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

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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 ℃ 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-backteria ekstrak ulat *L. cuprina* terhadap tujuh jenis bacteria pathogenik 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) disimpankan pada -70℃ 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

Symbols	Definitions
%	percent
˚C	degree Celsius
µl	microliter
µm	micrometer
$BaCl_2.2H_2O$	barium chloride dihydrate
g	gram
H_2SO_4	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 *Staphylococcus aureus*

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