

CYTOTOXICITY EFFECTS AND CELL DEATH MECHANISMS OF  
*Avicennia alba* AND *Bruguiera gymnorrhiza*  
EXTRACTS ON HUMAN BREAST CANCER CELL LINE  
(MCF-7)

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MASTER OF SCIENCE  
UNIVERSITI MALAYSIA TERENGGANU

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Perpustakaan Sultanah Nur Zahirah  
Universiti Malaysia Terengganu



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NURUL HUDA BINTI AB RAHMAN

Thesis Submitted in Fulfillment of the Requirement for the Degree  
of Master of Science in the Institute of Marine Biotechnology  
Universiti Malaysia Terengganu

March 2016

This Thesis is Dedicated to:

- ❖ Prof. Dr. Tengku Sifzizul Tengku Muhammad
  - ❖ Assoc. Prof. Dr. Aziz Ahmad
  - ❖ Assoc. Prof. Dr. Habsah Mohamad
  
- ❖ My beloved parents Abdul Rahman and Fauziah
  - ❖ My beloved husband Mohd Solihin
  
- ❖ My beloved siblings:
  - Mohd Shahazlan
  - Nor Azura
  - Mohamad Faiz
  - Muhammad Amir
  - Nurul Suraya
  - Mohd Zamarul Hisyam
  - Nurul Natasya
  
- ❖ My beloved friends

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science

**CYTOTOXICITY EFFECT AND CELL DEATH MECHANISM OF *Avicennia alba* AND *Bruguiera gymnorrhiza* EXTRACTS ON HUMAN BREAST CANCER CELL LINE (MCF-7)**

**NURUL HUDA BINTI AB RAHMAN**

**March 2016**

**Main Supervisor : Professor Tengku Sifzizul Tengku Muhammad, Ph.D**  
**Co-Supervisor : Associate Professor Aziz Ahmad, Ph.D**  
**Associate Professor Habsah Mohamad, Ph.D**  
**Institute : Institute of Marine Biotechnology**

Breast cancer is the second leading cause of cancer death and the most common form of cancer affecting women worldwide. Currently, over 50% of drugs used in clinical trials for cancer treatments were isolated from natural sources. Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanism of action. Apoptosis plays a crucial role for killing of tumor cells in response to cytotoxic agents used in the treatment of cancer patients. Therefore, the main aim of this study was to determine the cytotoxicity effect and mechanism of cell death of the extracts prepared from mangrove plants on human breast carcinoma cell line, MCF-7. In this study, two mangrove species were used, *Avicennia alba* and *Bruguiera gymnorrhiza*. From six extracts (methanol, butanol and diethyl ether) prepared from the leaves of both species, *B. gymnorrhiza* butanol extract showed the highest cytotoxicity activity against MCF-7 cell with  $IC_{50}$  value of 4.39  $\mu\text{g/ml}$ , followed by diethyl ether and methanol extract of the same species ( $IC_{50}$  at 16.22  $\mu\text{g/ml}$  and 24.15  $\mu\text{g/ml}$ , respectively), and, diethyl ether, butanol and methanol extracts of *A. alba* ( $IC_{50}$  at 25.12  $\mu\text{g/ml}$ , 27.12  $\mu\text{g/ml}$  and 28.91  $\mu\text{g/ml}$ , respectively) at 72 hr. The DeadEnd™ Colometric Apoptosis Detection System showed all six extracts exerted cytotoxicity effect on MCF-7 cells via apoptosis. The level of caspase-3 was found to increase in cells treated with both butanol and diethyl ether extracts of *B. gymnorrhiza*, albeit at different levels, indicating apoptosis was mediated by the activation of caspase-3. Interestingly, *B. gymnorrhiza* diethyl ether extract induced higher level of caspase-9 as compared to caspase-8 suggesting that the intrinsic pathway was the major route used by diethyl ether extract to induce apoptosis in MCF-7 cells. In contrast, there was no significant difference between the level of expression of caspase-8 and -9 (1.51 and 1.3-fold, respectively) when the cells were treated with butanol extract of *B. gymnorrhiza* (at  $IC_{50}$  72 hours) suggesting the extract triggered apoptosis via both intrinsic and extrinsic pathways. The low expression levels of both caspases in butanol-treated cells may indicate that other mechanism(s) may play a major role in inducing apoptosis. TLC profiling demonstrated the presence of phenolic, terpene and alkaloid compounds in crude methanol, diethyl ether and butanol extracts of *B. gymnorrhiza* and *A. alba*. In conclusion, methanol, diethyl ether and butanol extracts

of *B. gymnorhiza* and *A. alba* may contain potential compounds to be developed as anti-cancer agents against breast cancer.

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**KESAN SITOTOKSIK DAN MEKANISME KEMATIAN SEL  
OLEH EKSTRAK DARIPADA *Avicennia alba* DAN *Bruguiera gymnorrhiza*  
TERHADAP SEL KANSER PAYUDARA MANUSIA  
(MCF-7)**

**NURUL HUDA BINTI AB RAHMAN**

**March 2016**

**Penyelia Utama : Profesor Tengku Sifzizul Tengku Muhammad, Ph.D**

**Penyelia Bersama : Profesor Madya Aziz Ahmad, Ph.D**

**Profesor Madya Habsah Mohamad, Ph.D**

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Kanser payudara merupakan punca kedua utama kematian yang disebabkan oleh kanser yang kerap melanda wanita di seluruh dunia. Pada masa ini, lebih 50% daripada ubat-ubatan yang digunakan dalam ujian klinikal untuk aktiviti anti kanser telah dipencilkan daripada sumber semula jadi. Oleh itu, pencarian produk semula jadi yang akan digunakan dalam terapi kanser telah menarik minat yang besar di mana tumbuh-tumbuhan telah menjadi sumber yang paling penting, membekalkan pelbagai agen anti-tumor dengan struktur baru dan tindakan mekanisme yang unik. Laluan Apoptosis memainkan peranan penting untuk pembunuhan sel-sel tumor dalam tindak balas kepada agen sitotoksik yang digunakan dalam rawatan pesakit kanser. Oleh itu, tujuan utama kajian ini adalah untuk menentukan kesan sitotoksik dan cara kematian sel oleh ekstrak yang disediakan dari pokok bakau keatas sel karsinoma payudara manusia (MCF-7). Dalam kajian ini, dua spesis pokok bakau telah digunakan, iaitu *Avicennia alba* dan *Bruguiera gymnorrhiza*. Daripada enam ekstrak (metanol, butanol dan dietil eter) yang disediakan daripada daun kedua-dua spesis tumbuhan, ekstrak butanol *B. gymnorrhiza* telah menunjukkan kesan sitotoksiti yang paling tinggi terhadap sel MCF-7 dengan nilai  $IC_{50}$  sebanyak 4.39  $\mu\text{g/ml}$ , diikuti oleh ekstrak dietyl eter dan metanol daripada spesies yang sama (masing-masing pada  $IC_{50}$  pada 16.22  $\mu\text{g/ml}$  dan 24.15  $\mu\text{g/ml}$ ), ekstrak dietyl eter, butanol dan metanol daripada *A. alba* (masing-masing pada  $IC_{50}$  pada 25.12  $\mu\text{g/ml}$ , 27.12  $\mu\text{g/ml}$  dan 28.91  $\mu\text{g/ml}$ ) pada 72 jam. DeadEnd™ Colometric Apoptosis Detection System menunjukkan keenam-enam ekstrak dengan aktiviti sitotoksiti teraruh apoptosis yang menunjukkan bahawa apoptosis memainkan peranan utama dalam mendorong kematian sel MCF-7. Tahap caspase-3 didapati telah meningkat pada sel-sel yang telah dirawat dengan kedua-dua ekstrak butanol dan dietil eter *B. gymnorrhiza*, walaupun pada tahap yang berbeza, menunjukkan apoptosis telah dipengantarakan oleh pengaktifan caspase-3. Menariknya, ekstrak dietil eter *B. gymnorrhiza* mengaruh tahap caspase-9 lebih tinggi berbanding caspase-8, ini menunjukkan bahawa ekstrak dietil eter mengaruhkan apoptosis melalui intrinsik sebagai laluan utama dalam mekanisme kematian sel. Manakala, tiada perbezaan ketara diantara tahap pengaktifan caspase-8 dan -9 (masing-masing pada 1.51 dan 1.3-fold) apabila sel-sel dirawat dengan ekstrak butanol *B. gymnorrhiza* (pada  $IC_{50}$  72 jam) menunjukkan ekstrak mengaruhkan apoptosis melalui kedua-dua laluan intrinsik dan ekstrinsik. Kadar pengaktifan yang rendah bagi kedua-dua caspase



dalam sel-sel yang dirawat dengan ekstrak butanol menunjukkan mungkin mekanisme yang lain memainkan peranan utama dalam mengaruhi apoptosis. Profil TLC menunjukkan kehadiran sebatian fenolik, terpenoid dan alkaloid dalam ekstrak mentah metanol, ekstrak dietil eter dan butanol *B. gymnorrhiza*, dan *A. alba*. Kesimpulannya, kesemua ekstrak metanol, dietil eter dan butanol *A. alba* dan *B. gymnorrhiza* mungkin mempunyai bahan yang berpotensi tinggi untuk dikembangkan sebagai agen anti-kanser untuk rawatan kanser payudara.