Anticancer Activity of Methanol and Hexane Extract of *Pereskia grandifolia* Haw Leaves Against Human Cervical (HeLa) Cells Line

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Abstract

Pereskia grandifolia *Haw belongs to the Cactaceae family. This plant is claimed by local community in Malay to have medicinal properties to treat a variety of illnesses including diabetes, gastric pain, cancer, anti-inflammation, ulcer and hypertension. The objective of this research is to examine the anticancer activity of* P. grandifolia *leaves methanol and hexane extract against cervical cancer cell line (HeLa) culture. Leaves powder were extracted using methanol and hexane. Anticancer activity was measured by MTT [3-(4,5-dimetilthiazol-2-il)-2,5-difeniltetrazolium bromida) assay method. The result indicated that* P. grandifolia *leaves methanol extract possessed low anticancer activity against HeLa cell line with the* IC_{50} *value of 826.13 µg/ml, 928.42 µg/ml 899.90 µg/ml respectively and leaves hexane extract with the* IC_{50} *values of 816.85 µg/ml, 1,416 µg/ml, and 749.70 µg/ml respectively after incubated 24, 48 and 72 hours. The result indicated the possible use of medical plant,* P. grandifolia *as a source of chemopreventive agents.*

Key word: *Pereskia grandifolia Haw, cytotoxic, HeLa cell, MTT assay*

INTRODUCTION

Cervical cancer is an important area of action for any cancer control program because of the burden of disease, and the potential for effective prevention via screening. Cervical cancer comprises approximately 12% of all cancers in women. It is the second most common cancer in women worldwide, but the commonest in developing countries such as Indonesia.

Annual global estimates around the year 2000 are for 470,600 new cases and 233,400 deaths from cervical cancer annually. Eighty percent of these cases occur in developing countries (WHO, 2002). Incidence of cancer in Indonesia was estimated to be 100 per 100.000 people per years or about 200.000 population per years and cervical cancer is the first most common cancer in women Indonesia (Puspitasari *et al.,* 2003; Aziz *et al.,* 2006).

Cervical cancer is malignant neoplasm of the cervix uteri or cervical area. It may present with vaginal bleeding but symptoms may be absent until the cancer is in its advanced stages. The most important risk factor in the development of cervical cancer is infection with a high-risk strain of Human Papilloma virus. Symptoms of advanced cervical cancer may include: loss of appetite, weight loss, fatigue, pelvic pain, back pain, leg pain, single swollen leg, heavy bleeding from the vagina, leaking of urine or feces from

the vagina, and bone fractures (Ramli *et al.,* 2005; Aziz *et al.,* 2006; Rasjidi, 2007).

Plant natural compounds have provided many effective anticancer agents in current use. Currently, over 50% of drugs in clinical trials for anticancer activity were isolated from natural sources (Newman and Gragg, 2007). The cacti are well-known desert plants, widely recognized by their specialized growth form and essentially leafless condition. Very primitive cacti have leaves and are generally not considered succulent. They are deciduous shrubs, small trees or even climbers and the cactaceae contained 1500-1800 species renowned for their remarkable morphological and physio-logical adaptations to drought (Edwards *et al.,* 2005; Butterworth and Edwards, 2008)**.**

Pereskia grandifolia commonly known as *jarum tujuh bilah* in Malay or *cak sing cam* in Chinese, *daun tujuh jarum* in Indonesia and with common name in English are leaf cactus, perescia, rose cactus, or wax rose, belongs to Cactaceae family (Sri Nurestri *et al.,* 2008; Wahab *et al.,* 2009). This plant commonly used by the local community in Malay and Chinese for its medicinal properties. This plant is claimed to have medicinal properties to treat a variety of illnesses including blood pressure, diabetes, cancer, inflammation, ulcer and hypertension (Goh, 2000).

According to the previous reports several active components have been isolated from *P. grandifolia* for example β-sitosterol, vitamin E, 2,4-ditert-butyl-phenol, phytone, and a mixture consisting of methyl palmitate, 2,4-ditert-butylphenol, methyl oleate and methyl stearate which showed cytotoxicity against several cancer cell lines and this is the first report of the cytotoxic activity of *P. grandifolia* (Sri Nurestri *et al.,* 2009)

Takeiti *et al.,* (2009) revealed other species *Perereskia* such as *P. aculeate* leaves showed remarkable levels of total dietary fiber (39.1% dry basis), minerals (calcium, magnesium, manganese and zinc) and vitamins (vitamin A, vitamin C and folic acid). Among amino acids, tryptophan was the most abundant (20.5%) of the total amino acids.

Crude methanol and its fractionated (hexane, ethyl acetate and water) of *P. grandifolia* leaves extracts showed high antioxidant activity. Leaves extract of *P. grandifolia* contains gallic acid equivalents (GAEs) (phenolic compounds). The highest amount was found in the ethyl acetate extract with 45.99 mg of GAEs/g of extract. 2,4-di-tert-butylphenol, α-tocopherol and β-sitosterol were isolated and identified from the ethyl acetate extract of *P. grandifolia* (Sim *et al.,* 2010).

Cytotoxic activity has been reported from other plant extract. Bioactive compound from methanol extract of *P. bleo* showed to kill breast carcinoma cell (T-47D) by the activation of caspase-3 and c-myc pathways in apoptosis mechanism (Tan *et al.*, 2005) and study by Sri Nurestri *et al.,* (2009) reported that ethyl acetate fraction from *P. grandifolia* leaves showed high cytotoxic activity against KB (nasopharyngeal epidermoid carcinoma cell line) and MCF7 cell (hormone-dependent breast carcinoma cell line).

Therefore, the current study focused on examining of anticancer activity of methanol and hexane extract of *P. grandifolia* leaves *in vitro* against HeLa cancer cell line.

MATERIALS AND METHODS

Preparation of extract

P. grandifolia plant was collected from garden of LPPT Universitas Gadjah Mada (UGM). The plant was identified by Taxonomy Laboratory, UGM. HeLa cell line was obtained from Laboratory stock of LPPT Unit III, UGM. The leaves of *P. grandifolia* were washed, dried and ground to fine powder. The dried ground leaves were extracted with methanol and hexane. The methanol and hexane extract was filtered and concentrated using rotary evaporator and then evaporated to dryness.

Preparation of cervical cancer cell line (HeLa)

Cervical cancer cell line was grown on RPMI 1640 (Sigma) medium containing 5% v/v Fetal Bovine Serum (FBS) (Sigma) and 1% v/v fungison (Sigma) in the presence of 1% w/v of penicillin-streptomycin (Sigma) at 37°C in humidified atmosphere of 5% CO_2.

In vitro assay for cytotoxic activity

The cell suspension 2.10^4 cell mL^{-1} (100µl) was plated into 96 well microplate (Nunc, Germany) and treated with different concentration of methanol and hexane extract isolated from *P. grandifolia* leaves, in a serial dilution (1000, 500, 250, 125, 62.50, 31.25, 15.625 and 7.8125 µg/ml). Following treatment, plates were incubated in CO_2 incubator at 37°C for 24h, 48h and 72h. After incubation, media was removed and MTT reagent (10µl) in PBS (5mg/ml) was added to each well and the plates were further incubated for 4h. Reaction was stopped by the addition of 100µl sodium dodecylsulfate (SDS) 10% and incubation was continued in CO_2 incubator at 37°C for 15h. The absorbance was read at wavelength of 540 nm using ELISA reader. The percentage cellular viability was calculated with appropriate control taken into account. The concentration which inhibited 50% of cellular growth (IC_{50}) was determined. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth Inhibition (\%)} = \frac{\text{OD Control-OD treated}}{\text{OD Control}} \times 100$$

The cytotoxicity of sample on cancer cell was expressed as IC_{50} values (the drugs concentration reducing the absorbance of treated cell by 50% with respect to untreated cells).

RESULT AND DISCUSSION

In this study, *in vitro* anticancer activity of methanol and hexane extract from *P. grandifolia* leaves examined using MTT Assay. MTT was also used to evaluate the effects of the extracts. Tetrazolium salt is metabolically reduced by viable cells to yield a blue formazan product measurable in a multiwell scanning spectrophotometer. This technique permitted to evaluate dose-dependent-effect, by linear regression analysis. These techniques are considered quick and inexpensive for the evaluation cytotoxic effect. Toxicity data are expressed as the IC_{50} (concentration of extracts that causes 50% inhibition or cell death), and was obtained by plotting the inhibition percentage versus concentration of plant samples. Inhibitory concentration of the extract in HeLa cell line were calculated by linear regression analysis (Doyle and Griffiths, 2000).

The result demonstrated that methanol extract of *P. grandifolia* leaves possessed low cytotoxic activity against Hela cell line presented with IC_{50} values of 826.13 µg/ml, 928.42 µg/ml, and 899.90 µg/ml after incubated for 24h, 48h and 72h, respectively, while hexane extract with IC_{50} value of 816.85 µg/ml, 1,416 µg/ml and 749.70 µg/ml after incubated 24h, 48h and 72h, respectively (figure 1 and 3).

The result suggest that methanol and hexane extract of *P. grandifolia* leaves revealed low anticancer activity. According to the National cancer institute, in order for the crude extract of plant to considered cytotoxic against the treated cell, the IC_{50} value obtained should be less than 20 µg/ml (Tan *et al.,* 2005).

Our result is almost similiar to study by Sri Nurestri et al., (2009). The crude methanol and its fractionated (hexane, ethylacetat dan water) have no cytotoxic activity against CasKi (cervical carcinoma cell), HCT-116 (colon carcinoma cell) and A549 (human lung carcinoma cell).

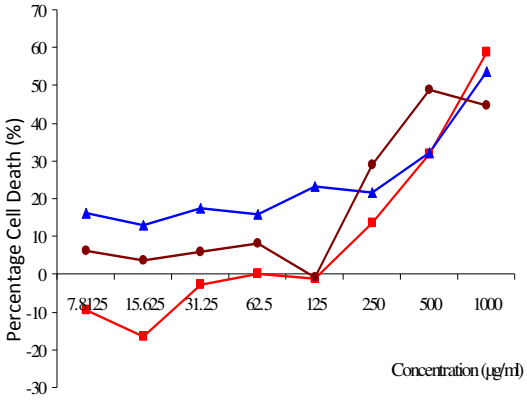

Figure 1. Correlation concentration of *P. grandifolia* leaves methanol extract with percentage death cell (HeLa). Cells were treated with several concentration of extract during 24h(■), 48h(▲) and 72h(●).

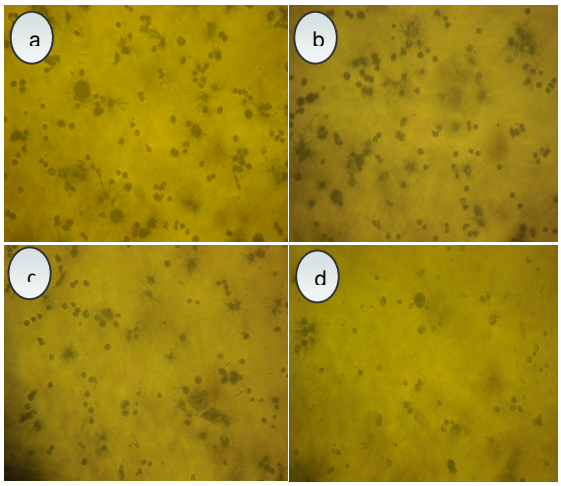

Figure 2. Morphology Hela cell line after treated with MTT (a) Untreated cell (b) treated with 15.625µg/ml extract (c). treated with 125µg/ml extract and (d) treated with 500µg/ml extract. Cell treated with methanol extract of *P. grandifolia*.

In addition, Sri Nurestri *et al.* (2009) reported that crude methanol and its fractionated (hexane, ethylacetate and water) have no cytotoxic activity towards non-cancer human fibroblast cell line (MRC-5) but hexane fraction of *P. grandifolia* leaves extract possessed cytotoxic activity against KB cell line (nasopharyngeal epidermoid carcinoma) with IC_{50} values of 5.0 µg/ml, and

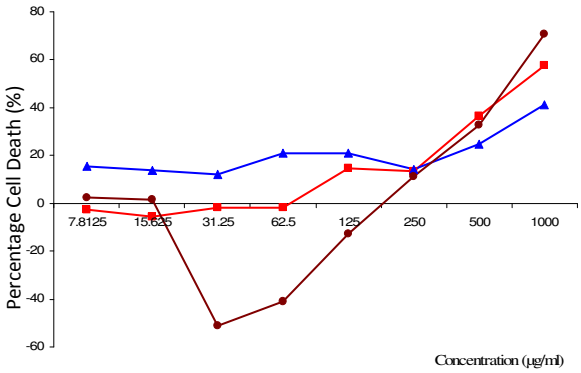

Figure 3. Correlation concentration of *P. grandifolia* leaves hexane extract with percentage death cell (HeLa). Cells were treated with several concentration of extract during 24h(■), 48h(▲) and 72h(●).

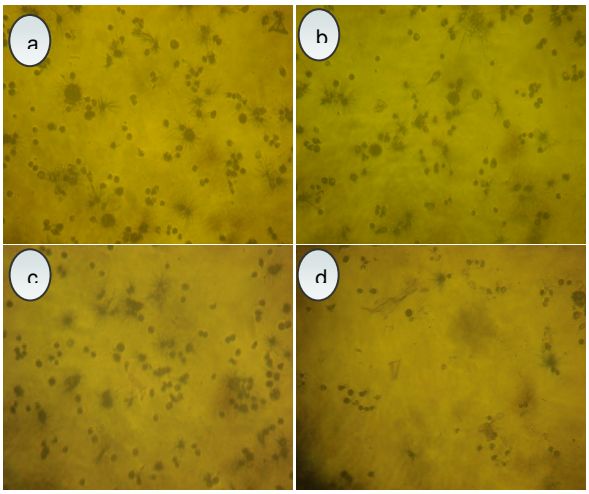

Figure 4. Morphology Hela cell line after treated with MTT (a) Untreated cell (b) treated with 15.625µg/ml extract (c). treated with 125µg/ml extract and (d) treated with 500µg/ml extract. Cell treated with Hexane extract of P. grandifolia.

ethylacetate fraction showed cytotoxic activity against KB and MCF7 cells with IC_{50} values of 16.0 µg/ml and 20.0 µg/ml.

Cytotoxic activity reported on other species showed that the aqueous and methanol extracts of *Pereskia bleo* leaves have no significant anti-proliferative effect against the mouse mammary cancer cells (4T1) or the normal mouse fibroblast cells (NIH/3T3) (Er *et al.*, 2007).

Crude methanol extract and ethyl acetate fraction of *P. bleo* possessed cytotoxic activity against KB cell line with

IC_{50} values of 6.5 and 4.5 µg/ml respectively and have no cytotoxic against MRC-5 (Sri Nuretsri *et al.* 2008). It means that bioactive compounds in methanol extract and ethyl acetate have selective cytotoxic effect againts KB cell line.

The result showed that, in control cell, it can be seen that the cells are growing normally as indicated by the presence of formazan dye formed. The increase of the extract concentration, is followed by the decrease of formazan dye (Figure 2 and 4). The formation of formazan dye directly correlates to the number of metabolically active cells in the culture. In cell after treatment with MTT, it appeared that in concentration 500µg/ml and 1000µg/ml, formation of formazan decrease. It indicated that both of methanol and hexane extract *P grandifolia* leaves are proved to possess low anticancer properties against HeLa cell lines tested. This result supported the MTT experiment as shown in figure 1 and 3.

The result suggested that this plant might be containing complex chemical constituent having anticancer activity but in low concentration or selectively toxic against cancer cell line. Methanol and hexane extract of *P. grandifolia* leaves showed less activity on HeLa cell line in our study, but caused changes of HeLa cell morphology in highly concentration. Methanol and hexane extracts used in this study were still in crude plant extract. In addition, a variety bioactive compounds from plant can show different biological activities in different cancer cell line, selectively toxic against cancer cell line and that might be due to different methods of extraction.

Some studies have reported that cytotoxic activity of methanol extract of *P. bleo* using MTT assay is not effective in inducing cell death in MCF-7 (human breast cancer cell line) after 72h incubation (Wahab *et al.,* 2009), its different reported by Tan *et al.* (2005), *in vitro* cytotoxic assay used methylene blue assay showed that methanol extract of *P. bleo* induce apoptosis in T-47D cell line (human breast cancer cell line) after incubated 72h.

The selection of crude plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure compounds isolated from natural products.

CONCLUSION

The methanol extracts of *P. grandifolia* leaves possessed low cytotoxic activity against HeLa cell with IC_{50} values of 826.13 µg/ml, 928.42 µg/ml, and 899.90 µg/ml respectively and hexane extract with IC_{50} values of 816.85 µg/ml, 1416 µg/ml and 749.70 µg/ml, respectively after incubated 24h, 48h and 72h.

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REFERENCES

Aziz, M.F., Andrijono, dan Saifuddin, A.B., 2006, Onkologi dan Ginekologi Buku Acuan Nasional. Yayasan Bina Pustaka Sarwono Prawirohardjo. Jakarta.

Butterworth, C and Edwards, E.J., 2008, Investigating Pereskia and The Earliest Divergences in Cactaceae, *Haseltonia*., **14**, 46-53.

Edwards, E.J., Nyffeler, R., and Donoghue, M.J., 2005, Basal Cactus Phylogeny: Implications of *Pereskia* (Cactaceae) Paraphyly for The Transition to The Cactus Life Form, *American J. Bot*., **92**, 1177-1188.

Er, H.M., Cheng, En-Hsiang., and Radhakrishnan, A.K., 2007, Anti-Proliferative and Mutagenic Activies of Aqueous and Methanol Extract of Leaves from *Pereskia bleo* (Kunth) D.C (Cactaceae*)., J.Etnopharm*., **113**, 448-456.

Goh, K.L., 2000, Malaysian Herbacous Plants. Milllennium edn. Advanco Press. Malaysia.

Newman, D.J., and Gragg, G.M. 2007. Natural product as Sources of New Drugs Over the Last 25 Years, *J.Nat.Prod*., **70**, 461-477.

Sri Nurestri, A.M., Wahab, N.A., Yacob, H., Shin, S.K., Lai, H.S., Serm, L.G., and Rahman, N.S.A., 2008, Cytotoxic Activity of *Pereskia bleo* (Cactaceae) Againts Human Cell Lines. *Inter.J.Cancer.Res.,* **4**(1), 20-27.

Sri Nurestri, A.M., Sim, K.S., and Norhanom, A.W., 2009, Phytochemical and Cytotoxic Investigations of *Pereskia grandifolia* Haw. (Cactaceae) Leaves, *J.Bio. Scie*., **9**(5), 488-493.

Sim, K.S., Sri Nurestri, A.M., and Norhanom, A.W., 2010, Phenolic content and antioxidant activity of *Pereskia grandifolia* Haw. (Cactaceae) extracts, *Pharmacogn.Mag.,* **6**(23), 248-254.

Puspitasari, H.P., Sukardiman, and Aty, W., 2003, Uji Aktivitas Sitotoksik Ekstrak Metanol Herba *Ageratum conyzoides* L Pada Kultur Sel Mieloma Mencit, *Majalah Farmasi Airlangga*., **3**(3), 93-96.

Ramli, M., Rainy, U., and Sonar, S.P., 2005, Deteksi Dini Kanker. Eds. Balai Penerbit FKUI. Jakarta.

Rasjidi, I., 2007, Panduan Penatalaksanaan Kanker Ginekologi Berdasarkan Evidance Base. Penerbit Buku Kedokteran EGC. Jakarta.

Tan, M.L., Sulaiman, S.F., Najimuddin, N., Samian, R.M., and Muhammad, T.S., 2005, Methanolic Extract of *Pereskia bleo* (Kunth) D.C. (Cactaceae) nuces Apoptosis in Breast Carcinoma, T47D Cell Line, *J.Ethno.pharm*., **96**, 287-294.

Takeiti, C.Y., Graziella, C., Antonio, G.C., Motta, E.M.P., Fernanda, P.C.Q., and Park, K.J., 2009, Nutritive Evaluation of a Non-Conventional Leafy Vegetable (*Pereskia aculeata* Miller), *Inter.J.Food.Scie.Nutr.,* **60**(1), 148-160.

WHO, 2002. Cervical Cancer Screening in Developing Countries : Report of a WHO Consultation. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. 75p.

Wahab, S.I.A., Abdul, A.B., Mohan, S.M., Al-Zubairi, A.S., Elhassan, M.M., and Ibrahim, M.Y., 2009, Research Article Biological Activities of *Pereskia bleo* Extracts, *Inter.J.Pharm*., **5**(1), 71-75.