

UNIVERSITI TEKNOLOGI MARA

**STUDIES ON ISOLATED CRUDE
BIOACTIVE COMPOUND(S)
FROM *Ficus deltoidea* FOR SMOOTH
MUSCLE CONTRACTILITY
POTENTIAL**

MUHAMMAD NASRUL HADI BIN AZHAR

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Natural Product Chemistry)

Faculty of Applied Sciences

September 2019

ABSTRACT

The isolation of the bioactive compound(s) from *Ficus deltoidea* was done by performing two different methods; traditional extraction technique using boiling step and conventional chemistry method. The former produced the crude water extract which will then partitioned with ethyl acetate producing water and ethyl acetate fractions. The latter involved sequential partitioning of the crude methanol extract with solvent of different polarity (hexane, chloroform, ethyl acetate and distilled water). Angiotensin converting enzyme (ACE) and acetylcholinesterase (AChE) enzyme assays were used as bio-guided assays to search for the bioactive compound(s). Samples that have good activity were further separated and purified using size exclusion-, silica gel- and Celite® gel- column chromatography. Samples with good activity were also subjected to protein identification, using SDS – PAGE and LC/Q-ToF MS approaches. From the traditional extraction method, water fraction (WF) and water fraction subfraction 2 (WFSF2) showed good inhibitory activity towards ACE ($60.360 \pm 0.508\%$ and $72.646 \pm 15.012\%$ respectively). Both samples have very minimal inhibitory activity towards AChE ($5.535 \pm 0.849\%$ and $13.835 \pm 3.249\%$ respectively). Water fractions showed the presence of rubber elongation factor protein, cdc2 kinase and non-specific serine/threonine protein kinase. Meanwhile, water fraction subfraction 2 only showed the presence of actin. The extract and fractions from the conventional solvent based method did not show good response towards the assays. Hexane soluble extract fraction 5 subfraction 1 (HSEF5SF1) and chloroform soluble extract fraction 1 subfraction 1 (CSEF1SF1) were sent for GCMS analysis. Only HSEF5SF1 showed the desired result with presence of palmitic acid, oleic acid, and petroselinic acid. All of the identified proteins and fatty acids demonstrated smooth muscle contractility potential as reported by other researches. However, none of these findings were reported from *F. deltoidea*. Currently only vitexin reported to have smooth muscle contractility potential. Thus, this study provides new data in term of protein and non – polar compounds with smooth muscle contractility potential for the benefit of future research.

ACKNOWLEDGEMENT

Firstly, I want to praise Allah for giving me the opportunity to complete my Master degree study. My gratitude goes to my supervisor Professor Dr. Yamin Bin Yasin and my co-supervisor Associate Professor Dr. Norhaniza Binti Aminudin from University of Malaya. Thank you for the opportunity, your guide and most important believing me to complete this study.

I like to thank Ministry of Education and Universiti Teknologi MARA, Shah Alam for their research grant FRGS (600-RMI/ST/FRGS 5/3/Fst (10/2011). Without this grant, this study is impossible to be completed. This journey also became much easier with the scholarship by Malaysian government under MyBrain15 programme and High Impact Research Grant from University of Malaya, Kuala Lumpur.

I also received great help from the entire staff of Proteomics Laboratory, Biochemistry Laboratory and University Malaya Centre for Proteomics Research (UMCPR). With their involvement, I managed to conduct my study and completed this challenging journey. Not to forget, to all staff of Centre of Postgraduate Studies from Faculty of Applied Sciences and Universiti Teknologi MARA Postgraduate Institute for their assistance throughout completing this study. Special mention of thanks goes to Ken from Advanced Chemistry Solution, for his excellent service to analyze my sample that is importance for my research findings.

A special thanks to my fellow labmates, Madam Khaniza Khasliza, Atique, Fathin, Faridah, Ai Theng, Chuo, Hanisah, Nurul Huda and every single member of Proteomics Laboratory and Biochemistry Laboratory of University of Malaya for helping me and work together efficiently so that every one of us could complete their postgraduate journey successfully.

I would like to extend my gratitude to my fellow friend/brother/sister, Nizam, Aizat, Izzudin, Helmy, Aina, Faiz, Ijat, Meor, Khalil, Ann Nazira, Izlina, Patrick Jones, Iera, Ayu, and Akma who are very supportive and always feed me with positive vibe throughout my Master degree journey. I like also to dedicate this study to my dear senior who I look up like my sister, Miss Izzatul Huda Abd Ghaffar that passed away in 2014 because of lymphoma cancer and she couldn't finish her Master study. She was my mentor for natural product research during my degree at University Malaysia Terengganu. Her passion, kindness and hardship will not be forgotten and always be remembered in my heart.

Finally, this journey would not start without my parent blessing. Special big thanks to Mr Azhar Bin Abdul Rahman and [redacted] for raising me and always have faith in me. Their struggle and hardship in life makes me stronger and motivate me to move forward in every aspect of my life and most important to not give up. To my brother, Muhammad Najmi Bin Azhar seeing you growing up and shape your career and took risks make me believe nothing is impossible as long as you work hard and not give up. I will remember all helps and support that make this dream come true. Thank you.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF PLATES	xvi
LIST OF SYMBOLS	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER ONE: INTRODUCTION	1
1.1 Dissertation Outline	1
1.2 Background of Study	2
1.3 Problem Statement	4
1.4 Objectives of Study	5
1.5 Significance of Research	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 <i>Ficus</i>	6
2.2 <i>Ficus deltoidea</i> Jack	7
2.3 Smooth Muscle Contractions by Autonomic Nervous System	13
2.4 Smooth Muscle Contractions by the Rennin – Angiotensin Aldosterone System	20
2.5 <i>In Vitro</i> Smooth Muscle Contractility Related Assays	22
2.5.1 Acetylcholinesterase Enzyme Inhibition Assay	22
2.5.2 Angiotensin Converting Enzyme Inhibition Assay	22
2.6 Isolation of Bioactive Compounds from Plant	22
2.6.1 Plant Extraction	23

2.6.2 Protein Estimation by BCA Protein Assay	24
2.6.3 Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis	24
2.6.4 Chromatography and Mass Spectrometry	25
2.6.4.1 Thin Layer Chromatography (TLC)	25
2.6.4.2 Column Chromatography	26
2.6.4.3 Thin Layer Chromatography and Column Chromatography for Phytochemical Study	28
2.6.4.4 Gas Chromatography Mass Spectrometry (GCMS)	29
2.6.4.5 The Advantages of Gas Chromatography Mass Spectrometry for Non Polar Compound Identification	32
2.6.4.6 Liquid Chromatography Mass Spectrometry (LCMS)	34
2.6.4.7 Matrix Assisted Laser Desorption – Time - of – Flight Mass Spectrometry (MALDI –ToF MS)	35
2.6.4.8 Protein Identification by Mass Spectrometry	37
CHAPTER THREE: RESEARCH METHODOLOGY	39
3.1 Chemicals, Solvents and Reagents	39
3.2 Equipments	41
3.3 Apparatus and Consumables	41
3.4 Kits	42
3.5 Softwares	42
3.6 Sample Collection and Preparation	43
3.7 Preparation of Plant Extracts	43
3.7.1 Crude Water Extract (CWE) Preparation	43
3.7.1.1 Further Purification of CWE	43
3.7.2 Solvent Extract Preparation	44
3.8 Methodological Approach for Isolation of Bioactive Compound(s) From FDML	46
3.9 Protein Estimation by Bichoninic Acid (BCA) Protein Assay	47
3.9.1 Protein Precipitation by Cold Acetone	47
3.9.2 BCA Protein Assay	47
3.10 Thin Layer Chromatography (TLC)	47