

**ISOLATION OF ANTIBACTERIAL AND
ANTIOXIDATIVE COMPOUNDS FROM STEMS OF
*Entada spiralis***

NURFARAH FARINI BINTI MUHAMAD KAMARAZZAMAN

**BACHELOR OF SCIENCE (Hons.) CHEMISTRY
FACULTY OF APPLIED SCIENCES
UNIVERSITI TEKNOLOGI MARA**

JANUARI 2020

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF ABBREVIATIONS	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	1
1.1 Background of The Study	1
1.2 Problem Statement	3
1.3 Significant of Study	3
1.4 Objective	5
CHAPTER 2 LITERATURE REVIEW	6
2.1 Family of Leguminecae and Its Antioxidative Activities	6
2.2 Antioxidative compound from <i>E. spiralis</i>	9
2.3 Antimicrobial Activity from <i>E. spiralis</i>	13
2.4 Phytochemical Screening of <i>Entada sp.</i>	17
2.5 Function of Different Class of Phytochemical	17
2.6 Standard Method of Phytochemical Screening	20
CHAPTER 3 METHODOLOGY	22
3.1 Materials	22
3.2 Chemicals	22
3.3 Apparatus	22
3.4 Instrument	23
3.4.1 A rotary evaporator	23
3.4.2 Infrared spectroscopy (IR)	23
3.5 Methods	23
3.5.1 Collection of samples	23
3.5.2 Soaking and extraction	23
3.5.3 Thin Layer Chromatographic (TLC) analysis	24
3.5.4 Expected positive observations for various visualizing methods /reagents	26
3.5.5 Agar overlay bioautographic assay	27
3.6 Purity Test	27
3.6.1 Isolation of antioxidant and antimicrobial constituents from preparative thin layer chromatography	28
3.6.2 Setting up TLC developing tank	28
3.7 Structure Interpretation by Using ¹ H NMR	29

3.6.3	Preparing and developing TLC plates	28
3.8	Liquid Chromatography Mass Spectrophotometer (LCMS).	29
CHAPTER 4 RESULT AND DISCUSSIONS		30
4.1	Extraction	30
4.2	Thin Layer Chromatography (TLC) Screening Analysis	31
	4.2.1 Screening phytochemicals on TLC plates	33
4.3	FTIR Analysis	38
4.4	Isolation of antioxidative compounds by using preparative Thin Layer Chromatography (TLC)	41
	4.4.1 Isolation of antioxidative compounds	41
	4.4.2 Purity test	43
4.5	Proton NMR interpretation of isolated compound from EA extracts	44
4.6	LCMS Interpretation of isolated compound from EA extract	50
4.7	Antimicrobial activity analysis	52
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS		56
CITED REFERENCES		58
APPENDICES		64
CURRICULUM VITAE		72

3.7	Structure Interpretation by Using ^1H NMR	29
-----	--	----

LIST OF TABLES

Table	Caption	Page
2.1	Antioxidant activities from different extracts and constituents from the stem bark of <i>E. spiralis</i>	10
2.2	Results of phytochemical screening of roots, stem barks and leaves of <i>E. africana</i>	21
3.1	Expected observation for various visualizing methods/reagents	26
4.1	Mass and percentage yield of <i>E. spiralis</i> stem extract	31
4.2	TLC analysis	33
4.3	Phytochemicals analysis of PE extract	34
4.4	Phytochemicals analysis of DCM extract	35
4.5	Phytochemicals analysis of EA extract	36
4.6	The different intensity of yellow colour from three different types of extract.	38
4.7	IR spectrum for all crudes extract and interpretations	39
4.8	The information from spectral analysis of antioxidant labelled as H1	46
4.9	The information from spectral analysis of antibacterial labelled as H3	48
4.10	The detail of 11-O-p-Coumarylnepeticin from Mass Spectrometry analysis	51
4.11	The detail of Hordatine B from Mass Spectrometry analysis	51

ABSTRACT

ISOLATION OF ANTIBACTERIAL AND ANTIOXIDATIVE COMPOUNDS FROM STEMS OF *Entada spiralis*

The study was done to evaluate on the stem barks of *Entada spiralis* which belongs to Leguminosae family that can be found abundantly in Asia. This genus of species well known comprises variety of medicinal potential for many uses. The first objective of this study is to isolate and determine the preliminary structure of antioxidative compounds from the stems of *E. spiralis* by chromatographic and two spectroscopy methods. The next phytochemical analysis which used 2,2-diphenyl-1-picrylhydrazyl (DPPH) spraying reagent on three extracts, petroleum ether (PE), dichloromethane (DCM) and ethyl acetate (EA) were also done in order to identify the antioxidative compounds existed. The isolated compounds were labelled as H1 and H3 being further analysed using FTIR, ¹H NMR and LCMS to predict all the possible structures. As for the result, H1 have potent in antioxidative property and H3 possess both antioxidative and antimicrobial properties showed in best extract which is EA extract. As for the second objective, the effectiveness of the extracts toward plant disease-caused microbes by inhibiting the growth of *Erwinia sp.* was also measured through agar overlay bioautography. From the basis of spectroscopic analysis, the antioxidative compound, H1 was suggested as 11-O-p-Coumarylnepechin while antimicrobe compound, H3 suggested as Hordatine B. Based on these findings, *E. spiralis* is the best alternative plant that can be utilized globally as its bioactive compounds are expected to give major contribution as a remedies in medicinal field.