Antiproliferative Effect of *Strobilanthes crispus* on MCF-7 Cell Line

Norasyidah Gordani, Bo Eng Cheong, Peik Lin Teoh#
Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, MALAYSIA
*Corresponding author. E-Mail: peiklin@ums.edu.my; Tel: +6088-320000.

ABSTRACT Despite the advancement of chemical biology and combinatorial chemistry, natural products remain a potent source of the anticancer drug development today. *Strobilanthes crispus* (*S. crispus*) which is from the Acanthaceae family has been traditionally used as medicine in several countries and reported to have anticancer, antioxidant, free radical scavenging, antidiabetic, antimicrobial, wound healing and antiulcerogenic activities. Thus, the aim of this study was to investigate the antiproliferative properties of *S. crispus* towards breast cancer cell line. The chemical compounds were extracted from various parts of the plant using methanol then followed by liquid-liquid partition. The antiproliferative effects of these extracts were tested on MCF-7. Among the extracts, only five showed inhibition of cell proliferation in MCF-7. The best antiproliferative activity was observed in stem ethyl acetate and leaf water extract with the IC_{50} value of 38 µg/ml and 23 µg/ml respectively. However, the IC_{50} values for stem chloroform, leaf methanol and leaf chloroform extracts were at the range of 70-90 µg/ml. Treatment with *S. crispus* extracts also caused morphological changes on MCF-7 cells. Chromatin condensation and peripheral aggregation of nuclear chromatin were observed in the treated cells. However, further investigation is needed to understand its underlying mechanisms.

KEYWORDS: *Strobilanthes crispus*; anticancer property; Breast cancer; MCF-7; antiproliferation

Full Article - *Medical biotechnology*
Received 30 August 2017 **Online** 28 November 2017

INTRODUCTION

Breast cancer is the most common cancer among women and listed as one of ten leading cancer among population of Malaysia. The highest incidence of breast cancer was in 2006 and 2007. The number of breast cancer cases was continuously increased each year from 2006 to 2012 (Omar & Ibrahim Tamin, 2011). Surgery, chemotherapy, radiation therapy and hormone therapy are among the treatments available for cancer patients. Although surgery remains the main choice for cancer treatment (Yip *et al*., 2014), some patients prefer to consume medicine for prolonged period of time as adjuvant therapy. However, most of the medicines are synthetic, very expensive and tend to give many side effects.

Natural cancer treatment with the use of natural products plays a relevant role in cancer therapy today as they have minimal side effects and capable of reducing the side effects caused by synthetic drugs (Ho, 2015). *Strobilanthes crispus* is also known as pecah beling, pecah kaca, bayam karang or jin batu in Malaysia. *S. crispus* is from the Acanthaceae family and has been used as traditional medicine in Malaysia and Indonesia. The *S. crispus* is reported to be traditionally consumed by orang asli in Kampung Bawong, Perak of West Malaysia to enhance the immune system (Samuel *et al*., 2010). Previous studies showed that *S. crispus* has anticancer, antioxidant, free radical scavenging, antidiabetic, antimicrobial, wound healing and antiulcerogenic activities (Nurraihana & Norfarizan, 2013). Thus, the aim of this study was to examine the antiproliferative activity of Sabah *Strobilanthes crispus* against breast cancer cell line.

METHODOLOGY

Plant extracts preparation

The *Strobilanthes crispus* plants were purchased from herbal supplier in Kota Kinabalu and were verified by a botanist from Tropical Biology and Conservation Institute, University Malaysia Sabah (Voucher no. ACSC 001/2013). The plants were washed with tab water and left to dry before the leaves and stem were separated. Then, the plant parts were freeze dried separately for few days before ground into powder. Powdered plant was stored at -80°C for further use. The chemical compounds were first extracted using methanol solvent for 13 hours followed by liquid-liquid partition using hexane, chloroform, ethyl acetate and water solvents.

Cell culture

The *S. crispus* extracts were tested on hormone-dependent breast cancer cell line (MCF-7). The MCF-7 cell line was maintained in RPMI 1640 medium (Nacalai Tesque, Japan) containing 10% fetal bovine serum (Gibco, South America). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

MTT assay

The antiproliferative activity of *S. crispus* was screened using MTT Cell Proliferation Kit I (Roche Diagnostics, Germany). First, cell line was grown in 96-well mirotiter plates and treated with *S. crispus* extracts for 3 days. Then, the cultured cells were added with 10 µl of 3(4, 5-dimethyl-thiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) solution and incubated for 4 hours. Next, 100 µl of solubilization buffer was added to each well and incubated for overnight before being analyzed. Each extract was tested in triplicate and in three independent tests. The percentage of cell viability was calculated using the following formula.

Cell viability (%) = (Absorbance of treated cells/ Absorbance of untreated cells) x 100%

Morphological study

After treatment, medium from the treated cell culture were discarded and the cells were washed with phosphate-buffered saline (*P*BS) for two times. Then, 20 µl of m*e*thylene blue solution was added in each well and incubated for 20 minutes. Finally, the cells were washed with PBS before being observed under inverted microscope.

RESULT AND DISCUSSION

Figure 1 & 2 showed the effect of *S. crispus* extracts on the proliferation of MCF-7 cell line. Some *S. crispus* extracts had caused the decrease of MCF-7 cell viability after treatment. They were leaf water, leaf methanol, leaf chloroform, stem chloroform and stem ethyl acetate extracts.

The leaf water and stem ethyl acetate extracts showed the best inhibition against MCF-7 with the IC_{50} values of 23 µg/ml and 38 µg/ml respectively. However, the other three extracts showed lower inhibition with higher IC_{50} values of 86 µg/ml, 74 µg/ml and 80 µg/ml respectively for stem chloroform, leaf methanol and leaf chloroform extracts. All the IC_{50} values were summarized in Table 1. Minimal or no inhibition was observed in leaf hexane, leaf ethyl acetate and stem water extracts. The stem methanol extract inhibited cell growth at about 25% at 90 µg/ml. Besides that, stem hexane extract showed 20-40% inhibition when the concentration was gradually increased to 90 µg/ml.

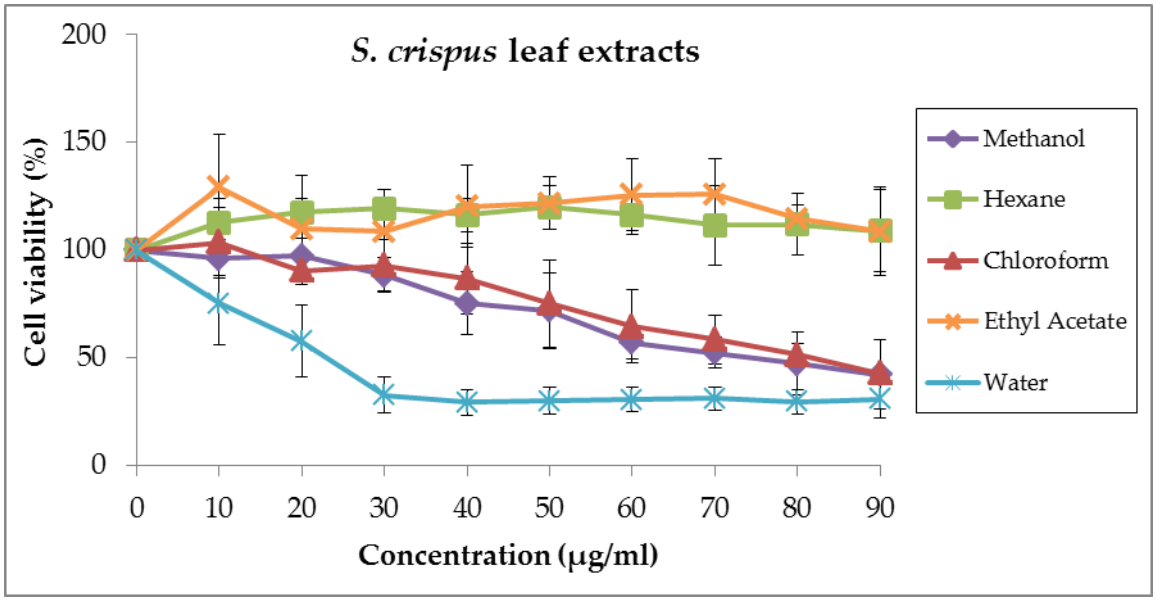


Figure 1. Effect of *S. crispus* leaf extracts on MCF-7 cells.

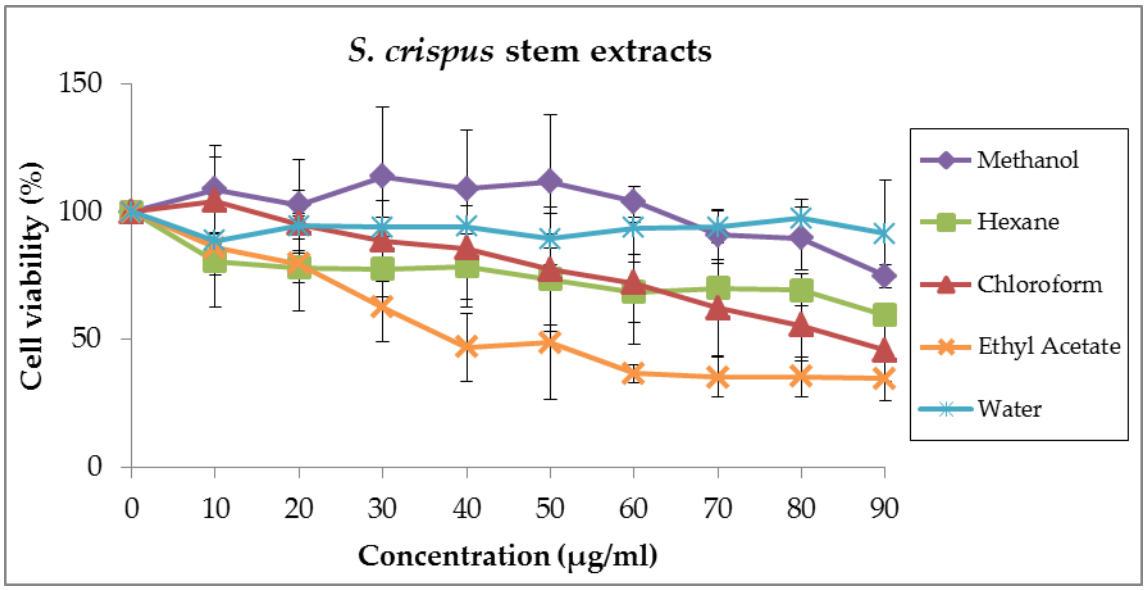


Figure 2. Effect of *S. crispus* stem extracts on MCF-7 cells.

Table 1. The IC_{50} values of *S. crispus* extracts against MCF-7 cell line.

Extracts	IC_{50} value (µg/ml)	
	S. crispus leaf	*S. crispus* stem
Methanol	74	-
Hexane	-	-
Chloroform	80	86
Ethyl acetate	-	38
Water	23	-

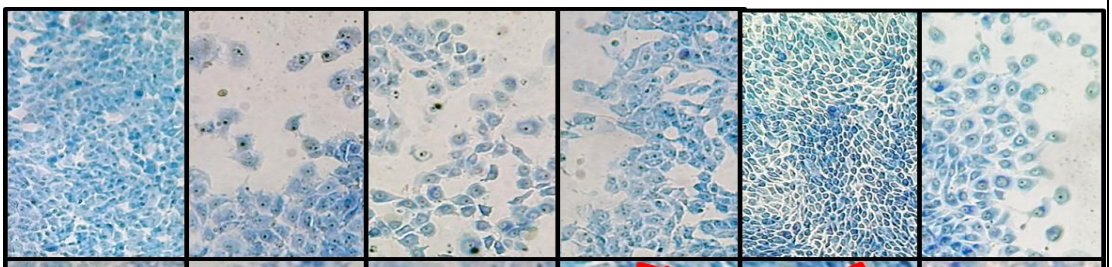
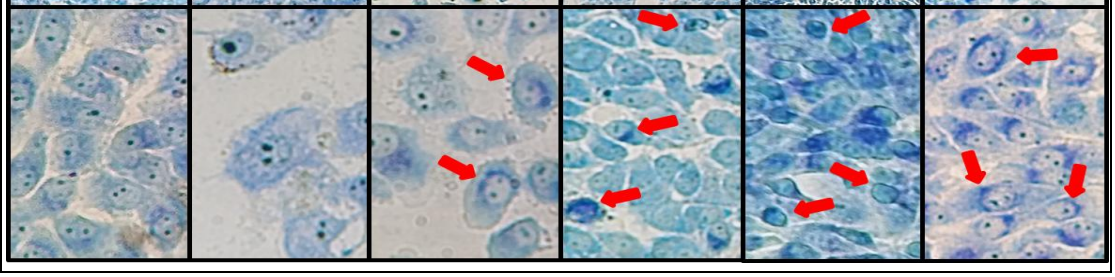
Magnification	Extracts					
	Control	Leaf methanol (40 µg/ml)	Leaf chloroform (80 µg/ml)	Leaf water (20 µg/ml)	Stem chloroform (80 µg/ml)	Stem ethyl acetate (40 µg/ml)
10x						
40x						

Figure 3. Effect of *S. crispus* extracts on the cell morphology of MCF-7.

Figure 3 showed the morphology of MCF-7 cell line treated with leaf methanol, leaf chloroform, leaf water, stem chloroform and stem ethyl acetate extracts with their respective IC_{50} values. Treatment with *S. crispus* extracts caused significant decrease in cell density of the MCF-7 cells. The *S. crispus* extracts were also caused morphological changes to MCF-7 cells such as the induction of chromatin condensation and peripheral aggregation of nuclear chromatin (red arrows). This indicates that these extracts might induce the occurrence of apoptosis in MCF-7 cells.

To sum up, in this study the leaf water and stem ethyl acetate extracts showed significant inhibitory effects towards MCF-7. However, the leaf methanol extract of *S. crispus* was reported to exhibit cytotoxic effects towards MDA-MB-231 and MCF-7 with IC_{50} values of 27.2 µg/ml (Asmah *et al*., 2006) and 160.16 µg/ml respectively (Muslim *et al*., 2010). Previous studies have also shown that *S. crispus* extracts possessed antiproliferative activities against other cancer cell lines such as HepG-2, Caco-2 (Asmah *et al*., 2006; Susi *et al*., 2007) and T-47D (Muslim *et al*., 2010). While Chong *et al*. (2012) reported that ethanol extracts induced apoptosis in MCF-7 with the IC_{50} value of 30 µg/ml.

The discrepancies in the cytotoxic studies could be due to the difference in extraction methods as well as geographical region. The geographical region is one of the factors that can influence the plant chemical constituents profile (Kole, 2011). For instance, *S. crispus* plants used in Asmah *et al*. (2006) and Chong *et al*. (2012) studies were from Peninsular Malaysia while Muslim *et al*. (2010) used the *S. crispus* plants originated from Padang, Indonesia. Whereas *S. crispus* used in this study was sourced from Sabah. The choice of extraction methods is also a very important step in the bioactive component discovery. It has been known that extraction process and solvent composition affected the yield and phytochemical constituents of the plant (Bimakr *et al*., 2011; Moreno *et al*., 2003; Sulaiman *et al*., 2015). Chong *et al*. (2012) used exhaustive solvent extraction method with absolute ethanol while Muslim *et al*. (2010) used maceration method with methanol. In this study, two steps extraction method was used which involved soxhlet extraction using methanol then followed by liquid-liquid partition with solvents of increasing polarity.

CONCLUSION

The leaf water and stem ethyl acetate extracts of *S. crispus* showed antiproliferative activity against MCF-7 cell line which indicates its potential use in chemotherapy. Therefore, further investigate is needed to understand the mechanism of how these extracts prevent cancer cell growth.

ACKNOWLEDGEMENTS

Authors thank Universiti Malaysia Sabah and Ministry of Higher Education (FRGS0360-SG2/2013) for the financial support

REFERENCES

[1] Al-Henhena, N., Mahmood, A. A., Al-Magrami, A., Nor Syuhada, A. B., Zahra, A. A., Summaya, M. D., Suzi, M. S. & Salmah, I. (2011). Histological study of wound healing potential by ethanol leaf extract of *Strobilanthes crispus* in rats. *Journal of Medicinal Plants Research*, **5**(16), 3660-3666.

[2] Asmah, R., Susi, E., Abdah, M. A., Patimah, I., Taufiq Yap, Y. H. & Mohd Fadzelly, A.B. (2006). Anticarcinogenic properties of *Strobilanthes crispus* extracts and its compounds *in vitro*. *International Journal of Cancer Research*, **2**(1), 47 - 49.

[3] Bimakr, M., Rahman, R. A., Taip, F. S., Ganjloo, A., Salleh, L. M., Selamat, J., Hamid, A. & Zaidul, I. S. M. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata L.*) leaves. *Food and bioproducts processing*, **89**(1), 67-72.

[4] Chong, H. Z., Asmah, R., Yeap, S. K., Abdah, M. A., Noorjahan, B. A., Fauziah, O. & Gwendoline-Ee, C. L. (2012). *In vitro* cytotoxicity of *Strobilanthes crispus* ethanol extract on hormone dependent human breast adenocarcinoma MCF-7 cell. *BMC Complementary and Alternative Medicine*, **12** (1), 35.

[5] Ho, W. S. (2015). *Active phytochemicals from chinese herbal medicines: anti-cancer activities and mechanisms*. CRC Press, 6.

[6] Kole, C. (Ed.). (2011). Wild crop relatives: genomic and breeding resources: tropical and subtropical fruits. *Springer Science & Business Media*, 159.

[7] Moreno, A. O., Dorantes, L., Galíndez, J. & Guzmán, R. I. (2003). Effect of different extraction methods on fatty acids, volatile compounds, and physical and chemical properties of avocado (*Persea americana Mill*.) oil. *Journal of Agricultural and Food Chemistry*, **51**(8), 2216-2221.

[8] Muslim, N. S., Ng, K. W., Itam, A., Nassar, Z. D., Ismail, Z. & Abdul Majid, A. M. S. (2010). Evaluation of cytotoxic, anti-angiogenic and antioxidant properties of standardized extracts of *Strobilanthes crispus* leaves. *International Journal of Pharmacology*, **6**(5), 591 - 599.

[9] Nohara, K., Wang, F. & Spiegel, S. (1998). Glycosphingolipid composition of MDA-MB-231 and MCF-7 human breast cancer cell lines. *Breast cancer research and treatment*, **48**(2), 149-157.

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

[11] Omar, Z. A. & Ibrahim Tamin, N. S. (2011). National cancer registry report: Malaysia cancer statistics-data and figure. *Malaysia: National Cancer Registry*, 85-87.

[12] Samuel, A. J. S. J., Kalusalingam, A., Chellappan, D. K., Gopinath, R., Radhamani, S., Husain H. A, Muruganandham, V. & Promwichit, P. (2010). Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. *Journal of Ethnobiology and Ethnomedicine*, **6**(1), 1.

[13] Sulaiman, N., Idayu, M. I., Ramlan, A. Z., Fashya, M. N., Farahiyah, A. N., Mailina, J. & Azah, M. N. (2015). Effects of extraction methods on yield and chemical compounds of gaharu (*Aquilaria malaccensis*). *Journal of Tropical Forest Science*, 413-419.

[14] Susi, E., Asmah, R., Patimah, I. & Taufiq-Yap, Y.H. (2007). Comparing of the cytotoxicity properties and mechanism of *Lawsonia inermis* and *Strobilanthes crispus* extract against several cancer cell lines. *Journal of Medical Science*, **7**(7), 1098 - 1102.

[15] Yip, C. H., Bhoo, P. N. & Teo, S. H. (2014). A review of breast cancer research in Malaysia. *Med J Malaysia*, **69**, 8-12.