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Artocarpus altilis extract effect on cervical cancer cells

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Abstract

This paper elucidate on the effects of Artocarpus Altilis Pulp part on cervix HeLa cancer cell. IC50 values of pulp extract were determined on HeLa cell with different concentration (12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml). Cell viability and cell growth were observed up to 72 hours with comparative to control cells. The results obtained in this research quantitatively revealed the dependence of cell proliferation on extract concentration. Control, 12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml of concentration showed 100%, 90%, 80%, 50%, 44% cell viability after 72 hours in culture respectively. This study result demonstrates that Artocarpus Altilis has the ability to inhibit cervical cancer cell proliferation.

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Keywords: Artocarpus altilis; cervical cancer cell

1. Introduction

Medicinal plants are known as rich resources of ingredients where an impressive number of modern drugs have been isolated from natural sources and occupy an important role in herbal medicine, allopathic medicine, homeopathy and aromatherapy [1]. Countless natural products like plant extracts and spices have been utilized in traditional medicines in the Native American, Indian and Chinese and research validate that these traditional

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products capable of having a potent anticancer effects . More than 85,000 plant species have been documented for medical use globally and research into the anticancer effects by using natural products has yielded many promising results.

An extract from the Mayapple, scientifically known as Podophyllum peltatum, was traditionally used by Native Americans to oppose skin cancers. The major constituents of this extract was podophyllotoxin, which was the first in a series of effective anticancer agents called podophyllins [2].Curcuma Longa or well known as turmeric is another natural product which has been studied widely for its anti-cancer properties due to the presence of active ingredient called curcumin. Previous studies proved that curcumin has the ability to suppress cancerous cell growth in a wide variety of cancer types [3-4]. Similarly, existence of polyphenol in grapes shows its potentiality as both preventative and an anti-cancer agent. One study claims that mix of natural extracts of turmeric, ginger and garlic has the potential in suppressing breast cancer cell [5].

Artocarpus Altilis, is said to be one of the medicinal plants. Artocarpus Altilis belongs to Moraceae family and commonly recognised as sukun in Malaysia and Indonesia. It is the staple food of South Pacific. Artocarpus Altilis is a huge evergreen tree with the roundish fruits having a white fibrous pulp [6-8]. It is the staple food in South Pacific. Each of every part of Artocarpus Altilis occupies an important role in traditional medicines. For an instances, leaves of sukun been used in treating hypertension, high blood pressure and diabetes. Toasted flowers of sukun used for tooth ache while the latex commonly used to treat skin ailments and fungal diseases. Apart from this, recent studies showed that sukun extract had cytotoxic effects on some human cancer cell lines including lung cancer cell, leukaemia cells, breast cancer cells and others, thus indicating that the extract can be a potential anti-cancer agent [9, 10].

Therefore, this preliminary research focus on investigating sukun extract in inhibiting viability of cervical cancer cells HeLa cell.

2. Methodology

In this section materials, preparation and treatment procedure were explained in detail.

2.1. Materials

The Pulp extract of Sukun were collected from International Islamic University Malaysia (IIUM).RPMI1640, Penicillin Streptomycin, Fetal bovine serum (FBS), Phosphate buffered saline (PBS), Tryple Express all product of Gibco, US.

2.2. Cell Culture

Cells were grown in RPMI 1640 enhance 10 % of Fetal Bovine Serum and 1% of Penicillin Streptomycin. Cells were cultured in humidified incubator at 37^{o} C containing 5% of CO_2. Cells were sub- cultured and used for treatment once reached 80% - 90% confluency.

2.3. Pulp (Sukun) Extract Preparations

A stock solution prepared by dissolving 37.8mg of pulp extract into 378µl of methanol (Fig. 1). To minimize the methanol effect in the sample extract, 10µl aspirated from the stock solution and further diluted into 990µl of complete growth media. Serial dilutions were prepared ranging from 12.5-100µg prior to use.

2.4. IC50 Determination

Cells were seeded cells in 6-well plate in 2 ml of complete growth media for attachment overnight. Prior to treatment with serial concentration (12.5-100 µg/ml) of pulp extract, cells were washed with PBS twice and pulp extract were added. After 72 hours of the treatment the medium was cleared off and the cells viability were counted using haemocytometer. IC50 was determined using Microsoft Excel.

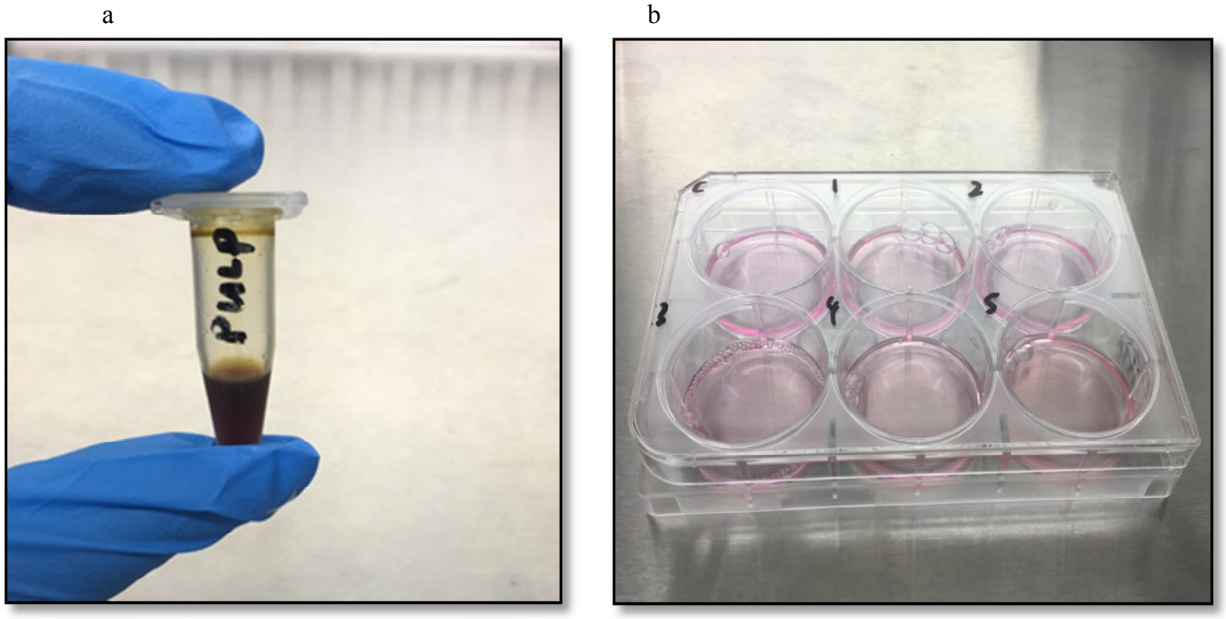

Fig. 1. (a) stock solution; (b) serial dilution.

4. Results and Discussion

Both Figure 2 and 3 showing image of HeLa cell after treatment of pulp part of sukun with different concentration ranging from 12.5µg/ml -100µg/ml. Figure 2 is the cell image obtained by 10X/0.25 while figure 3 by 4X/0.13 of TS100 objective lens. HeLa cell image as in Figure 3 shows that cells proliferation decreases as the concentration of treatment increasing. Cells in the control well (without pulp extract) proliferates more as compared to the highest concentration inhibit the cell proliferation as the well is not fully attached by the cells. Table 2 and Figure 5 quantitatively revealed that dependence of cell proliferation on extract concentration. Cells demonstrate a significant reduce in proliferation rate compared to untreated cells. Inhibition of cell growth was after 24 hours by when extract was added. Cell viability counted with the aid of haemocytometer and trypan blue staining, where the live cells possess intact membrane which excludes the trypan blue whereas the dead cells do not. It is found that viability of cell decreases in a concentration dependent manner as parameters shown in table 1 and graph illustration in figure 4. Lower cell viability induced through apoptosis process due to the flavonoid compound found in Artocarpus Altilis / Sukun.

Flavonoids are large class of plant pigments plant chemicals found in almost all fruits and vegetables and quercetin said to be one of the best known flavonoids. Tara et.al., 2015 reported that pulp part of Artocarpus altilis contains high percentage of quercetin which is about 78%.Quercetin induced apoptosis by decreasing mitochondrial membrane potential by releasing Cytochrome C and activate Caspase-3 and generate reactive oxygen species which leads to DNA fragmentation in the cell nucleus resulting in cell death [11-13].

A previous study mentioned that quercetin induced apoptosis in both the human breast cancer and the human epidermoid carcinoma (A-431) cell lines with concentrations starting with 50 and 75 µg/m, while in gastric cancer cell lines (BGC-832) the concentration was 90 or 120 µg/mL. Quercetin is an excellent antioxidant while smallest amount of quercetin activates a pro-apoptotic cascade in HeLa cells. Moreover, studies proved that quercetin and other flavonoids might influence protective effects against cancer cell growth [14-17]. Methanol been chosen as the solvent for the pulp extract as the previous research proved. It was found that methanol extract of pulp play a more effective role in inducing apoptosis in the cancer cell lines as methanol extract of pulp contains the highest percentage of quercetin [18-20]. IC50 value obtained was 50µg/ml (concentration at which growth or activity of cells is inhibited by 50%).

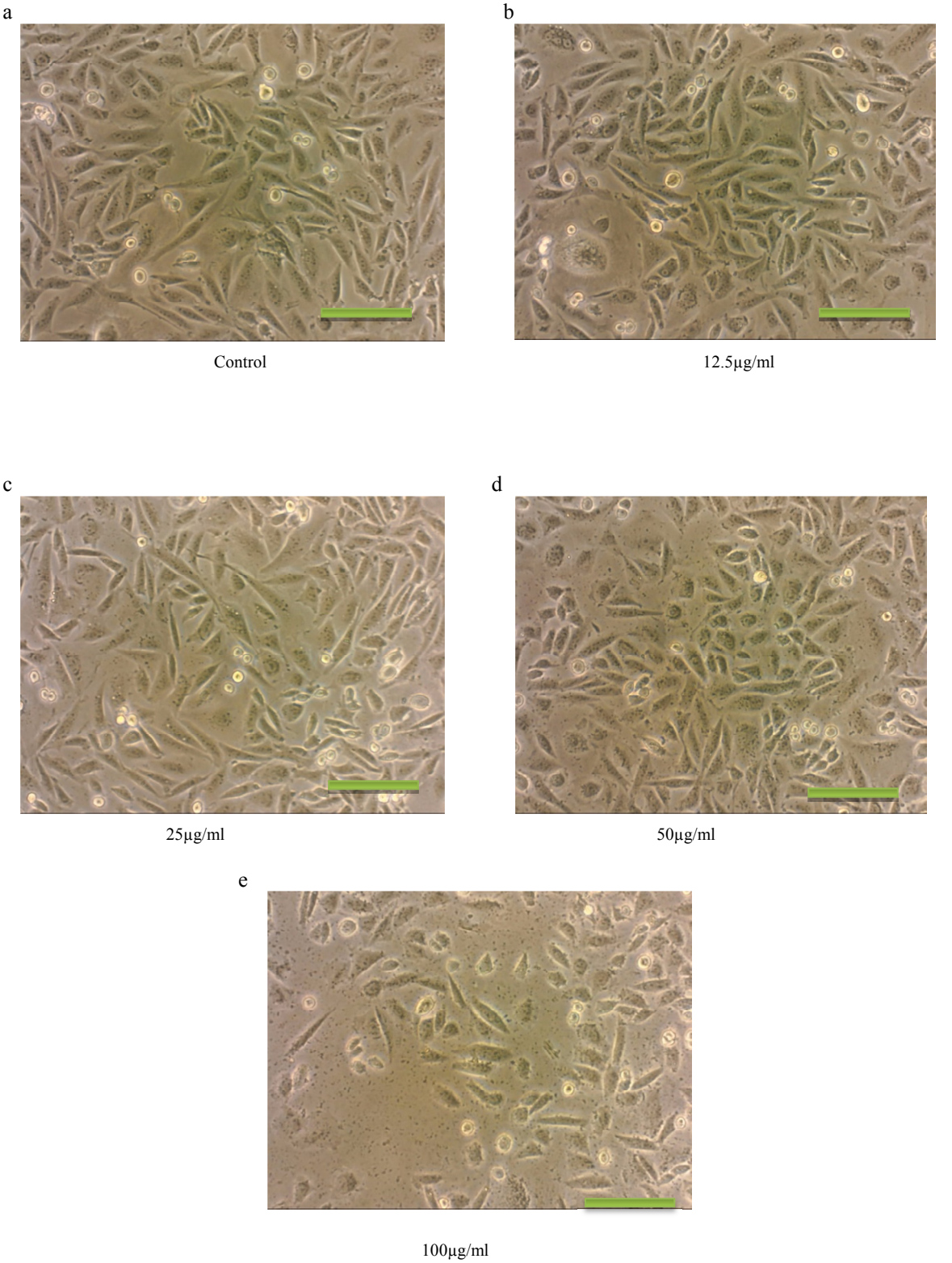

Fig. 2. Photomicrograph of HeLa cell with different concentration of treatment.
The image represents one of the several field views captured for analysis (10X/0.25 objective lens, scale bar -50µm)

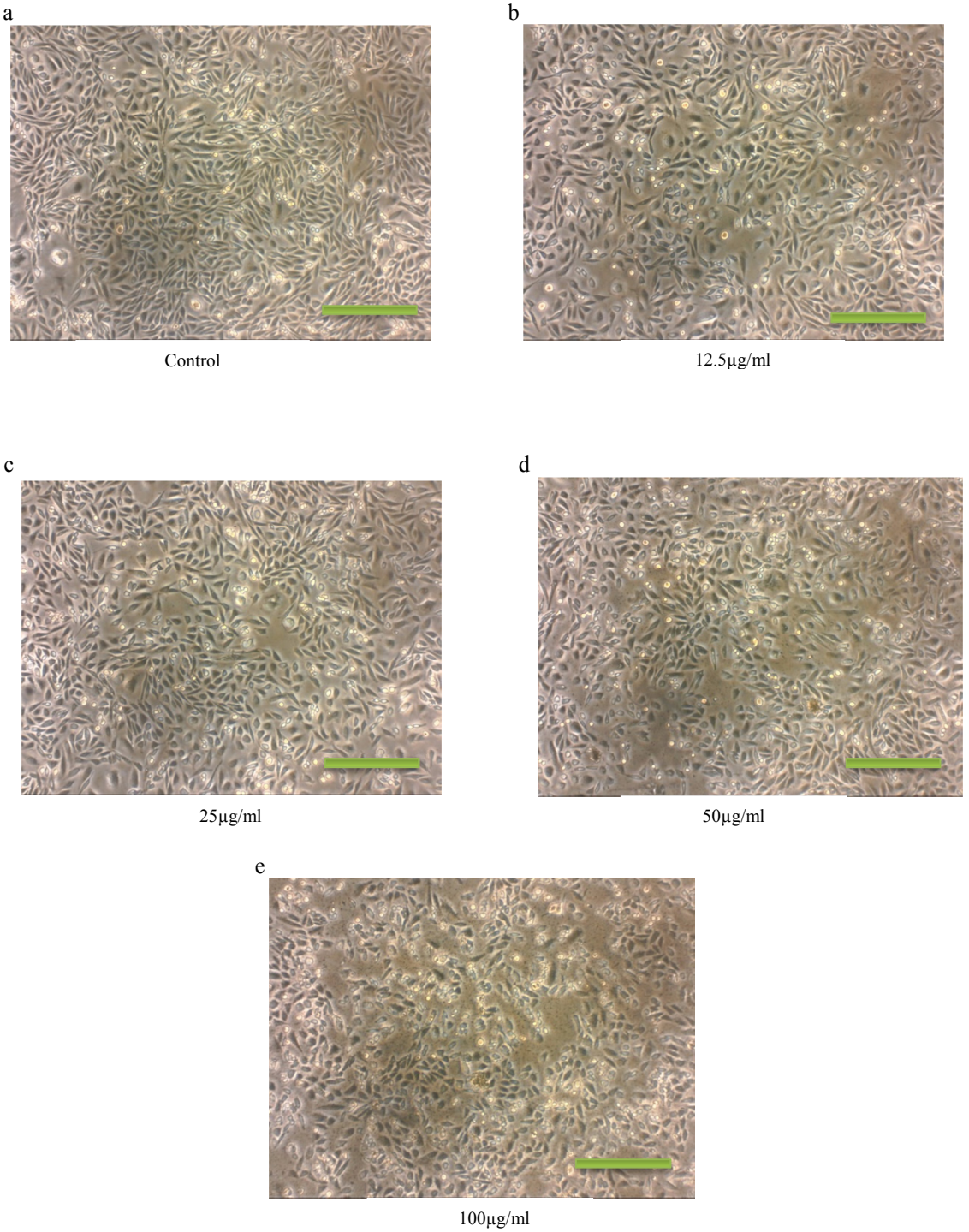

Fig. 3. Photomicrograph of HeLa cell with different concentration of treatment
(4X/0.13 objective lens, scale bar -50µm)

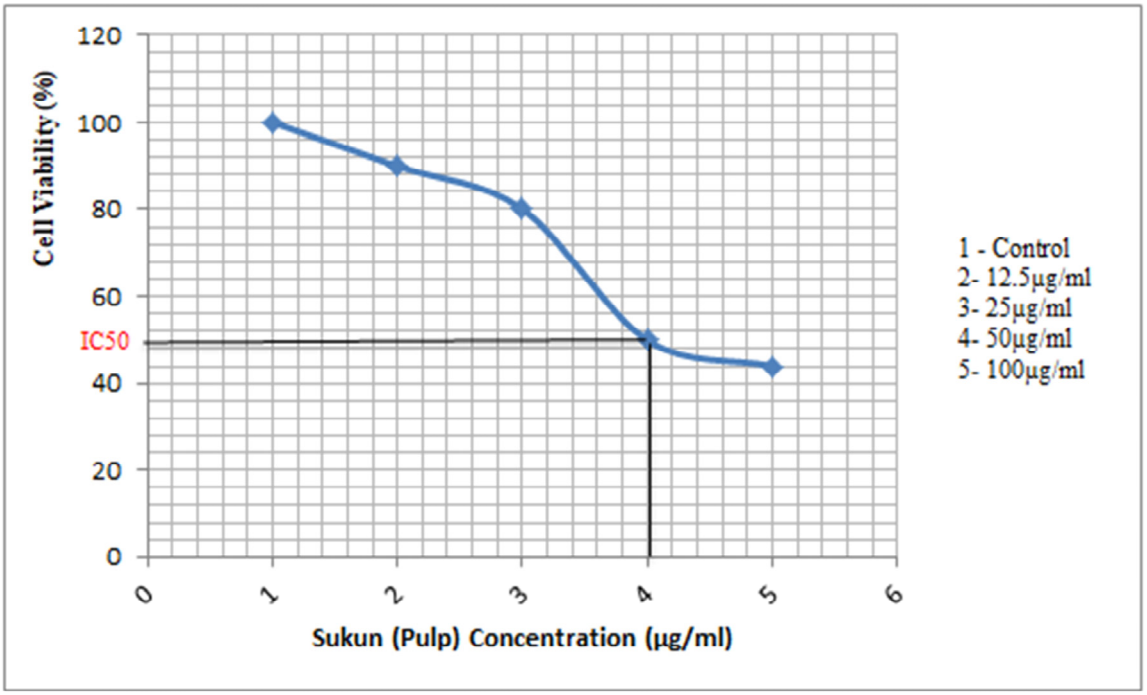

Fig. 4. Cell Viability against Pulp (Sukun) Concentration

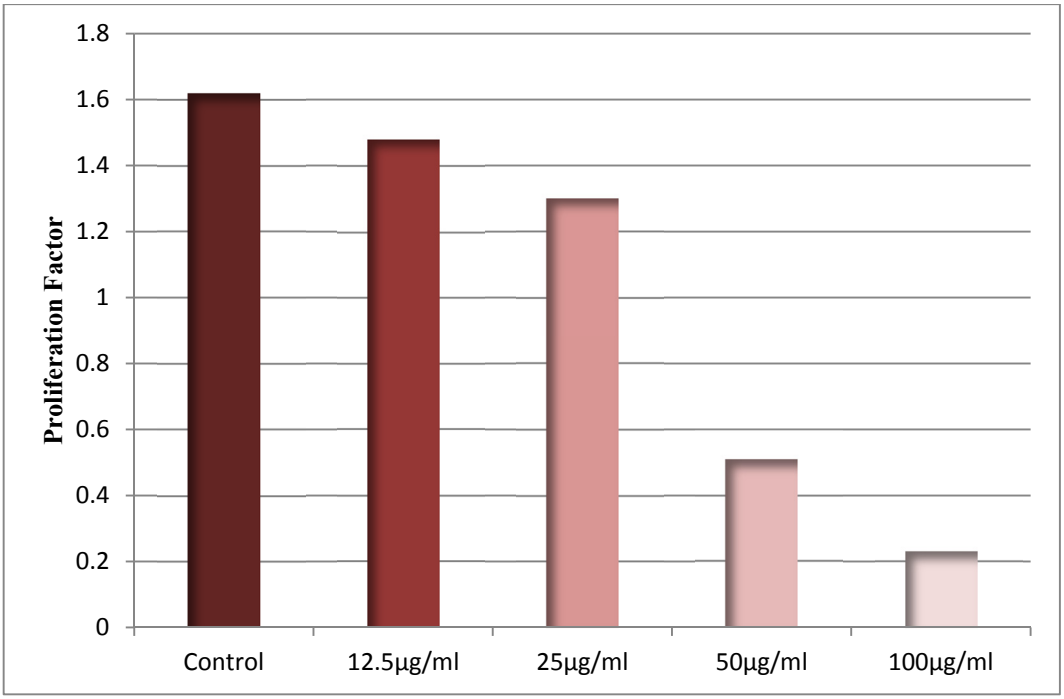

Fig. 5. Proliferation Factor with Different Sukun (Pulp) Concentration

Table 1. HeLa cell viability with different concentration.

Extract Concentration (μg/ml)	Cell Viability (%)
Control	100
12.5	90
25	80
50	50
100	44

Table 2. Proliferation Factor with Different Sukun (Pulp) Concentration

Extract Concentration (μg/ml)	Proliferation Factor
Control	1.62
12.5	1.48
25	1.30
50	0.51
100	0.23

3. Conclusion

Pulp part of artocarpus altilis works in dose dependent manner towards cervix HeLa cancer cell. Cell viability measurement proves that pulp part of artocarpus altilis extracts inhibit the growth of HeLa cancer cells when compared to untreated cell. As reported from previous research sukun extracts demonstrated that it was selective in inhibiting cell growth, where it only inhibit cancer cells but not toward normal cells even at highest concentration tested. Thus, pulp extract of artocarpus altilis has the ability in becoming anti-cancer agents. The result of this study can be used for further investigation in order to study the combination effect of electroporation method and sukun extract in inhibiting maximum HeLa cancer cell proliferation.

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