

**FRACTIONATION OF METHANOL EXTRACT FROM THE LEAVES OF  
*ENTADA SPIRALIS* RIDL. (SINTOK) USING VACUUM LIQUID  
CHROMATOGRAPHY AND ITS PRELIMINARY PHYTOCHEMICAL  
INVESTIGATION OF AN ACTIVE COMPONENTS**

**MOHD ARDYKA BIN YAHYA**

**BACHELOR OF SCIENCE (Hons.) CHEMISTRY  
FACULTY OF APPLIED SCIENCES  
MARA UNIVERSITY OF TECHNOLOGY**

**JAN 2015**

## **ACKNOWLEDGEMENT**

Bismillahirrahmanirrahim

In the name of Allah, the Most Gracious and the Most Merciful. Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. In this opportunity I would like to express my profound gratitude and deep regards to my supervisor and co-supervisor, Dr. Aiza Bt. Harun and Miss Siti Suhaila Bt. Harith for exemplary guidance, monitoring and constant encouragement throughout the course of this thesis. Thanks a lot for giving me an advice and suggestion throughout this project until the final form. Thanks to them because they supporting me and also for time in advising which made this work possible. I have been very fortunate to be under her guidance.

I am highly indebted to the authorities of MARA University of Technology (UiTM) for providing me various infrastructures like library, Internet access and permission include copyright which have enabled me to complete my research work successfully. I am obliged to staff members, laboratory assistant especially Mr. Fauzi and Mr. Mohamad Zahir Ismail for the guidance and valuable information provided by them in their respective fields. I am grateful for their cooperation during the period of my assignment.

## TABLE OF CONTENTS

	<b>PAGE</b>
<b>ACKNOWLEDGEMENT</b>	i
<b>TABLE OF CONTENTS</b>	iii
<b>LIST OF TABLES</b>	v
<b>LIST OF FIGURES</b>	vi
<b>ABSTRACT</b>	viii
<b>ABSTRAK</b>	ix
<b>CHAPTER 1 INTRODUCTION</b>	1
1.1 Background and problem statement	4
1.2 Objective of study	4
1.3 Significance of study	4
1.4 Scope of study	5
<b>CHAPTER 2 LITERATURE REVIEW</b>	6
2.1 Leguminosae	7
2.2 <i>Entada</i> species	7
2.2.1 <i>Entada scandens</i>	7
2.2.2 <i>Entada abyssinica</i>	8
2.2.3 <i>Entada spiralis</i>	8
2.3 Chromatography techniques	9
2.3.1 Thin Layer Chromatography	10
2.3.2 Vacuum Liquid Chromatography	12
2.4 Bio-activity	13
2.4.1 Disc-diffusion method	14
2.4.2 Antimicrobial and antibacterial assay	15

<b>CHAPTER 3 METHODOLOGY</b>	17
3.1 Materials	17
3.1.1 Raw materials	17
3.1.2 Chemicals	17
3.1.3 Apparatus	18
3.2 Methods	19
3.2.1 Leaves sample extraction	19
3.2.2 Fractionation by Vacuum Liquid Chromatography (VLC)	19
3.2.3 Determination by Thin Layer Chromatography (TLC)	20
3.2.4 Preparation of spraying reagent	20
3.2.4.1 Vanillin/H <sub>2</sub> SO <sub>4</sub>	20
3.2.4.2 Ferric Chloride (FeCl <sub>3</sub> )	20
3.2.4.3 DPPH	21
3.3 Antimicrobial activity assay	21
3.3.1 Preparation for microbial suspension	21
3.3.2 Preparation of test solution and disc	21
3.3.3 Chemicals for antimicrobial assay	22
3.4 In-vitro Antimicrobial Activity Assay	22
3.4.1 Disc diffusion assay	22
<b>CHAPTER 4 RESULTS AND DISCUSSIONS</b>	23
4.1 Extraction and fractionation	23
4.2 Thin-layer chromatography profile	26
4.3 Phytochemical screening	29
4.3.1 Determination of phenolic compound	32
4.3.2 Determination of terpenoid	33
4.3.3 Detection of antioxidants	35
4.4 In-vitro antimicrobial activity	37
4.5 Relation between phytochemical compounds and antimicrobial activity	42
4.6 Determination of functional group using FTIR	45
<b>CHAPTER 5 CONCLUSION AND RECOMMENDATIONS</b>	51
<b>CITED REFERENCES</b>	52
<b><i>CURRICULUM VITAE</i></b>	56

## ABSTRACT

### FRACTIONATION OF METHANOL EXTRACT FROM THE LEAVES OF *ENTADA SPIRALIS* RIDL. (SINTOK) USING VACUUM LIQUID CHROMATOGRAPHY AND ITS PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF AN ACTIVE COMPONENTS

This study was performed to investigate the preliminary phytochemicals and in-vitro antimicrobial properties against *Staphylococcus aureus* ATCC 33591 and *Streptococcus epidermis* ATCC 12228 from the leaves extract of *Entada spiralis* Ridl which can cause skin diseases to human. The fine-powdered leaf samples of *E. spiralis* were soaked with methanol. The soaking solution was evaporated using rotary evaporator at 75°C for 90 (rpm) to get the crude extract. Next the crude was undergoing fractionation process using two different solvents of dichloromethane (DCM) and methanol (MeOH) as the binary system. The fraction system used was fraction F1 (9:1); (DCM:MeOH), (fraction F2 (5:5)); (DCM:MeOH), and fraction F3 (3:7)); (DCM:MeOH). After fractionated, the solution was evaporated once again to get the crude extract based on the fractionated polarities. The crude extract was investigated with antimicrobial assay. The sample containing 400mg/mL of concentration from each three fraction system was pipetted onto a sterile paper disc on a Mueller-Hinton agar plate that had been spread with both *S. aureus* and *S. epidermis*. For the next method, the crude extract was undergoing TLC profiling to determine the compound present inside the leaves. The developing solvent for TLC profiling consisted of chloroform (CHCl<sub>3</sub>) and methanol (MeOH) solvent with different ratios. The best developing solvent for fraction F1 and F3 was 9:1; (CHCl<sub>3</sub>: MeOH) and 7:3 for fraction F2. And the last method, the crude extract for the three fraction systems was identified its functional groups using FTIR. As for the result, fraction F2 had the most active compound for both phytochemical investigation and antimicrobial properties. In TLC profiling, fraction F2 had all the compounds responsible for antimicrobial properties which were the antioxidant, terpenoid and phenolic compounds. For fraction F1 and F3, both fractions indicated the absence of phenolic compound. gave a negative result towards FeCl<sub>3</sub> only for fraction F3. While for the antimicrobial assay, fraction F2 had the biggest inhibition zone for both *S. aureus* and *S. epidermis* and proved to be the most susceptible compound towards both of the bacteria. As for the other two fractions, both had weak susceptibility towards *S. aureus* and *S. epidermis* due to small inhibition zone. Based on the result for both TLC profiling and antimicrobial assays, it was proved that fraction F2 was the most active fraction from the leaves extract of *E. spiralis* towards phytochemical screening and also antimicrobial properties.