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Testing of *Kaempferia galanga* extracts against cancer cell lines

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Abstract. Extracts of *Kaempferia galanga*, from the Zingerberaceae family, were screened for anti-tumour promoting and anti-tumour activities. The bioassay involved the inhibition of 12-*O*-tetradecanoylphorbol-13-acetate induced Epstein-Barr virus early antigen activation in Raji cells. KB cells were used for the anti-tumour screening. Chloroform extracts showed strong anti-tumour promoting activity against Raji cells (ED_{50} 38 μg mL^{-1}) and anti-tumour activity against KB cells (ED_{50} 7.6 μg mL^{-1}). Petroleum ether extracts were active against the KB cells (ED_{50} 8.2 ug/mL) but the methanol extracts was not active .

Abstrak. Ekstrak dari tumbuhan *Kaempferia galanga*, daripada famili Zingerberaceae, telah disaring untuk aktiviti anti-penggalak tumor dan aktiviti anti-tumor. Bioasei melibatkan perencatan antigen awal virus Epstein-Barr dalam sel Raji yang telah diaktifkan dengan 12-*O*-tetradekanoilforbol-13-asetat. Sel KB digunakan untuk penyaringan anti-tumor. Ekstrak kloroform menunjukkan aktiviti anti-penggalak tumor terhadap sel Raji (ED_{50} 38 μg mL^{-1}) dan aktiviti anti-tumor terhadap sel KB (ED_{50} 7.6 μg mL^{-1}). Estrak petroleum eter adalah aktif terhadap sel KB (ED_{50} 8.2ug/mL) tetapi ekstrak metanol adalah tidak aktif .

Introduction

Anti-tumour substances from plants have played an important role in the development of anti-tumour agents and these compounds are effective against carcinogens as well as against tumour promoters. Vegetables and fruits contain some of these useful chemical compounds [1,2].

Several members of the Zingerberaceae family, namely, *Zingiber mioga* and *Zingiber officinale*, have been reported to exhibit anti-tumour and anti-tumour promoter activity [1]. In the present study, the edible parts of *Kaempferia galanga*, locally known as cekur or cekur jawa, were tested for their anti-tumour promoting and anti-tumour properties. Chloroform, petroleum ether and methanol extracts were tested against the human lymphoblastoid Raji cell line and the human laryngeal carcinoma KB cell line. The performance of the extracts were compared by using the indirect immonufluorescence assay (IFA) results from the inhibition of the Epstein-Barr virus (EBV) early antigen (EA) expression in Raji cells as well as the ED_{50} value from the KB cells mortality curves.

Experimental

Chemicals. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co., St. Louis, USA. Sodium *n*-butyrate was obtained from BDH Chemicals Ltd, England. TPA was dissolved in DMSO and kept in liquid nitrogen (-196°C). The TPA solution was further diluted to final a concentration of 10 ng mL^{-1} for the experiments.

Cell lines. The human lymphoblastoid Raji cells were obtained from the National Cancer Institute. Bethesda, USA, and were grown in RPM1 1640 culture medium. The KB cells of the laryngeal carcinoma were provided by the Institut de Chimie des Substances Naturelles (ICSN), Centre National de la Recherche Scientifique (Cnrs) of Paris, France, and were grown in Medium 199 with Earle's salt.

Plant extracts. Edible parts of *Kaempferia galanga* were air dried and ground to a fine powder. The extraction was carried out on a soxlet apparatus and the solvents used were petroleum ether, chloroform followed by methanol. The three extracts were then dried and dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution of 20 mg mL^{-1} and stored at –20°C.

Raji cells bioassay. The Raji cells were maintained in RPMI 1640 supplemented with 10% inactivated foetal calf serum (FCS), 0.2g L^{-1} L-glutamine, 100 µg mL^{-1} streptomycin, 100 IU mL^{-1} penicillin and 50 µg mL^{-1} fungizone. The tissue culture reagents were obtained from Flow Lab., Australia. The medium was filter sterilized by using a 0.22 µm filter membrane (Gelman Sc., Australia) and stored at 4°C up to 3 weeks.

Raji cells (1 x 10^6 cell mL^{-1}) in the rapidly dividing log phase at a density were incubated with the plant extracts at concentrations ranging from 10 to 200 µg mL^{-1} in the presence of 20 ng mL^{-1} TPA and 4 mM mL^{-1} sodium *n*-butyrate. The Raji cells were incubated for 72 hours in a 5% CO_2 humidified incubator at 37°C, and were harvested after the third day by centrifugation at 1000 rpm for 5 minutes. The cells were washed twice with phosphate buffered saline (PBS). The cells were re-suspended in PBS and dispensed onto the wells of teflon-coated multi-well slides. The slides were air dried and later fixed in cold acetone at –20°C for 10 minutes. EBV-EA expression was detected by means of the IFA [3-5]. EBV-EA positive serum, obtained from nasopharyngeal carcinoma (NPC) patients, was dispensed onto the well of the acetone fixed slides. The slides were incubated in a humidified chamber at 37°C for 45 minutes and rinsed three times with PBS for 5 minutes. Fluorescein isothiocynate conjugated IgG (Behringwerke AG, France), was added to each well, which was re-incubated in the humidified chamber for 45 minutes. At the end of the incubation, the slides were again washed in PBS. The slides were dried and mounted with glycerol-PBS buffer (9:1) and the number of positive (fluorescing) cells were counted under a UV-microscope (Olympus, Japan).

KB cells bioassay. The KB cells were maintained in Medium 199 with supplemented 10% FCS. The medium was changed a day before testing. The confluent growth of cells was washed twice with PBS and treated with 0.25% trypsin (Amresco, USA) for 5 minutes [6]. The plant extracts at the concentrations of 1, 10 and 100 µg mL^{-1} were added to the cells [7]. The cells were then incubated at 37°C in a humidified atmosphere with 5% CO_2 for 72 hours. At the end of the incubation, Neutral Red dye was added to each flask [6,8]. The cells were then rinsed twice with PBS and 1% sodium dodecyl sulphate (SDS) was added to each flask. After one hour of contact, the optical density was read at 540 nm on a UV/VIS spectrophotometer. The ED_{50} value for each extract was determined from the mortality curve.

Results

In the present study, TPA, a tumour promoter, was used as an inducer of EBV-EA [9]. The inhibitory activity of the crude extracts of Kaempferia galanga on EBV EA expression in Raji cells is shown in Figure 1. The chloroform extract showed stronger anti-tumour promoter activity when compared with the petroleum ether and methanol extracts. The chloroform extract exerted 50% inhibitory activity when tested at 38 µg mL^{-1} and 100% inhibitory activity at 100 µg mL^{-1}. The petroleum ether and methanol extracts exerted 50% inhibitory activity at 63 and 102 µg mL^{-1}.

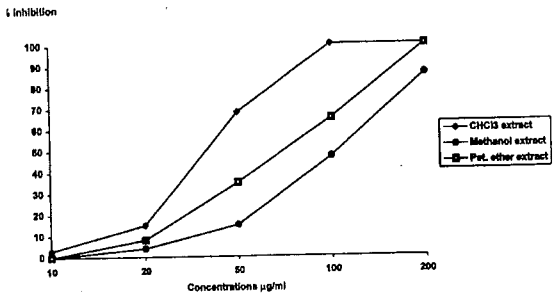

Figure 1. Inhibition of Epstein-Barr virus early antigen expression in Raji cells by extracts of *Kaempferia galanga.*

The results of the ED_{50} for the three extracts on KB cells are shown in Figure 2. The chloroform and petroleum ether extracts demonstrated ED_{50} values of 7.6 and 8.2 μg mL^{-1}. According to Geran *et al.* [7], an activity against KB cells at 20 μg mL^{-1} and below can be considered as the compound being active. The methanol extract demonstrated an ED_{50} value of 77 μg mL^{-1}, i.e, the chloroform extract was most active when in the two bioassays.

The results of the present study showed that the crude petroleum ether and chloroform extracts of *Kaempferia galanga* demonstrated *in vitro* anti-tumour promoter activity when tested against EBV EA expression in Raji cells and anti-tumour activity when tested against KB cells. The petroleum ether extract showed some activity against KB cells. The present results compare well with other studies that reported that *Kaempferia galanga* strongly inhibited the TPA stimulated phospholipid synthesis in Raji cells [10].

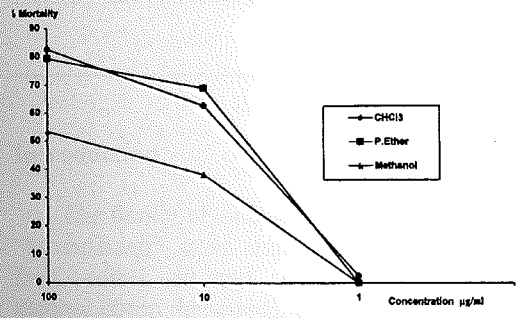

Figure 2: Cytotoxic effect of *Kaempferia galanga* extracts on KB cells.

Kaempferia galanga is a herb of Indian origin and it cultivated throughout Malaysia. The plant is popular among the Malays as its carminative expectorant juice can be used in many medicaments. The leaves are prepared ass lotions and poultices for ailments such sore throats, fevers, swellings, rheumatisms and sore eyes [11]. The plant is also used to flavour rice or eaten fresh as a salad. However, there has been no study in Malaysia to correlate the intake of Kaempferia galanga with the reduction of incidence of cancer.

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