Anticancer study of *Carissa carandas* extracts

Shaida Fariza Sulaiman, Wong Soo Teng, Ooi Kheng Leong, Siti Rafidah Yusof and Tengku Sifzizul Tengku Muhammad

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang.

ABSTRACTS

Carissa carandas extracts were screened for their anticancer activity. A three step extraction protocol using *n*-hexane, chloroform and methanol as the solvent systems was carried out on the leaves, the unripe and ripe fruits of *Carissa carandas*. In the present study, plant extracts were tested for their effectiveness as anticancer agent on the human ovarian carcinoma, Caov-3 and the lung cancer cells, NCI. Chloroform extract from leaves showed good anticancer activity against the Caov-3 with the EC_{50} value of 7.702µg/ml while the *n*-hexane extract of the unripe fruit exhibit a remarkable activity towards the NCI with the EC_{50} value of 2.942µg/ml when assayed using the methylene blue assay (MBA).

INTRODUCTION

Ovarian cancer is the fourth leading cause of cancer death and the most frequent cause of death from gynecologic malignancy (Greene *et al.*, 1984). The annual worldwide incidence of ovarian cancer exceeds 140 000. Ovarian cancer rates vary enormously between countries and appear to relate to their respective reproductive patterns (Byrom and Davies, 2003). Meanwhile, lung cancer death is the most common cause of cancer death in the world for both men and women. It is estimated that 163 510 people will die from lung cancer in 2005 worldwide and the major risk factor for lung cancer is evidently cigarette smoking (http://www.oncolink.upenn.edu).

Plants have many phytochemicals with various bioactivities, including anticancer activity. For example, some studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer, compared with chemotherapy or recent hormonal treatments (Pezzuto, 1997; Wu *et al.*, 2002). Studies also indicated that β-carotene, may be of benefit in the treatment of precancerous conditions such as leukoplakia (precursor of oral cancer) (Langseth, 1995). Many other plants have been examined to identify new and effective anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis (Swammy and Tan, 2000). Therefore, the aim of the present study was to evaluate the anticancer activity of *Carissa carandas* extracts.

MATERIALS AND METHODS

Preparation of extracts

Leaves, unripe and ripe fruits of *Carissa carandas* were cleaned and dried in the drying cabinet (Protech, Malaysia). The materials (30g) were subjected to *n*-hexane, followed by chloroform and methanol extraction using soxhlet apparatus. The extracts were concentrated using rotary evaporator (Eyela, U.K.) and evaporated to dryness in a fume cupboard. Fresh samples of ripe fruits were squeezed to get the juice. The extracts were dissolved in DMSO to a final concentration that ranging from 1.5625 to 100μg/ml.

Cell lines and culture medium

The Caov-3 (human ovarian carcinoma) and NCI (lung cancer) cell lines were purchased from American Type Culture Collection (ATCC, Rockville, USA). Caov-3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) while the NCI cells were cultured in the RPMI 1640 Medium. All media were supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 100U/ml penicillin, 100μg/ml streptomycin solution and 4mM L-glutamine. The cell cultures were maintained in an incubator containing 5% (v/v) CO_2 at 37°C.

In-vitro cytotoxicity assay

Confluent stock cultures of cells were harvested with 0.05% (v/v) Trypsin-EDTA and plated onto 96-well plates (Costar, USA) at cell density of approximately 6000 cells/well. Cell viability before plating was routinely determined by Trypan blue exclusion test. The cells were allowed to attach and incubated for 24-48 hours. When the cells reached confluency between 80-90%, the medium was replaced with fresh medium containing only 0.5% (v/v) FBS. The cells were incubated for another 4 hours, for the cells to achieve quiescent state. The cells were then treated with different concentrations of *n*-hexane, chloroform and methanol extracts from *Carissa carandas* (1.5625 to 100 μg/ml). Control cells were cultured in 0.5% (v/v) FBS-containing medium. Vincristine sulphate was used as positive control. After treatment, the plates were incubated for 72 hours.

Cell survival was determined by using methylene blue staining method (Yamazaki *et al.*, 1986). Briefly, glutaraldehye was added to each well to a final concentration of 2.5% (v/v) and the surviving cells were fixed for 15 min. After washing with 0.15M sodium chloride and removing the dead cells, the fixed cells were stained with 0.1ml of 0.05% (w/v) methylene blue solution for 15 minutes. After washing off the excess dye with 0.15M sodium chloride solution, dye elution was carried out with 0.2ml of 0.33M HCl. Absorbance was then read at 650nm using Vmax Kinetic Microplate Reader (Molecular Devices, USA).

Calculations and statistics

Experiments were performed in six replicates. Results were expressed as percentage growth inhibition of control. EC_{50} values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using Graphpad Prism version 3.00, Graphpad Software (San Diego, USA). Data were expressed as mean ± S.E.M.

RESULTS AND DISCUSSION

The inhibition of Caov-3 and NCI cells poliferation after treatment with extracts by using methylene blue assay (MBA) (Yamazaki *et al.*, 1986) were analyzed in order

to study the potential anticancer effect of *Carissa carandas* extracts. The effective concentrations required for 50% inhibition of cell poliferation (EC_{50}) were measured. The criterion of cytotoxic or non-cytotoxic was adapted from the guidelines set by the National Cancer Institute, United States of America. The screening protocol has indicated that plant or animal extracts with $EC_{50} \leq 20\mu g/ml$ were considered to be cytotoxic and non-cytotoxic if otherwise (Geran *et al.*, 1972).

The *n*-hexane and methanol extracts of *Carissa carandas* leaves produced a very low level of growth inhibitory activity against Caov-3 cell lines with less than 10% growth inhibition for every concentration used. However, only chloroform leaves extract appeared to be cytotoxic with EC_{50} at 7.702μg/ml (**Figure 1**). As for ripe fruits of *Carissa carandas*, all extracts except chloroform exhibited poor activity with less than 30% growth inhibition and undetermined EC_{50}. Chloroform extract performed the best activity among the ripe fruit extracts with EC_{50} value of 23.42μg/ml. Meanwhile, the *n*-hexane extract of the unripe fruits act as the best anticancer agent towards the NCI cell lines with the EC_{50} value of 2.942μg/ml (**Figure 2**) in comparison with chloroform and methanol extracts of the unripe fruits which EC_{50} values were 8.422μg/ml and 24.220μg/ml respectively.

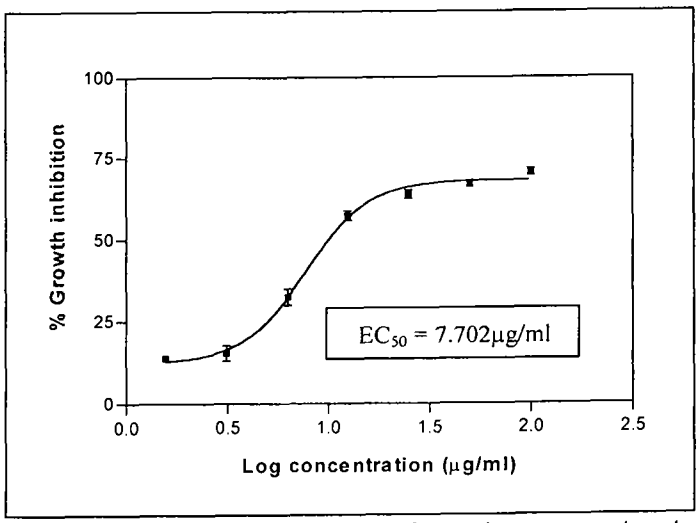

Figure 1 Growth inhibition curve of chloroform leaves extract against Caov-3. Each value represents mean ± S.E.M. of six replicates (n=6).

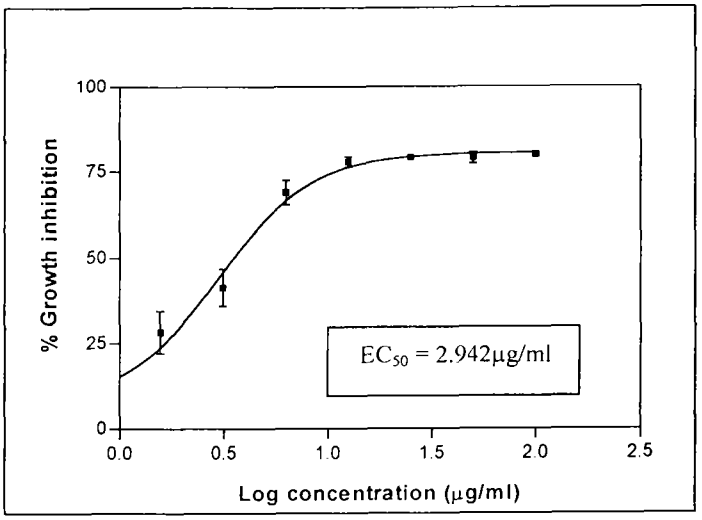

Figure 2 Growth inhibition curve of *n*-hexane unripe fruits extract against NCI. Each value represents mean ± S.E.M. of six replicates (n=6).

CONCLUSION

The study has shown that the chloroform extract of *Carissa carandas* leaves exhibited cytotoxicity on human ovarian carcinoma cell line (Caov-3) with EC_{50} value of 7.702µg/ml, while the *n*-hexane extract of the unripe fruits is cytotoxic towards the lung cancer cell line (NCI) with EC_{50} value of 2.942µg/ml when assayed using methylene blue assay (MBA). The chloroform extract of the unripe fruits is also cytotoxic on NCI with slightly higher EC_{50} value than the *n*-hexane extract of the same organ. Other extracts were non-cytotoxic.

REFERENCES

Byrom, J. and Davies, Q. 2003. Cancer of the ovary. *Current Obstetrics & Gynaecology* **13** : 88-94.

Geran, R.I., Greenberg, N.H., MacDonald, M.M., Schumacher, A.M. and Abott, B.J. 1972. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemotherapy Reports* **3(2)** : 1-16.

Greene, M., Clark, J. and Blayney, D.W. 1984. The epidemiology of ovarian cancer. *Semin. Oncol.* **11** : 209-226.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. *Journal of Food Science* **58(6)** : 1407-1410.

Langseth, L. 1995. *In Oxidants, antioxidants, and disease prevention.* International Life Sciences Institute, ILSI Press, pp 1-23.

Lee, J.Y., Hwang, W.I. and Lim, S.T. 2004. Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De Candolle roots. *Journal of Ethnopharmacology* **93** : 409-415.

Mohd Zin, Z., Abdul Hamid, A. and Osman, A. 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry* **78** : 227-231.

Ottolenghi, A. 1959. Interaction of ascorbic acid and mitochondria lipids. *Arch. Biochem. Biophys.* **79** : 355-363.

Pezzuto, J.M. 1997. Plant-derived anticancer agents. *Biochemical Pharmacology* **53** : 121-133.

Swammy, S.M.K. and Tan, B.K.H. 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *Journal of Ethnopharmacology* **70** : 1-7.

Wu, J., Wu, Y. and Yang, B.B. 2002. Anticancer activity of *Hemsleya amabilis* extract. *Life Sciences* **71** : 2161-2170.

Yamazaki, S., Onishi, E., Enami, K., Natori, K., Kohase, M., Sakamoto, H., Tanouchi, M. and Hayashi, H. 1986. Proposal of standardized methods and reference for analyzing recombinant human tumor necrosis factor. *Japanese Journal of Medical Sciences and Biology* **39** : 105-118.

http://www.oncolink.upenn.edu