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**EFFECT OF CENTELLA ASIATICA ETHANOLIC EXTRACT ON ALKYL  
HYDROPEROXIDE REDUCTASE C (AhpC) OF STAPHYLOCOCCUS  
AUREUS (ATCC 25923)**

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**Thesis Submitted in Partial Fulfillment of The requirement for Bachelor of  
Medical Laboratory Technology (Hons), Faculty of Health Science,**

**UNIVERSITI TEKNOLOGI MARA**

**2016**

## ACKNOWLEDGEMENT

Praise to Allah S.W.T because of His guidance and blessing I enable to complete my final year project successfully in time. I received a lot of helps in completing this paper and my project. I would like to thank everyone that have been encouraged and supporting me directly and indirectly during my final year project because without them I would not be able to finish and complete this.

Firstly, million thanks to my beloved supervisor, Dr. Roslinah binti Mohamad Hussain for guidance, supervision, motivation and supports for finishing my project successfully.

I also would like to appreciate helps and guidance from Miss Zayyan Nabilah in conducting and performing tests for my project.

My team members, lab staffs of Medical Laboratory Department and Pharmacy department for compromised and cooperate with me for me finishing my lab work.

Last but not least, I would like to appreciate and thanks to my parents, En. Ramezan bin Abdul Aziz and \_\_\_\_\_ for their support, motivation and time for me to complete this course.

## TABLE OF CONTENT

Research article	i
Declaration	ii
Intellectual properties	iii-v
Acknowledgement	vi
Abstract	xiii
Chapter 1: INTRODUCTION	
1.1. Background of study	1
1.2. Problem statement	2-3
1.3. Objectives of the study	3
1.4. Scope and limitations of study	3-4
1.5. Research hypothesis	4
Chapter 2: LITERATURE REVIEW	
2.1 <i>Centella asiatica</i>	5-6
2.2 <i>Staphylococcus aureus</i>	6-7
2.3      Multidrug resistance of <i>Staphylococcus aureus</i>	7-8
2.4      Alkyl hydroperoxide reductase C (AhpC)	8-9
2.5      Reactive Oxygen Species (ROS)	9-10
2.6      Diamide	10
3. MATERIALS AND METHOD	
3.1. Materials	
3.1.1. Media culture, Chemicals and Reagent	11
3.1.2. Instrument and Equipment	11
3.1.3. Bacterial culture	11
3.2. Culture media, chemicals preparation	
3.2.1. Nutrient agar	12
3.2.2. Brain Heart Infusion Broth	12
3.2.3. Brain Heart Infusion Agar	12
3.2.4. PBS	12
3.2.5. Preparation of SDS-PAGE Buffer	12-14
3.2.6. Preparation and formulation of gel	14-15

## ABSTRACT

*Centella asiatica* plant has many different properties such as antimicrobial, potential antifungal and antioxidants which is of interested for studies. *Staphylococcus aureus* is a major problem in the community as it causes a variety of infections and develops resistance towards antibiotics rapidly. The aims of this study is to identify the antimicrobial effect of *C. asiatica* ethanolic extract against *S. aureus* (ATCC 25923), to investigate the effect of diamide and *C. asiatica* ethanolic extract treatment on killing rate of *S. aureus* (ATCC 25923) and the effect of *C. asiatica* ethanolic extract on the expression of AhpC protein in *S. aureus* (ATCC 25923). The antimicrobial effect of *C. asiatica* ethanolic extract against *S. aureus* (ATCC 25923) was determined by Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) methods which MIC and MBC value were 16 mg/ml and 32 mg/ml respectively. *S. aureus* treated with MIC of *C. asiatica* extract and 10 mM diamide showed decreasing survival to 7.23 % at 30 minutes and almost completely killed at 150 minutes. *S. aureus* challenged with MIC of *C. asiatica* extract alone showed slower death rate compared to the treatment of MIC of *C. asiatica* extract together with 10 mM diamide which only about 70 % of *S. aureus* killed at first 30 minutes and were killed completely at 150 minutes. 10 mM diamide treatment alone showed slowest killing rate compared with other treatment which only about 37 % *S. aureus* were killed at 30 minutes and at 180 minutes, 80 % of *S. aureus* were killed. The expression of AhpC protein in *S. aureus* (ATCC 25923) was visualized by SDS-PAGE analysis. There was no reduced expression of Ahpc in treated with 1 hour and 2 hour of MIC *C. asiatica* extract compared to untreated *S. aureus*. *S. aureus* treated with 32 µg/ml penicillin also showed same intensity colour band corresponding to Ahpc of untreated *S. aureus*. Thus, it is found that *C. asiatica* ethanolic extract has antimicrobial effect against *S. aureus* (ATCC 25923), and diamide can enhance *C. asiatica* ethanolic extract killed *S. aureus* (ATCC 25923) but *C. asiatica* ethanolic extract alone killed better *S. aureus* (ATCC 25923). The expression AhpC protein of untreated *S. aureus* (ATCC 25923) and treated *S. aureus* (ATCC 25923) with *C. asiatica* ethanolic extract and penicillin do not showed any reduced expression. It can be concluded AhpC does have oxidative stress resistance alongside with catalase in *S. aureus*.

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of study

Natural resources such as fruit, plant, grain, flowers is of great interest for researchers to study their properties and medical benefits. One of these is *Centella asiatica* plant which is also classified as a herb. In light of the emergence of multi drug resistant strains of groups of bacteria, natural product can serve as remedy against infectious disease due to less toxicity, their availability and fewer side effects compared to modern antibiotics (Firdaus Jahan, Lawrence, Kumar and Mohd Junaid, 2011). Natural antibiotics or drugs have another great advantage in that the mechanism of the active compound in plants are usually have random binding sites which lead to difficulty of the bacterial cell to develop resistance against the natural drugs (Pitindhipat and Yasurin,2012). The different properties of *Centella asiatica* extract may have potential antimicrobial activity against microorganism especially *Staphylococcus aureus*. *Staphylococcus aureus* is a common and major problem in the community as it has rapid development of multidrug resistant strains. It is known that there are proteins in *S. aureus* that serve as survival mechanisms of the bacteria such as catalase, alkyl hydroperoxide reductase subunit F and C, thioredoxin and others. Bacterial alkyl hydroperoxide reductase C (AhpC) protein involve in protection of enteric bacteria against oxidative metabolism (Armstrong-Buisseret, Cole and Stewart, 1995). Previously, Alkyl hydroperoxide reductase (Ahp) system (AhpC and AhpF) was found associated with the detoxification process of organic hydroperoxides (Carbona, Sauvageot, Giard, Benachour, Posteraro, Auffray, Sanguinetti and Hartke, 2007).

The aim of this study is to investigate the antimicrobial activity of *Centella asiatica* ethanolic extract against *Staphylococcus aureus* and to investigate the effect of *Centella asiatica* ethanolic extract on alkyl hydroperoxide reductase C (AhpC) protein in *S. aureus* by using SDS-PAGE analysis.