EFFECT OF *ANDROGRAPHIS PANICULATA* METHANOLIC LEAF EXTRACT ON ALKYLHYDROPEROXIDE REDUCTASE C (AhpC) OF *STAPHYLOCOCCUS AUREUS* (ATCC 25923) *in vitro*

By

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ABSTRACTS

S.aureus infection nowadays has becomed more difficult to treat because of the emergence of multidrug- resistant strains. To overcome this problem, there is a need to find alternative sources of antimicrobials from non-synthetic sources such as natural plants extracts. *Andrographis paniculata* which is commonly found in India and Thailand is a plant that has been proven to have antibacterial, antifungal, and antioxidant properties. The aims of this study are to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Andrographis *paniculata* methanolic extract against Staphylococcus *aureus,* to determine the killing potential of methanolic extract of *A. paniculata* against *S.aureus* in reference to hydrogen peroxide (H_2O_2) and to study the effect of *A. paniculata* methanolic extract on expression of alkyl hydroperoxide reductase (AhpC) in *S.aureus* by using SDS- PAGE analysis. Antimicrobial effect of *A.paniculata* extract was studied by determination of their minimum inhibitory concentration and minimum bactericidal concentration. The killing potential of extract against *S.aureus* was determined by killing assay test with hydrogen peroxide as reference. There are significantly difference (p <0.05) in percentage of survival of cells when added three different components which are 7.5mm hydrogen peroxide, 50mg/ml extract and both components. Percentage of survival of *S.aureus* when treated with 7.5mM hydrogen peroxide is 50.6% while when treated with extract is 3.8 % at 90 minutes incubation. This show that extract can kill *S.aureus* better than hydrogen peroxide alone. Methanolic extract of *A.paniculata* also can enhance the killing of *S.aureus* with 0% of cells survival after 90 minutes with presence of 7.5mM hydrogen peroxide. SDS PAGE is used to analysis the effect of AhpC enzyme in *S.aureus* after treat with methanolic extract of *Andrographis paniculata.* Methanolic extract of *A.paniculata* show antimicrobial effect with MIC and MBC values against *S.aureus* are 50 mg/ml and 100 mg/ml respectively. SDS PAGE analysis show that there are slightly decrease in intensity of 25.4kDA band of AhpC in *S.aureus* after treated with methanolic extract of *A.paniculata* compared with penicillin which is inhibit AhpC better than extract. Thus, there is slightly inhibition of AhpC in *S.aureus* when treated with *Andrographis paniculata* methanolic extract.

CHAPTER 1

INTRODUCTION

1.1 Background

Andrographis paniculata is a traditional Asian medical plant that has been used for centuries (Akbar, 2011). In Malaysia, it is known as "Hempedu Bumi". This plant belongs to the *Acanthaceae* family and is commonly found in India and Thailand. It has antibacterial, antifungal, and antioxidant properties. The main biological compound that is responsible for its antibacterial function is andrographolide which is a type of diterpenoids substance. (Premanath & Devi, 2011).

S.aureus is a member of Micrococcaceae family and gram positive bacteria (Lowy, 1998). It is a common organism found worldwide and has ability to colonize the anterior nares and other skin areas of healthy individuals (Pantosti & Venditti, 2009). It causes both community and hospital-acquired infections and is increasing steadily nowadays. Infections caused by *S.aureus* are bacteremia, endocarditis, metastatic infections, sepsis and toxic shock syndrome (Lowy, 1998).

AhpC is an oxidoreductase enzyme and an antioxidant in *S.aureus*. It functions to detoxify H_2O_2 and various hydroperoxide and peroxynitrite. Expression of AhpC in *S.aureus* is regulated by peroxide response regulator, Per R. AhpC antioxidant activity is important in protection of bacterial damage by ROS (Cosgrove, Coutss, Jonsson, Tarkowski, Kokai-Kun, Mond & Foster, 2007). Thus the aim of this study is to investigate the effect of methanolic extract of *A.paniculata*