Malaysian Journal of Science (24): 207-212 (2005)

Cytotoxic Activity of Selected Zingiberaceae

L. R. Ling[1], N. Abdul Wahab[2] and N. Zainal Abidin[1]

[1] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2] Centre for Foundation Studies in Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Abstract Zingiberaceae is a well-known plant family in Southeast Asia that have been widely used as spices and for medicinal purposes throughout the world. In this preliminary study, crude petroleum ether, chloroform and methanol extracts obtained from ten selected Zingiberaceae were screened for possible cytotoxic activity against CaSki cells by using an *in vitro* neutral red cytotoxicity assay. The results demonstrated that all extracts tested showed cytotoxic effect on CaSki cells, a cervical cancer derived cell line. The trend observed was that the percentage of killing increased with the concentration of extracts tested. Fifteen extracts (50%) were found to be cytotoxic with ED_{50} values equal to or less than 20 μg/ml. The petroleum ether extract of *Languas galanga* with an ED_{50} value of 3.8 μg/ml, chloroform extract of *Curcuma domestica* with an ED_{50} value of 2.0 μg/ml and methanol extract of *Curcuma xanthorrhiza* with an ED_{50} value of 13.8 μg/ml were the most active extracts for each solvent used. Overall, petroleum ether and chloroform extracts of Zingiberaceae showed greater cytotoxic activity against CaSki cells compared to methanol extracts. However, the mechanism of action has not been investigated in the present study. Zingiberaceae species have great potential to be exploited for the search of novel anticancer agents for the future.

Abstrak Zingiberaceae merupakan satu famili tumbuhan yang terkenal di Asia Tenggara yang kerap digunakan sebagai rempah dan untuk tujuan perubatan di seluruh dunia. Dalam kajian ini, penyaringan aktiviti sitotoksik terhadap sel CaSki bagi ekstrak petroleum eter, klorofom dan metanol yang diperoleh daripada sepuluh Zingiberaceae terpilih dengan menggunakan esei *in vitro* neutral merah telah dijalankan. Keputusan kajian menunjukkan bahawa kesemua ekstrak memberi kesan sitotoksik terhadap sel CaSki, iaitu sel yang terbitkan dari kanser servik. Tren keputusan menunjukkan bahawa kenaikan peratus membunuh selaras dengan kepekatan ekstrak kajian. Antara ekstrak Zingiberaceae yang diuji, lima belas ekstrak (50%) adalah sitotoksik dengan bacaan ED_{50} bersamaan atau kurang daripada 20 μg/ml. Ekstrak paling aktif untuk setiap pelarut yang digunakan adalah ekstrak petroleum eter daripada *Languas galanga* dengan nilai ED_{50} 3.8 μg/ml, ekstrak klorofom daripada *Curcuma domestica* dengan nilai ED_{50} 2.0 μg/ml dan ekstrak metanol daripada *Curcuma xanthorrhiza* dengan nilai ED_{50} 13.8 μg/ml. Secara keseluruhan, ekstrak petroleum eter dan klorofom Zingiberaceae menunjukkan aktiviti sitotoksik yang lebih tinggi terhadap sel CaSki berbanding dengan ekstrak metanol. Namun demikian, tindakan mekanisme yang berkaitan belum dikaji lagi. Zingiberaceae mempunyai potensi untuk digunakan sebagai agen anti-kanser yang baru pada masa akan datang.

(Zingiberaceae, Cytotoxicity, CaSki cells)

INTRODUCTION

In recent years, considerable attention has been focused on herbal medicine which is based on the premise that plants contain natural substances that can promote health and alleviate illness. The World Health Organization estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components [1].

Research reported that 80 to 90% of all cancers are attributable to environmental risk factors, such as chemicals, radiations and viruses [2]. Cervical cancer is the second most common

cancer in women worldwide [3]. According to the Malaysian National Registry launch report [4] cervical cancer was found to be the second most frequent neoplasm (12%) after breast cancer (30.4%) among Malaysian females in 2002. Therefore, the effort in search of new cancer chemopreventive compounds, particularly those derived from medicinal or edible plants is necessary to prevent the development of cervix carcinoma.

Cancer chemoprevention is a novel concept which involves the use of natural, dietary or pharmaceutical agents to delay, inhibit or reverse the development of cancer before malignancy occurs [5]. The primary goal of cancer chemoprevention research is to identify effective agents for clinical trials and ultimately, application to human populations in order to reduce the incidence of human cancer [2].

The Zingiberaceae is one of the largest family from the order Zingiberales and it is predominantly found in the tropical Asia. The Zingiberaceae comprises about 1200 species in which 30-40 species of the Zingiberaceae have long been used in traditional herbal medicine [6, 7]. In Malaysia, Zingiberaceae is frequently used as spices, food preservatives, colouring agents and cooking ingredients in the society. Various ginger rhizobia provide health-promoting effects and have been utilized to treat certain illnesses such as nausea, motion sickness, stomache, asthma, diarrhea, digestive disorder, vomiting, rheumatism, swelling, common cold, cough and other disorders [2, 8, 9].

Several studies have revealed that the members of the Zingiberaceae family consist of a wide variety of active phytochemicals and possess antioxidative, anti-inflammatory, anticancer and anti-tumour promoter activity [10, 11, 12, 13]. [6]-Paradol is a pungent phenolic compound present in the rhizome of ginger (*Zingiber officinale*) with marked anti-inflammatory effects. As inflammation is closely associated with carcinogenesis, particularly in the stage of promotion, thus this compound is considered a potent cancer chemopreventive agent. [6]-Paradol has been reported to attenuate promotion of mouse skin carcinogenesis and ear edema in female ICR mice. Besides, it also exerted ability to induce apoptosis in cultured human promyelocytic leukemia (HL-60) cells [12]. A few bioactive components isolated from nonvolatile fraction of the dichloromethane extract of ginger rhizomes have exhibited a strong antioxidative activity using linolenic acid as the substrate in ethanol-phosphate buffer solution. Among the five gingerol related compounds and eight diarylheptanoids purified by chromatographic techniques, 12 compounds exhibited higher antioxidative activity than α-tocopherol [14].

Curcumin, a yellow odorless pigment isolated from the rhizome of turmeric (*Curcuma longa*) has exerted significant inhibitory effects on 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamsters. The results showed that curcumin has retarded the progression of existing precancerous lesions and the growth of tumors in the oral mucosa [15]. Research also reported that *in vitro* curcumin induces apoptosis in myelogenous leukemia cell line HL-60 through mitochondrial pathway [13].

Recent researches have indicated the high potential of edible Southeast Asian plants for cancer chemoprevention. Among 135 methanol extracts from 48 plant families (107 species) of edible plants, the Zingiberaceae have been found to be one of the desirable sources of effective cancer-preventive agents because of the high frequency of inhibitory activity towards Epstein-Barr Virus activation induced by 12-o-hexadecanoylphorbol-13-acetate (HPA) in Raji cells [16]. Seven ginger rhizomes, namely *Curcuma domestica, Curcuma xanthorrhiza, Zingiber officinale, Zingiber officinale* (red variety), *Zingiber cassumunar, Zingiber zerumbet* and *Kaempferia galanga* were found to express Epstein-Barr Virus early antigen (EBV-EA) activation inhibitory activity, induced by 12-O-tetradecanoyl phrobol-13-acetate (TPA) in Raji cells. The inhibition of TPA-induced EBV-EA was detected by using the indirect immunofluroscence assay and Western blot technique [11].

Many of the consumable ginger rhizobia were used as folk medicine for centuries but yet have not been thoroughly investigated for their bioactive properties. Hence, the aim of the present study was to screen for the cytotoxic effect of crude petroleum ether, chloroform and methanol extracts of selected Zingiberaceae by using an *in vitro* neutral red assay using CaSki cells.

MATERIALS AND METHODS

Plant material

Rhizomes of ten species from the Zingiberaceae, namely *Zingiber officinale* (Halia), *Zingiber zerumbet* (lempoyang), *Zingiber montanum* (Bonglai), *Curcuma domestica* (kunyit), *Curcuma mangga* (Temu Pauh), *Curcuma xanthorrhiza* (Temu Lawak), *Curcuma aeruginosa* (Temu Hitam), *Languas galanga* (Lengkuas), *Kaempferia galanga* (Cekur) and *Boesenbergia pandurata* (Temu Kunci) were collected from a local market selling local herbs.

Preparation of crude extracts

The fresh Zingiberaceae rhizomes were washed, dried at 50°C and ground into fine powder. Twenty gramme of each sample was extracted using petroleum ether, chloroform and methanol successively by the Soxhlet extractor system. The resultant extract obtained was evaporated and dissolved in dimethylsulphoxide (DMSO) at a stock concentration of 20 mg/ml and stored at -20°C until use.

Neutral Red Cytotoxicity Assay

The cytotoxicity was evaluated against CaSki (human cervix carcinoma) cells according to protocol previously described [17, 18]. In brief, CaSki cells were maintained in RPMI 1640 medium supplemented with 10% foetal bovine serum, 100 µg/ml penicillin/streptomycin and 50 µg/ml of amphostat B. The cells were maintained in continuous exponential growth by twice a week passage. CaSki cells at a density of 5 x 10^4/ml were plated into 96 well microtitre plate and were incubated to achieve 60-70% confluence at the time of the addition of crude extracts in a humidified incubator at 37°C with 5% CO_2 in atmosphere. Different concentrations of the crude extracts (1, 10, 50 and 100 µg/ml) were added into each well (three wells per concentration, with untreated control wells). The CaSki cells were incubated at 37°C for additional 72 h. After the incubation period, the cells were stained with neutral red (50 µg/ml) and were incubated for 3 h to allow maximum uptake of dye by the surviving cells. The CaSki cells were rapidly washed with washing solution (mixture of 1.0 v/v% formaldehyde and 1.0 w/v% Calcium chloride). The neutral red dye was extracted from the viable cells with resorb solution (1.0 w/v% glacial acetic acid in a mixture of ethanol : distilled water) and the microtitre plate was left to stand at room temperature for 15 minutes. The microtitre plate was gently agitated on a microplate shaker for 30 minutes and the optical density (OD) was determined spectrophotometrically at 540 nm by using a multi-well plate reader.

RESULTS AND DISCUSSIONS

In the present study, thirty crude Zingiberaceae extracts were evaluated for their cytotoxic activities against CaSki cells by using *in vitro* neutral red assay. The advantages of *in vitro* cytotoxicity tests over *in vivo* studies, such as speed, economy in funds and animals, increased sensitivity and reproducibility of test conditions are apparent [19]. The CaSki cells were incubated with crude petroleum ether, chloroform and methanol extracts from selected Zingiberaceae ranging from 1 µg/ml to 100 µg/ml for 72 hours to determine the cytotoxic effect of these plant extracts. The negative control consisted of untreated CaSki cells with growth medium. The average data from triplicates were expressed in terms of killing percentage relative to negative controls. The dose-response curves for each extract was plotted in Figure 1-3 and ED_{50} values obtained were summarised in Table 1. The results were expressed as ED_{50} values which refer to the concentrations of crude extracts (µg/ml) that inhibited 50% of cell growth. The ED_{50} values, being determined from the centre of the dose-response curve for each crude extract (Figure 1-3), is considered to be more reliable than values based [18].

Based on the data presented in Table 1, all crude extracts tested showed some cytotoxic effect on CaSki cells. The trend observed was that the percentage of killing rose as the concentration of extracts increased. Among thirty extracts, only the methanol extract of *Kaempferia galanga* giving percentage of killing less than 50% at concentration of 100 µg/ml. Therefore, the ED_{50} value for this particular extract cannot be determined from the dose-response curve (Figure 5). Of these crude Zingiberaceae extracts, fifteen extracts (50%) tested were found active in which extracts having an ED_{50} equal to or less than 20 µg/ml are considered active for cytotoxicity assay against CaSki cells [20].

The petroleum ether extract of *Languas galanga* with ED_{50} value of 3.8 µg/ml, chloroform extract of *Curcuma domestica* with ED_{50} value of 2.0

µg/ml and methanol extract of *Curcuma xanthorrhiza* with ED_{50} value of 13.8 µg/ml were the most active extract for each solvent used. Results showed that *Curcuma xanthorrhiza* exhibited the most potent cytotoxic effect among ten species of Zingiberaceae chosen with ED_{50} values less than 20 µg/ml for all petroleum ether, chloroform and methanol extracts. Overall, petroleum ether and chloroform plant extracts of Zingiberaceae demonstrated greater cytotoxic activity against CaSki cells compared to methanol extracts. However, the mechanism of action has not been investigated in the present study.

Despite major advances being achieved in the treatment of malignancy through radiotherapy and chemotherapy, the search for naturally occuring cancer chemoprevention compounds is always the focus of research worldwide. Chemoprevention of cancer are often applied in a primary prevention setting aiming to inhibit occurrence of malignancy among healthy individuals. It was used in a secondary prevention setting hoping to reverse or delay the carcinogenesis process in individuals bearing premalignant tumors [12]. Naturally occurring substances that block or suppress the proliferation of tumour cells are potentially potent antitumour agents. Anticancer agents from edible plants would have an advantage in their clinical application on account of low toxicity [11]. Plants of ginger family is among the most frequently and heavily consumed dietary condiments throughout the world. Thus, further work on the isolation and identification of active compounds with potent antitumour properties from Zingiberaceae would be worthwhile to investigate.

Table 1. ED_{50} value of Zingiberaceae against CaSki cells

Plant		ED_{50} (µg/ml)
	Extract	
Halia *Zingiber officinale*	Petroleum ether Chloroform Methanol	25.3 24.7 17.8
Lempoyang *Zingiber zerumbet*	Petroleum ether Chloroform Methanol	12.2 14.1 79.4
Bonglai *Zingiber montanum*	Petroleum ether Chloroform Methanol	4.4 25.8 26.9
Temu Pauh *Curcuma mangga*	Petroleum ether Chloroform Methanol	21.3 13.8 88.8
Temu Hitam *Curcuma aeruginosa*	Petroleum ether Chloroform Methanol	10.6 16.6 47.5
Kunyit *Curcuma domestica*	Petroleum ether Chloroform Methanol	26.6 2.0 40.6
Temu Lawak *Curcuma xanthorrhiza*	Petroleum ether Chloroform Methanol	12.5 18.4 13.8
Cekur *Kaempferia galanga*	Petroleum ether Chloroform Methanol	45.6 31.3 -
Lengkuas *Languas galanga*	Petroleum ether Chloroform Methanol	3.8 4.1 83.8
Temu Kunci *Boesenbergia pandurata*	Petroleum ether Chloroform Methanol	8.1 20.0 27.8

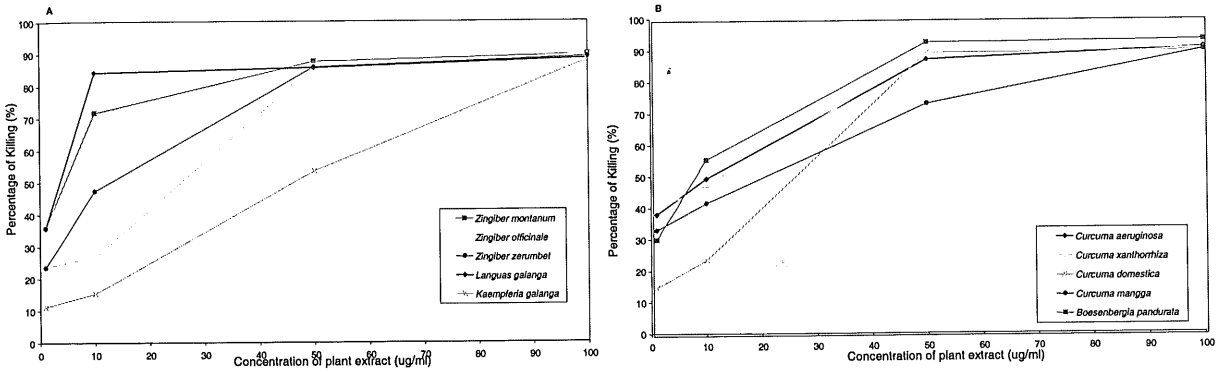

Figure 1. Cytotoxic activity of *Zingiberaceae sp.* petroleum ether extracts

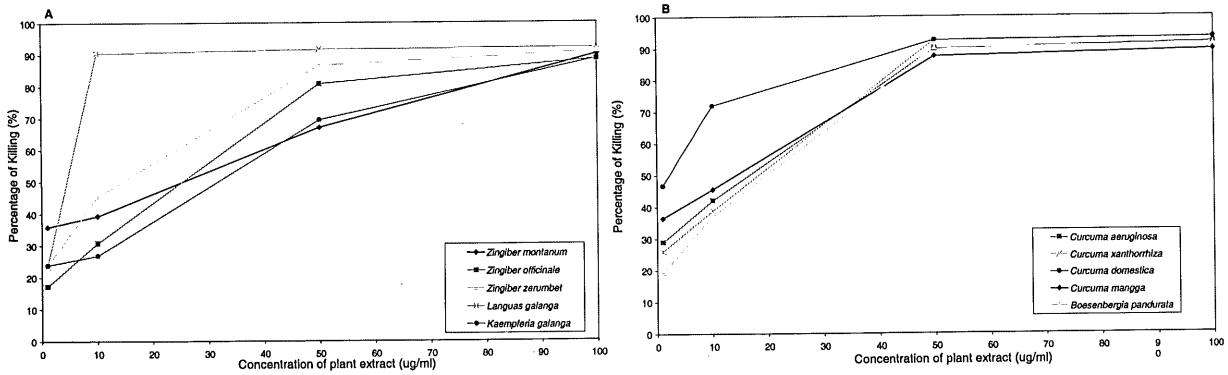

Figure 2. Cytotoxic activity of *Zingiberaceae sp.* chloroform extracts

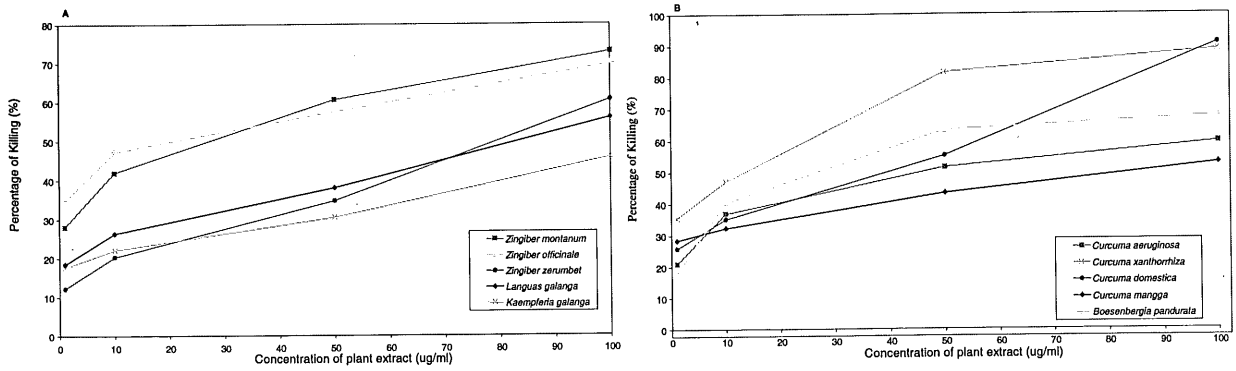

Figure 3. Cytotoxic activity of *Zingiberaceae sp.* methanol extracts

Acknowledgements We would like to thank Ms. Sujatha a/p Ramasamy, Mr. Mohd. Azrul and Ms. Chan Pek Yue for their technical assistance. This research was supported by Vote-F, University of Malaya.

REFERENCES

1. Craig, W. J. (1999). *The American Journal of Clinical Nutrition*. 70(3):491S-499S.
2. Surh, Y. J. (1999). *Mutation Research/ Genetic Toxicology and Environmental Mutagenesis*. 428(1-2):305-327.
3. Nobbenhuis M. A. E., Walboomers, J. M. M., Helmerhorst, T. J. M., Rozendaal, L., Remmink, A. J., Risse, E. K. J., Linden, H. C. V. D., Voorhorst, F. J., Kenemans, P. and Meijer, C. J. L. M. (1999). *The Lancet*. 354(9172):20-25.
4. National Cancer Registry Launch Report (2003) Ministry of Health, Malaysia.
5. Chang, L. C., Song, L. L., Park, E. J., Lumonadio, L., Lee, K. J., Farnsworth, N. R., Pezzuto, J. M. and Kinghorn, A. D. (2000). *Journal of Natural Product*. 63:1235-1238.
6. Halijah, I. and Ahmad A.R. (1988) *Proceedings Malaysian Traditional Medicine*. Kuala Lumpur,Malaysia, pp.159-161.
7. Larsen, K., Ibrahim, H., Khaw, S. H. and Saw, L. G. (1999). *Natural History Publications (Borneo), Malaysia*.
8. Kikuzaki, H. (2000). Pacific Agri-Food Research Centre, Summerland, BC, Canada.
9. Young, H. Y., Chiang, C. T., Huang, Y. L., Pan, F. P. and Chen, G. L. (2002). *Journal of Food and Drug Analysis*. 10(3):149-153.
10. Hasnah, M. S. (1991). *Proceedings of the medicinal products from tropical rain forests*. Kuala Lumpur, Malaysia, pp.299-304.
11. Vimala, S., Norhanom, A. W., Yadav, M. (1999). *British Journal of Cancer*. 80(1/2):110-116.
12. Chung W. Y., Jung, Y. J., Surh, Y. J., Lee, S. S. and Park, K. K. (2001). *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 496(1-2):199-206.
13. Anto, R. J., Mukhopadhyay, A., Denning, K. and Aggarwal, B. B. (2002). *Carcinogenesis*. 23(1):143-150.
14. Kikuzaki, H. and Nakatani, N. (1993). *Journal of Food Science*. 58(6):1407-1410.
15. Li, N., Chen, X. X., Liao, J., Yang, G. Y., Wang, S., Josephson, Y., Han, C., Chen J. S., Huang, M. T. and Yang C. S. (2002). *Carcinogenesis*. 23(8):1307-1313.
16. Murakami, A., Morita, H., Safitri, R., Ramlan, A., Koshimizu, K. and Ohigashi, H. (1998). *Cancer Detection and Prevention*. 22(6):516-525.
17. Knox, P., Uphill, P. F., Fry, J. R., Benford, J. and Balls, M. (1986). *Fd Chem. Toxic*. 24(6/7):457-463.
18. Riddell, R.J., Panacer, D.S., Wilde, S.M., Clothier, R.H. and Balls, M. (1986). *ATLA*. 14: 86-92.
19. Stark, D. M., Shopsis, C., Borenfreund, E. and Babich, H. (1986). *Fd. Chem. Toxic*. 24(6/7):449-455.
20. Geran, R. I., Greenberg, N.H., McDonald, M. M., Schumacher, A. M. and Abbott, B. J. (1972). *Cancer Chemotherapy Reports*. 3:1-103.