UNIVERSITI TEKNOLOGI MARA

ANALYSIS OF THE EFFECT OF ALLYLPYROCATECHOL (*Piper betle* Linn.) ON OXIDATIVE STRESS RESISTANCE ENZYMES IN *Staphylococcus aureus* AND NADPH OXIDASE IN POLYMORPHONUCLEAR LEUKOCYTES

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of 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. It is original and is the results 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 degree or qualification.

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

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ABSTRACT

Allylpyrocatechol (APC) is a major phenolic constituent in *Piper betle* L. leaves extract which consists of benzene ring with hydroxyl groups. It displays antimicrobial activity against *Staphylococcus aureus* (*S. aureus*), however, study about its effects on the stress response mechanism of this organism is limited. An optimised method was developed for APC isolation based on elution from analytical RP-HPLC in isocratic mode using ratio 45% acetonitrile: 55% aqueous phosphoric, where APC was proven to be the major constituent with yield of 78% and purity of 97%. In this study, expression of *katA, sodA*, *sodM,* and *ahpC* genes of *S. aureus* in response to APC (2 mg/mL) was analyzed by RT-qPCR in reference to *gyrA* and 16S rRNA. Corresponding activities of the superoxide dismutases (SOD), alkylhydroperoxide reductase C (ahpC) and catalase were also investigated. APC increased expression of *sodA* (2.478), *sodM* (1.667) and *ahpC* (6.53), but not *katA* (0.706) gene in comparison to untreated *S. aureus* cells Corresponding increased of total SOD (12.24 U/ml) and AhpC (A310 nm=0.672) activities but decreased catalase activity (1.8×10^4 U/l) were observed in APC-treated cells. APC reduced the generation of superoxide anion through the inhibition of NADPH oxidase. APC-treated PMNs had lower relative light unit (RLU) of 0.003 within 30 minutes ($P<0.05$) compared to PMNs with *S. aureus* (0.319) suggesting reduction in oxidative bursts. The NBT assay resulted on 0.330 of absorbance reading at 560 nm, which indicated lower extracellular superoxide level released by neutrophils with APC treatment. Similarly, expression of *CYBB* (gp91-phox) and *NCF2* (p67-phox) in APC-treated PMNs challenged with *S. aureus* were down regulated at 0.473-fold and 0.39-fold respectively. In addition, APC was able to inhibit *S. aureus* more effectively than 10 mM H_2O_2 with only 8.9% cells survived than H_2O_2 10 mM H_2O_2 (87%). Similarly, APC-treated cells showed significant difference ($p<0.05$) in the percentage of survival (10%) in comparison to diamide (10 mM) killing (15%) after 1 hour treatment. The treatment of paraquat (10 mM) with APC reduced the number of cells survived to 7%. APC more effectively reduced the number of viable *S. aureus* cells compared to hydrogen peroxide (10 mM), diamide (10 mM) and paraquat (10 mM). This study has provided insight on regulatory of stress responses, but the relevance of these genes in survival, repair of damage, or cellular recovery have not yet been revealed.

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