

**OPTIMIZATION OF LIPASE CATALYZED SYNTHESIS OF 3-O-  
BENZOYLBETULINIC ACID**

**By**

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in  
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Betulinic acid derivatives were successfully synthesized from reactions of betulinic acid with benzoyl chloride and acetic anhydride using enzymes as biocatalyst in an organic solvent system. Preliminary detection of the reaction products were conducted using thin layer chromatography (TLC) and subsequent quantitative studies were conducted using gas chromatography. Characterizations of the products were further conducted using (NMR), (MS) and (FTIR).

Initial screening of biological activities, revealed that the ester of the reaction of betulinic acid with benzoyl chloride showed a good cytotoxicity activity while no activity was detected for ester of reaction of betulinic acid and acetic anhydride. Thus, the reaction of betulinic acid and benzoyl chloride was chosen for further studies. Enzyme screening revealed that Novozyme was the most efficient biocatalyst for the reactions which produced a good yield of 3-O-benzoylbetulinic acid at 45.3%. The effects of various reaction parameters such as time of reaction, temperature, solvents

used, amount of enzyme, mole ratio of substrates and initial water activity ( $a_w$ ) was studied to determine optimal condition of 3-O-benzoylbetulinic acid synthesis. The optimal conditions 3-O-benzoylbetulinic acid synthesis was obtained at incubation time of 12 h; temperature 50°C; mole ratio of betulinic acid/benzoyl chloride, 4.0; amount of lipase, 150 mg; organic solvent, chloroform and initial water activity ( $a_w$ ), 0.12.

Response surface methodology (RSM) was then used to study the interactive effects of the reaction parameters. The best model for the reaction of 3-O-benzoylbetulinic acid was quadratic model. Generally, increasing both reaction temperature and amount of enzyme increased the percentage yields of products. However, a negative effect was detected at higher temperature due to denaturation of enzymes. Increasing reaction time and amount of enzyme improved the reactions, as did increasing reaction temperature and amount of enzyme. High amount of enzyme showed a slightly negative effect. In general, increasing the temperature and amount of enzyme resulted in negative effects of the response. Optimal conditions derived from the RSM differed slightly from those of the conventional “one-variable-at-a-time” approach due to the latter’s inability to consider interactions among the parameters. The percentage yield for the optimize reaction conditions derived from RSM was 48.5%.

Betulinic acid and its acylated product were subjected to cytotoxicity, anti-viral, anti-microbe and anti-fungal assays in order to study the biological activities of this compound. The cytotoxicity evaluation of these compounds against cancer cell showed that 3-O-benzoylbetulinic acid from reaction of betulinic acid and benzoyl

chloride (BCL) was the most active compound compared to betulinic acid (BA) and 3-acetylbetulinic acid (BAA). BCL showed  $IC_{50} < 5 \mu\text{g/ml}$  which indicated significant cytotoxicity activity against all cancer cells. However, no activities were detected against dengue viral and some microbes and fungi that were tested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**SINTESIS ASID BETULINIK ESTER MENGGUNAKAN ENZIM  
SEBAGAI MANGKIN**

Oleh

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3-O-benzoil asid betulitik berjaya dihasilkan melalui tindakbalas antara asid betulitik dengan benzoil klorida dan asetik anhidrida menggunakan enzim sebagai mangkin dalam sistem pelarut organik. Pengesanan awal produk dijalankan dengan cara kromatografi lapisan nipis (TLC). Seterusnya, gas kromatografi digunakan dalam analisis kuantitatif. Pengkelasan produk seterusnya dijalankan dengan menggunakan NMR, MS dan FTIR.

Kajian awal keaktifan biologi sampel ke atas sel kanser menunjukkan produk yang dihasilkan melalui tindakbalas antara asid betulitik dengan benzoil klorida adalah aktif sementara produk yang dihasilkan melalui tindakbalas diantara asid betulitik dan asetik anhidrida adalah tidak aktif. Seterusnya, tindakbalas diantara asid betulitik dan benzoil klorida dipilih untuk kajian seterusnya. Antara beberapa enzim yang dikaji, lipase tersekat-gerak Novozyme didapati memberikan hasil yang terbaik dengan

penghasilan produk sebanyak 45.3%. Kesan pelbagai parameter tindakbalas seperti masa, suhu, pelarut organik, kuantiti lipase, pecahan mol reaktan dan aktiviti air awal ( $a_w$ ) telah dikaji untuk menentukan keadaan tindakbalas maksimum bagi sintesis tersebut. Keadaan optimum telah dicapai pada masa 12 j; suhu 50°C; pelarut organik, kloroform; kuantiti lipase 150 mg; pecahan mol reaktan, 4.0 dan aktiviti air awal ( $a_w$ ), 0.12.

Seterusnya, kaedah permukaan respon (RSM) digunakan untuk mengkaji interaksi antara kesan-kesan parameter atas tindakbalas tersebut. Model yang terbaik untuk tindakbalas asid betulitik adalah model kuadratik. Secara keseluruhannya, peningkatan suhu dan jumlah enzim akan meningkatkan peratusan hasil. Walau bagaimana pun kesan yang negatif akan dapat dilihat pada penggunaan suhu yang agak tinggi yang mungkin disebabkan oleh ketidakaktifan enzim pada suhu yang tinggi. Peningkatan masa tindakbalas dan jumlah enzim juga meningkatkan peratusan hasil. Pada jumlah enzim yang terlalu tinggi kesan negatif kepada peratusan hasil telah dilihat. Pada amnya, peningkatan suhu dan jumlah enzim pada tahap yang terlalu tinggi akan menghasilkan kesan negatif kepada respon. Keadaan optimum yang dirumuskan melalui kaedah RSM didapati berlainan daripada keadaan optima yang dihuraikan melalui kaedah lama “mengganti-parameter-satu-demi-satu”. Perbezaan ini berpunca dari kegagalan kaedah lama untuk mempertimbangkan interaksi-interaksi antara parameter-parameter ujikaji. Peratusan hasil yang diperolehi pada keadaan optimum menggunakan kaedah RSM adalah 48.5%.

Keaktifan biologi asid betulnik dan esternya diuji dengan kajian kesitotoksikan, anti-virus, anti-mikrob dan anti-fungus. Kesitotoksikan sampel ini keatas sel kanser menunjukkan produk dari tindakbalas diantara asid betulnik dan benzoil klorida (BCL) adalah paling aktif jika dibandingkan dengan asid betulnik (BA) dan asid betulnik asetat (BAA). BCL menunjukkan  $IC_{50} < 5 \mu\text{g/ml}$  yang menerangkan kesesuaian untuk aktiviti kesitotoksikan ke atas sel kanser. Walau bagaimana pun, tiada keaktifan ke atas virus denggi dan sebahagian mikrob dan fungus yang diuji menggunakan sampel ini.

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I certify that an Examination Committee met on 6<sup>th</sup> September 2004 to conduct the final examination of Yamin Yasin on his Doctor of Philosophy thesis entitled “Optimization of Lipase Catalyzed Synthesis of 3-O-benzoylbetulinic Acid” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

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

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**YAMIN YASIN**

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