

**PARTIAL PURIFICATION AND CHARACTERIZATION OF A
MONOTERPENE SYNTHASE EXTRACTED FROM YOUNG LEAVES
OF *MICHELLIA ALBA***

By

LEE YUAN CHERN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Chairman : Associate Professor Radzali Muse, PhD

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This study was conducted to partially purify linalool synthase from the young leaves of *Michellia alba* (Cempaka Putih) from the Magnoliaceae family. The technique used to determine the amount of linalool produced from enzyme activity was the combination of solid-phase microextraction (SPME) and gas chromatography with flame-ionization detector (GC/FID) technique. The substrate used for this enzyme activity assay was geranylpyrophosphate (GPP). Optimal conditions such as temperature and incubation time for SPME technique were also determined. The linalool synthase exhibited a strict requirement for a divalent metal cofactor with a preference for Mg^{2+} , Mn^{2+} and K^+ ions. The optimal pH and temperature of the enzyme was 6.0 and 30°C respectively. The enzyme was inhibited by 1,2-Di(2-aminoethoxy)ethane-N,N,N',N'-tetra-acetic acids (EGTA). Three steps of partial purification of enzyme were carried out, including the crude extraction of young

leaves, ultra centrifugation and Mono-Q anion exchange chromatography. The partially purified linalool synthase was characterized and studied for its enzyme kinetic properties. The linalool synthase has a K_m of $83\mu\text{M}$ for substrate GPP. The SPME-GCFID technique was later proved to be reliable and sensitive in determination of monoterpene products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENULENAN SEPARA DAN PENGKAJIAN SIFAT SATU
MONOTERPENE SINTASE DARIPADA DAUN MUDA *MICHELLIA
ALBA***

Oleh

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Kerja penyelidikan ini telah dijalankan bagi penulenan separa satu enzim, iaitu Linalool Sintase daripada daun muda *Michellia alba* (cempaka putih) daripada famili Magnoliaceae. Satu teknik hasil gabungan antara teknik ‘Solid-phase microextraction’ (SPME) dan ‘gas chromatography with flame ionization detector’ (GCFID) telah digunakan untuk mengkaji jumlah linalool yang dihasilkan daripada aktiviti enzim tertentu. Substrat yang telah digunakan untuk aktiviti enzim ini adalah geranil pirofosfat (GPP). Keadaan optimum suhu dan masa pengeraman untuk kaedah SPME juga telah dikaji. Linalool sintase memerlukan ion Mg^{2+} , Mn^{2+} dan K^{+} sebagai kofaktor kation dwivalent. pH dan suhu optimum bagi aktiviti enzim adalah 6.0 dan 30°C masing-masing. Enzim ini juga didapati direncat oleh 1,2-Di(2-aminoethoxy)ethane-N,N,N’N’-tetra-acetic acids (EGTA). Tiga langkah penulenan telah dijalankan, iaitu ekstrak kasar, pengemparan-ultra dan kromatografi pertukaran anion ‘Mono-Q’ anion. Sifat-sifat dan kajian kinetik enzim yang separa tulen itu juga

telah dikaji. Enzim ini mempunyai nilai K_m 83 μ M untuk substrat GPP. Teknik SPME-GCFID kemudiannya telah dibukti sensitif dan berguna di dalam menentukan produk monoterpene yang dihasilkan.

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I certify that an Examination Committee has met on 6th February 2006 to conduct the final examination of Lee Yuan Chern on his Master of Science thesis entitled " Partial Purification and Characterization of a Monoterpene Synthase Extracted from Young Leaves of *Michellia alba* " 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 UPM or other institutions.

LEE YUAN CHERN

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