EXTRACTION, CHARACTERISATION AND BIOASSAY ACTIVITIES OF ALLELOCHEMICALS FROM 11 ALLELOPATHIC SPECIES

BY

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2019

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Halal Industry

International Institute for Halal Research and Training
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MAY 2019

ABSTRACT

This study established that allelopathic species from different plant groups of tree, fern, sedge and herbaceous differ greatly with respect to types and concentrations of allelochemical content. A total of 11 allelopathic species were evaluated for quantitative and qualitative phenolic compounds composition in different plant groups, extraction method, solvents and bioassay activities. The allelopathic effect towards weeds germination and growth were also tested by developing an *in vitro* model system. The main phenolic compounds identified in 11 allelopathic species were 4-Hydroxybenzoic acid, Caffeic acid, Vanillic acid, *trans-p*-Coumaric acid, Ferulic acid, 3-Coumaric acid and 2-Coumaric acid. The ratio of these phenolic compounds varies between plant groups. Tree group was found to have the highest total phenolic content followed by a group of ferns, herbaceous and sedges. 4-Hydroxybenzoic acid (3.00 to 1169.53 µg/g DW), Vanillic acid (1.88 to 1594.08 µg/g DW), *trans-p*-Coumaric acid (1.50 to 1171.28 µg/g DW), Ferulic acid (1.68 to 1876.0 µg/g DW), 3-Coumaric acid (15.58 to 933.63 µg/g DW) and 2-Coumaric acid (230.30 µg/g DW) were detected predominantly in tree group whereas Caffeic acid was found predominantly in herbaceous group (1.95 to 3375.23 µg/g DW). Marked differences were observed between the trees, ferns, herbaceous and sedges. Therefore, 11 allelopathic species were further analysed over bioassay activities to assess antimicrobial and weeds inhibitory. Antimicrobial activities of 11 allelopathic species in this study confirmed that the antifungal assay showed lower inhibitory than antibacterial assay. *A. auriculiformis*, *M. cajuputi*, *D. linearis* and *H. malayana* showed great antibacterial activity towards *S. aureus*, *S. epidermis*, *E. coli*, MRSA and *P. aeruginosa* whereas for antifungal only *C. albicans* and *Fusarium sp* were inhibited. A high-throughput *in vitro* model system for investigating allelopathic effect in weeds was developed and validated by assessing the allelopathic effects at a different concentration, a period of time and type of weed. The most influential factor appeared to be allelochemical extract concentration. The higher the concentration, the greater the inhibitory effect regardless type of weed.

ملخص البحث

أثبتت هذه الدراسة أن الأنواع الأليلوباثية من مجموعات نباتية مختلفة، من أشجار، وسراخس، وسعديات، وعشبيات، تختلف اختلافًا كبيرًا تبعا لأنواع وتراكيز الأليلوكيميائيات. تم تقييم 11 نوعا من الأليلوباثيات لفحص المحتوى الكمي والنوعي للمركبات الفينولية بناء على مجموعات نباتية مختلفة، وطرق الاستخلاص، ونوع المذيب، وأنشطة الفحص الحيوي. تم أيضا فحص التأثير الأليلوباثي تجاه إنبات ونمو الأعشاب من خلال تطوير نظام نموذجي خارج الجسم الحي. تألفت المركبات الفينولية الرئيسية المحددة في أنواع الأليلوباثيات الـ11 كلا من: 4-حمض الهيدروكسي بنزوويك، وحمض الكافيين، وحمض الفانيليك، ترانس-p-حمض الكوماريك، وحمض الفيروليك، و3-حمض الكوماريك، و2-حمض الكوماريك، حيث تختلف نسب هذه المركبات الفينولية بين المجموعات النباتية. أظهرت النتائج أن مجموعة الأشجار احتوت على أعلى نسبة للمحتوى الفينولي الكلي، تلتها مجموعة السراخس، والعشبيات، ومن ثم السعديات. تم الكشف عن كل من: 4-حمض الهيدروكسي بنزوويك (3.00 إلى 1169.53 مكروجرام/جرام من الوزن الجاف)، وحمض الفانيليك (1.88 إلى 1594.08 مكروجرام/جرام من الوزن الجاف)، وترانس-p-حمض الكوماريك (1.50 إلى 1171.58 مكروجرام/جرام من الوزن الجاف)، وحمض الفيروليك (1.68 إلى 1876.0 مكروجرام/جرام من الوزن الجاف)، و3-حمض الكوماريك (15.58 إلى 933.63 مكروجرام/جرام من الوزن الجاف، و2-حمض الكوماريك (230.30 مكروجرام/جرام من الوزن الجاف) بنسب عالية في مجموعة الأشجار، وكان حمض الكافيين الأعلى في مجموعة العشبيات (1.95 إلى 3375.23 مكروجرام/جرام من الوزن الجاف). لوحظت فروق كبيرة بين الأشجار، والسراخس، والسعديات، والعشبيات، ولذلك تم اختبار الأليلوباثيات بفحص الأنشطة الحيوية لتقييم نشاطها المضاد للميكروبات وفي تثبيط الأعشاب الضارة. أكدت الأنشطة المضادة للميكروبات في الأليلوباثيات في هذه الدراسة أن فحص النشاط المضاد للفطريات أظهر تثبيطا أقل مقارنة بالنشاط المضاد للميكروبات. أظهر كل من أكاكيا أوريكوليفورميس، وميلالالوكا كاجوبوتي، ودروسيرا لينياريس نشاطًا قويا ضد المكورات العنقودية الذهبية، والعنقودية البشروية، والإشريكيية القولونية، والمكورات العنقودية الذهبية المقاومة للميثيسيلين، والزائفات الزنجارية، أما بالنسبة لتثبيط مضادات الفطريات فقد ثبطت فقط كلا من المبيضات البيضاء والمغزلاويات. تم تطوير نظام نموذجي خارج الجسم الحي عالي الإنتاجية للتحقيق في تأثير الأليلوباثيات على الأعشاب الضارة ومن ثم التحقق من صلاحية هذا النظام من خلال تقييم آثار الأليلوباثيات بتراكيز، وفترات، وأنواع مختلفة من الأعشاب الضارة. وبدى أن العامل الأكثر تأثيرا هو تركيز المستخلص الأليلوكيميائي، حيث كلما زاد التركيز زاد التأثير المثبط، بغض النظر عن نوع العشب الضار.

APPROVAL PAGE

The thesis of Razanah Binti Ramya @ Abd Rahim has been approved by the following:

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DECLARATION

I hereby declare that this thesis 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.

Razanah Binti Ramya @ Abd Rahim

Signature …………………………..………. Date …………………………….

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This dissertation is dedicated to my beloved parents

ACKNOWLEDGEMENTS

In the name of Allah, the Most Beneficent, the Most Merciful

Alhamdulillah thumma Alhamdulillah.

First of all, I praise Allah, the Almighty for providing me this opportunity and granting me the capability to accomplish this study successfully. May the Peace and Blessings of Allah be upon His Prophet Muhammad (peace be upon him).

Special appreciation goes to my supervisor, Assoc. Prof. Dr. Rashidi Othman for his supervision and support throughout the process of completing this research. My journey in developing this research study began from my first degree. Assoc. Prof. Dr. Rashidi has supported and guided me every step of the way. He continues to inspire me to gain more knowledge for the benefit of the *ummah*. His encouragement, thoughtful guidance and assistance over the past three years exceed all possible verbal appreciation.

The phrase '*man jadda wajada, wa man zara'a hasada, wa man yajtahid yanjah'* has been a guiding principle in my search for knowledge. The vital lesson that he taught was the importance of one's parent's blessing and *niyyah* (intention) in seeking knowledge. Secondly, I would like to express my deep gratitude to my co-supervisor, Asst. Prof. Dr. Norazian Mohd Hassan for offering valuable advice and comments throughout the duration of this study.

My deepest gratitude goes to my beloved parents, *Abah,* Mr. Ramya @ Abd Rahim Ariffin and *Mak,* Mrs. Rohani Rashid for their unwavering support and encouragement during the pre and post-research phases of the study. I would also like to express my warmest appreciation to my husband, Muhammad Redzuan Zainol Abidin for the support and motivation along this journey, specifically during hard times. To my family in law and siblings (Mohammad Azhar, Mohammad Azri, Rafidah, Rauhah and Siti Raudhah), I thank you for your endless love, *dua'* and encouragement.

I am also thankful to my colleagues in the INHART Laboratory and Herbarium Laboratory research team for their useful discussions, critical comments and assistance. Special thanks to Dr. Shirwan, Sr. Farah Ayuni, Sr. Alifah, Sr. Eyna, Br. Akram, Sr. Ruhul, Sr, Hanie, Sr. Azlen, Sr. Masyitah, Sr. Syibrah, Sr. Hidayah, Sr. Aina, Sr. Widad and Sr. Aliya who assisted through advice. I wish them every success in their Ph.D and Master degrees. I am grateful to the assistance rendered me by my beloved lab officer, Sr. Norhafizana Mat Jusoh. Last but not least, I am thankful for the support of my close friends, Sr. Rosfazaton Mat Sarif and Sr. Akifah Alamin who were a source of constant support, advice, and direction. Only Allah will reward all of you for your invaluable assistance. It is my sincere hope that this study will be of benefit to all who read and pursue its many implications.

This thesis is humbly dedicated to the Ministry of Higher Education Malaysia who granted me the scholarship necessary to pursue a Doctor of Philosophy in Halal Industry and International Institute for Halal Research and Training, International Islamic University Malaysia.

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LIST OF ABBREVIATIONS

%	Percentage	L	Litre
<	Less than	LLE	Liquid-liquid extraction
>	More than	M	Molar
°C	Degree celcius	m^{-2} s^{-1}	Metre squared per second
µg	Microgram	MHA	Muller-Hinton agar
µL	Microliter	MHB	Muller-Hinton broth
µmol	Micromole	MICC	Minimum inhibitory concentration
4CL	Coumarate: coenzyme A ligase	mL	Millilitre
ABA	Abscisic acid	mm	Millimetre
ANOVA	Analysis of variance	MOA	Modes of action
ATCC	American type culture collection	MS	Mass spectrometry
B	Butanol	MS	Murashige and Skoog
C	Final concentration of solution	MW	Molecular weights
C4H	Cinnamic Acid 4-hydroxylase	ND	Not detected
cm^{-1}	Reciprocal wavelength	NIST	National Institute Standard and Technology
DAD	Diode array detection	OPPP	Oxidative pentose phosphate pathway
DAHP	3-deoxy-D-arabino-heptulosonic acid 7-phosphate	P	Potency of antibiotic base
DW	Dry weight	PAL	Phenylalanine ammonia lyase
E4P	Etrythose-4-phospate	PDA	Potato Dextrose Agar
EA	Ethyl acetate	PDAD	Photodiode array detection
EtOH	Ethanol	PE	Petroleum ether
FT-IR	Fourier transform infrared	PEP	Phosphoenolpyruvate
g	Gram	pH	A figure to expressing the acidity or alkalinity of a solution on logarithm scale which 7 is neutral, lower values are more acid and higher values more alkaline
GAE	Gallic acid equivalent	Phe	L-phenylalanine
GCTOF-MS	Gas chromatography-time of flight mass spectrometry	ROS	Reactive oxygen species
GM	Genetic modified	RP	Reversed phase
H	Hour	RT	Retention time

HEX	Hexane	SA	Salicylic acid
HPLC	High performance liquid chromatography	SEM	Standard error of the mean
IAA	Indole-3-acetic	TPC	Total phenolic compound
USEPA	United States Environmental Protection Agency	Trp	L-tryptophan
W	Weight of antibiotic to be dissolved in V	Tyr	L-tyrosine
		V	Volume in ml required

CHAPTER ONE

INTRODUCTION

1.1 ALLELOCHEMICALS AS NATURAL HERBICIDES: PRESENT PERSPECTIVES AND PRACTICES

In the global scenario, a principal approach to controlling weeds relies on herbicides (Hong et al., 2004) where the cost of using these products was in excess of US$ 4.6 billion and about US$ 3 million tonnes of herbicides per year are used to control weeds (Bhadoria, 2011). More than 350 confirmed instances of weed resistance have been reported in 197 weed species globally and more than one-third of these are found in the United States (David et al., 2012).

Meanwhile in Malaysia, for rice alone, an estimated US$ 4.10 million is spent annually on herbicides for broadleaf weeds (Rezaul, 2004). The 3rd National Agricultural policy (1998-2010) aimed to maximise the utilisation of agriculture fields and production while at the same time supporting sustainable agriculture in Malaysia (Faridah, 2001). Furthermore, during 1991-1999 the use of herbicides accounted for approximately RM 220–230 million/year in Malaysia (Bakar, 2004). Despite such expenditures, the lack of weed control is among the most pressing concerns expressed by farmers (Stokstad, 2013).

Many reports support the use of herbicides as alternative weed management, yet the continued use of herbicides in heavy doses of chemicals creates environmental pollution and increases the number of herbicide-resistant weeds (Bhadoria, 2011; Setia et al., 2007). Approximately 99% of applied herbicides released into the air, water and soil and only 1% reaches its target (Botelho & Cury, 2009). Such changes become crucial to preserving natural resources and product quality. Since triazine herbicides and photosynthesis inhibitors are widely implemented, inappropriate application of herbicides has contributed to the accumulation of active compounds in the soil as well as weed species (Soltys et al., 2013).

On top of that, from 1996 until 2014, genetic modified (GM) crops rapidly expanded. The United State (US), Brazil and Argentina populate about 77% of total GM crops of which 95% contain two traits (herbicide tolerance and insect resistance) (Bonny, 2015) and currently applied to 41 million hectares as herbicide-resistant crop (Bonny, 2008; Owen & Zelaya, 2005). Among the risks of GM are contamination of grain, herbicide-resistant biotypes and other implications on the ecology which are interrupting arable (crop) land biodiversity, increasing weed populations and contributing to the evolution of herbicide-resistant biotypes that interfere with land ecology and human health (Conner et al., 2003; Watkinson et al., 2000).

Based on the current statistics of natural product-based practices, almost 70% of registered active pesticide ingredients have their origins in natural products research, and only 8% of conventional herbicides are derived from natural compounds. Moreover, only 7% of the biochemicals approved by the United States Environmnetal Protection Agency (USEPA) are bioherbicides (Cantrell et al., 2012). These statistics underscore the critical situation and the need for research that produces herbicides from natural compounds. In order to solve this problem, several factors affect the performance of herbicides as mentioned in Figure 1.1.

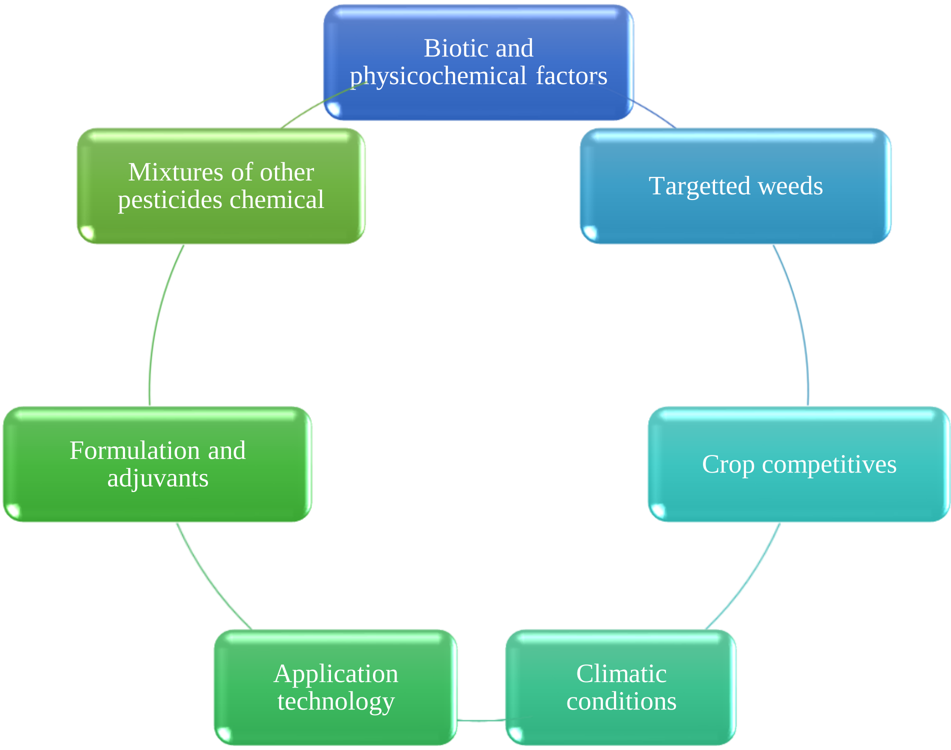

Figure 1.1 Factors affecting herbicides performance (Kudsk, 2008)

Due to these highlighted issues, exploiting the allelopathy of plant-plant interaction as a promising alternative to control weed evokes a new prospect (Duke et al., 1986; Kato-Noguchi et al., 2013). The “eco-friendly” slogan forces scientists to explore innovative solutions and tools for weed management. Discovery of natural compounds for new environmentally safe herbicides known as bioherbicides produced by living organisms, where 24 million organic compounds are found in a large group of secondary plant metabolites (Soltys et al., 2013). In recent decades, there has been an increase in the allelopathic or weed suppressive approach as a green alternative that involves plant-plant interaction to produce secondary metabolites called allelochemicals (Bhowmik, 2003; Bich & Kato-Noguchi, 2012; Dayan et al., 1999; Durán-Serantes et al., 2002; Voltarelli et al., 2012).