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Full Length Research Paper

Antiproliferative activity of aqueous extract of *Piper betle* L. and *Psidium guajava* L. on KB and HeLa cell lines

A. R. Fathilah[1]*, R. Sujata[2], A. W. Norhanom[2] and M. I. Adenan[3]

[1]Department of Oral Biology, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[3]Malaysia Institute of Pharmaceuticals and Nutraceuticals, 11900 Bukit Jambul, Penang, Malaysia.

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The aim of the present study was to assess the effect of the aqueous extracts of *Psidium guajava* and *Piper betle* plants on the proliferation of cancerous cell lines, that is, KB and HeLa cell line. Using the neutral red cytotoxicity assay, the IC_{50} of *P. guajava* and *P. betle* were determined at 29.0 ± 0.4 and 29.5 ± 0.3 µg/ml, respectively, indicating both plant extracts equally potent for the treatment of cancerous oral epidermal lesions. However, a less potent anti - proliferative activity was recorded by *P. guajava* towards HeLa cell line with an IC_{50} of 51.0 ± 0.6 µg/ml, whereas *P. betle* extract did not affect the proliferation of HeLa.

Key words: Antiproliferative, KB cells, heLa cells, *Psidium guajava, Piper betle.*

INTRODUCTION

Piper betle L. and *Psidium guajava* L. are popularly regarded as medicinal plants in the South East Asia region. The leaves of these plants are often used in the form of wet paste for external application or decoctions for internal consumption. Better known as betel leaf vine, *P. betle* come under the family of *Piperaceae*. In the tropical Asia and East Indies, betel leaves are often chewed together with a little quicklime and areca nut. An alkaloid in the nut is said to act as stimulant and tonic to the chewers (Indu et al., 2000). The leaves extract of *P. betle* have been reported to exhibit biological capabilities of detoxication, antioxidation and antimutation that suggested the chemopreventive potential of the extract against various ailments including liver fibrosis (Shun et al., 2007). Other activities include bacteriostatic and fungistatic effects against *Candida albicans, Pseudomonas aeruginosa, Aspergillus flavus* and *E. coli* (Indu et al., 2000). Some of the chemical constituents isolated from betel leaves include estragole, catechols, eugenols, terpenes, lominene and cardinene (Ponglux et al., 1987). *Psidium guajava* L. is the plant that bears the guava fruit and comes under the family of *Myrtaceae*. Various parts of the guava tree have been traditionally used as astringent for skin diseases, antidiarrheal, antidysentric and also as deodorant (Ponglux et al., 1987). Among the biological activities displayed by *P. guajava* extract includes antibacterial, antiviral, antiplaque, hypoglycaemic, antiscorbutic, estrous cycle disruption and antigonadotropin (Fathilah, 2005; de Oliveira et al., 2003; Hobert et al., 1998; Ponglux et al., 1987). Its chemical constituents include among others, tannins, vitamin C, proteins, cellulose, carbohydrates, acids, enzymes and essential oils components of acetone, zeatin, zeatin nucleotides and zeatin ribosides (Norlia, 1992; Ponglux et al., 1987). The aqueous and ethanolic extracts of *P. guajava* showed antioxidative activities in inhibiting lipid peroxidation (Wang et al., 2007) and has been associated with the decreased risk of cancer and cardiovascular diseases (Rahmat et al., 2006). In a recent study, essential oil from the leaves of the plant was reported to exhibit anticancer activities on

*Corresponding author. E-mail: fathilah@um.edu.my. Tel: +603-79674851. Fax: +603-79674536.

KB and P388 cell lines (Manosroi et al., 2006).

In the field of dentistry, the aqueous extracts of *P. betle* and *P. guajava* leaves have been shown to exhibit antiplaque activities that suggested their potential to be used as adjuvant in oral health care products. Both extracts were found to disrupt the early phase of plaque formation by altering the adhering properties of the acquired pellicle and at the same time reduces the cell - surface hydrophobicity of plaque bacteria that are involved in assisting the adhesion process (Fathilah et al., 2006; Fathilah, 2005; Fathilah et al., 2003). Anticaries property was also suggested by Nalina and Rahim (Nalina et al., 2007) for the extract of *P. betle*. In this study the aqueous extracts of *P. betle* and *P. guajava* leaves were tested for their effect on the proliferation of human nasopharyngeal epidermoid carcinoma (KB) and HeLa cell lines.

MATERIALS AND METHODS

Materials

P. betle leaves were purchased from a local market while the leaves of *P. guajava* were collected from a local farm. Aqueous extraction procedures were performed by boiling cleaned, fresh leaves of the plants in distilled water to about one tenth of the volume. The concentrated decoctions were aliquoted into microvials and dried overnight using a speed-vaccum concentrator (Heto LabEquipment). The dried *P. betle* leaves (PBL) and *P. guajava* leaves (PGL) extracts were then pooled, reweighed and prepared into stocks of 20 mg/ml for use in the experiments.

Cell cultures

The human nasopharyngeal epidermoid carcinoma (KB) and HeLa cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (FlowLab, Australia) supplemented with 10% foetal bovine serum (FBS) (FlowLab, Australia) and incubated in a humidified atmosphere of 5% CO_2 at 37ºC. After confluent, the cells were washed in phosphate-buffered saline (PBS) and then resuspended in 1 ml of the culture medium. The viable cells were then accounted using tryphan.

The cell proliferation assay

The cell proliferation was assessed by a colorimetric assay using neutral red as a dye as previously described (Borenfreund et al., 1984). Briefly, the cell lines at the concentration of 3×10^4 cells/well were plated in 24-wells culture plate. Using the stock extract (20 mg/ml), dilutions of PBL and PGL were made to varying concentrations of 0.1, 1, 10, 50 and 100 µg/ml in separate wells using dimethylsulfoxide (DMSO) (FlowLab, Australia) as diluents. The effect of the extracts on KB and HeLa cells was observed after an exposure period of 72 h at 37ºC and 5% CO_2. Wells containing HeLa and KB cells in the absence of the extracts represented the negative control for the test. After the culture medium was discarded, 100 µl neutral red (1% v/v) was added and the cell cultures were again incubated for 2 h. After washing, 1 ml of solution containing 1% sodium dodecyl sulphate was added and the plate was placed on a rocker for 30 min. The optical density of the cells in each well was measured at a wavelength of 540 nm by using a spectrophotometer (Shimadzu, Japan). The concentration of extract causing 50% cell death known as the inhibition concentration (IC_{50}) was determined by a graph of percentage of cell death versus concentrations of the plant extracts. The experiment was repeated three times in triplicates (n=9).

Statistical analysis

SPSS 17.0 was used in the statistical analysis and the Mann Whitney non-parametric test was performed to compare the antiproliferative activities of the two plants. The significance level was set at $p<0.05$.

RESULTS

As seen in Figure 1, both *P. betle* and *P. guajava* extracts exhibited equivalently potent antiproliferative activity towards KB cells with IC_{50} values of 29.5 ± 0.3 and 29.0 ± 0.4 µg/ml, respectively ($p = 0.013$). Comparatively, the antiproliferative activity of *P. guajava* on HeLa cells was significantly lower at an IC_{50} value of 51.0 ± 0.6 µg/ml ($p = 0.000$). Cytotoxic activity of *P. betle* on HeLa cells was not observed at 100 µg/ml, ($IC_{50} > 100$ µg/ml) (Figure 2).

DISCUSSION

Many plant components have been screened in the search for anticancer agents. It was reported that essential oil from the leaves of *P. guajava* exhibited the highest antiproliferative activity towards KB (IC_{50} = 37.9 µg/ml) and P388 (IC_{50} = 45.4 µg/ml) cells compared to sixteen other Thai medicinal plants screened (Monosroi et al., 2006). In this study, no such activity was recorded on the aqueous leaves extract of *P. betle* even when tested at a very high concentration of 4.96 mg/ml. In this study, both PBL and PGL aqueous leaves extracts were found to exhibit stronger antiproliferative activity towards human nasopharyngeal epidermoid carcinoma (KB) cells compared to their essential oils (Monosroi et al., 2006).

The anticancer properties of *P. betle* and *P. guajava* have been widely reported by many research groups. Significant anticancer activities displayed by *P. betle* have suggested its potential to be developed as drug and neutraceutical for the management of liver fibrosis. Shun et al. (2007) has reported that *P. betle* extract was able to significantly inhibit elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities caused by carbon tetrachloride-induced liver injury in a rat model. *Piper betle* extract was suggested to have acted by decreasing α-smooth muscle actin (α-sma) expression, inducing active matrix metalloproteinase-2 (MMP2) expression through Ras/Erk pathway, and eventually inhibiting tissue inhibitor of metalloproteinase 2 (TIMP2) levels that consequently attenuated the fibrosis of the liver. In addition to that the *P. betle* also attenuated total glutathione S-transferase (GST) and GST α isoform

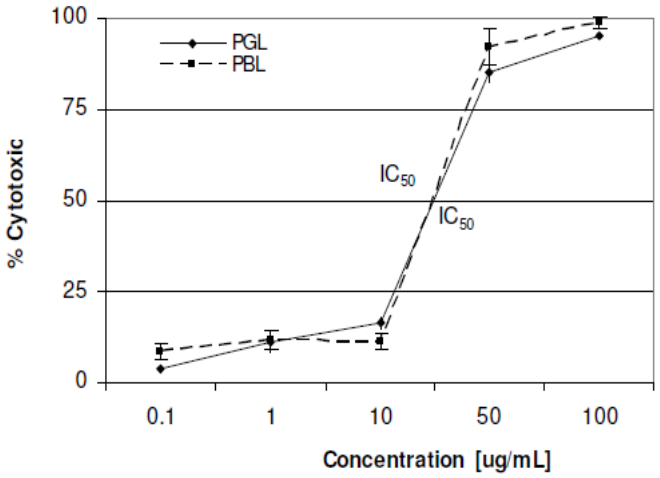

Figure 1. Cytotoxic effect of PBL (■) and PGL (♦) aqueous extracts on KB cells. Both extracts exhibited equivalent strength of antiproliferative activity towards KB cells with IC_{50} of 29.5±0.3 and 29.0±0.4 µg/ml, respectively.

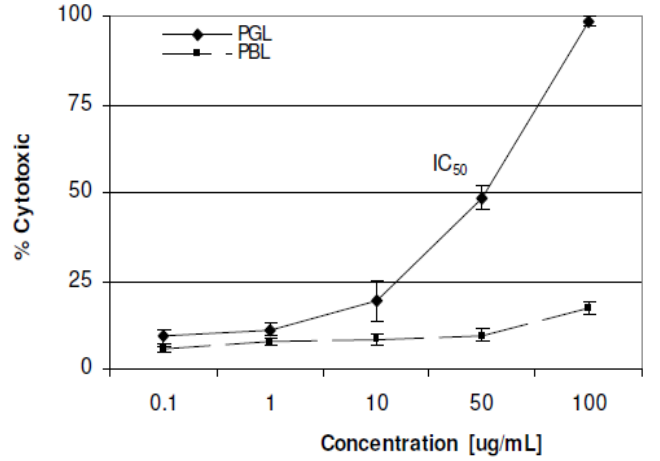

Figure 2. Cytotoxic effect of PBL (■) and PGL (♦) aqueous extracts on HeLa cells. PGL displayed moderate antiproliferative activity of 51.0±0.6 µg/ml on HeLa cells while PBL did not show any cytotoxic activity even at 100 µg/ml (IC_{50} > 100 µg/ml).

activities enhances superoxide dismutase (SOD) and catalase (CAT) activities (Shun et al., 2007). Another research group in Eastern Indian city has also claimed that a molecule in the PBL extract identified as chlorogenic acid targets and kills myeloid and lymphoid cancer cells but leaves other normal cells unaffected (IICB Report, 2004). The acid was shown to induce programmed cell death in human cancer cells transplanted in experimental nude mice and at the same time, showed no effect on the growth of non-cancerous cells. This has gives PBL extract great potential to be developed as a target-specific, therapeutic drug for blood cancer.

As for *P. guajava*, its organic extract has been reported to exhibit cytotoxic effect on human breast cancer cells (MCF7) at 24 h with an IC_{50} of 55 µg/ml. Immunomodulatory activity was also reported by this extract on NFκB, a nuclear transcription factor that regulates the expression of various genes involved in apoptosis and immunomodulation (Kaileh et al., 2007). The extract of this plant has also been reported to exert antitumor effect by inhibiting the function of T regulatory cells in mice (Seo et al., 2003).

Conclusion

The antiproliferative activity showed by both *P. guajava* and *P. betle* aqueous extracts toward nasopharyngeal epidermoid carcinoma cells is indicative of their potential to be developed as neutraceuticals for the prevention and management of cancerous oral epidermal lesions. This cytotoxic property would be an added value that would complement the use of *P. guajava* and *P. betle* as adjuncts in the formulation of oral health care products.

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