



**UNIVERSITI PUTRA MALAYSIA**

**LIPASE-CATALYSED SYNTHESIS AND OPTIMIZATION OF  
BIOLOGICALLY ACTIVE AMIDES OF CINNAMIC ACID DERIVATIVES**

**MOHAMMAD HESHAM ABDELRAHMAN ABU ALRUB**

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**By**

**MOHAMMAD HESHAM ABDELRAHMAN ABU ALRUB**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**July 2013**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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July 2013

**Chair: Professor Mahiran Basri, PhD**

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Natural products compounds are very important source of materials for the pharmaceutical industry. Amides are in general present in natural products. Amides play an essential role in virtually all biological processes such as enzymatic catalysis and medical chemistry. Natural products which contain amide group play an important role in modern drug discovery, especially in cancer treatment. The extracts from Litsea plants have been reported to have significant cytotoxic activity against human tumor cells. The compounds which are the major components of Litsea plants are so valuable and important to be synthesized and studied. Litsea plant consists of many cinnamic acid amides. There are no suggested methods for the synthesis of *N-trans*-sinapoylmethoxytyramine and *N-trans*-sinapoyldimethoxytyramine. The compounds *N-trans*-feruloylmethoxytyramine, *N-trans*-sinapoyltyramine and *N-trans*-feruloyldimethoxytyramine have only one method for synthesis with a low yield. *N-trans*-feruloyltyramine needs hydrazine as a reagent which is very

dangerous. The extraction method gives very low yield and the process is not economic. Thus, it is important to find a new direct method to synthesize these compounds. Six cinnamic acid amides derivatives were successfully synthesized from the reaction of cinnamic acids with tyramine derivatives in a one-step lipase-catalyzed reaction. The use of immobilized lipase, Lipozyme TL IM as the catalyst in the reaction provides an easy isolation of the enzyme from the products and other components in the reaction mixture.

All the amides were characterized using Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance ( $^1\text{H}$  NMR) and carbon-13 ( $^{13}\text{C}$  NMR) techniques. The optimized percentage yield obtained was 93.5 % when the process was carried out for 48 h, at molar ratio of tyramine HCl: cinnamic acid - 1:6 at 40 °C.

Enzymatic synthesis of *N-trans*-feruloyltyramine was optimized by Response Surface Methodology (RSM) using 4-hydroxy-3-methoxycinnamic acid and tyramine hydrochloride in a one-step lipase catalyzed reaction using Lipozyme TL IM. RSM based on five-level, four-variable central composite rotatable design (CCRD) was used to evaluate the interactive synthesis with variables consisting of reaction time (24-96 h), temperature (30-50 °C), amount of enzyme (2.5-25 mg/mL) and substrate molar ratio [cinnamic acid:tyramine HCl, (1:1 - 8:1 mmol)] on the percentage yield of *N-trans*-feruloyltyramine. The optimum conditions derived via RSM were; reaction time, 52 h, temperature, 43 °C, amount of enzyme, 13 mg/mL and substrate molar ratio (cinnamic acid:tyramine HCl) 6.2:1. The actual

experimental yield was 96.3 % under the optimum condition, which compared well to the maximum predicted value of 97.2 %.

The anticancer activities for all compounds were evaluated against human colorectal (HT-29), human estrogen-receptor positive breast cancer (MCF-7), human estrogen-receptor negative breast cancer (MDA-MB-231) and human hepatocellular carcinoma (HepG2) cell lines. It can be concluded that all the amides normalized the rate of cell growth.

The antibacterial properties of the synthesized amide compounds were evaluated on gram negative bacteria, gram positive bacteria and yeast. It was found that all synthesized amides inhibited the growth of the tested bacteria and yeasts with good zone diameter. *N-trans*-Sinapoyldimethoxytyramine inhibited the growth of the tested gram negative with 24 mm zone. *N-trans*-Sinapoyltyramine inhibited the growth of the tested gram positive MRSA and yeast CA with 30 mm and 40 mm zone, respectively against the corresponding microorganisms.

Antioxidant activities were studied for all the compounds using DPPH and ATBS methods. In both methods, all compounds showed potential activity. *N-trans*-sinapoylmethoxytyramine showed the highest effective activity for all the compounds. DPPH Inhibition Ratio (%) was at 69.2 % and ABTS Inhibition Ratio (%) at 79.6 %. In addition, *N-trans*-feruloyldimethoxytyramine showed relatively lower activity with DPPH Inhibition Ratio (%) at 59.4 % and ABTS Inhibition Ratio (%) at 72.0 %. However, these results are considered as good antioxidant activity.

Kinetic study using Lipozyme TL IM in the amidation of cinnamic acid with tyramine HCl was carried out. The effect of both substrates on the initial reaction rate was studied. The initial rates of the reaction were calculated and the results showed that the amidation reaction obeyed the Ping-Pong Bi-Bi mechanism. Lineweaver-Burk plots of amidation reaction were determined. The kinetic constants of reaction were studied whereby  $V_{\max}$  (mmol/L/min) was 0.0328 and  $K_m$  (CA) (mmol/L) was 0.8955.



Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk mendapatkan ijazah Doktor Falsafah

**SINTESIS BERMANGKIN-LIPASE DAN PENGOPTIMUMAN TERBITAN  
AMIDA ASID SINAMIK AKTIF DARI SEGI BIOLOGI**

Oleh

**MOHAMMAD HESHAM ABDELRAHMAN ABU ALRUB**

Julai 2013

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Produk hasilan semulajadi adalah sumber yang sangat penting untuk industri farmaseutikal. Amida secara umumnya terdapat di dalam produk hasilan semula jadi. Amida memainkan peranan penting dalam hampir semua proses biologikal seperti pemangkinan enzim dan kimia perubatan. Produk hasilan semulajadi yang mengandungi amida memainkan peranan penting dalam penemuan ubatan moden, terutamanya dalam rawatan kanser. Tumbuhan *Litsea* telah dilaporkan mempunyai aktiviti sitotoksik yang penting bagi menentang sel-sel tumor manusia. Tumbuhan *Litsea* telah dipertimbangkan sebagai sumber utama untuk produk hasilan semulajadi yang penting bagi menentang sel-sel tumor manusia. Tumpuan kajian ini ialah komponen utama tumbuhan *Litsea* yang dianggap sebatian yang sangat berharga dan penting untuk disintesis dan dikaji. Tumbuhan *Litsea* terdiri daripada banyak amida asid sinamik. Tiada kaedah yang dicadangkan untuk sintesis *N-trans*-sinapoilmetoksitiramin dan *N-trans*-sinapoildimetoksitiramin. Bagi sebatian *N-trans*-



feruloilmetoksitiramin, *N-trans*-sinopoitiramin dan *N-trans*-feruloildimetoksitiramin hanya mempunyai satu kaedah sintesis dengan hasil yang rendah. *N-trans*-feruloiltiramine memerlukan hidrazin iaitu sejenis reagen yang amat berbahaya. Kaedah pengestrakan memberikan hasil yang sangat rendah dan proses itu tidak berekonomi. Oleh itu, adalah penting untuk mencari kaedah langsung baru yang untuk mensintesis sebatian-sebatian ini.

Enam asid sinamik amida terbitan telah berjaya dihasilkan daripada tindak balas asid sinamik dengan tiramin terbitan dalam satu langkah tindak balas pemangkinan lipase. Penggunaan lipase yang telah dipegunkan, Lipozyme TL IM sebagai pemangkin dalam tindak balas menyediakan pengasingan mudah enzim daripada produk dan komponen lain dalam campuran tindak balas.

Semua amida telah dicirikan menggunakan spektroskopi infra merah transformasi Fourier (FTIR). Proton resonan magnetik nuklear ( $^1\text{H-NMR}$ ) dan teknik karbon -13 ( $^{13}\text{C-NMR}$ ). Peratusan optimum yang diperolehi ialah 93.5 % apabila proses telah dijalankan selama 48 jam, pada nisbah molar tiramin HCl:asid sinamik – 1:6 dan suhu 60 °C.

Tindakan enzim terhadap *N-trans*-feruloiltiramin telah di optimumkan oleh Kaedah Respon Permukaan (RSM) menggunakan asid 4-hidroksi-3-metoksisinamik dan tiramin hidroklorik di dalam satu langkah tindak balas pemangkinan lipase menggunakan Lipozyme TL IM.

RSM berdasarkan lima-peringkat, empat-faktor rekaan pusat komposit berputar (CCRD) telah digunakan untuk mengkaji interaktif sintesis dengan pemalar seperti masa tindak balas (24 – 96) h, suhu (30 – 50) °C, jumlah enzim (2.5-25 mg/mL) dan nisbah molar substrat (asid sinamik : tiramin HCl = 1:1- 8:1) mmol, terhadap peratusan hasil N-trans-feruloiltiramine. Keadaan optimum dihasilkan melalui RSM adalah; masa tindakbalas 52 h, suhu 43 °C, jumlah enzim 13.0 mg/mL dan nisbah molar substrat (asid sinamik:tiramin HCl= 6.2:1). Peratusan sebenar hasil kajian ialah 96.3 % di bawah keadaan optimum, telah dibandingkan sesuai dengan nilai anggaran maksimum iaitu 97.2 %.

Aktiviti anti kanser untuk semua sebatian telah ditentukan terhadap kolorekral (HT-39), estrogen manusia-reseptor positif kanser payudara (MCF-7), estrogen manusia-reseptor negatif kanser payudara (MDA-MB-231) dan sel hepatoselular karsinoma manusia (HepG2). Kesimpulan dapat dibuat bahawa kesemua amida menormalkan kadar pertumbuhan sel.

Ciri-ciri antibakteria sebatian amida yang disintesis dinilai berdasarkan bakteria gram negatif, bakteria gram positif dan yis. Didapati bahawa semua amida yang disintesis menghalang pertumbuhan bakteria yang diuji dan yis dengan garis pusat zon yang baik. *N-trans*-Sinapoildimetoksitiramin menghalang pertumbuhan gram negatif yang diuji dengan zon 24 mm. *N-trans*-sinopoltiramin menghalang pertumbuhan MRSA gram positif dan yis CA diuji dengan zon 30 mm dan 40 mm, masing-masing, terhadap mikroorganisma yang diuji.

Aktiviti antioksidasi telah dikaji untuk semua sebatian menggunakan kaedah DPPH dan ABTS. Dalam kedua-dua kaedah tersebut, semua sebatian menunjukkan potensi aktiviti. *N-trans*-sinapoilmetoksitiramin menunjukkan aktiviti berkesan tertinggi untuk semua kompaun. Nisbah Perencatan DPPH (%) berada pada 69.2 % dan Nisbah Perencatan ABTS (%) pada 79.6 %. Di samping itu, *N-trans*-feruloildimetoksitiramin menunjukkan aktiviti yang lebih rendah dengan Nisbah Perencatan DPPH (%) pada 59.4 % dan Nisbah Perencatan ABTS (%) pada 72.0 %. Walau bagaimanapun, keputusan ini dianggap sebagai aktiviti antioksidasi yang baik.

Kajian kinetik menggunakan Lipozyme TL IM dalam amidasi asid sinamik dengan tiramin HCl telah dijalankan. Kesan kedua-dua substrat ke atas kadar tindak balas awal telah dikaji. Kadar awal tindak balas telah dikira dan keputusan menunjukkan bahawa tindak balas amidasi itu menuruti mekanisma Ping-Pong Bi-Bi. Plot Lineweaver-Burk bagi tindakbalas amidasi telah ditentukan dimana  $V_{max}$  (mmol/L/min) adalah 0.0328 dan  $K_m$  (CA) (mmol/L) adalah 0.8955.

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I certify that a Thesis Examination Committee has met on the 8 July 2013 to conduct the final examination of Mohammad Hesham Abdelrahman Abu Alrub on his thesis entitled "Lipase-Catalysed Synthesis and Optimization of Biologically Active Amides of Cinnamic Acid Derivatives" in accordance the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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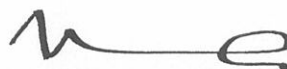
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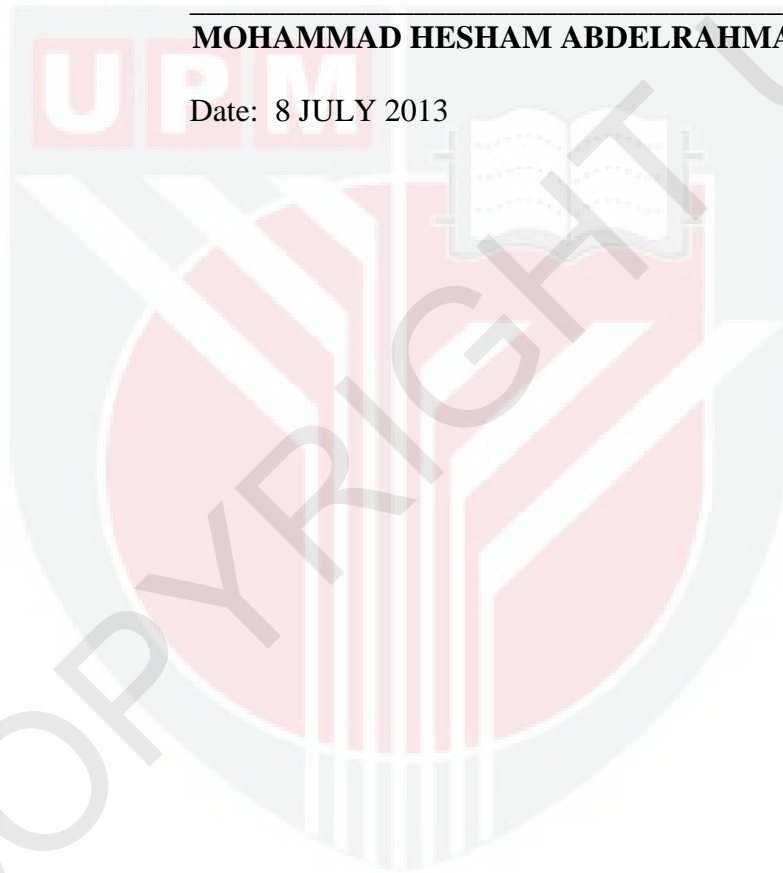
## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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**MOHAMMAD HESHAM ABDELRAHMAN ABUALRUB**

Date: 8 JULY 2013





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