

## RESEARCH ARTICLE

# Apoptosis Induction in MV4-11 and K562 Human Leukemic Cells by *Pereskia sacharosa* (Cactaceae) Leaf Crude Extract

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### Abstract

**Background:** *Pereskia sacharosa* is a genus of cacti widely used in folk medicine for cancer-related treatment. Anti-proliferative effects have been studied in recent years against colon, breast, cervical and lung cancer cell lines, with promising results. We here extended study of anti-proliferative effects to a blood malignancy, leukemia. **Materials and Methods:** Two leukemic cell lines, MV4-11 (acute myeloid leukemia) and K562 (chronic myeloid leukemia), were studied. IC<sub>50</sub> concentrations were determined and apoptosis and cell cycle regulation were studied by flow cytometric analysis. The expression of apoptosis and cell-cycle related regulatory proteins was assessed by Western blotting. **Results:** *P. sacharosa* inhibited growth of MV4-11 and K562 cells in a dose-dependent manner. The mode of cell death was via induction of intrinsic apoptotic pathways and cell cycle arrest. There was profound up-regulation of cytochrome c, caspases, p21 and p53 expression and repression of Akt and Bcl-2 expression in treated cells. **Conclusions:** These results suggest that *P. sacharosa* induces leukemic cell death via apoptosis induction and changes in cell cycle checkpoint, thus deserves further study for anti-leukemic potential.

**Keywords:** *Pereskia sacharosa* - leukemia - intrinsic pathway - cell cycle arrest - FLT3-ITD - BCR-ABL

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### Introduction

Leukemia is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells which alter normal mechanisms of self-renewal, proliferation and differentiation (Fröhling et al., 2005). Typically, the treatment of leukemia involves combined chemotherapy, molecular-targeted therapy, biological therapy, radiation therapy and stem-cell transplantations. Genetic alterations such as FLT3-(FMs-like tyrosine kinase 3) internal tandem duplication (ITD) or FLT3-ITD in acute myeloid leukemia (AML) (Fathi and Chen, 2011; Takahashi, 2011) and breakpoint cluster region (BCR)-Abelson (ABL) or BCR-ABL mutation in chronic myeloid leukemia (CML) causes genetic instability that give poor response to treatment by increased proliferation and decreased apoptosis (Patel et al., 2010).

Phytochemical study is established as an intervention strategy for alternative or complementary medicine for cancer prevention. World Health Organization (WHO) reported that at least one-third of cancer death can be prevented, and consumption of fruits and vegetables provide chemopreventive activities (Bode and Dong, 2009). Therapeutic action of phytochemicals may be directed on molecular targeted mechanism involved in

neoplastic transformation, cell proliferation and signaling pathway in apoptosis cells or in damaged and transformed cells (William et al., 2009). Studies demonstrated that phytochemicals constituents commonly found in fruits and vegetables may have complementary mechanism of actions, including antioxidant activity and scavenging free radicals (Chu et al., 2002). In this study, leaves of *P. sacharosa* were used to screen for chemopreventive property.

*P. sacharosa* (Cactaceae) is a spiny shrub having substantial leaves and thin stems which can reach up to 2 to 8 m (Wahab et al., 2009). Its leaves can be either eaten raw or taken as a concoction brewed from fresh plant. It has been found to have cytotoxic activity against various cancer cell lines, such as Human nasopharyngeal epidermoid carcinoma cell line (KB), Human colon carcinoma cell line (HCT 116), Human hormone dependent breast carcinoma (MCF7), Human cervical carcinoma cell line (Hela) and Human lung carcinoma cell line (A549). It is also believed to have anti-tumor, anti-inflammatory, anti-rheumatic and anti-ulcer properties (Malek et al., 2009). Previous study shown that methanol extract of *P. sacharosa* possessed cytotoxic effects against human ductal breast epithelial tumor cell line (T-47D) and the cell death was found to be apoptotic in nature, mainly via the activation of the caspase-3 and c-myc pathways (Tan et al., 2005).

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The aims of this study were to determine the potential inhibitory effects of *P. sacharosa* leaves crude extract on leukemic cell lines that carry a FLT3-ITD and BCR-ABL mutations, respectively.

## Materials and Methods

### Reagents

The following reagents were purchased from respective suppliers, Roswell Park Memorial Institute (RPMI) 1640, fetal bovine serum (FBS), streptomycin penicillin were purchased from Gibco® (CA, USA), phosphate buffered saline (PBS) and 0.4% Trypan blue from Sigma-Adrich, (MO, USA), propidium iodide (PI), Annexin- V FITC apoptosis kit and cell cycle kit from Becton and Dickinson and company (BD) (NJ, USA). Primary antibodies against caspase 9, caspase 7, caspase 3, cytochrome c, bax, bcl-2, p21, p53 and akt were purchased from Cell Signaling Technology (MA, USA), secondary antibodies, anti-rabbit and anti-mouse from Santa Cruz Biotechnology (CA, USA). All chemicals and reagents were analytical graded and purchased locally.

### Cell culture

MV4-11 cells (human acute myeloid leukemia cell line) and K562 cells (human chronic myeloid leukemia cell line) were donated by Department of Hematology, Universiti Sains Malaysia, originally purchased from American Type Cell Culture (ATCC) (MD, USA). Both MV4-11 and K562 cells were culture in complete RPMI 1640 media, containing 10% (v/v) of fetal bovine serum (FBS) and 1% (v/v) of penicillin-streptomycin in humidified temperature containing 5% carbon dioxide (CO<sub>2</sub>) at 37°C in incubator.

### Plant materials and extraction protocols

Fresh plants of *P. sacharosa* were collected from the herb garden in Pasir Tumboh, Kelantan, Malaysia. The plant samples species were identified by the Malaysian Agricultural Research and Development Institute (MARDI), Strategic Resource Research Centre, Serdang, Malaysia. The samples were washed with distilled water and cut into pieces and freeze dried (at temperature -140°C), to excessively remove their water contents. The dried leaves of *P. sacharosa* were ground for lyophilized powder using grinding machine. The dried *P. sacharosa* leaves were extracted according to Tan et al. (2005) extraction method with some modifications. 50 g of dried leaves were extracted using soxhlet extractor in 500 ml of 60% (v/v) ethanol for 20 to 30 hours with boiling temperature of 60°C. Dark green extract solution produced was filtered using Whatman filter paper (Camlab, Cambridge, UK) and evaporated under reduce pressure in a rotary evaporator (Buchi, Lausanne, Switzerland) adjusted at 50°C to remove residual ethanol content in the extract. The delicate paste product was freeze dried for lyophilized powder (Tan et al., 2005). The final extract in powder form obtained was designated as '*P. sacharosa* leaves extract (PSLE)'. The percent recovery of pure product was 10% from crude extract used. The extract was redissolved in culture medium and diluted to

indicated concentration (0, 100, 200, 400, 600 and 800 µg/ml), stored at -4°C for immediate usage and under -20°C for later experimentation.

### Phytochemical screening

The phytochemical tests were carried out to detect the presence of important phytochemical constituents that usually exhibit biological activities such as alkaloids, saponins, flavonoids, tannins, triterpenes and steroids. Lyophilized plant samples on portions of 20 grams as requested were sent to the Forest Research Institute of Malaysia (FRIM) (Reference number; P sacharosa: 0711012) for screening procedure.

### Cell viability assay

Approximately 1x10<sup>5</sup> cells of MV4-11 and K-562 were seeded at 37°C in 5% CO<sub>2</sub> atmosphere for 24h and treated with different PSLE concentration (0, 100, 200, 400, 600 and 800 µg/ml) for another 72h. The cell viability was determined by Trypan blue exclusion assay (TBEA). Equal volume of Trypan blue dye and treated cell in suspension were mixed and filled under chamber slide of hemocytometer. Result was expressed as a percentage of viable cells, with 100% representing untreated control cells. All experiments were performed in triplicate.

### Detection of apoptosis by flow cytometry analysis

Apoptosis induced by the PSLE was carried out using Annexin V labeling BD Annexin-V-FITC assay kit (BD, USA). BDFac Canto II flow cytometer (NJ, USA) was used for acquiring and BD FacDiva software (NJ, USA) for analyzing test samples. Briefly, the treated cells were washed twice with PBS. A total of 5x10<sup>5</sup> cells were collected by centrifugation at 1500 rpm, 24°C for 5 min. Cells were stained with 5 µl annexin-V conjugated with fluorescein isothiocyanate (FITC) and 5 µl propidium iodide (PI) at room temperature in the dark for 15 min. The cells were analyzed by flow cytometry to measure the fluorescence intensity to detect FITC and PI. Untreated cells served as the negative control. A minimum of 10,000 events were collected per sample. All experiments were performed in triplicate.

### Cell cycle progression study by flow cytometric analysis

The cell cycle distribution of the cell treated with PSLE was examined by measuring DNA contents using flow cytometry. The treated cells were collected by centrifugation and constituted in cold PBS. Cells then were stained with PI solution (20 µg mL<sup>-1</sup> of PI and 10 µg mL<sup>-1</sup> of RNase A) at 37°C in the dark for 30 min. The stained cells were examined by flow cytometry to score DNA content of cells (Roy et al., 2008). Percentage of G1, S and G2/M cell cycle phases were calculated by Modfit LT 3.2 software (Verity Software House, ME, USA). All experiments were performed in triplicate.

### Immunoblotting

After 72h of PSLE treatment, cell pellets were collected by centrifugation at 1500 rpm for 5 min at 24°C, washed twice with cold PBS, and suspended in 1 ml (sufficient to lyse cells from one 100 mm culture dish

containing 0.5 to  $5 \times 10^7$  cells) of RIPA buffer [50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS)] (Sigma-Adrich, MO, USA) containing protease inhibitor (GE Healthcare, Buckinghamshire, UK), diluted in 1:1000, for protein extraction. The pellets were incubated in refrigerator for at least 5 min to let the buffer reacts. Unbroken cells, large plasma membrane pieces and nuclei were removed by centrifugation at 1500 rpm for 5 min at 24°C. The protein samples containing cytosolic fraction and mitochondria were used immediately or stored at -20°C for storage. Protein concentration were measured based on Bradford method and analyzed by photocyotometer at 595 nm wavelength.

For western blotting, the total protein amount of 10 µg/ml was mixed with an equal volume of laemmli sample buffer and was incubated in thermocycler (Applied Biosystem, CA, USA) for 10 min. The samples were loaded to each lane of 10-12% SDS polyacrylamide gel (PAGE). The gel electrophoresis was run at 70 Volts, 140 miliAmpere (mA), for 90 min. Separated proteins were transferred to PVDF membrane (Amersham, GE, Buckinghamshire, UK) using Transblot SD semi-dry transfer (Bio-Rad, CA, USA) at 15 Volts, 100 miliAmpere (mA), for 90 min. After blotting, the membrane was blocked with blocking agent using 5% of skim milk for 1 hour and membrane was probes with various primary antibodies (anti-bax, anti-bcl-2, anti-cytochrome c, anti-caspase 9, anti-caspase 3, anti-caspase 7, anti-p21Waf1/Cip1, p53, Akt and anti-β-actin) (Cell signaling, MA, USA) overnight at 4°C in shaker, according to manufacturer instructions and incubated with HRP-linked rabbit and mouse secondary antibodies (Cell signaling, MA, USA) for 1 h at room temperature. Bound proteins were detected using Amersham ECL Detection Agent (GE, Buckinghamshire, UK) and were exposed to Hyperfilm (GE, Buckinghamshire, UK) in the dark room. The films were inserted into image developer machine (Fujifilm, Tokyo, Japan) for image enhancement.

#### Statistical analysis

All the results were expressed as mean  $\pm$ SD of three independent experiments. Man-Whitney U test was employed to determine the statistical significance between untreated and treated groups with significant level of

$p < 0.05$ .

## Results

#### Phytochemical screening

The phytochemical screening was carried out using standard test methods as described in Table 1. Result demonstrated that PSLE contained slight amount of saponins, flavonoids and tannins and polyphenols compounds but no alkaloids present. In addition, triterpines and steroids were present abundantly in PSLE (Table 2).

#### Anti-proliferative effect of PSLE

There was marked growth inhibition after 72h of PSLE treatment on MV4-11 and K562 cells. The growth inhibition was in dose dependent manner with the  $IC_{50}$  value of  $110 \pm 0.04$  µg/ml in MV4-11 treated cells and  $130 \pm 0.03$  µg/ml in K562 treated cells, respectively (Figure 1). MV4-11 cells promoted lower  $IC_{50}$  value compared with K562 cells after PSLE treatment. This finding demonstrated that MV4-11 cells give higher response to PSLE treatment rather than K562 cells.

#### PSLE trigger apoptosis mechanism in leukemic cell lines

To study the mode of cancer cell death exhibited by the extracts, apoptosis analysis was conducted to evaluate the presence of apoptotic cells based on phospholipid phosphatidylserine (PS) translocations on cell surface using FITC-conjugated annexin-V. The cells treated with anti-leukemic drugs, CEP-140 (AML drug) and Imatinib mesylate or IM (CML drug) acted as a reference for apoptosis induction. The  $IC_{50}$  concentration of MV4-11 and K562 treated cells were employed for apoptosis, cell cycle and western blot studies. As shown in Figure 2, PSLE induced apoptosis in both cell lines, MV4-11 and K562 with total apoptosis inductions of 18.5% and 14.4%,

**Table 2. Preliminary Phytochemical Screening of PSLE Endorsed by FRIM**

Test of active substances	Pereskia sacharosa (leaves)
Alkaloids	-
Saponins	1+
Flavonoids	1+
Tannins and Polyphenolic compounds	1+
Triterpines/Steroids	3+

\*- =not present, 1+ =Modererate amount, 3+ =High amount

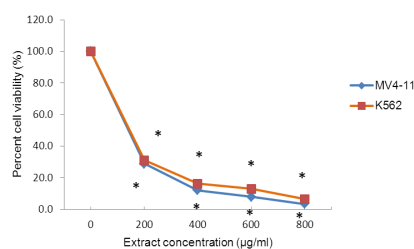
**Table 1. Standard test Procedures for Preliminary Phytochemical Screening Endorsed by FRIM**

Test	Procedures
Alkaloids	The sample was partition with chloroform followed by addition of ammoniacal chloroform. The mixture was then treated with 10% sulphuric acid and tested with Mayer' reagent. Alkaloid was obtained as formation of white precipitate.
Saponins	The methanol extract of the sample was mixed with distilled water in a test tube. The formation of stable froth for at least 15 minutes indicates the presence of saponins.
Flavonoids	The chloroform extract of sample was dissolved in ether and shaken in 10% ammonia solution. The formation of yellow colour in ammonia layer indicates the presence of flavonoids.
Tannins/ Polyphenolic compounds	The methanolic extract of sample was mixed with 1% ferric chloride solution. The formation of blue colour indicates the presence of hydrolysable tannins, while brownish-green indicates that of condensed tannins.
Triterpines/ Steroids	The chloroform extract of sample was tested using Liebermann-Buchard reagent. The formation of reddish colour indicates the presence of triterpines and greenish colour for steroids.

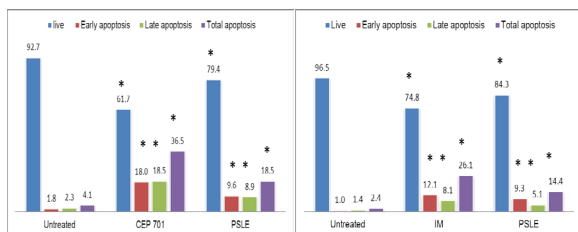
respectively. In addition, the pro-apoptotic effects of the extracts were significantly stronger in cell expressing FLT3-ITD mutation in MV4-11, compared to K562.

### Cell cycle progression

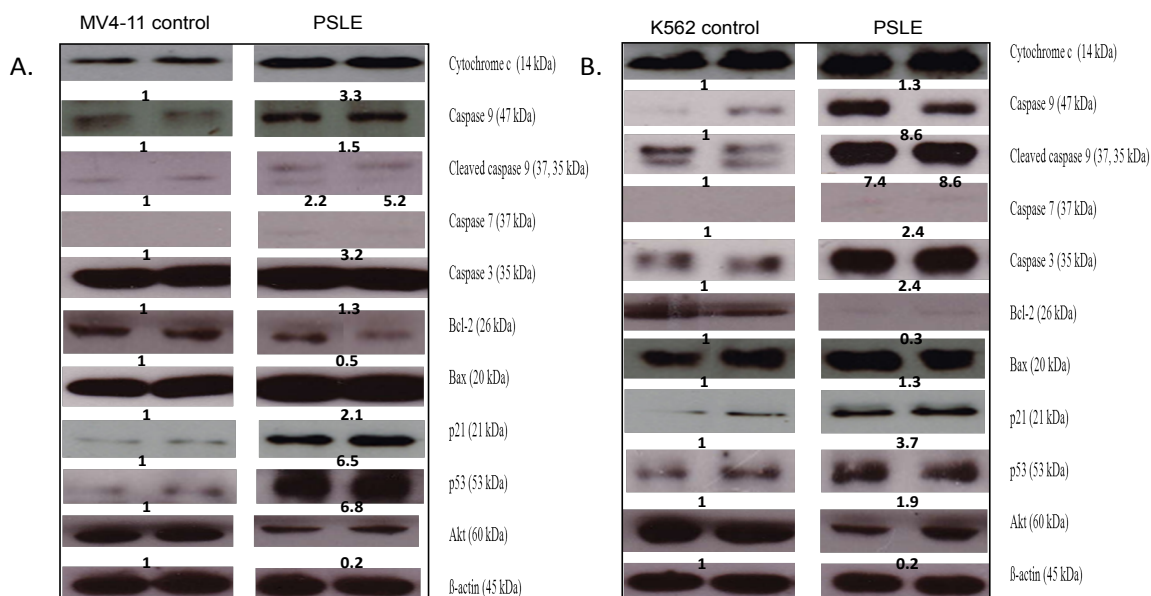
The effects of PSLE on cell cycle progression of MV4-11 and K562 cells were examined by flow cytometric analysis. A representative data of DNA ploidy histogram for MV4-11 and K562 cells after PSLE and PGPE treatment at  $IC_{50}$  concentration were shown in Figure 3. Result demonstrated that difference cell lines promoted



**Figure 1. Cell Viability Assay of PSLE Extract Treated with Various Extract Concentration (72 h) on MV4-11 and K562 Cells.** The cell viability was expressed as a percentage of cell viability (mean±SD of triplicate) determined by Trypan blue exclusion assay with significant value of  $p < 0.05$



**Figure 2. Apoptotic Inductions of PSLE on MV4-11 (A) and K562 (B) Cells.** Results are expressed as percentage in apoptosis (mean in triplicates). Untreated cells served as negative control while anti-leukemic drug, CEP 701 and IM served as reference drug for apoptosis activity. Statistical significant different from the negative control are indicated as  $*p < 0.05$  non-parametric Man-Whitney U test.

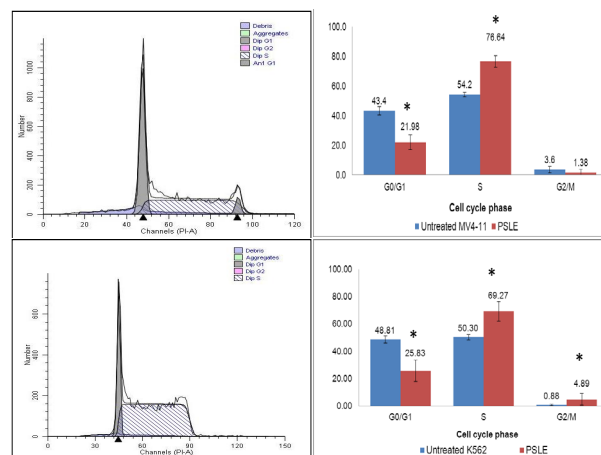


**Figure 4. Effects of PSLE on the Targeted Proteins Related to Apoptosis and Cell Cycle by Western Blotting.** MV4-11 and K562 cells were treated with PSLE (A and B) at  $IC_{50}$  concentration for 72 h. The values below the figures represent the fold change (fold  $\Delta$ ) in protein expression of the bands normalized to  $\beta$ -actin, quantified by densitometer [Adj.Vol.OD (mm<sup>2</sup>)]

difference responses on cell cycle progression. PSLE increased DNA contents at S phase and a corresponding decrease in G2/M phase leads to S phase arrest in MV4-11 cells. Interestingly, in K562 cells, PSLE did not arrest the cell cycle progression at S phase due to continuous increased of cells at G2/M phase and subsequent diminished cells at G0/G1 phase (Figure 3).

### The expression of cytochrome c and caspases

The regulatory mechanism of PSLE induced apoptosis was studied by examining the effect of the extract on the expression of cytochrome-c, caspase-9, caspase-7 and caspase-3 proteins in MV4-11 and K562 cells. Untreated MV4-11 and K562 cells served as negative control and  $\beta$ -actin served as internal control. As shown in Figures 4 (A and B), MV4-11 and K562 cells treated with PSLE



**Figure 3. Cell Cycle Progression of PSLE Treated Cells.** Representative data of Flow Cytometric analyzed by Modfit Software on the Effects of PSLE on MV4-11 Cells (A) and on K562 Cells (B). The bar graph illustrated percentage of cell population of each cell cycle phases in MV4-11 (C) and K562 cells (D). Untreated MV4-11 and K562 cells served as negative control. The cell cycle analysis was performed by flow cytometric analysis in three independent experiments



increased the cytochrome c expression by 3.3 fold and 1.3 fold, respectively, compared with control untreated cell. The fold changes were greater in MV4-11 cells compared with K562 cells, which mean the higher rate of cytochrome c execution occurred in AML cells, and lower rate in CML cells. PSLE significantly increased the expression of caspase 9 and cleaved caspase 9 in MV4-11 and K562 cells compared with untreated cells. The fold different of full length caspase 9 (47 kDa) of PSLE treated MV4-11 cells were 1.5 fold while in K562 give rise to 8.6 fold change. There was a great induction of caspase 9 in PSLE treated of K562 cells. Cleaved caspase 9 (35 kDa and 37 kDa) were increased in both treated MV4-11 and K562 cells (Figures 4). Treatment of PSLE also increases the expression of executioner caspase 7 by 3.2 fold in MV4-11 and 2.4 fold in K562 cells compared to untreated cells (Figure 4). As predicted, PSLE also caused an increment of caspase 3 activities in both cell lines by 1.3 and 2.4 folds changes, respectively. The induction of caspase 3 was greater in K562 cells and lower in MV4-11 cells.

#### *The expression of bax, Bcl-2 and akt activation*

To study which pathways responsible for inhibition in cell number of both leukemia cells treated with PSLE, we targeted the pro-apoptotic protein, bax protein and anti-apoptotic protein, Bcl-2 and akt proteins. Result showed that, the bax expression was up-regulated in both MV4-11 and K562 treated cells, correlated with their Bcl-2 repression. Meanwhile, akt were down-regulated in MV4-11 and K562 treated cells which shows lower cell survival and prone to cell death (Figure 4).

#### *The expression of CDKs inhibitor activity*

To detect the changes in cell cycle associated with growth arrest, p21 Waf1/Cip1 and its regulator, p53 protein were studied. Result showed upon 72h of treatment with PSLE at IC<sub>50</sub> concentration elevated level of p21 were seen in MV4-11 and K562 treated cells, by 6.5 and 3.7 folds, respectively (Figure 4). The expression of p53 protein was correlated with increased expression of p21 by 6.8 fold in MV4-11 and 1.9 fold in K562 cells compared to untreated cells (Figure 4).

## Discussion

The cancer chemoprevention study started as early in the 1920s by works of Berenblum on the effects of ether extract of a horizontal retort tar treated with lime on tumor induction (Berenblum, 2005). Three strategies in cancer chemoprevention have been proposed comprised of primary, secondary and tertiary chemoprevention. The primary chemoprevention strategy involved intervention strategy to prevent cancer development in healthy individual. The secondary and tertiary chemoprevention are designed to prevent the cancer progression and to prevent the onset of second primary cancer (Bonovas et al., 2008). The prospective studies has provide a new definition of effective chemopreventive agent that should be nontoxic, effective at lower doses, easily available and economical (Amin et al., 2009). Natural phytochemicals can be classified into carotenoids, phenolics, alkaloids,

nitrogen-containing compounds and organosulfur compounds that were shown to have anticancer properties. Their mechanism of action was responsible for their anti-proliferative effect through inhibition of tumor growth and induction of tumor cell differentiation (Liu, 2004). It previously been reported that phytochemicals found in fruits and vegetables has health promoting and disease preventing properties based on epidemiological studies, experimental studies by the use of animals and cells models and evidences from human intervention studies (Traka and Mithen, 2011).

Our results showed phytochemicals constituent such as flavonoids, saponins, tannins and polyphenolic compounds found from *P. sacharosa* leaves extract (PSLE) may inhibit cancer cell proliferation and apoptosis through various signaling pathways. Soxhlet extraction of *P. sacharosa* exerted a high amount of triterpines/steroids and substantial amount of saponins, flavonoid and condensed tannins. Triterpines is a precursor of steroids distinct by their structure and function. Triterpines found has been actively investigated for their chemopreventive activity in the previous study and many positive results produced. The most promising studies of triterpene was lupeol which displayed an anti-inflammatory, anti-arthritic, anti-mutagenic, anti-malarial activity and most recently anti-carcinogenic (Chaturvedi et al., 2008). Lupeol also caused a dose-dependent cell growth inhibition and induce apoptosis in human pancreatic adenocarcinoma cell line, asPC-1 (Saleem et al., 2005). In addition, lupeol also showed cytotoxic activity against human leukemias, melanomas, neuroblastomas and normal fibroblast cell. In chronic myeloid leukemia cell line, K562, luteolin inhibit cell growth in K562/ADM (adriamycin resistant cell) and K562/VCR (vincristine resistant cell) to the same extent by induction of apoptosis (Hata et al., 2003). Previous phytochemical and biological studies of *P. sacharosa* lead to the isolation of 2,4-Di-terbutylphenol,  $\alpha$ -tocopherol,  $\beta$ -sitosterol and a mixture of sterol extracted from ethyl acetate extraction of *P. sacharosa* (Malek et al., 2009). The example of plant-derived steroidal compounds, such as C<sub>21</sub> steroidal, cardiac glycosides, steroidal saponin and plant sterol which possessed the ability to induce differentiation in leukemic cell lines (Wang et al., 2006). It is due the nature of leukemic cells which fail to differentiate to their normal counterpart caused by various molecular abnormalities, such as point mutations (T315I) and deletions of amino acid bases.

A pro-apoptotic effect of the PSLE was significantly higher in MV4-11 cells, compared to K562 cells. The pattern of apoptosis activity in K562 cells was probably due to endogenous expression of BCR/ABL fusion gene they carried. The preserved feature of BCR-ABL mutation is inhibition of apoptosis mechanism with enhanced proliferation (Wong and Witte, 2004). This explained why the apoptotic stimulus of PSLE was higher in MV4-11 than in K562 cells. In addition, the same compound may activate different signaling pathways depending on the cell type (Amin et al., 2009).

The importance of cell cycle regulation is to maintain normal proliferation and differentiation of a cell. Our result showed that, different types of cells promoted different

responses on cell cycle progression. PSLE caused S phase arrest by an increased in DNA contents at S phase and a corresponding decrease in G2/M phase in MV4-11 cells. S phase arrest occurs when cells were exposed to genotoxic insult by means of cytotoxic agent giving rise to lesion in DNA (DNA damage) while cells were in synthesizing phase. Due to the arrest, there was a reduced rate of DNA synthesis, thus limit amount of sister chromatids DNA formed, therefore growth was retarded and cannot proceed to G2 phase (Bartek and Lukas, 2001). PSLE did not affect cell cycle progression at the same phase in K562 cells expressing BCR-ABL mutation. In a study by Dierov et al. (2004) shows that despite of causing an increased in DNA damage in stressful K562 cells, this BCR-ABL expressing cells were able to repair DNA damage quickly, suggesting that the role of BCR-ABL in altering DNA repair is reversible (Dierov et al., 2004). Moreover, the BCR-ABL kinase prone to induce prolong G2/M phase activation lead to their arrest (Skorski, 200-7).

To further characterize the effects of this PSLE treatment on leukemia cells, we examined the level of expression of apoptosis and cell cycle related proteins by western blot analysis. We found that cytochrome-c expression was increased after the treatment. This elevation was in conjunction with increase expression of bax protein as pro-apoptotic protein and inactivation of akt as anti-apoptotic protein. Akt inhibits bax conformational change due to apoptotic stimuli caused by its translocation to mitochondria (Parikh et al., 2007).

Initial execution of pro-apoptotic, bax protein from the mitochondria promotes the release of cytochrome-c into intracellular spaces (Boatright et al., 2003). An apoptosome, a multicomplex protein triggered caspase 9 activation and subsequent effector caspase activation, caspase-3 and caspase-7 (Pop et al., 2006). In this study, caspase-9 and caspase-3 transcription level were up-regulated in MV4-11 and K562 cells following 72 h of PSLE treatment. The involvement of pro-apoptotic family and the released of cytochrome-c and caspases proved that the mechanism of cell death was via mitochondria (intrinsic pathway). p53, a tumor suppressor was known for controlling the cell cycle, apoptosis, DNA repair and genomic integrity in stressful cells due to external stimuli (Bode and Dong, 2004). In this study, PSLE treatment induces p53 and p21 protein expression which higher induction in MV4-11 cells compared to K562 cells. These differences may provide early evidence of impaired p53 function in cell expressing a BCR-ABL mutation. Previous studied has shown the cells expressing BCR-ABL mutation influenced p53 status. p53 negative cells produced 10-folds more BCR-ABL expressing colonies than in p53 positive cells which demonstrated decrease proliferative capacity in p53 positive cells (Wendel et al., 2006). Up-regulation of p53 was associated with proliferation attenuating that diverts incipient cancer cells to undergone cell cycle arrest or apoptosis (Stiewe, 2007). In response to DNA damage, p53 expression were directly proportional with their downstream target, p21 expression to stop the damage cell from dividing, thus shuts down the cell cycle progression (Sperka et al., 2012).

In summary, this study highlights the anti-proliferative

properties exhibited by *P. sacharosa* leaves on MV4-11 and K562 leukemic cells model with FLT3-ITD and BCR/ABL mutation, respectively. These properties have proliferative-attenuating, apoptosis-inducing effect and promote cell cycle changes in leukemia cells. The expressions of bax, cytochrome-c and caspases proteins are the conserved features of apoptosis that are proved to be involved in AML and CML apoptosis activity. Whilst the candidature of CDKs molecule, p21 and its regulator, p53 predominantly influenced the growth pattern of leukemic cells. Although the apoptotic rate is not such enormous compared with standard chemotherapeutic agent, we generate a new insight of potential anti-leukemic agent of *P. sacharosa* into consideration. Our previous study demonstrated *P. sacharosa* did not cause harm to normal mononuclear cells and mouse fibroblast, L929 cells (data not shown). Therefore *P. sacharosa* might be a new candidature in the therapeutic strategy of AML and CML.

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