zone (5 mm), followed by BA and BC (4 mm), while combination with DW showed the smallest inhibition zone (2 mm). These findings suggest that haemolymph from *C. septempunctata* potentially exhibits antibacterial properties against *E. coli*, with buffer composition affecting its efficacy. Thus, the results highlight the potential of haemolymph derived from insects as a natural antimicrobial agent for future study.

Keywords: *Coccinella septempunctata, Escherichia coli, Haemolymph, Antibacterial, Inhibition zone*

INTRODUCTION
Antibiotic resistance has emerged as one of the global health threats, compromising the efficacy of conventional antibiotics and increasing the urgency to discover novel antimicrobial agents (World Health Organization, 2020). As bacteria such as *E. coli* evolve to resist commonly used drugs, the search for alternative antibacterial sources has shifted toward natural and sustainable bioresources. Among these, insects have shown growing scientific interest due to their innate immune systems, which produce potent AMPs within their haemolymph, a circulatory fluid like vertebrate blood (Morejon & Michel, 2023).

C. septempunctata, commonly known as the seven-spotted ladybird, is a beneficial predatory beetle of the family Coccinellidae, widely recognized for its ecological role in pest control. Its haemolymph, which is abundance in immune-related compounds and defensive enzymes, has been suggested to possess antimicrobial properties that can inhibit bacterial growth. Studies on insect-derived AMPs from species such as *Hermetia illucens* and *Philosamia ricini* have demonstrated inhibitory effects against various pathogens (Singma et al., 2025). However, scarce evidence exists regarding the antibacterial potential of *C. septempunctata* haemolymph.

Exploring the bioactivity of *C. septempunctata* haemolymph is therefore relevant, not only to expand scientific understanding of insect immune defenses but also to identify natural bioactive molecules that could be developed into sustainable antimicrobial agents. This preliminary study investigates the antibacterial activity of haemolymph extracted from *C. septempunctata* against *E. coli*, to provide fundamental insights into the species' potential as a source of natural antibacterial compounds.

METHODOLOGY
Adult specimens of *C. septempunctata* were manually collected from a garden within the Universiti Teknologi MARA (UiTM) Tapah Campus, Perak. The insects were maintained under controlled laboratory conditions until the haemolymph extraction process.

Buffers (BA, BB, and BC) were prepared under sterile conditions with varying pH and compositions as below.

- BA (pH 6.0): NaCl, glucose, trisodium citrate, citric acid
- BB (pH 6.5): NaCl, glucose, trisodium citrate, citric acid, Na_2EDTA
- BC (pH 5.0): NaOH, NaCl, EDTA, citric acid

Haemolymph was extracted from live adult ladybirds by making a small incision using a sterile needle. The exuding fluid was immediately mixed with one of the prepared buffers to prevent clotting, collected with a micropipette, and stored in sterile microcentrifuge tubes on ice. The mixtures were gently vortexed to ensure homogenization.

A fresh culture of *E. coli* was prepared by inoculating a single colony into nutrient broth and incubating at 37°C for 18–24 hours. The optical density (OD) was adjusted to 0.143 to standardize bacterial concentration. Nutrient agar plates were prepared, autoclaved, and solidified under aseptic conditions.

The antibacterial assay was performed using the agar well diffusion method. Wells of 6 mm diameter were created in agar plates seeded with *E. coli* using a sterile cork borer. Each well received 20 µL of the haemolymph-buffer mixture. A standard antibiotic served as the positive control (PC), while sterile distilled water served as the negative control (NC). Plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters to evaluate antibacterial activity. Each treatment was conducted in triplicates, and mean inhibition zone diameters were calculated.

FINDINGS

The findings from this preliminary study demonstrated that the haemolymph extracted from *C. septempunctata* exhibited antibacterial activity against *E. coli*, thus supporting the hypothesis that insect haemolymph contains bioactive compounds capable of inhibiting bacterial growth. The variation in inhibition zone diameters among the treatments indicates that buffer composition significantly influenced haemolymph stability and antibacterial efficacy. The combination of haemolymph with buffer B (BB) (pH 6.5, containing Na_2EDTA) resulted the largest inhibition zone diameter (5 mm), suggesting that a slightly alkaline environment and the presence of chelating agents such as EDTA may enhance the antimicrobial activity by maintaining protein integrity and destabilizing bacterial cell membranes (Lee et al., 2021).

In contrast, haemolymph mixed with distilled water produced the smallest inhibition zone diameter (2 mm), possibly due to protein denaturation. Buffers A (BA) and C (BC) showed moderate inhibition zone diameter (4 mm), This suggests the importance of balanced ionic composition and pH to maintain the functionality of antimicrobial peptides (AMPs). These findings align with previous studies reporting that the bioactivity of insect haemolymph is influenced by environmental factors and buffer systems (Caccia et al., 2019; Morejon & Michel, 2023).

The antibacterial effect observed can be attributed to the presence of AMPs and immune-related proteins within the haemolymph. AMPs such as cecropins, defensins, and attacins are small, cationic peptides known to disrupt bacterial cell membranes and interfere with essential metabolic pathways (Bulet & Stöcklin, 2005; Ruiu, 2015; Wu et al., 2018). Insects produce these peptides as part of their innate immune defense, often regulated through signaling cascades such as the Toll and Imd pathways (Hoffmann, 2022; Hanson & Lemaitre, 2020). The measurable inhibition of *E. coli* growth therefore confirms that *C. septempunctata* haemolymph contributes to innate immune defense and may possess novel AMPs with therapeutic potential (Sahoo et al., 2023).

Furthermore, the positive control (antibiotic) exhibited a 6 mm inhibition zone, validating the sensitivity of *E. coli* under the assay conditions. Although the antibacterial activity of haemolymph was lower than that of the synthetic antibiotic, its efficacy remains notable given that it was unpurified and tested in crude form. With proper purification and concentration optimization, stronger antibacterial effects could likely be achieved, as demonstrated in similar studies involving purified peptides from *Hermetia illucens* and *Harmonia axyridis* and other insect species (Röhrich C.R et al., 2012; Park et al., 2015).

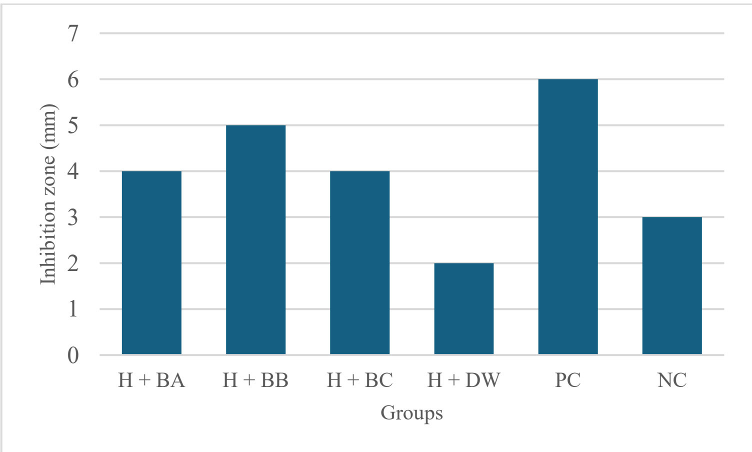

Figure 1 Antibacterial activity of different combination of haemolymph from *C. septempunctata* (H) and buffer A (BA), B (BB), C (BC) and distilled water (DW).

CONCLUSIONS

This preliminary study demonstrated that the haemolymph extracted from *C. septempunctata* possesses antibacterial activity against *E. coli*. Buffer B enhanced its activity the most, suggesting the importance of buffer composition in maintaining the stability of antibacterial compounds. The results highlight the potential of insect haemolymph as a natural source of antimicrobial agents. Its bioactive compounds could be developed into sustainable alternatives against conventional antibiotics, reducing dependency on synthetic antimicrobials and mitigating the spread of antibiotic resistance. Future studies should focus on isolating and characterizing the bioactive compounds responsible for these effects and expanding tests to wider range of bacterial species.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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Sekian, terima kasih.

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