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# Anti-tumor Promoting Activity of Some Malaysian Traditional Vegetables (Ulam)

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**Abstract** – Ethanolic extracts of different parts of 10 local traditional vegetables (ulam) (*Amaranthus gangeticus, Jussiaea linifolia, Eugenia polyantha, Trapa incisa, Trichosanthes anquina, Mangifera indica, Pachyrrhirus erosus, Barringtonia mcarostachya, Carica papaya*, and *Coleus tuberosus*) were screened for *in vitro* anti-tumor promoting activity using the inhibition test of Epstein-Barr virus (EBV) activation in Raji cells induced by phorbol 12-myristate 13-acetate and sodium-*n*-butyrate. All the extracts were found to have strong inhibition activity toward EBV-activation, except for leaf extract of *T. anquina*. The extracts were non-cytotoxic to the Raji cells except for the extracts of *A. gangeticus* (leaves), *B. macrostachya* (leaves), *E. polyantha* (young leaves), and *J. linifolia* (leaves) where the viability of the cells were decreased significantly.
**Key words** – Cancer chemoprevention, Anti-tumor promotion, Epstein-Barr virus activation, Raji cells, Traditional vegetables

## Introduction

Studies on medicinal properties of food plants, i.e. plants consumed as food by humans or used for culinary purposes, is of growing importance. The main reasons for this are firstly, the propagation of the concept of functional foods, *viz.* foods that cure, ameliorate or prevent disease, and secondly, the biologically active components in food plants are presumed to be of low toxicity relative to non-edible, usually toxic, medicinal plants. The chemical constituents of many common food plants have been extensively reported. Consequently, chemical investigations would be expected to yield known constituents and thus, from a chemistry perspective, be of limited significance. The flora of Malaysia comprises about 15,000 species of higher plants, which includes a group of food plants classified as traditional vegetables, locally known as ulam (Kiew & Lajis, 1996). There are more than 120 species of ulam belonging to several families, ranging from herbs to trees (Mansor *et al.*, 1988). The roles of ulam as functional food plants or a source of functional food phytochemicals are attractive in the Malaysian context because of its wide consumption among the local people especially of the Malay and indigenous communities (Mackeen *et al.*, 1997). The 'ulam' is mostly eaten raw as salad, particularly the leaves, or otherwise blanced, sauteed, curried and fried (Bautista *et al.*, 1988; Mansor *et al.*, 1988; Mackeen *et al.*, 1997).

Cancer prevention research has been based on the concept of multistage carcinogenesis: initiation, promotion and progression. Among these stages, tumor promotion takes a long time to occur and is the only reversible stage in the multistage carcinogenesis (Murakami *et al.*, 1997). Research in recent years has strengthened the inverse association between cancer rates and the consumption of fruits and/or vegetables (Wei *et al.*, 1990). As an intensive screening test to search for the suppressive potentials of vegetables and fruits for tumor promotion using a convenient short-term *in vitro* assay, the Epstein-Barr virus

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(EBV) induced by phorbol 12-myristate 13-acetate and sodium *n*-butyrate activation has been conducted by many researchers (Ito *et al.*, 1981). The screening test done for the edible Thai plants suggested higher cancer preventive potentials of edible Thai plants, particularly, the plant families of Rubiaceae, Zingiberaceae, Labiatae and Piperaceae were demonstrated to contain potent anti-tumor promoters (Murakami *et al.*, 1995). For instance, auraptene, coumarin-related compound has been isolated from *Citrus natsudaidai* and shown to have strong anti-tumor promoting activity (Murakami *et al.*, 1997).

Therefore, the objective of this study is to highlight on the inhibition of the promotion stage of some Malaysian traditional vegetables using short-term *in vitro* assay system.

## Materials and Methods

**Chemicals** – Phorbol 12-myristate 13-acetate (PMA) (Sigma) was used as an inducer of the EBV activation. Sodium *n*-butyrate was added to enhance the EBV activation. Early antigen (EA)-positive sera from nasopharyngeal carcinoma (NPC) patients were obtained from Prof. Dr. Umapati Prasad of University of Malaya and FITC-labeled IgG was purchased from Sigma.

**Sample preparation** – Fresh vegetables were obtained from wet market around Universiti Putra Malaysia. The samples were cut into small pieces and macerated in 80% ethanol at room temperature for a week. The crude extracts were then filtered and evaporated *in vacuo* at 45°C using rotary evaporator. The extracts were dissolved in 100% ethanol or dimethyl sulfoxide (DMSO) at the concentrations of 200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, and 6.25 μg/ml.

**Inhibitory assay of Epstein-Barr virus activation** – Raji cells ($5\times10^5$ cells/ml) were maintained in 1 ml of RPMI 1640 medium supplemented with 10% fetal calf serum containing sodium *n*-butyrate (3 mM), phorbol 12-myristate 13-acetate (0.05 μM) and test extract (5 μl) at 37°C under 5% $CO_2$ for 48 hours. EA expressed in Raji cells were detected by an indirect immunofluorescence method with EA-positive sera from NPC patients and FITC-labeled anti-human IgG as described by Murakami *et al.* (1995). The average EA induction was compared to a control with only PMA and sodium *n*-butyrate, the induction rate of the control was less than 40%.

## Results and Discussion

In the present study, the phorbol 12-myristate 13-acetate (PMA, 5 μM) and 3 mM of sodium *n*-butyrate were used as an inducer of the EBV-EA activation in Raji cells. The inhibitory effect (IE) of each tested crude extract towards the EBV activation was classified into four ranks by the inhibitory rate of early antigen (EA)-induced cells. These are strongly active (+++; IE > 69%), moderately active (++; 70% > IE > 49%), weakly active (+; 50% > IE > 29%), and inactive (–; 30% > IE). Additionally, cell viability (CV) was also classified as non-toxic (CV > 69%), moderately toxic (70% > CV > 29%), and highly toxic (CV < 30%) (Murakami *et al.*, 1993).

The inhibitory activity of the 17 crude extracts of the leaf, stem, root, tuber, flesh, skin of fruit, fruit and flower was assayed in the ethanol solution (Table 1). The plants tested were *A. gangeticus* (leaf, root, stem), *J. linifolia* (leaf, root, stem), *E. polyantha* (old leaf, young leave), *T. incisa* (leaf), *T. anquina* (fruit), *M. indica* (skin of fruit, flesh), *P. erosus* (tuber), *B. macrostachya* (leaf), *C. papaya* (flower) and *C. tuberosus* (tuber). All the samples except *T. incisa* exhibited the strongest inhibitory activity at the concentration of 200 μg/ml. The inhibition rates and the cell viability of the 17 samples were 74.76%, 98.5% (*A. gangeticus,* leaf), 73.46%, 98.96% (*A. gangeticus,* root), 82.70%, 0% (*A. gangeticus,* stem), 78.59%, 96.3% (*J. linifolia,* root), 71.94%, 94.53% (*J. linifolia,* stem), 95.49%, 57.59% (*J. linifolia,* leaf), 72.28%, 98.33% (*E. polyantha,* old leaf), 100%, 33.7% (*E. polyantha,* young leaf), 59.50%, 93.1% (*T. incisa,* leaf), 78.76%, 95.54% (*T. anquina,* fruit), 82.86%, 97.11% (*M. indica,* flesh), 100%, 81.9% (*M. indica,* skin of fruit), 73.80%, 100% (*P. erosus,* tuber), 100%, 0% (*B. macrostachya,* leaf), 87.55%, 92.75% (*C. papaya,* flower), and 94.41%, 97.1% (*C. tuberosus,* tuber).

On the other hand, extracts from *A. gangeticus, J. linifolia, E. polyantha,* and *M. indica* were found to show different rank of inhibitory activity attained from the different part of the same species. This suggested that plants may synthesize the organic compounds during particular stages of growth and development (Mann, 1986). The anti-tumor promoting substances are thus distributed unevenly within the same species. The inhibition rates of the extracts were compared at the minimum concentration of 6.25 μg/ml. The crude extract of *A. gangeticus* (stem) dis-

**Table 1.** Anti-tumor promoting properties of traditional vegetables (Ulam) and fruits

| Family/ Scientific name | Vernacular name | Part used | Amount of crude extract (µg/ml) | Inhibitory effect (%) | Cell viability (%) |
|---|---|---|---|---|---|
| **Amaranthus** | | | | | |
| *Amaranthus gangeticus* Linn. | Bayam merah | Root | 200 | 74.76 | 98.50 |
| | | | 100 | 61.02 | 99.03 |
| | | | 50 | 57.21 | 99.20 |
| | | | 25 | 55.25 | 100 |
| | | | 12.5 | 51.58 | 100 |
| | | | 6.25 | 40.24 | 100 |
| | | Stem | 200 | 73.46 | 98.96 |
| | | | 100 | 65.71 | 100 |
| | | | 50 | 54.22 | 100 |
| | | | 25 | 53.38 | 100 |
| | | | 12.5 | 51.55 | 100 |
| | | | 6.25 | | 50.52 |
| | | Leaves | 200 | 82.70 | 0 |
| | | | 100 | 76.98 | 50.75 |
| | | | 50 | 63.38 | 61.67 |
| | | | 25 | 46.93 | 86.08 |
| | | | 12.5 | 29.21 | 95.18 |
| | | | 6.25 | 6.07 | 98.65 |
| **Onagraceae** | | | | | |
| *Jussiaea linifolia* Vahl | Maman pasir | Root | | 200 | 78.59 |
| | | | 100 | 69.54 | 97.71 |
| | | | 50 | 65.17 | 97.83 |
| | | | 25 | 63.56 | 95.45 |
| | | | 12.5 | 63.39 | 92.24 |
| | | | 6.25 | 59.04 | 94.05 |
| | | Stem | 200 | 71.94 | 94.53 |
| | | | 100 | 68.35 | 97.54 |
| | | | 50 | 64.75 | 97.44 |
| | | | 25 | 62.04 | 97.12 |
| | | | 12.5 | 52.77 | 95.06 |
| | | | 6.25 | 48.20 | 91.78 |
| | | Leaves | 200 | 95.49 | 57.59 |
| | | | 100 | 32.59 | 67.72 |
| | | | 50 | 29.81 | 74.68 |
| | | | 25 | 24.20 | 86.21 |
| | | | 12.5 | 20.75 | 94.78 |
| | | | 6.25 | 15.00 | 90.91 |
| **Myrtaceae** | | | | | |
| *Eugenia polyantha* Wight | Serai kayu | Old leaves | 200 | 72.28 | 98.33 |
| | | | 100 | 62.58 | 96.00 |
| | | | 50 | 53.91 | 100 |
| | | | 25 | 34.56 | 100 |
| | | | 12.5 | 26.16 | 100 |
| | | | 6.25 | 17.37 | 100 |

**Table 1.** Continued

| Family/ Scientific name | Vernacular name | Part used | Amount of crude extract (μg/ml) | Inhibitory effect (%) | Cell viability (%) |
|---|---|---|---|---|---|
| | | Young leaves | 200 | 100 | 33.70 |
| | | | 100 | 66.87 | 82.40 |
| | | | 50 | 55.33 | 95.30 |
| | | | 25 | 33.16 | 97.50 |
| | | | 12.5 | 15.85 | 95.50 |
| | | | 6.25 | 7.03 | 97.90 |
| **Onagraceae** | | | | | |
| *Trapa incisa* | Tomior | leaves | 200 | 59.50 | 93.10 |
| | | | 100 | 56.23 | 100 |
| | | | 50 | 55.98 | 100 |
| | | | 25 | 55.67 | 99.27 |
| | | | 12.5 | 45.24 | 100 |
| | | | 6.25 | 42.80 | 98.18 |
| **Cucurbitaceae** | | | | | |
| *Trichosanthes anguina* Linn. | Tola ular | Fruit | 200 | 78.76 | 95.45 |
| | | | 100 | 76.22 | 97.18 |
| | | | 50 | 75.45 | 98.78 |
| | | | 25 | 70.72 | 98.41 |
| | | | 12.5 | 66.74 | 98.95 |
| | | | 6.25 | 66.57 | 99.14 |
| **Anacardiaceae** | | | | | |
| *Mangifera indica* Linn. | Mempelam epal | Flesh | 200 | 82.86 | 97.11 |
| | | | 100 | 79.43 | 97.96 |
| | | | 50 | 75.03 | 97.76 |
| | | | 25 | 73.04 | 99.11 |
| | | | 12.5 | 71.90 | 96.95 |
| | | | 6.25 | 68.51 | 99.11 |
| | | Skin of fruit | 200 | 100 | 81.90 |
| | | | 100 | 77.37 | 98.90 |
| | | | 50 | 73.62 | 98.70 |
| | | | 25 | 59.31 | 100 |
| | | | 12.5 | 57.46 | 100 |
| | | | 6.25 | 25.44 | 97.70 |
| **Leguminosae** | | | | | |
| *Pachyrrhizus erosus* Urban | Sengkuang | Tuber | | | |
| | | | 100 | 53.54 | 100 |
| | | | 50 | 46.06 | 97.44 |
| | | | 25 | 45.08 | 97.87 |
| | | | 12.5 | 40.11 | 97.83 |
| | | | 6.25 | 38.82 | 100 |
| **Lecythidaceae** | | | | | |
| *Barringtonia macrostachya* Kurz | Putat | Leaves | 200 | 100 | 0 |
| | | | 100 | 75.93 | 91.60 |
| | | | 50 | 54.54 | 97.70 |
| | | | 25 | 52.21 | 97.50 |
| | | | 12.5 | 50.30 | 97.70 |
| | | | 6.25 | 41.17 | 98.60 |
| **Caricacease** | | | | | |
| *Carica papaya* Linn. | Betik rampai | Flower | 200 | 87.55 | 92.75 |
| | | | 100 | 72.46 | 92.41 |
| | | | 50 | 71.56 | 96.47 |
| | | | 25 | 65.45 | 95.19 |
| | | | 12.5 | 52.35 | 93.85 |
| | | | 6.25 | 42.06 | 98.51 |
| **Labiatae** | | | | | |
| *Coleus tuberosus* Benth. | Ubi kemili | Tuber | 200 | 94.41 | 97.10 |
| | | | 100 | 83.01 | 99.26 |
| | | | 50 | 78.57 | 100 |
| | | | 25 | 69.65 | 100 |
| | | | 12.5 | 62.40 | 99.05 |
| | | | 6.25 | 56.99 | 99.16 |

played the strongest inhibition activity, followed by the crude extract of the root and leaves. The values of the inhibition rates were 50.52%, 40.24%, and 6.07%, respectively. The cell viability of each sample was attained at 100%, 100%, and 98.65%. The crude extract of the root and stem of *J. linifolia* exhibited higher inhibitory activity than the crude extract of leaves of *J. linifolia,* the inhibitory activity at 6.25 µg/ml were 59.04%, 48.2%, and 15.00%, and the cell viability were obtained at 94.05%, 91.78%, and 90.91%, respectively. The young leaves of *E. polyantha* was indicated to have lower inhibition activity compared to the old leaves, the inhibition rates at 6.25 µg/ml of crude extract were 7.03% and 17.37%. Cell viability of both crude extracts were 97.9% and 100%. *M. indica*, the common fruit among Malaysians was also demonstrated to contain the potent anti-tumour promoting agents. The flesh which are eaten most showed stronger anti-tumor promoting activity than the skin of fruit. The inhibition rate and cell viability attained from the flesh were 68.51%, 99.11% at 6.25 µg/ml of crude extract, whilst the skin of fruit only showed 25.44%, 97.7% at 6.25 µg/ml of crude extract. Although the skin of *M. indica* fruit was always assumed not containing any valuable nutrient, yet this experiment has indicated that it showed 100% inhibitory activity at 200 µg/ml of crude extract, this suggested that it has the possibility to contain the anti-tumor promoting agents.

Among these plants, comparison between species demonstrating that species of *A. gangeticus* (stem), *J. linifolia* (root), *T. anquina* (fruit), *M. indica* (flesh) and *C. tuberosus* (tuber) showed very strong inhibitory activity towards EBV-EA activation. At the minimum concentration of 6.25 µg/ml, the inhibition activity of these species was more than the 50%. The rate of the inhibition activities were 50.52%, 59.04%, 66.57%, 68.51%, and 56.99%, respectively. The cell viability was attained at the percentage of 100, 94.05, 99.14, 99.11 and 99.16, respectively.

The plants that showed very strong anti-tumor promoting activity in this assay was also reported by Burkill (1966) to have medicinal value and other uses. The roots of *A. gangeticus* can be used to control haemorrhage. *J. linifolia* is one of the most generally stocked in Chinese herbalists shops. The infusion of the root may be swallowed by the Malays for syphilis. The very young, purple-brown leaves of *M. indica* are eaten by the Javanese with rice. Indian is using the mango flowers to cure diarrhoea. The seed that is bitter is used as a vermifuge in India, China and Malaysia. It is also used as an astringent, which is given for the obstinate diarrhoea and for bleeding piles. Tubers of *C. tuberosus* are starchy and have aromatic flavor. The leaves could be used as flavoring agent.

Epstein-Barr virus activation assay that directed in this study has revealed the anti-tumor promoting properties of traditional vegetable. Sixteen out of the 17 plant extracts were found to exhibit very strong inhibitory activity, in which their inhibition rate was more than 70%. In addition, the MIC values of the plant extracts demonstrated that the active agents of anti-tumor promoting activity distributed unevenly throughout the plant. Thereby, the MIC data obtained can assist the isolation of the active compounds from the major part of the plant that has been screened to contain the active compound. As reported by Murakami research group, besides giving us the nutrient and minerals, the daily intake of traditional vegetables and fruits also can partly provide the anti-cancer properties.

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