# BIOASSAY GUIDED ISOLATION AND CHARACTERIZATION OF COMPOUNDS WITH ANTIOXIDANT ACTIVITIES FROM THE LEAVES OF *Entada spiralis* RIDL. (SINTOK)

BY

SHARIFAH NURUL AKILAH BINTI SYED MOHAMAD

A thesis submitted in fulfillment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Chemistry)

Kulliyyah of Pharmacy
International Islamic University Malaysia

JULY 2017

# ABSTRACT

*Entada spiralis* Ridl. known as Sintok or Akar Beluru is the woody climber plant belong to Leguminoceae family. *E. spiralis* is an excellent foaming agent due to the presence of saponin. Thus, *E. spiralis* stem bark is established used as natural body soap, shampoo and washing agent. The possession of antioxidant activity has been revealed by *E. spiralis* ability to treat syphilis, insect bites and superficial skin diseases. This research aims to screen the major compounds by phytochemical screening test, verify the antioxidant compounds from crude extracts and fractions via *in-vitro* antioxidant activity assays, isolate the antioxidant compounds through chromatographic methods and characterize the structure of antioxidant compounds by using various spectroscopic techniques. The crude extract of *E. spiralis* leaves was prepared by macerating the leaves with petroleum ether, chloroform, and methanol solvent sequentially. Determination and evaluation of antioxidant activity was done through *in-vitro* study by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation. Folin-Ciocalteu's reagent method was used to measure total phenolic content and flavonoid content was evaluated by calorimetric method. The crude extract of *E. spiralis* leaves was fractionate by vacuum liquid chromatography (VLC) and liquid-liquid extraction. Isolation of antioxidant compounds was further with column chromatography technique and characterized by spectroscopic analysis. Methanol, chloroform, and petroleum ether crude extracts were analyzed with 5.53 %, 2.05 % and 2.55 % yield. Saponin and flavonoid were the major components. Methanol extract exhibited the highest antioxidant activity with $IC_{50}$ (DPPH); 40.23 ± 2.66 ug/mL and $IC_{50}$ (ABTS); 5.09 ± 0.53 ug/mL. The highest possession of phenolic content and flavonoid content; 124.67 ± 6.63 mg GAE/g and 51.67 ± 2.17 mg QE/g were also displayed by the methanol extract. Isoscutellarein, kaempferol-3-C-rutinoside, kaempferol-3-O-glucoside, and kaempferol glycoside were isolated from liquid–liquid extraction fraction while stearic acid and ethyl oleate were isolated from the fraction of vacuum liquid chromatography. $IC_{50}$ (DPPH) of isoscutellarein; 112.18 ± 2.21 ug/mL, kaempferol-3-C-rutinoside; 136.04 ± 0.52 ug/mL, kaempferol-3-O-glucoside; 301.01 ± 3.02 ug/mL, and stearic acid; 187.49 ± 1.37 ug/mL. Ethyl oleate and kaempferol glycoside were showed negative results towards DPPH. Whereas, $IC_{50}$ (ABTS) of isoscutellarein; 17.50 ± 0.26 ug/mL, kaempferol-3-C-rutinoside; 34.10 ± 1.13 ug/mL, kaempferol-3-O-glucoside; 14.65 ± 0.11 ug/mL, stearic acid; 5.69 ± 0.06 ug/mL, ethyl oleate; 474.37 ± 2.91 ug/mL, and kaempferol glycoside; 124.82 ± 6.60 ug/mL. In conclusion, the active crude extract and fraction was verified by several *in-vitro* antioxidant assays. Six antioxidant compounds were successfully isolated from this research study.

# خلاصة البحث

يرجع أصل نبتة إنتادا سبيراليس،أو المعروفة باسم سينتوك أو أكار برلورو،إلى فصيلة البقوليات.تعتبر هذه النبتة عامل إرغاء ممتاز نظرا لاحتوائها على الصابونين،ولذلك يستعمل لحاء سيقانها كصابون،وشامبو، وغسول طبيعي للجسم.تم التأكد من حيازة هذه النبتة على نشاط مضاد للأكسدة من قدرتها على علاج مرض الزهري،ولدغات الحشرات،وأمراض الجلد السطحية.تهدف الدراسة لفحص المركبات الرئيسية بواسطة الفحص الكيميائي النباتي،والتحقق من المركبات المضادة للأكسدة في المستخلصات الخامة وأجزاء المستخلصات عن طريق اختبارات النشاط المضاد للأكسدة في الوسط المخبري،وعزل المركبات المضادة للأكسدة من خلال الطرق الكروماتوغرافية،وتحديد أشكال المركبات باستخدام تقنيات طيفية مختلفة.تم استخراج المستخلصات الخامة عن طريق نقع الأوراق في أثير البترول،والكلوروفورم،والميثانول بالتتابع.تحديد وتقييم النشاط المضاد للأكسدة بفحوصات الوسط المخبري باستخدام2،2-ثنائيالفينيل-1-بيكريلهيدرازيل(DPPH)الجذرية،2،2-أزينو بيس(3-إيثيلبنزينثولين-6-حمض السلفونيك)(ABTS)الموجبة الجذرية.استخدام طريقة كاشف فولين-سيو كالتيو لقياس المحتوى الفينولي الكلي وتم تقييم محتوى الفلافونويد بالطريقة السعرية.تمت تجزئة المستخلص الخام عن طريق الكروماتوغرافيا السائلة المفرّغة،وطريقة استخلاص السائل-سائل.تم عزل المركبات المضادة للأكسدة كذلك بتقنية الكروماتوجرافيا العمودية وتمييزها بالتحليل الطيفي.تحليل المستخلصات الخامة للميثانول،وا لكلوروفورم،وأثير البترول بنسب إنتاج 5.53٪و2.05٪و2.55٪.السابونين،والفلافونويد كانتا الأكثر كمية .أظهر مستخلص الميثانول أعلى نشاط مضاد للأكسدة بنسبة $IC_{50}$(DPPH)قدرت بـ40.23±2.66 مكروغرام/مل و$IC_{50}$(ABTS)،و5.09±0.53مكروغرام/مل.أعلى محتوى للفينول والفلافونويد أيضا في مستخلصات الميثانول وبنسبة124.67±6.63ملغ GAE/غم،و51.67±2.17ملغ QE/غم.تم عزل الإيسوسكوتلارين،كامبفيرول-3-C-روتينوسيد،كامبفيرول3-O-غلوكوسيد،وكايمبفيرول جليكوسيد من السائل-سائل للأجزاء،بينما الحامض الدهني،وأوليت الإيثيل من جزء الكروماتوجرافيا السائلة المفرّغة. $IC_{50}$ (DPPH) للإيسوسكوتلارين112.18±2.21مكروغرام/مل،والكامبفيرول-3-C-روتينوسيد136.04 ±0.52مكروغرام/مل،والكامبفيرول-3-O-غلوكوسيد301.01±3.02مكروغرام/مل،والحامض الدهني 187.49±1.37مكروغرام/مل.أظهرت أوليت الإ يثيل والكامبفيرول جليكوسيد نتائج سلبية نحو DPPH $IC_{50}$(ABTS) للإيسوسكوتلارين17.50 ±0.26 مكروغرام/مل ،والكامبفيرول -3-C-رتينوسيد 34.10±1.13مكرغرام/مل،والكامبفيرول-3-O-غلوكوسيد14.65±0.11مكروغرام/مل،والحامض الدهني5.69±0.06مكروغرام/مل،وإيثيل الأوليت474.3± 2.91مكروغرام/مل،والكامبفيرول جليكوسيد 124.8± 6.6مكروغرام/مل.في الختام، التحقق من نشاطات المستخلصات الخامة وأجزائها من قبل عدد من اختبارات مضادات الأكسدة في المختبر.تم عزل ستة مركبات مضادة للأكسدة بنجاح في هذه الدراسة البحثية.

# APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Sciences (Pharmaceutical Chemistry).

……..
Siti Zaiton Mat So'ad
Supervisor

……..
Norazian Mohd Hassan
Co-Supervisor

……..
Aiza Harun
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Sciences (Pharmaceutical Chemistry).

……..
Alfi Khatib
Internal Examiner

……..
Ibtisam Abdul Wahab
External Examiner

This thesis was submitted to the Department of Pharmaceutical Chemistry and is accepted as a fulfillment of the requirement for the degree of Master of Pharmaceutical Sciences (Pharmaceutical Chemistry).

……..
Siti Zaiton Mat So'ad
Head, Department of
Pharmaceutical Chemistry

This thesis was submitted to the Kulliyyah of Pharmacy and is accepted as a fulfillment of the requirement for the degree of Master of Pharmaceutical Sciences (Pharmaceutical Chemistry).

……..
Juliana Md. Jaffri
Dean, Kulliyyah of Pharmacy

# DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Sharifah Nurul Akilah Binti Syed Mohamad

Signature ..... Date .....

**INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA**

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH**

**BIOASSAY GUIDED ISOLATION AND CHARACTERIZATION OF COMPOUNDS WITH ANTIOXIDANT ACTIVITIES FROM THE LEAVES OF *Entada spiralis* RIDL. (SINTOK)**

I declare that the copyright holders of this dissertation are jointly owned by the student and IIUM.





By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Sharifah Nurul Akilah Binti Syed Mohamad

……..…….. ……..

Signature Date

# ACKNOWLEDGEMENTS

Firstly, I wish to express my high gratitude to The Creator for His Blessings and every step that He has planned for me.

Secondly, my utmost pleasure to dedicate this work to my supportive supervisor, Assoc. Prof. Dr. Siti Zaiton Mat So'ad, my co-supervisor, Asst. Prof. Dr. Norazian Mohd Hassan and Dr. Aiza Harun for their continuous support, encouragement and leadership, and for that, I will be forever grateful.

I wish to express my appreciation and thanks to the Ministry of Science and Technology (MOSTI) and Ministry of Higher Education (MOHE) for providing the research grant (e-Science : SF13-005-0055) and MyBrain scholarship to support my research financially.

A special appreciation dedicated to my researchmates; who have always stay by my side through thick and thin; Sr. Farah Dayana, Sr. Hawa Mas Azmar, Sr. Huda Jamilah, Sr. Ain Ayob, Sr. Fadila, Sr. Asween, Sr. Fatima Bukolami, and Bro Faris Osman. I am grateful having all of you during my research journey.

I would also thank to those who provided their time, effort and support for this project especially staff from Pharmaceutical Chemistry Department and Pharmaceutical Technology Department, Kulliyyah of Pharmacy, IIUM, Kuantan Campus, Universiti Teknologi MARA, Puncak Alam Campus staff, and Universiti Malaya staff and colleagues. To the members of my dissertation committee, thank you for your full cooperation, guidance and motivational supports.

Lastly, a special thanks to my dear parents and my family, who granted me the gift of their unwavering belief in my ability to accomplish this goal: thank you so much for your support and patience.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF GRAPHS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| LLE | Liquid-liquid extraction |
| VLC | Vacuum liquid chromatography |
| TLC | Thin layer chromatography |
| Prep-TLC | Preparative thin layer chromatography |
| BDE | Bond dissociation energy |
| SET | Single electron transfer |
| HAT | Hydrogen atom transfer |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| ABTS | 2, 2’- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) |
| FRAP | Ferric reducing antioxidant power |
| ORAC | Oxygen radical absorbance capacity |
| TRAP | Total radical-trapping antioxidant parameter |
| CUPRAC | Cupric reducing antioxidant capacity |
| TPC | Total phenolic content |
| TFC | Total flavonoid content |
| NMR | Nuclear magnetic resonance spectrometer |
| FTIR | Fourier transform infrared spectrometer |
| LC-MS | Liquid chromatography-mass spectroscopy |
| GC-MS | Gas chromatography-mass spectroscopy |
| HPLC | High performance liquid chromatography |
| UV-Vis | Ultraviolet visible |
| HMBC | Heteronuclear multiple bond correlation |
| COSY | H-H correlation spectroscopy |

| | |
|---|---|
| ESI | Electronspray ionization |
| oops | Out of plane stretching |
| SEM | Standard error of mean |
| $CHCl_3$ | Chloroform |
| MeOH | Methanol |
| EA | Ethyl acetate |
| DCM | Dichloromethane |
| Hex | Hexane |
| PE | Petroleum ether |
| $H_2O$ | Water |
| $H_2O_2$ | Hydrogen peroxide |
| $AlCl_3$ | Aluminium trichloride |
| $CDCl_3$ | Deutereted chloroform |
| $CD_3OD$ | Deutereted methanol |
| $^{1}H$ | Proton |
| $^{13}C$ | Carbon |
| Ar | Benzene |
| O-H | Hydroxyl |
| C=O | Carbonyl |
| C-H | Alkyl |
| C=C | Alkene |
| C-O | Ether |
| glu | Glucose |
| rha | Rhamnose |
| ppm | Parts per million |

| | |
|---|---|
| ug/mL | Microgram per liter |
| mg | Milligram |
| mL | Milliliter |
| uL | Microliter |
| nm | Nanometer |
| Hz | Hertz |

# LIST OF SYMBOLS

| | |
|---|---|
| J | Coupling constant |
| $\delta$ | Chemical shift |
| k | Force constant value |
| s | Singlet |
| d | Doublet |
| dd | Doublet of doublet |
| m | Multiplet |
| m/z | Mass to charge ratio |
| *n* | Nonbonding orbitals |
| $\pi$ | Pi bonding |
| $\sigma$ | Sigma bonding |
| $\pi^*$ and $\sigma^*$ | Antibonding orbitals |
| p | Significant value |
| $R^2$ | Coefficient of determination |
| $IC_{50}$ | Inhibition concentration at 50% |
| $R_f$ | Retention factor value |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

Nature's healing power has been explored across centuries and regarded as an ancient concept (Teke et al., 2011). Plants are recognized as being a main source of promising drugs to variety of biological activities such as antioxidants, anticancer, antimicrobials, antidiuretics etc. (Das et al., 2014). Across decades, humans have used different extracts of plant and created their own remedies and formulations in alleviating and treating number of diseases. Generally, the medicinal properties possessed by plants have been contributed from various parts of plants including stem bark, leaves, fruit, and seeds. Most of plants have been declared in possessing their own ethnopharmacological properties which have extensively used by the tribal people across the world since centuries. Therefore, it is believed that nature has provided us the best medicine with their own benefits for treating every human disease (Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011).

Plants with medicinal properties are known to be rich in source of raw drugs, and therapeutic agents in prevention number of diseases and symptoms. The medicinal, nutraceutical, and cosmeticeutical properties exerted by plant are due to the different phytochemicals, bioactive constituents from various parts of the plant. Recently, substances and products originated from plants showing a great interest market caused by their versatile applications (Tiwari et al., 2011). Medicinal plants are managed to synthesize a large number of organic compounds which are categorized into two classes, namely primary and secondary metabolites (Seifu,

Assefa, & Abay, 2012). Mostly, the biological activities that are exhibited by medicinal plants are caused by secondary metabolites which are expressed during plants metabolic pathway (El-Toumy, Mohamed, Hassan, & Mossa, 2011). Plant secondary metabolites can be classified into three main groups according to their biosynthetic origin. They are phenolic and polyphenolic compounds, terpene and alkaloids chemically nitrogen or sulphur bonded (Crozier, Clifford, & Ashihara, 2006).

Primary metabolites are chemical components essential for the growth, development, respiration, and photosynthesis of plant. Examples of primary metabolites are carbohydrate, amino acids, polysaccharides, lipids, and organic acids. Many of them are well distributed among the number of species within plant kingdom. Even though primary metabolites are seen not to be prominent in research field, their role nowadays have captivated researchers' view and mind as being a significant element for plants against herbivores and infections of microbial, act as ultraviolet (UV) protectants as well as provide signal molecules for root nodules to form nitrogen-fixing in legumes. Besides that, primary metabolites also act as seed-dispersing animal's attractants and pollinators and agents for allopathic (Seifu et al., 2012).

Besides primary metabolites, plants also accumulate high concentration of secondary metabolites. Secondary metabolites have been well known due to their potential as sources of new natural drugs, insecticides, antibiotics, and herbicides and their use as flavoring agents, dyes, perfumes, fibers, oils, waxes, and glues (Seifu et al., 2012). Across years, most of researchers have given their full attention on exploring these metabolites and trying so much to isolate as many as possible the