

**UNIVERSITI TEKNOLOGI MARA**

**ELICITATION OF ASIATIC ACID IN *CENTELLA*  
*ASIATICA* USING *KAPPAPHYCUS ALVAREZII***

**PUTRI NUR HALIMAH BT ABDUL RAHMAN  
PUTRA**

**BACHELOR OF PHARMACY (Hons.)**

**FACULTY OF PHARMACY**

**2016**

## **ACKNOWLEDGEMENT**

First and foremost, I would like to thank to the Almighty for His blessing toward accomplishing this project. His Protection and Guidance had enabling me to complete this thesis.

I was so indebted to many people who helped me toward accomplishing this project. It is impossible for me to acknowledge every one of them individually, but several in particular deserve recognition. I wish to deepest appreciation and thanks to Madam Noor Anilizawatima Bt Sulong as supervisor for my research project, for her invaluable concern, sustained guidance and diligent support which enable me to bring this project to complete. Her continuous review, guidance, ideas and suggestion has been precious to this project.

I also would like to take this opportunity to show my gratitude to Dr Humera Naz and Dr Alwani Bt Ariffin as my co-supervisor for their kind ideas and guidance. Next, I am particularly grateful to laboratory staffs of Plant Tissue Culture Laboratory and Analytical Unit Laboratory for their guidance and effort to help in fulfilling my laboratory needs and supplies during this research.

I also would like to express my appreciation to my family and friends for their support during my research final project because their support had given me strength to continue and complete this research.

Lastly, special thanks to Faculty of Pharmacy, UiTM Puncak Alam for giving me the opportunity to learn and experience such a wonderful knowledge and provide a good research environment.

# TABLE OF CONTENTS

	Page
TITLE PAGE	
APPROVAL SHEET	
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vii
LIST OF GRAPHS	ix
LIST OF ABBREVIATIONS	x
ABSTRACT	
 CHAPTER ONE (INTRODUCTION)	
1.1 Background of Study	1
1.2 Problem statement	4
1.3 Objective of study	5
1.4 Hypothesis of study	5
1.5 Significance of study	6
1.6 Limitations	6
 CHAPTER TWO (LITERATURE REVIEW)	
2.1 The biology and characteristics of <i>Centella asiatica</i>	8
2.1.1 Morphological characteristics of <i>Centella asiatica</i>	10
2.1.1.1 Macroscopic description	10
2.1.1.2 Microscopic description	13
2.1.2 Organoleptic properties	14
2.2 Metabolites in plants	15
2.2.1 Primary metabolites	15
2.2.2 Secondary metabolites	17
2.2.2.1 Alkaloids	19
2.2.2.2 Phenols	20
2.2.2.3 Terpenoids	21
2.2.2.3.1 Terpenoids in <i>Centella asiatica</i>	24
2.2.2.3.1.1 Asiatic acid	28
2.2.2.3.1.2 Madecassic acid	29
2.2.2.3.1.3 Asiaticoside	31
2.2.2.3.1.4 Madecassoside	33
2.3 The Uses of <i>Centella asiatica</i>	34
2.3.1 Traditional uses of <i>Centella asiatica</i>	34
2.3.2 Pharmacological uses of <i>Centella asiatica</i>	35
2.4 Plant tissue culture technique	37
2.4.1 Micropropagation technique	38

## ABSTRACT

*Centella asiatica* is an endangered medicinal herb which used in the preparation of herbal drugs mainly due to the presence of four pentacyclic triterpene which are asiatic acid, asiaticoside, madecassic acid and madecassoside. It's over exploitation necessitates the development of conservation strategies and enhanced the production of secondary metabolites. In present study, the effect of various concentration of seaweed *Kappaphycus alvarezii* elicitor was used to increase the amount of asiatic acid production in *C. asiatica*. Four difference concentration of seaweed elicitor was treated in *C. asiatica* which are 0 g/L, 2g/L, 4g/L and 8g/L. They are harvested at day 0, 7, 14 and 21 and soak with methanol to obtain crude brown extract. The amount of asiatic acid containing in the samples was analyzed by HPLC and area under the curve of retention peak was calculated by using a formula. The diameter of leaf, number of new shoots and flowers were measured during successive stages of development. In conclusion, *K. alvarezii* can increase the production amount of asiatic acid in *C. asiatica* at shorter time, which at day 14, concentration of 4g/L and 8 g/L are the best while the additional of certain concentrations of seaweed elicitor can induce flowering in *C. asiatica*.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of study

*Centella asiatica* also known as ‘Pegaga’ or ‘Gotu Kola’ which come from the family of *Apiaceae*, a subfamily of the *Hydrocotyle* and genus of *Centella*. It is a slender creeping perennial herb that found in shady, moist area and usually as an annual plant in India, Sri Lanka, Malaysia, and other parts of Asia (Gandi & Giri, 2013). It also grows very well in sandy and clay soil rich in humus and organic matter (Jamil, Qudsia, & Salam, 2007). It produces a group of bioactive compounds collectively called ‘Centellosides or Centelloids’ which are ursane type of pentacyclic triterpenoid saponins and sapogenin that act as secondary metabolites. According to Gandi *et al.*, (2013), the Centellosides include asiaticoside, centelloside, madecassoside, brahmoside, asiatic acid, madecassic acid, thankuniside and others. However, the main bioactive compounds in *C. asiatica* are asiatic acid, asiaticoside, madecassic acid, and madecassoside (Kim *et al.*, 2009). It has been established that the climatic conditions, soil texture, and agronomic work will highly