



**UNIVERSITI PUTRA MALAYSIA**

***INDUCTION OF APOPTOSIS BY *cis*-3-(3',4'-DIMETHOXYPHENYL)-4-[(E)-3'',4''-DIMETHOXYSTYRYL]CYCLOHEX-1-ENE ISOLATED FROM THE RHIZOME OF *Zingiber cassumunar* Roxb. ON HUMAN T-LYMPHOBLASTIC LEUKEMIA CELL LINE, CEMss***

**THEEBAA ANASAMY**

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By

**THEEBAA ANASAMY**

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

**May 2013**

## DEDICATION

*To my late parents, Anasamy Rajoo and Asothda Muthiah. Their words of inspiration and encouragement in pursuit of excellence, still lingers on.*



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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**INDUCTION OF APOPTOSIS BY *cis*-3-(3',4'-DIMETHOXYPHENYL)-4-[(*E*)-3''',4'''-DIMETHOXYSTYRYL]CYCLOHEX-1-ENE ISOLATED FROM THE RHIZOME OF *Zingiber cassumunar* Roxb. ON HUMAN T-LYMPHOBLASTIC LEUKEMIA CELL LINE, CEMss**

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**May 2013**

**Chair: Ahmad Bustamam Abdul, PhD**

**Faculty: Institute of Bioscience**

*Zingiber cassumunar* Roxb. is one of the most widely cultivated species of Zingiberaceae family and commonly known as 'plai' in Thailand and 'bonglai' in Malaysia. *cis*-3-(3',4'-Dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl]cyclohex-1-ene (ZC-B11) is a phenylbutenoid dimer isolated from the rhizomes of *Z. cassumunar*. The objective of this study is to investigate the antiproliferative activities of this compound on human T-lymphoblastic cell line, CEMss and the mechanism by which apoptosis is triggered. *In vitro* cytotoxic effect of ZC-B11 was determined using MTT assay in several human cancer cell lines including leukemia (CEMss). ZC-B11 showed selectivity towards CEMss with an IC<sub>50</sub> value of 7.11 ± 0.24 µg/ml. The antiproliferative activity of ZC-B11 was also tested against non-tumorigenic human blood mononuclear cells and ZC-B11 does not show cell growth inhibition of human blood mononuclear cells (IC<sub>50</sub> > 50 µg/ml).

Various microscopy techniques used in this study showed distinctive morphological changes corresponding to typical apoptosis. Cell cycle analysis revealed significant ( $p < 0.05$ ) S phase arrest in a time-dependent manner whilst DNA fragmentation of ZC-B11 treated CEMss cells was detected using 1.2% agarose gel. Decrement of mitochondrial membrane potential was also observed in treated CEMss cells in time-dependent manner using the Rh123 staining. To evaluate further the mechanisms of apoptosis induction by ZC-B11 towards CEMss cells, screening of several proteins implicated to apoptosis induction were done using the human apoptosis proteome profiler array, in which, proteins such as Bax, caspase 3, cytochrome c and SMAC showed significant increase ( $p < 0.05$ ) compared to untreated control cells, whilst proteins such as Bcl-2, HSP70 and XIAP decreased significantly. On the other hand, caspase 8, p53 and BID remain unaffected ( $p > 0.05$ ). Caspase bioluminescent assay and Western blot analysis were done to further confirm these results. Collectively, results presented in this study demonstrate that ZC-B11 isolated from the rhizome of *Z. cassumunar* inhibited the proliferation of CEMss selectively, leading to the programmed cell death via mitochondrial signaling pathway and has the potential to be developed as an antileukemic and chemotherapy agent.

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

**PENGARUHAN APOPTOSIS OLEH *cis*-3-(3',4'-DIMETOKSIFENIL)-4-[(*E*)-3''',4'''-DIMETOKSISTIRIL]SIKLOHEKS-1-ENA YANG DIPENCILKAN DARIPADA RIZOM *Zingiber cassumunar* Roxb. KE ATAS TITISAN SEL LEUKEMIA T-LIMFOBLASTIK MANUSIA, CEMss**

Oleh

**THEEBAA ANASAMY**

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*Zingiber cassumunar* Roxb. merupakan salah satu spesies tanaman paling meluas dalam famili Zingiberaceae dan juga dikenali sebagai 'plai' di Thailand dan 'bonglai' di Malaysia. *cis*-3-(3',4'-Dimetoksifenil)-4-[(*E*)-3''',4'''-dimetoksistiril]siklohex-1-ena (ZC-B11) ialah satu dimer fenilbutenoid yang dipencilkan dari rizom *Z. cassumunar*. Objektif kajian ini ialah untuk mengkaji aktiviti antiproliferasi sebatian ini ke atas titisan sel leukemia T-limfoblastik manusia, CEMss dan mekanisme pengaruh apoptosis. Kesan sitotoksik *in vitro* ZC-B11 ke atas beberapa titisan sel kanser manusia termasuk leukemia (CEMss) ditentukan dengan menggunakan pengasaian MTT. ZC-B11 menunjukkan kesan sitotoksik selektif ke atas CEMss dengan nilai  $IC_{50}$   $7.11 \pm 0.24$   $\mu\text{g}/\text{ml}$ . Aktiviti antiproliferasi ZC-B11 ke atas sel darah mononuklear manusia juga dikaji dan ZC-B11 tidak menunjukkan perencatan pertumbuhan sel darah mononuklear manusia ( $IC_{50} > 50$   $\mu\text{g}/\text{ml}$ ). Perubahan morfologi sel yang ditentukan dengan menggunakan beberapa teknik mikroskopi menunjukkan ciri-ciri apoptosis yang nyata. Analisis kitar sel menunjukkan

penahanan yang signifikan ( $p < 0.05$ ) pada fasa S selari dengan tempoh masa perlakuan manakala fragmentasi DNA juga dibuktikan melalui elektroforesis gel agaros 1.2%. Susutan ketelapan membran mitokondria juga diperhatikan pada sel-sel CEMss selari dengan peningkatan tempoh masa perlakuan ZC-B11 dengan bantuan pewarna Rh123. Untuk menilai seterusnya mekanisme aruhan apoptosis oleh ZC-B11 ke atas sel-sel CEMss, penglibatan beberapa protein yang berkaitan dengan pengaruh apoptosis dikaji dengan menggunakan tatasusun pemprofil protein apoptosis manusia di mana, protein-protein seperti Bax, kaspase 3, sitokrom c and SMAC menunjukkan peningkatan signifikan ( $p < 0.05$ ) berbanding sel-sel kawalan manakala protein-protein seperti Bcl-2, HSP70 and XIAP menunjukkan penurunan aras pengekspresan yang signifikan. Aras pengekspresan kaspase 8, p53 and BID pula tidak terjejas ( $p > 0.05$ ). Asai biolumenesen kaspase dan pemblotan Western dilakukan untuk mengesahkan hasil yang diperolehi. Secara kolektifnya, hasil kajian yang diperolehi menunjukkan bahawa ZC-B11 yang dipencil dari rizom *Z. cassumunar* berupaya menghalang proliferasi sel-sel CEMss secara terpilih dan seterusnya menyebabkan kematian sel secara terprogram melalui tapak jalan pengisyaratan mitokondria dan berpotensi untuk dibangunkan sebagai agen antileukemia dan kemoterapi.



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Last but not least, I would like to thank my fellow friends and staffs of UPM-Makna Cancer Research Laboratory for all their help and cooperation.

I certify that a Thesis Examination Committee has met on 7 May 2013 to conduct the final examination of Theebaa a/p Anasamy on her thesis entitled “Induction of Apoptosis by *cis*-3-(3',4'-Dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl]cyclohex-1-ene Isolated from the Rhizome of *Zingiber cassumunar* Roxb on Human T-lymphoblastic Leukemia Cell Line, CEMss” in accordance with 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 degree of Master of Science.

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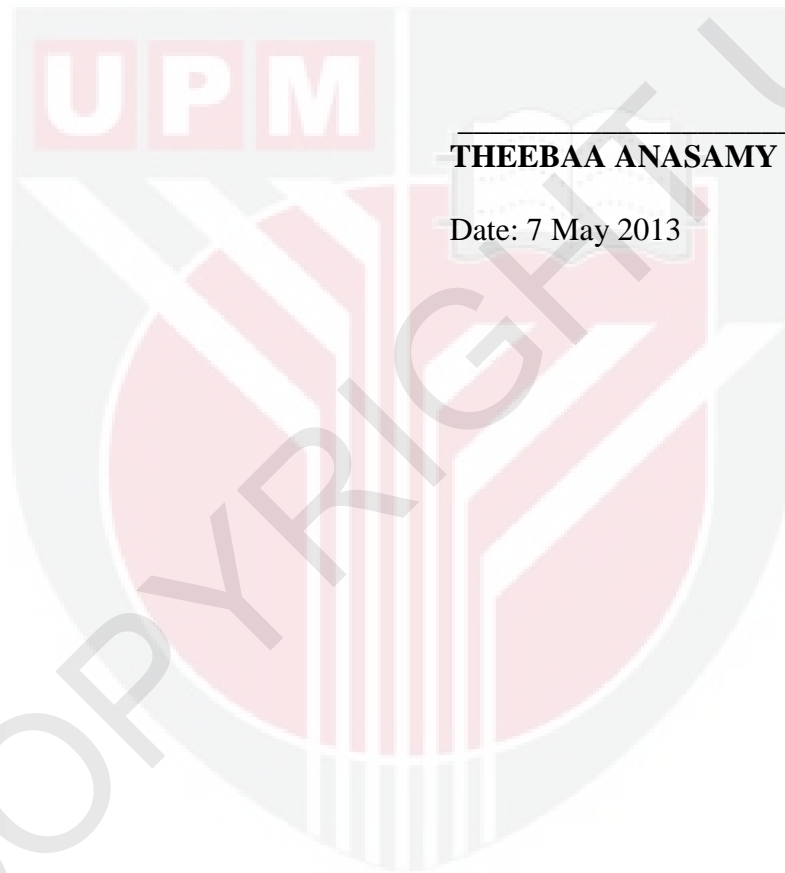
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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotation 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.



**THEEBAA ANASAMY**

Date: 7 May 2013

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