UNIVERSITI TEKNOLOGI MARA

ANTIMICROBIAL AND ANTIOXIDATIVE EFFECTS OF *PIPER BETLE* LEAVES EXTRACT ON NEUTROPHIL SURVIVAL AND katA EXPRESSION IN *STAPHYLOCOCCUS AUREUS*

NOR ANIS NADHIRAH BT MD NASIR

Thesis submitted in fulfilment
of the requirements for the degree of
Master of Science

Faculty of Health Science

September 2012

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 result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institutions or non-academic institution for any other degree or qualification.

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

Name of Student	:	Nor Anis Nadhirah bt Md Nasir
Student's I.D. No.	:	2009607226
Programme	:	Master of Science
Faculty	:	Health Science, UiTM, Puncak Alam Campus
Thesis Title	:	Antimicrobial And Antioxidative Effects of *Piper betle* Leaves Extract on Neutrophil Survival and katA Expression in *Staphylococcus aureus*

Signature of Student : .....

Date : September 2012

ABSTRACT

P. betle leaves are known to have antimicrobial and antioxidant activities widely used as traditional medicine in Asian countries. This study investigated the antimicrobial activities of ethanolic and aqueous extract of *P. betle* against *S. aureus* (ATCC 25923) by determining the Minimum Inhibitory Concentration (MIC) , Minimum Bactericidal Concentration (MBC) and its effects on the *katA* expression in *S.aureus*. The antioxidative activities were tested against H_2O_2 and oxidative burst in neutrophil. Hydrogen peroxide sensitivity test was performed to determine antioxidant of the ethanolic extract against H_2O_2. Catalase expression of *S.aureus* was analyzed by the SDS-PAGE , NATIVE-PAGE analysis and further confirmed using ferric chloride and potassium ferricyanide. Its effect on oxidative burst was determined using chemiluminescence assay. Ethanolic extract of *P.betle* leaves showed significant higher antimicrobial activity compared to the aqueous extract where $p<0.05$. MICs values of ethanolic and aqueous extracts were 5mg/ml and 10mg/ml respectively. The hydrogen peroxide test showed the ethanolic extract able to detoxify hydrogen peroxide resulting in thirteen percent survival of cells. Interestingly, *P.betle* extract itself showed effective killing of *S.aureus* within thirthy minutes. The RLU in the presence of ethanolic extract showed significant reduction ($p<0.05$) in oxidative burst by neutrophils. Catalase (58.3kDa) encoded by *katA* in *S.aureus* showed significant reduction after one hour treatment with ethanolic extract. The possible mechanism by which ethanolic extract of *P.betle* is inhibitory to *S.aureus* by downregulation of an important protein, catalase. Further work is required to quantitate the mRNA of *katA* expression following treatment with ethanolic of *P.betle* to confirm mechanism involved.

TABLE OF CONTENTS