



UNIVERSITI PUTRA MALAYSIA

ANTI CANCER ACTIVITY OF PLANT EXTRACTS ON MDA-MB231

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FBSB 2015 128

**ANTI CANCER ACTIVITY OF PLANT EXTRACTS ON
MDA-MB-231**

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**Dissertation submitted in partial fulfillment of the requirement for the course
BMY 4999 Project in the Department of Microbiology
Universiti Putra Malaysia
JUNE 2015**

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MDA-MB-231**

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UNIVERSITI PUTRA MALAYSIA
2015**

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “ANTI CANCER ACTIVITY OF PLANT EXTRACTS ON MDA-MB-231” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Seri Wahyuni Binti Yussoff (163384) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Local plants of *Annona squamosa* L., *Strobilanthes crispus*, *Cinnanomum iners*, *Premna cardifolia*, *Barringtonia racemosa* L., *Acalypha indica*, *Ipomoea aquatica*, *Gynura procumbers*, *Vigna unguiculata* L., *Piper betle* L. and *Piper nigrum* L. were selected for their potential anti-cancer property. The leaves of plants were extracted using methanol and tested on breast cancer cell, MDA-MB-231. The MTT cytotoxicity assay has been carried out to calculate the IC₅₀. The result shown *Annona squamosa* L. and *Piper betle* L. has potential anti cancer activity with IC₅₀ of 500.234 µg/ml and 375.710 µg/ml respectively. Meanwhile, *Piper nigrum* L. was cytotoxic to Chang liver cell with IC₅₀ of 721.770 µg/ml.



ABSTRAK

Tumbuhan-tumbuhan tempatan iaitu *Annona squamosa* L., *Strobilanthes crispus*, *Cinnanomum iners*, *Premna cardifolia*, *Barringtonia racermosa* L., *Acalypha indica*, *Ipomoea aquatica*, *Gynura procumbers*, *Vigna unguiculata* L., *Piper betle* L. dan *Piper nigrum* L.) telah dipilih untuk mengkaji potensi ciri anti-kanser. Daun tumbuhan-tumbuhan tersebut telah diekstrak dengan menggunakan metanol and diuji dengan sel kanser payudara, MDA-MB-231. Analisis MTT sitotoksik telah dijalankan untuk mengira IC_{50} (perencatan sel). Hasil kajian menunjukkan *Annona squamosa* L. and *Piper betle* L. mempunyai ciri anti-kanser yang berpotensi dengan IC_{50} 500.234 $\mu\text{g/ml}$ dan 375.710 $\mu\text{g/ml}$. Sementara itu, *Piper nigrum* L. adalah sitotosik terhadap sel hati Chang dengan IC_{50} 721.770 $\mu\text{g/ml}$.



ACKNOWLEDGEMENT

Assalamualaikum W.B.T. First of all, I would like to send my gratitude to my project supervisor, Prof. Madya Dr. Muhajir Hamid for all your guidances and advices. I am very grateful to have such kind and supportive supervisor during my final year project. I am also wants to thank Department of Microbiology and Animal Tissue Culture Lab for the equipment and facilities provided during my project.

A big thank you to Ms. Khoo Li Teng, postgraduate student in Animal Tissue Culture Lab for helping, guiding and sharing knowledge with me in order to assist me to complete my project. Your contributions mean a lot to me.

In addition, I am glad to have such good lab mates which are Mohamad Zulhafiz Shafiq Bin Zulhilmi Cheng and Lau Ee Hong for assisting and sharing valuable information during the project. Lasty, thank you to my parents, family, friends and lecturers for giving moral support and advices to me along my final year project.

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CHAPTER 1

INTRODUCTION

In advanced world today, cancer has become the second highest factor contributed to the people deaths (Amin *et al.*, 2009). The top cancer cases were breast cancer, lung cancer, colon or rectum cancer, stomach cancer and prostate cancer (Cragg and Newman, 2005). Cancer can be classified into two types which are benign and malignant. Benign cancer is normally referred to tumor. Uncontrollable growth of cancerous cells which affect the basal membrane, attack and invade surrounding tissue and may metastasize is known as malignant cancer (de Melo *et al.*, 2011). Destruction of apoptotic mechanism is the crucial reason the normal cell transformed to cancerous cell (Hu and Kavanagh, 2003). Cancer also may be undefined and may refer to conditions such as hard swellings, abscesses, calluses, corns, warts, polyps and tumors. The symptoms mentioned before generally involve the skin, tangible or clear conditions and sometimes related to cancerous state (Cragg and Newman, 2005).

To overcome this disease, anti cancer agents and chemotherapy are invented by scientists. Chemotherapy is treatment by using synthetic or natural agents to counter or stop the process of carcinogenesis (Sporn *et al.*, 1976). The active agents may be extracted from plants, microorganisms or marine organisms and commonly altered to enhance the potent effect. The case of drug resistance is common in therapeutic field. Hence, many cytotoxic drugs are applied in combination to overcome this problem although it is still not thoroughly effective. So, the

researchers need to find some other ways to encounter this problem such as searching for other potential active compound available in nature.

In addition, the available drugs generally are not specific which lead to many side effects to the body (Ophardt, 2003). The main focus of chemotherapy is to lower or totally stop the growth level of cancerous cells which also affect the normal cells in the body. The cells that have rapid turnover such as hair, gastrointestinal and bone marrow cells regularly affected (Ophardt, 2003). This is the reason why the chemotherapy patients have common symptoms such as nausea and vomiting, loss of hair, digestive distress, pale skin and many more which can worsen the patient's health. Hence, the requirement to invent the effective but specific drugs is very significant.

The objectives of this experiment are:

1. To extract potential plants for anti-cancer activity
2. To determine the cytotoxicity of plant extract towards breast cancer cell line

REFERENCES

Ahmed, F. N. (2011). *Examination of Mediator composition and p53 in distinct breast cancer lines: MCF7 and MDA-MB-231*. Unpublished Undergraduate Honors Theses, University of Colorado Boulder, United States.

Amin, A., Gali-Muhtasib, H., Ocker, M., & Schneider-Stock, R. (2009). Overview of major classes of plant-derived anticancer drugs. *International Journal of Biomedical Science: IJBS*, 5(1), 1.

Baguley, B. C. (2002). A brief history of cancer chemotherapy. In *Anticancer Drug Development*. B. Baguley & D. J. Kerr, pp 1-9. Academic Press, London.

Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology*, 100(1), 72-79.

de Melo, J. G., Santos, A. G., de Amorim, E. L. C., Nascimento, S. C. D., & de Albuquerque, U. P. (2011). Medicinal plants used as antitumor agents in Brazil: an ethnobotanical approach. *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, 1-14.

Freshney, R. I. (2010). *Culture of Animal Cells: A Manual of Basic Technique and Specilized Application*, 6th Edition. New Jersey (USA): Wiley-Blackwell Inc.

Gottesman, M. M. (2002). Mechanisms of cancer drug resistance. *Annual Review of Medicine*, 53(1), 615-627.

Holliday, D. L., & Speirs, V. (2011). Choosing the right cell line for breast cancer research. *Breast Cancer Research*, 13(4), 215-222.

Hu, W., & Kavanagh, J. J. (2003). Anticancer therapy targeting the apoptotic pathway. *Lancet Oncology*, 4(9), 721-729.

Kaewseejan, N., Puangpronpitag, D., & Nakornriab, M. (2012). Evaluation of phytochemical composition and antibacterial property of *Gynura procumbens* extract. *Asian Journal of Plant Sciences*, 11(2), 77-82.

Kathirvel, A., & Sujatha, V. (2012). Phytochemical analysis and antioxidant activity of *Barringtonia acutangula* (L.) Gaertn. leaves. *International Journal of Pharmacy and Pharmaceutical Science*, 4(2), 277-281.

Kenny, P. A., Lee, G. Y., Myers, C. A., Neve, R. M., Semeiks, J. R., Spellman, P. T., Lorenz, K., Lee, E. H., Barcellos-Hoff, M. H., Peterson, O. W., Gray, J. W., & Bissell, M. J. (2007). The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. *Molecular Oncology*, 1(1), 84-96.

Levenson, A. S., & Jordan, V. C. (1997). MCF-7: the first hormone-responsive breast cancer cell line. *Cancer research*, (57), 3071-3078.

Manvar, M. N., & Desai, T. R. (2013). Phytochemical and pharmacological profile of *Ipomoea aquatica*. *Indian Journal of Medical Sciences*, 67(3), 49-60.

Nahak, G., & Sahu, R. K. (2011). Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum*. *Journal of Applied Pharmaceutical Science*, 1(8), 153-157.

Nirmala, M. J., Samundeeswari, A., & Sankar, P. D. (2011). Natural plant resources in anti-cancer therapy-A review. *Research in Plant Biology*, 1(3), 01-14.

Nurraihana, H., & Norfarizan-Hanoon, N. A. (2013). Phytochemistry, pharmacology and toxicology properties of *Strobilanthes crispus*. *International Food Research Journal*, 20(5), 2045-2056.

Ophardt, C. E. (2003). Anti-cancer Drugs I. In *Virtual Chembook*, Elmhurst College, Chicago.

Payne, S., & Miles, D. (2008). Chapter 4: Mechanisms of anticancer drugs. In *Scott-Brown Otorhinolaryngology: Head and Neck Surgery*, 7th Edition. M. J. Gleeson, & R. C. Clarke, Vol 1. pp 34-46. US: CRC Press.

Pengelly, A. (2004). *Constituents of Medicinal Plants*, pp. 66. Cambridge: CABI Publishing.

Pradhan, D., Suri, K. A., Pradhan, D. K., & Biswasroy, P. (2013). Golden Heart of the Nature: *Piper betle* L. *Journal of Pharmacognosy and Phytochemistry*, 1(6), 147-164.

Prasad, R., & Koch, B. (2014). Antitumor activity of ethanolic extract of *Dendrobium formosum* in T-cell lymphoma: an *in vitro* and *in vivo* study. *BioMed Research International*,

Riss, T. L., Moravec, R. A., Niles, A. L., Benink, H. A., Worzella, T. J., & Minor, L. (2013). Cell Viability Assays. In *Assay Guidance Manual* [Internet]. G. S. Sittampalam, N. P. Coussens, & H. Nelson. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.

Sandeep, D. (2014). Evaluation of Antibacterial Activity of Seed Extracts of *Vigna unguiculata*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(1), 75-77.

Selvan, R. T., Mohideen, A. K. S., Sheriff, M. A., & Azmathullah, N. M. (2012). Phytochemical screening of *Acalypha indica* L. leaf extracts. *International Journal of Applied Biology and Pharmaceutical Technology*, 3(2), 158-161.

Shukri, M. M., Alan, C., & Noorzuraini, A. S. (2011). Polyphenols and antioxidant activities of selected traditional vegetables. *Journal of Tropical Agriculture and Food Science*, 39(1), 1-15.

Sporn, M. B. (1976). Approaches to prevention of epithelial cancer during the preneoplastic period. *Cancer Research*. 36, 2699–2702.

Tandon, R., Gupta, A., & Ray, A. (2013). Mechanism of action of anti-diabetic property of cinnamic acid. A principal active ingredient from the bark of *Cinnamomum cassia*. *International Journal of Therapeutic Applications*. 9, 39-45.

Vats, S. (2012). Antioxidant activity of callus culture of *Vigna unguiculata* (L.) Walp. *Researcher*, 4(6), 22-24.

Wang, D. S., Rizwani, G. H., Guo, H., Ahmed, M., Ahmed, M., Hassan, S. Z., Hassan, A., Xu, Z. S., & Xu, R. H. (2014). *Annona squamosa* Linn: Cytotoxic activity found in leaf extract against human tumor cell lines. *Pakistan Journal of Pharmaceutical Sciences*, 27(5), 1559-1563.