# **UNIVERSITI TEKNOLOGI MARA**

# LC PROFILING AND DETERMINATION OF STEVIODISE AND REBAUDIOSIDE A IN TEN Stevia rebaudiana BERTONI ACCESSIONS

## AMANINA ALWANI ARSHAD

Thesis submitted in fulfillment of the requirements for the degree of **Master of Science** 

## Faculty of Plantation and Agrotechnology

May 2019

#### ABSTRACT

Stevia rebaudiana Bertoni leaves contain non-caloric natural sweet compounds which are steviol glycosides. Stevioside and rebaudioside A are types of steviol glycosides that are the most abundant in the leaves. The aims of this study are (1) to characterize and evaluate morphological characteristics of ten stevia accessions. (2) to obtain an LC-MS profiling of stevia leaves extract and quantify major constituents of steviol glycosides and identify other major compounds that presented in the stevia leaves extract (3) to determine the effect of different harvesting stages towards stevioside and rebaudioside A. The leaves were extracted using Soxhlet extractor and analysed using LC-MS for profiled. Due to environmental factors, ten accessions showed different morphological characteristics. Before flowering stage, accession Bangi had showed high dried yield leaves, 0.72±0.01g. Accession Bertam produced number of leaf 25±2.0 per plant. Accession MS012 (4X) had a good characteristic on the plant height, 22.6±1.0cm before flowering stage. Most of accession showed the length of leaf varied from 6.5cm to 7.3cm. The profiles of stevia extracts showed that presence of major constituents steviol glycosides in stevia extracts which were  $[M-H]^2 m/z$  803 (stevioside) and 965 (rebaudioside A). The content of stevioside was found abundant in the extract accession MS023 before until late flowering stage, respectively with 2.14% and 2.40%, respectively. Meanwhile rebaudioside A content was found abundant in the extract of accession Bertam that harvested from before until late flowering stage, respectively with 0.60% and 0.69% respectively. Based on the LC-MS profiling, accession Bertam and MS023 are considered to have high stevioside and rebaudioside A contents and can be applied for future breeding programme to enhance the economical value of stevia for farmers.

## ACKNOWLEDGMENT

Alhamdulilah, Thanks to Allah S.W.T., whom with His willing giving me the opportunity to complete this master research. Firstly, I would like to express my highest gratitude to my supervisor, Dr. Nor Azma Binti Yusuf and my co-supervisor Professor Datin Dr. Rohaya Binti Ahmad for giving me a lot of new experiences, exposures and knowledge from beginning till the end of this master research.

Their suggestion, guidelines and advices in learning process are highly appreciated. They taught and helped me a lot for the smoothness and progression of my research. I also would like to thank all staff of Faculty of Plantation and Agrotechnology, Faculty of Applied Sciences and Atta'ur Rahman Institute for Natural Products Discovery (RiND) science officers, and all laboratory assistants last but not least Agilent Technologies for their efforts, time and sharing knowledge in helping me throughout this research. Deepest appreciation also goes to my former supervisor Professor Dr. Mohamad Osman for inspired, cooperation, and helping during progression of my research. It was an honour for me to extend my special thanks to my beloved parents, Arshad Yaacob and **Composition** and my friends for their loves, encourage and endless support to help me in overcome the tribulation, sacrifice and problems arise throughout completing this research.

Last but not least, I offer my regards and blessing to all those people who had contributed either directly or indirectly in this project and helping me in any respect during the completion of this project. Thank you for all your kindness. May Allah bless all of us in this world and hereafter.

## **TABLE OF CONTENTS**

Page

CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF SYMBOLS	xiv
LIST OF ABBREVIATIONS	XV
LIST OF NOMENCLATURE	xvii
CHAPTER ONE: INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	4
1.3 Objectives	4
1.4 Limitation of Study	5
1.5 Significance of Study	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Stevia rebaudiana Bertoni	6
2.1.1 Cultivation Technology	10
2.2 Steviol Glycosides	12
2.2.1 Effect of Flowering on Contents Stevioside and Rebaudioside	15
А	
2.3 Extraction af Steviol Glycosides	17
2.4 Liquid Chromatography Mass Spectrometry	20
2.4.1 Liquid Chromatography	20
2.4.2 Mass Spectrometry	22

2.4.3 Application of Liquid Chromatography Mass Spectrometry	25
CHAPTER THREE: MATERIALS AND METHODS	29
3.1 Stevia Accessions	29
3.2 Extraction Method	32
3.3 Quantification Using Liquid Chromatography Mass Spectrometry	33
3.4 Statistical Analysis	34
CHAPTER FOUR: RESULTS AND DISCUSSIONS	35
4.1 Characteristics of Ten Stevia Accessions	35
4.1.1 Plant Height	35
4.1.2 Dried Weight of Stevia Leaves	37
4.1.3 Number of Leaves	38
4.1.4 Leaf Length	39
4.2 Liquid Chromatography Mass Spectrometry Profiling Oof Stev	ria 44
Extracts	
4.2.1 Liquid Chromatography Mass Spectrometry Analyses	of 45
Stevioside and Rebaudioside A Standards	
4.2.2 Profiling of Ten Stevia Accessions	46
4.2.2.1 Accession Bangi	47
4.2.2.2 Accession Bertam	48
4.2.2.3 Accession Langat	50
4.2.2.4 Accession Mergong	52
4.2.2.5 Accession MS007	54
4.2.2.6 Accession MS012	55
4.2.2.7 Accession MS012 (4X)	57
4.2.2.8 Accession MS023	58
4.2.2.9 Accession Souq Bukhory	61
4.2.2.10 Accession Taman Pertanian	62
<ul> <li>4.3 Quantification of Stevioside and Rebaudioside A Content</li> <li>Different</li> <li>4.3.1 Stevioside</li> </ul>	at 67
$4.3.2$ Rebaudioside $\Delta$	60
T.J.2 Rebaudioside A	09 77
	11