UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF *Chromolaena odorata* ETHANOLIC EXTRACT ON *Pseudomonas aeruginosa* BIOFILM FORMATION

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the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA (UiTM). It is original and is the result of my own work. Unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA (UiTM), regulating the conduct of my study and research.

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ABSTRACT

Chromolaena odorata is known to possess antimicrobial effects against wide range of microorganisms including *Pseudomonas aeruginosa*. However, the inhibitory effects of *C. odorata* extracts against the biofilm growth mode of *Pseudomonas aeruginosa* remain uncertain. Therefore, this study was carried out to determine the antibiofilm activity of *C. odorata* extracts against *P. aeruginosa* under aerobic and anaerobic conditions. Phytochemical screening using Gas Chromatography Mass Spectrometry (GCMS) revealed the major constituents in *Chromolaena odorata* ethanolic extracts (COEE) as Germacrene D, Caryophyllene and δ- Cadinene. Microbroth dilution assay showed that absence of oxygen did not affect MIC and MBC of COEE against *P. aeruginosa*. However, antibacterial susceptibility test showed that the size of inhibition zone of COEE against *P. aeruginosa* were slightly different between the aerobic and anaerobic conditions. Colony forming unit counting of biofilm cells showed inhibition greater than 50% at COEE test concentration of 50mg/ml and 200mg/ml under both aerobic and anaerobic conditions. Also, COEE treatment resulted in changes in the biochemical composition of *P. aeruginosa* biofilm extracellular matrixes under both experimental conditions as indicated by variation in the infrared spectra in the region between 1700 and 900cm^{-1}. A combination of SDS polyacrylamide gel electrophoresis and densitometry was used to analyze the total protein profile of *P. aeruginosa* biofilm under aerobic and anaerobic conditions. Treatment with COEE was found to alter the total protein profile of *P. aeruginosa* biofilm under aerobic and anaerobic conditions. Densitometric analysis between 400 and 750 nm demonstrated higher expression of proteins of 16 kDa, 34 kDa and 44 kDa under aerobic condition whilst lower expression of 25 kDa, 41 kDa and 55 kDa proteins was observed under anaerobic condition. Meanwhile, two dimensional polyacrylamide gel electrophoresis (2D PAGE) combined with MALDI TOF-TOF successfully identified 19 biofilm proteins under both experimental conditions. The three highest functional categories of identified *P. aeruginosa* biofilm proteins were found to be responsible for translation, ribosomal structure and biogenesis (56%), energy production and conversion (22%) and redox homeostasis (11%).

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