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MOLECULAR ANALYSIS OF EFFECTS OF *Andrographis paniculata* (HEMPEDU BUMI) ON OXIDATIVE STRESS ENZYMES AND LIPASE IN *Staphylococcus aureus*

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ABSTRACT

A.paniculata leaves are known to have antimicrobial activities and is widely used in traditional medicine worldwide. This study investigated the anti-oxidative properties of methanolic leaves extract of *A.paniculata* (0.06 mg/ml, MIC) against *S.aureus* (ATCC 25923) (3.8 x 10^8 cfu/ml) by determining the ability of the extract to reduce toxicity of hydrogen peroxide (H_2O_2) against *S.aureus*. Effects of the extract on gene expressions were determined by RT-qPCR and corresponding activity assays of catalase (*katA*), superoxide dismutases (*sodA* and *sodM*), alkylhydroperoxide reductase C (*ahpC*) and lipase (*geh*) in *S.aureus* were also perfomed. For hydrogen peroxide (H_2O_2) sensitivity test, *S.aureus* cells (3.8 x 10^8cfu/ml) were challenged separately with 7.5 mM H_2O_2 only, 7.5 mM H_2O_2 and methanolic leaves extract of *A.paniculata* (0.06 mg/ml) and methanolic leaves extract of *A.paniculata* (0.06 mg/ml) only. Cells challenged with H_2O_2 only showed 0% survival in 30 minutes whereas 25% cells survived after treatment with the extract and H_2O_2. *S.aureus* cells that were treated with extract only had 43% survival in the same exposure period. Nitroblue tetrazolium (NBT) reduction assay was performed to determine intracellular and extracellular levels of O_2^- after treatment with the extract. Extract –treated *S.aureus* cells had significantly lower intra- and extracellular O_2^- levels with absorbance readings (A_{575nm}) of 0.340 and 0.524 respectively compared to untreated cells which were 0.516 and 0.928 respectively (P<0.05). Cells treated with the extract showed 3.3-fold increase of *katA* expression compared to untreated cells (P<0.05) with corresponding catalase activity of 1.828 U compared to untreated cells which was 1.248 U, (P<0.05). Similarly, *ahpC* expression was significantly increased by 61-fold in extract-treated cells, (P<0.05) with corresponding increase in AhpC activity of 0.018 U compared to untreated cells, 0.012 U, (P<0.05). Expressions of *sodA* and *sodM* genes in extract-treated cell were decreased at 0.8-fold and 0.7-fold respectively (P<0.05) with corresponding decrease in total SOD activity of 26.8 U in treated cells compared to untreated cells, 32.4 U (P<0.05). Expression of lipase (*geh*) was significantly decreased at 0.01-fold in extract-treated cells compared to untreated cells which was 1-fold, (P<0.05) with corresponding decrease in lipase activity at 31.58 U compared to untreated cells, 132.55 U, (P<0.05). Therefore, *A.paniculata* was suggested to possess anti-oxidative properties with respect to killing of *S.aureus*.

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CHAPTER ONE
INTRODUCTION

S.aureus is established as a pathogen that causes both community and hospital-acquired infections. Multidrug resistant strain such as methicillin-resistant *S.aureus* (MRSA) for example, is a persistent problem that causes life-threatening conditions such as sepsis, necrotizing pneumonitis and necrotizing fasciitis and accounts for a majority of skin diseases and soft tissue infections (Harding et al., 2014). Oxidative stress is a state of imbalance between the production and the removal of reactive oxygen species (ROS) that may cause harm to the cell. In order to combat ROS, *S.aureus* uses its defensive molecules including catalase (CAT), superoxide dismutase (SODs) and alkylhydroperoxide reductase C (AhpC) (Painter, Strange, Parkhill, Bamford, Armstrong-James & Edwards, 2015) and others. In my previous study, methanolic extract of *Andrographis paniculata* (*A.paniculata*) showed high antimicrobial activity against *Staphylococcus aureus* (ATCC 25923) with minimum inhibitory concentration (MIC) of 0.06mg/ml (Zayan, 2015, Bachelor Degree Dissertation). According to Al-alusi, Kadir, Ismail & Abdullah (2010), MIC of an agent in the range between 0.02 to 0.078 mg/ml is considered to have potential for development as an alternative treatment for bacterial infections. Hence, *A.paniculata* extract shows good potential for its development into an alternative antistaphylococcal agent as it showed an MIC of 0.06 mg/ml against *S.aureus*. In addition, *A.paniculata* inhibited expression of a putative protein of 76 kDa which corresponds to lipase, one of the virulence enzymes in *S.aureus* (Zayan, 2015, Bachelor Degree Dissertation). It is therefore of significant relevance to determine the mechanism on antimicrobial activity of *A.paniculata* against *S.aureus*. In this study, effects of methanolic leaves extract of *A.paniculata* were investigated focusing on the molecular analysis of its effects on gene expressions and activities on oxidative stress related enzymes and lipase in *S.aureus* (ATCC 25923).