ANTI-PROLIFERATIVE ACTIVITIES OF 32 MALAYSIAN PLANT SPECIES IN BREAST CANCER CELL LINES

M. Y. Nurhanan*, O. Asiah, M. A. Mohd Ilham, M. M. Siti Syarifah, I. Norhayati & H. Lili Sahira

Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia

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NURHANAN, M. Y., ASIAH, O., MOHD ILHAM, M. A., SITI SYARIFAH, M. M., NORHAYATI, I. & LILI SAHIRA, H. 2008. Anti-proliferative activities of 32 Malaysian plant species in breast cancer cell lines. Anti-proliferative activities of 143 crude extracts obtained from 32 plants collected from the rain forests of Pahang, Malaysia, were evaluated in two breast cancer cell lines, i.e. MCF-7 and T47D, obtained from the American Type Culture Collection, USA. The objective of this study was to bioprospect a list of plants to find out their potential to be developed into anti-cancer drug(s), by means of bioassay guided isolation procedure. After performing sulforhodamine-B assay, 13 crude extracts from 11 plant species were found to have active anti-proliferative effects ($IC_{50} \leq 100$ μg ml^{-1}).

Keywords: MCF-7 and T47D cells, active plant extracts, forest reserves, Pahang

NURHANAN, M. Y., ASIAH, O., MOHD ILHAM, M. A., SITI SYARIFAH, M. M., NORHAYATI, I. & LILI SAHIRA, H. 2008. Aktiviti anti-proliferasi 32 spesies tumbuhan Malaysia terhadap sel kanser buah dada. Aktiviti anti-proliferasi 143 ekstrak mentah daripada 32 tumbuhan yang diperoleh daripada hutan hujan Pahang, Malaysia dikaji terhadap dua jenis sel kanser buah dada iaitu MCF-7 dan T47D yang diperoleh daripada Koleksi Kultur Tip Amerika, Amerika Syarikat. Kajian ini menjalankan bioprospek terhadap tumbuhan bagi menilai potensi mereka untuk dibangunkan menjadi ubat anti-kanser menggunakan kaedah pengasingan biocerakinan. Selepas cerakinan sulforhodamina-B, 13 ekstrak mentah daripada 11 spesies tumbuhan dikenal pasti mempunyai kesan anti-proliferasi yang aktif ($IC_{50} \leq 100$ μg ml^{-1}).

INTRODUCTION

Cancer is the fourth leading cause of death in Malaysia (Lim 2002) and the second highest cause of death in developed countries after cardiovascular diseases (WHO 2005). The Ministry of Health, Malaysia (1998) reported that breast cancer ranks among the top 10 deaths in cancer-related deaths in Malaysia. One of the modalities in cancer treatment is via chemotherapy. About 65% of drugs used in chemotherapy are of natural origin (Newman *et al.* 2003).

Malaysia has an immense wealth of plant species, both endemic and non-endemic. The most extensive screening for anti-cancer compounds obtained from plant species was done by the National Cancer Institute (NCI) in the United States of America. More than 35 000 species (or about 14% of the estimated number of higher plant species on earth) collected from different rain forests in Asia, Africa and the Amazon were screened by the NCI alone more than two decades ago (Fabricant & Farnsworth 2001). From the screening process, taxol from *Taxus brevifolia* was discovered and developed into one of the most successful plant-based anti-cancer drug. Other anti-cancer drugs originated from plants include vinblastine and vincristine derived from *Catharantus roseus* and etoposide, from *Podophyllum peltatum*, to name a few. Although chemotherapy is effective in detecting cancer at a very early stage, the side effects and resistance towards drugs are a problem. Hence, new drug(s) or treatment(s) are needed.

This paper reports the anti-proliferative activities of 32 plant species collected from different rain forests in Pahang, Malaysia, in two breast cancer cell lines. Due to ethical concerns, human breast cancer cell lines were established about three decades ago and are widely used as experimental models in breast cancer research especially in pre-clinical phase (Burdall *et al.* 2003). The most widely used cell lines for breast

*E-mail: hanan@frim.gov.my

cancer research include the estrogen receptor positive (ER+) breast cancer cell lines: MCF-7 and T47D. These cell lines are good models for identifying molecular events that are likely to be important in some ER+ human breast cancers (Zhu *et al.* 2006).

MATERIALS AND METHODS

Plant collection

All the 32 plant species from 21 plant families (Table 1) were identified by FRIM botanist. At least two plant parts from each species were collected from Mencali, Bebar and Endau-Rompin Forest Reserves in Pahang, Malaysia. The voucher specimens were kept at FRIM.

Plant extraction

Each plant part was dried and ground into powder. The samples were weighed to between 200 and 300 g and immersed in 1500 ml methanol. The samples were refluxed at 60 °C for three hours to obtain methanolic extract. The methanol was removed using rotary evaporator.

Preparation of extract for treatment

All methanol extracts was dissolved in ethanol (1% v/v) and diluted by 10 fold dilutions to produce five different concentrations (final concentrations ranged from 1 to 100 μg ml^{-1})

Anti-proliferative assay

The anti-proliferative assay used was Sulforhodamine B (SRB) assay (Skehan *et al.* 1990). Before performing the assay, MCF-7 and T47D cell lines were cultured in Dubelcco's Modified Eagle's Medium (DMEM), supplemented with 5% Foetal Calf Serum, 1% fungizone, 1% penicillin streptomycin and 0.125% gentamycin sulphate until it reached sub-confluent state. Cell plating was performed in 96-well plate at a density of 7000–10000 cells/well and left overnight in the incubator set at 37 °C and 5% carbon dioxide in air. The medium was renewed the next day and the cells in different wells were treated with a series of plant extracts (1% v/v) prepared at five different concentrations (1 to 100 μg ml^{-1} in ethanol) in duplicate. The control wells contained cells and medium only. Another set of wells on the same plate contained cells that were not treated with any plant extracts but were treated with ethanol (1% v/v) as a vehicle control since ethanol had been used to dissolve and dilute the plant extracts. The cells were incubated for 72 hours in the same incubator. SRB assay (Skehan *et al.* 1990) was then performed and the OD values were read at 492 nm using Magellan V.4 Elisa reader and software. The percentage of living cells were calculated based on the OD values as below:

$$\frac{\text{OD of plant-treated cells} - \text{OD of ethanolic-treated cells}}{\text{OD of control} - \text{OD of ethanolic-treated cells}}$$

IC_{50} values (inhibition concentration of the plant extract when 50% of the cells were killed) of each plant extract in MCF-7 and T47D breast cancer cell lines were obtained from the dose-response curve (percentage of living cells versus the concentrations of plant extracts used). The experiment was repeated at least three times. The cut-off point used to dictate the effectiveness of the plant extract in inhibiting proliferation of breast cancer cell lines was when its IC_{50} value was less than and equal to 100 μg ml^{-1}. This assessment was employed in our study to select the extract for future fractionation and isolation of bioactive compounds.

RESULTS AND DISCUSSION

No standard cut-off point had been outlined for determining active extract for all types of cancer cell lines since different cells probably have different sets of molecular targets. For example, the National Cancer Institute, USA assigned the extracts with IC_{50} value less than 20 μg ml^{-1} in epidermoid KB cancer cell line (Boik 1996). However, this cut-off point need not necessarily be followed since it depends on the objectives of the research (Boyd & Paull 1995). In our lab, crude extracts with IC_{50} values less than or equal to 100 μg ml^{-1} in breast cancer cell lines (MCF-7 and T47D) were considered as active so as to widen the chance of discovery of novel anti-cancer compound(s). This is based on the fact that in these initial stages, only the degree of cell growth inhibition is necessary to judge whether to discard some of these plant extracts or to continue with

Table 1 The anti-proliferative effects of methanol extracts (IC_{50} (μg ml^{-1}) ± SE < 5%) of 32 Malaysian plant species in MCF-7 and T47D breast cancer cell lines

Species	Family	Part	Anti-proliferative activity IC_{50} (μg ml^{-1})	
			MCF-7	T47D
Bouea oppositifolia	Anacardiaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Cerbera odollam	Apocynaceae	Leaf	8.49	10.99
		Fruit	> 100	> 100
Avicennia alba	Avicenniaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Casuarina equisetifolia	Casuarinaceae	Leaf	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Terminalia catappa	Combretaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Dryobalanops aromatica	Dipterocarpaceae	Leaf	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Shorea marestalis	Dipterocarpaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Aporusa prainiana	Euphorbiaceae	Leaf	> 100	> 100
		Stem	> 100	> 100
Macaranga pruinosa	Euphorbiaceae	Leaf	17.52	40.10
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Macaranga triloba	Euphorbiaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	65.01	65.55
Mallotus macrostachyus	Euphorbiaceae	Leaf	14.69	12.64
		Stem + bark	> 100	> 100
Calophyllum inophyllum	Guttiferae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Calophyllum sclerophyllum	Guttiferae	Root + bark	51.68	53.43
Cratoxylum arborscens	Guttiferae	Leaf	16.66	41.98
Garcinia hombroniana	Guttiferae	Leaf	30.97	34.47
		Stem	20.25	34.80
		Bark of stem	64.57	69.81
		Fruit	> 100	> 100
Stemonurus secundiflorus	Icacinaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Ixonanthes icosandra	Ixonanthaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
Neolitsea zeylanica	Lauraceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
Barringtonia macrostachya	Lecythidaceae	Leaf	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Leea indica	Leeaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Pongamia pinnata	Leguminosae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Hibiscus tiliaceus	Malvaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
Streblus elongates	Moraceae	Leaf	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Maesa ramentacea	Myrsinaceae	Leaf	> 100	> 100
		Stem + bark	49.09	46.25
Melaleuca cajuputi	Myrtaceae	Leaf	20.38	18.38
		Stem	> 100	18.38
		Bark of stem	> 100	18.38

(continued)

Table 1 (continued)

Rhodamyrtus tomentosa	Myrtaceae	Leaf	> 100	> 100
		Fruit	> 100	> 100
Syzygium grande	Myrtaceae	Leaf	> 100	> 100
		Stem + bark	40.74	42.42
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Tristaniopsis whiteana	Myrtaceae	Leaf	32.86	49.64
		Stem + bark	> 100	> 100
Bruguiera sp.	Rhizophoraceae	Leaf	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
		Fruit	> 100	> 100
Rhizophora apiculata	Rhizophoraceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Guettarda speciosa	Rubiaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100
		Bark of stem	> 100	> 100
Guioa pleuropteris	Sapindaceae	Leaf	> 100	> 100
		Stem + bark	> 100	> 100
		Stem	> 100	> 100

the fractionation, isolation and characterization processes (Mans *et al.* 2000).

A total of 13 extracts from 11 plant species were active in causing anti-proliferative effects in both breast cancer cell lines (Table 1). A student's t-test analysis was performed to measure if there was any significant difference between the activities exerted by these extracts in both MCF-7 and T47D cell lines. The results showed that there was no significant difference.

The most active extracts with IC_{50} values less than 20 µg ml^{-1} were extracted from *Cerbera odollam* (Apocynaceae), followed by *Mallotus macrostachyus* (Euphorbiaceae) and *Melalueca cajuputi*. In other studies, Laphookhieo *et al.* (2004) managed to isolate a few compounds from *C. odollam* seeds and they were proven to have anti-proliferative effects on human epidermoid carcinoma (KB), human breast cancer cell (BC) and human lung cancer (NCI-H187) cell lines. These results showed that different plant parts of *C. odollam* contain phytochemicals that can potentially develop into anti-cancer agents that can inhibit the proliferation of different types of cancers.

Besides *M. macrostachyus,* leaves from other *Mallotus* species were also found to have anti-proliferative effects on different cancer cell lines (Xu *et al.* 1991, Kiem *et al.* 2005). Hence, bioactivity guided isolation procedure can be done to obtain the bioactive compounds in *M. macrostachyus* and other *Mallotus* species.

Not many anti-proliferative studies in cancer cells were done on *Melaleuca* species including *M. cajuputi*. Other species with anti-proliferative effect on cancer cells was known to be *Melaleuca alternifolia* (tea tree) oil. Terpinen-4-ol, the main active compound of *M. alternifolia* oil was able to induce caspace-dependent apoptosis of melanoma cells (Calcabrini *et al.* 2004). Hence, these *Melaleuca* species are worth further investigations to determine whether any of their fractions and compounds have anti-proliferative effects on cancer cells.

Other active extracts were shown to have moderate and low anti-proliferative activities in either one or both breast cancer cell lines (Table 1). Extracts with moderate activities were leaf extracts from *C. arborscens* and *M. pruinosa* and stem extract from *G. hombroniana*. The IC_{50} values for MCF-7 were 16.66, 17.52 and 20.25 µg ml^{-1} and T47D were 41.98, 40.10 and 34.80 µg ml^{-1} respectively. Seventy five per cent of the plants from the Euphorbiaceae and Guttiferae families were found to be active but with moderate anti-proliferative activities. Possibly, future screening could include more of the plant species from both families. One of the plant species from Guttiferae family, namely *G. hombroniana* had all its plant parts tested active in both breast cancer cell lines except for its fruit.

These active extracts which consisted of more than a few compounds will be purified first through bioassay guided fractionation and

isolation procedures (Norhayati *et al.* 2006) before the mass and molecular weight could be identified and exact doses in molar concentration could be used for the next treatment in breast cancer cell lines. The results will be compared with results of existing chemotherapy drug treatments such as taxol and tamoxifen in both breast cancer and normal cell lines. Since the existing drug (tamoxifen) has been reported to benefit women with ER positive breast tumour (Jordan & Morrow 1999, Colleoni *et al.* 2006), we will also expand our research using ER negative breast cancer cell lines such as MDA-MB-231 in order to search for anti-breast cancer agents with wider spectrum of inhibitions in different types of breast cancer cells.

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REFERENCES

Boik, J. 1996. *Cancer & Natural Medicine: a Textbook of Basic Science and Clinical Research.* Oregon Medical Press, Princeton.

Boyd, M. R. & Paull, K. D. 1995. Some practical considerations and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. *Drug Development Research* 34: 91–109.

Burdall, S. E., Hanby, A. W., Lansdown, M. R. J. & Speirs, V. 2003. Breast cancer cell lines: friend or foe. *Breast Cancer Research* 5: 89–95.

Calcabrini, A., Stringaro, A., Toccacieli, L., Meschini, S., Marra, M., Colone, M., Salvatore, G., Mondello, F., Arancia, G. & Molinari, A. 2004. Terpinol-4-ol, the main component of *Melaleuca alternifolia* (tea tree) oil inhibits the *in vitro* growth of human melanoma cells. *The Journal of Investigative Dermatology* 122: 349–360.

Colleoni, M., Gelber, S. & Goldhisch, A. 2006. Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: international breast cancer study group trial 13–93. *Journal of Clinical Oncology* 24: 1332–1341.

Fabricant, D. S. & Farnsworth, N. R. 2001. The value of plant used in traditional medicine for drug discovery. *Environmental Health Perspective* 109: 69–75.

Jordon, V. C. & Morrow, M. 1999. Tamoxifen, raloxifene, and the prevention of breast cancer. *Endrocrine Reviews* 20: 253–278.

Kiem, P. V., Dang, N. H., Bao, H. V., Huong, H. T., Minh, C. V., Huong Le M., Lee, J. J. & Kim, Y. H. 2005. New cytotoxic benzopyrans from the leaves of *Mallotus apelta. Archives of Pharmacal Research* 28: 1131–1134.

Laphookhieo, S., Cheenpracha, S., Karalai, C., Chantrapromma, S., Rat-A-Pa, Y., Ponglimanont, C. & Chantrapromma, K. 2004. Cytotoxic cardenolide glycoside from the seeds of *Cerbera odollam. Phytochemistry* 65: 507–510.

Lim, G. C. C. 2002. Overview of cancer in Malaysia. *Japanese Journal of Clinical Oncology* 32: S37–S42.

Mans, D. R. A., Da Rocha, A. B. & Schwartsmann, G. 2000. Anti-cancer drug discovery and development in Brazil: targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. *The Oncologist* 5: 185–198.

Ministry of Health, Malaysia. 1998. 10 jenis penyebab utama kemasukan hospital kerajaan tahun 1998. http//www.dph.gov.my. 5 Disember 2000.

Newmann, D. J., Cragg, G. M. & Snader, K. M. 2003. Natural products as sources of new drugs over period 1981–2002. *Journal of Natural Products* 66: 1022–1037.

Norhayati, I., Mohd Ilham, A., Asiah, O., Syarifah, S. M. M., Lili S. H., Chang, L. Y. & Hafidz, H. A. 2006. Bioassay guided fractionation of active plant extract against breast cancer cells using sepbox 2D-250. Poster presented at the Seminar and Workshop on Forest Biotechnology 2006. 4–7 December 2006, Kepong.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. & Boyd, M. R. 1990. New colorimetric assay for anticancer-drug screening. *Journal of the National Cancer Institute* 82: 1107–1112.

WHO. 2005. Are the number of cancer cases increasing or decreasing in the world? http://www.who.int/features/qa/15/en/print.html.6/12/2006. 13 May 2005.

Xu, R. S., Tang, Z. J., Feng, S. C., Yang, Y. P., Lin, W. H., Zhong, Q. X. & Zhong, Y. 1991. Studies on bioactive components from Chinese medicinal plants. *Memorias do Instituto Oswaldo Cruz* 86: 55–59.

Zhu, Y., Wang, A., Liu, M. C., Zwart, A., Lee, R. Y., Gallagher, A., Wang, Y., Miller, W. R., Dixon, J. M. & Clarke, R. 2006. Estrogen receptor alpha positive breast tumors and breast cancer cell lines share similarities in their transcriptome data structures. *International Journal of Oncology* 29: 1581–1589.