

***IN VITRO* ANTIBACTERIAL ACTIVITY OF MEDICINAL *Lucilia cuprina* LARVAE (DIPTERA: CALLIPHORIDAE) AGAINST  
SELECTED PATHOGENIC BACTERIA**

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UNIVERSITY OF MALAYA  
KUALA LUMPUR**

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SELECTED PATHOGENIC BACTERIA**

**TEH CHIEN HUEY**

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Title of Dissertation ("this Work"):

***In Vitro* Antibacterial Activity of Medicinal *Lucilia Cuprina* Larvae (Diptera: Calliphoridae) Against Selected Pathogenic Bacteria**

Field of Study: **Medical Entomology**

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## ABSTRACT

Maggot Debridement Therapy (MDT) is a type of biosurgery involving the intentional application of live, disinfected fly larvae into the chronic non-healing wounds of human or animal to debride the necrotic tissue and disinfect the infected wounds. Many studies have demonstrated the potent antibacterial activity of *Lucilia sericata* larval excretions/secretions against bacteria, however, the antibacterial activity of the local strain of blowfly, *L. cuprina* (Wiedeman) larval extract against bacteria has never been determined, although MDT using *L. cuprina* larvae was successfully conducted. In view of this, the objectives of this study are to develop a procedure for the production of sterile *L. cuprina* larval extract as well as to study the *in vitro* antibacterial activity of *L. cuprina* larval extract against seven selected potentially pathogenic wound bacteria: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Larvae were sterilized using established procedures and sterile larval extract was produced successfully via subsequent methanol-homogenization of larvae, centrifugation of homogenate and vacuum-concentration of the resultant supernatant. The vacuum-concentrated product (larval extract) was kept at -70 °C and re-suspended in sterile distilled water prior to use. Turbidometric (TB), Colony-Forming Units (CFU), Agar Well-Diffusion and Minimum Inhibitory Concentration (MIC) assays were adopted to determine the *in vitro* antibacterial activity and properties (bactericidal and/or bacteriostatic) of larval extract against the seven selected bacteria. TB Assay has demonstrated significant growth inhibition of all bacteria tested ( $p < 0.001$ ). However, both CFU and well-diffusion assays have demonstrated the significant potent inhibitory effect of *L. cuprina* larval extract towards *P. aeruginosa* and these results were substantiated by the MIC assay

that as little as 0.78 mg/ml of larval extract was able to inhibit at least 50% of the growth of *P. aeruginosa*. *L. cuprina* larval extract has proven to withstand long-term storage (13 months) and was thermally stable. In conclusion, the highly robust *L. cuprina* larval extract exhibited broad-spectrum antibacterial activity and was particularly potent against the Gram-negative bacteria.

## ABSTRAK

Terapi Ulat merupakan sejenis bio-terapi yang melibatkan aplikasi ulat lalat hidup dan steril dalam luka kronik manusia atau binatang untuk membersihkan tisu nekrotik dan menyah-infeksikan luka terinfeksi. Kajian-kajian lepas telah membuktikan keberkesanan aktiviti anti-bakteria yang ditunjukkan oleh ulat lalat *Lucilia sericata*. Walau bagaimanapun, aktiviti anti-bakteria bagi ulat strain tempatan, iaitu *L. cuprina* tidak pernah dikaji sedangkan terapi ulat yang menggunakan ulat *L. cuprina* telah dilaksanakan dengan berjayanya. Oleh itu, objektif kajian ini adalah untuk mewujudkan prosedur penghasilan ekstrak ulat *L. cuprina* serta mengaji secara *in vitro* aktiviti anti-bakteria ekstrak ulat *L. cuprina* terhadap tujuh jenis bacteria patogenik yang kerap menginfeksi luka, iaitu *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* dan *Escherichia coli*. Ulat lalat dinyah-infeksikan dengan prosedur pembersihan tertentu dan kemudian di-homogenisasi dalam methanol, di-sentrifugasi dan akhirnya supernatant yang didapati dipekatkan melalui pengvakuman untuk menghasilkan ekstrak ulat yang steril. Produk akhir yang didapati (ekstrak ulat) disimpan pada -70 °C dan dilarutkan dalam air suling steril sebelum digunakan. Asai turbidometrik (TB), unit pembentukan-koloni (CFU) dan *Agar Well-Diffusion* telah digunakan untuk menentukan secara *in vitro* aktiviti dan ciri (bakterisidal dan/atau bakteriostatik) anti-bakteria ekstrak ulat terhadap tujuh spesis bacteria yang terpilih. Asai TB telah menunjukkan bahawa ekstrak ulat merencatkan pertumbuhan semua bacteria yang dikaji secara signifikan ( $p < 0.001$ ). Bagaimanapun, asai CFU dan *Agar Well-Diffusion* menunjukkan bahawa kesan perencatan ekstrak ulat adalah lebih signifikan dan berkesan terhadap *P. aeruginosa* dan keputusan ini pula disokong oleh data dari asai *Minimum Inhibitory Concentration* yang membuktikan

hanya sebanyak 0.78 mg/ml ekstrak ulat adalah mencukupi untuk merencatkan sekurang-kurangnya 50% pertumbuhan *P. aeruginosa*. Selain itu, ekstrak ulat *L. cuprina* telah dibukti dapat menahan masa penyimpanan yang panjang (13 bulan) dan amat stabil terhadap haba. Secara kesimpulannya, ekstrak ulat *L. cuprina* yang tahan lazak ini menunjukkan spektrum aktiviti anti-bakteria yang luas dan adalah secara khususnya berkesan terhadap bakteria Gram-negatif.

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**LIST OF SYMBOLS AND ABBREVIATIONS**

<b>Symbols</b>	<b>Definitions</b>
%	percent
°C	degree Celsius
μl	microliter
μm	micrometer
BaCl <sub>2</sub> ·2H <sub>2</sub> O	barium chloride dihydrate
g	gram
H <sub>2</sub> SO <sub>4</sub>	sulphuric acid
M	molar
mg	milligram
mg/ml	milligram/mililiter
ml	mililiter
w/v	weight/volume

**Abbreviations****Definitions**

ANOVA

Analysis of Variance

ATCC

American Type Culture Collection

BA

blood agar

BHI

brain heart infusion

BHIA

brain heart infusion agar

CFU

colony-forming unit

DNA

deoxyribonucleic acid

ECM

extracellular matrix

ES

excretions/secretions

HSD

honesty significant difference

IMR

The Institute for Medical Research

LPS

lipopolysaccharides

MDT

maggot debridement therapy

MICs

minimum inhibitory concentrations

MRSA

methicillin-resistant *Staphylococcus aureus*

MSSA

methicillin-susceptible *Staphylococcus aureus*

OD	optical density
PBS	phosphate-buffered saline
rpm	revolutions per minute
SPSS	Statistical Package for the Social Sciences
TB	turbidometric
VRSA	Vancomycin-resistant <i>Staphylococcus aureus</i>

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