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Camellia sinensis and *Phyllanthus amarus* Ethanol Extracts Induced Apoptosis and Cell Cycle Arrest on Human Leukemic Cell Lines

(Ekstrak Etanol *Camellia sinensis* dan *Phylanthus amarus* Mengaruh Apoptosis dan Hentian Kitaran Sel pada Titisan Sel Leukemia Manusia)

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ABSTRACT

Leukaemia is a heterogeneous hematologic malignancy characterized by unregulated proliferation of the early blood-forming cells which starts in bone marrow. The basic strategy of leukaemia therapy involves the induction of leukemic cells apoptosis. Research on natural products have shown that some plant derivatives have anticancer properties by inducing apoptosis of leukemic cells. Plants such as *Camellia sinensis* and *Phyllanthus amarus* are those that had gained a wide interest due to their anti-cancer effect. The aim of this study was to investigate the anti-cancer effects of *C. sinensis* and *P. amarus* extracts on human leukemic cell lines by analysing the cell cycle and determining the apoptotic state. The cell lines were treated with ethanolic plant extracts at the concentrations of 31.25 - 500 µg/mL for 24 h followed by MTT assay to determine the IC_{50}. The IC_{50} of *C. sinensis* and *P. amarus* on the U937 cells were 170±10.39 and 210±6.78 µg/mL, respectively. Flow cytometric analysis of apoptosis using Annexin V/propidium iodide (PI) staining was also performed. *C. sinensis* extract at 170 µg/mL significantly increase apoptosis in U-937 ($p<0.001$), Jurkat ($p<0.05$) and K-562 cells ($p<0.01$) when compared to untreated cells. Meanwhile, *P. amarus* extract at 210 µg/mL significantly induced apoptosis in both U937 and K562 cells ($p<0.05$) but not Jurkat cells and caused cell cycle arrest at S phase in U-937 cells ($p<0.001$) and at G0/G1 in K652 cells ($p<0.05$) when compared to control. Based on the findings, both *C. sinensis* and *P. amarus* extracts showed potential in inducing apoptosis in human leukemic cell lines. In addition, *P. amarus* has the capability to disrupt cell cycle.

Keywords: Apoptosis; *Camellia sinensis*; cell cycle arrest; leukemia; *Phyllanthus amarus*

ABSTRAK

Leukemia adalah malignansi hematologi heterogen, dicirikan oleh pemproliferatan sel pembentuk darah awal secara tidak terkawal, yang bermula di dalam sumsum tulang. Sel leukemia mengalami apoptosis teraruh yang merupakan strategi asas dalam terapi leukemia. Kajian yang melibatkan produk semula jadi ada menunjukkan ciri antikanser terhadap sel leukemia melalui apoptosis teraruh. Tumbuhan seperti *Camellia sinensis* dan *Phyllanthus amarus* adalah antara yang mendapat perhatian meluas kerana mempunyai ciri antikanser. Matlamat penyelidikan ini adalah untuk mengkaji kesan antikanser ekstrak *C. sinensis* dan *P. amarus* terhadap pelbagai jenis sel leukemia manusia dengan menganalisis kitaran sel dan menentukan keadaan apoptosis. Titisan sel dirawat dengan ekstrak etanol tumbuhan pada kepekatan 31.25 - 500 µg/mL selama 24 jam dan diikuti dengan ujian MTT untuk menentukan IC_{50}. IC_{50} *C. sinensis* dan *P. amarus* pada sel U937 masing-masing adalah 170±10.39 dan 210±6.78 µg/mL. Analisis apoptosis sitometri aliran menggunakan pewarnaan Annexin V/propidium iodida (PI) juga dilakukan. Ekstrak *C. sinensis* pada 170 µg/mL meningkatkan apoptosis pada sel U-937 ($p<0,001$), Jurkat ($p<0,05$) dan K-562 ($p<0,01$) secara signifikan jika dibandingkan dengan sel yang tidak dirawat. Sementara itu, ekstrak *P. amarus* pada 210 µg/mL telah menyebabkan apoptosis pada sel U937 dan K562 ($p<0.05$) secara signifikan tetapi tidak pada sel Jurkat serta menyebabkan hentian kitaran sel pada fasa S pada sel U-937 dengan peratusan 55.71±2.06% ($p<0.001$) dan pada fasa G0/G1 pada sel K652 dengan peratusan 56.77±4.69% ($p<0.05$) jika dibandingkan dengan kawalan. Berdasarkan penemuan ini, kedua-dua ekstrak *C. sinensis* dan *P. amarus* menunjukkan potensi dalam mengaruh apoptosis terhadap sel leukemia manusia dan *P. amarus* berupaya untuk mengganggu kitaran sel.

Kata kunci: Apoptosis; *Camellia sinensis*; hentian kitaran sel; leukemia; *Phyllanthus amarus*

Introduction

Leukemia is a type of cancer characterised by malignant progressive disease that originates from hematopoietic cells in the bone marrow or lymphoid organs. Although there are various modalities of treatment including chemotherapy, bone marrow transplant and radiotherapy used on leukaemia patients, these treatments can cause side effects such as increased risk of infections, fatigue, damages to intestinal mucosa, or bleeding gums that pose serious discomfort to the patients and greatly affect their daily lives (Vickers 2004). On that note, a lot of research have been carried out to discover new anticancer agents of plant origins containing natural compounds and derivatives with therapeutic benefits. Several studies have shown that plant derivatives are apoptotic and cell cycle arrest inducer that can inhibit cancer cells. In fact, more than 60% of currently available anticancer drugs such as topotecan, etoposide and paclitaxel were of plant origin (Fulda & Debatin 2006).

One of the plants studied is *Camellia sinensis* which is also known as green tea. Green tea is well known as having high amount of catechin epigallocatechin-3-gallate (EGCG) that can promote anti-cancer activity (Kim et al. 2014). This plant possesses powerful antioxidant, anti-angiogenic and antitumor activity and can act as a modulator of tumour cell response to chemotherapy. Much of the chemopreventive properties of green tea are mediated by EGCG which could induce apoptosis and promotes cell growth arrest by changing the expression of cell cycle regulatory proteins, activating killer caspases, and suppressing oncogenic transcription factors and pluripotency maintaining factors (Ghasemi-Pirbaluti et al. 2015). It was found that green tea tends to maintain cell viability and also able to induce apoptosis in many cancers cell lines, including leukemic cells (Forester & Lambert 2011).

Another well-known medicinal plant that also possess chemopreventive and antitumor effects is *Phyllanthus amarus*, commonly known as gale of the wind, display protective properties in preventing hepatocarcinogenesis and inhibiting the growth of murine leukemic cell line (Pettit et al. 1990). Moreover, the apoptotic inducing ability of *Phyllanthus* sp. plants on various types of cancer cells has been identified. Different species of *Phyllanthus* sp. have been proven to trigger activation of caspase-3 and -7, which lead to apoptosis induction in cancer cells (Harikumar et al. 2009; Lee et al. 2011). Furthermore, it has been found that *P. amarus* inhibits cancer growth by disrupting the cancer cell cycle at different phases which later induces apoptosis (Tang et al. 2010).

Apoptosis plays an important role in eliminating damaged or unwanted cells. Cancer therapies by most anticancer agents currently used in clinical oncology such as chemotherapy, γ-irradiation, suicide gene therapy or immunotherapy, are linked to the activation of apoptosis signal transduction pathways in cancer cells (Fulda & Debatin 2006). However, patients are sometimes inundated with unbearable side effects from the therapy which could force patients to live with the consequences of the treatment (Galligan 2020). Therefore, the pursuit of alternative anticancer therapies with less side effects is an ongoing effort aims at delivering the best treatment approach for cancer patients. This study focuses on such pursuit by studying the potential of *C. sinensis* and *P. amarus* in modulating cancer cell line's growth and proliferation.

Materials and Methods

Chemicals

Chemicals that were used in this study included Gibco™ Roswell Park Memorial Institute (RPMI) Medium 1640 (Life Technologies, USA), Gibco™ Iscove's Modified Dulbecco's Medium (IMDM) (Life Technologies, USA), Gibco™ Fetal Bovine Serum (FBS) (Life Technologies, USA), Penicillin Streptomycin Antibiotic (Amresco, USA), trypan blue solution 0.4% (Sigma-Aldrich, USA), phosphate buffered saline tablets (Sigma-Aldrich, USA), thiazolyl blue tetrazolium bromide Methylthiazolyldiphenyl-tetrazolium bromide (MTT) reagent powder (Sigma-Aldrich, USA), Ethanol 95% (HmbG® Chemicals, Germany), dimethyl sulfoxide (DMSO) (Merck Millipore, Germany), and CycleTEST™ PLUS DNA Reagent Kit (Becton Dickinson, California).

Extract Preparation

Dried crushed *C. sinensis* leaves were soaked in 80% ethanol for 7 days and filtered through filter paper with 11 mm pore size to collect the filtrate. For the *P. amarus* extract, the dried plant samples were soaked in 80% ethanol for 3 days prior to filtration through the filter paper. This step was repeated three times. Solvent was removed by evaporation using rotary evaporator to obtain the crude extract.

Cell Cultures

U937 human leukemic monocyte lymphoma cell line (ATCC® CRL1593.2™), Jurkat Clone E6-1 human acute

T cell leukemia cell line (ATCC® TIB152™) and K-562 human chronic myelogenous leukemia cell line (ATCC CCL243™) was purchased from American Type Culture Collection (ATCC), USA. U937 and Jurkat cells were cultured in RPMI 1640 (Gibco, OK, USA) supplemented with 10% fetal bovine Serum (FBS; Gibco, OK, USA) and 1% penicillin/streptomycin (Amresco, OH, USA). K562 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Gibco, OK, USA) supplemented with 10% fetal bovine Serum (FBS) and 1% penicillin/streptomycin.

MTT ASSAY

Cell viability was assessed by using methyl thiazolyl tetrazolium (MTT) assay. Cells in exponential growth state were seeded at a density of 5×10^5 cells/mL in a 96-well plate and treated with 100 µL of *C. sinensis* 75% ethanol and *P. amarus* 80% ethanol extracts at concentration ranging from 31.25 - 500 µg/mL prior to 24 h incubation at 37 °C. After 24 h, 20 µL of MTT (5 mg/mL) was added into each well and further incubated for 4 h at 37 °C. After completion of the incubation period, 150 µL of the media was carefully removed from each well and was added with 150 µL dimethylsulfoxide (DMSO) to dissolve the insoluble formazan formed at the bottom of the well. Absorbance was read at 565 nm using the microplate reader Infinite® 200 Pro (Tecan, Switzerland). Cell viability was determined using equation (1):

$$\text{Cell viability (\%)} = \frac{A_{\text{treatment}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} x\ 100 \quad (1)$$

Using the Graph pad prism statistical software, the percentage of cell viability was then plotted against the log concentration of extract used to determine the IC_{50} and the standard error mean (SEM). IC_{50} is the concentration of the extract that reduced cell viability by 50% from the total cell population. These values obtained were then used in subsequent analysis to study the anti-leukemic effects of *C. sinensis* and *P. amarus* extracts on U-937, Jurkat Clone E6-1 and K562 cell line.

APOPTOSIS ASSAY

K562, U937, and Jurkat cells were seeded at 1×10^6cells per well in a 6-well plate, and treated with the IC_{50} concentration of *C. sinensis* and *P. amarus* extracts at 170 and 210 µg/mL, respectively, at 37 °C for 24 h. At the end of the incubation period, cells were washed with cold phosphate-buffered saline (PBS) and re-suspended in Annexin V Binding buffer. FITC-conjugated Annexin V dye and propidium iodide (PI) were added to the cells and were subjected to analysis by flow cytometer (BD FacsCanto II, CA, USA) to identify necrotic and apoptotic cells.

CELL CYCLE ASSAY

Cell cycle assay was done according to the manufacturer's instructions. The cell cycle of U-937, Jurkat Clone E6-1 and K562 cell lines treated with *C. sinensis* (170 µg/mL) and *P. amarus* (210 µg/mL) extracts were analyzed using flow cytometer. The procedures of seeding and treatment were prepared under sterile condition. In a sterile 6-well plate, cells were seeded at 5×10^5 cells/mL in serum-free media with or without the extract and were incubated in 5% CO_2 at 37 °C for 24 h. After 24 h, the cells were prepared for staining to determine the effect of the plant extracts on cell cycle arrest using flow cytometer. The cell suspension was covered and incubated for 10 min at 4 °C before subjected to flow cytometry analysis.

STATISTICAL ANALYSIS

Graphpad Prism 5 was used to analyse the data obtained from each experiment. All data were presented as mean±SEM. Calculations and statistical tests were performed using one-way ANOVA with Tukey's Post Hoc test. All experiments were repeated at least three times unless indicated otherwise. A $p<0.05$ is considered as significant.

RESULTS AND DISCUSSION

CYTOTOXIC EFFECT OF *C. sinensis* AND *P. amarus* EXTRACTS ON LEUKEMIC CELL LINES

After 24 h treatment with increasing concentration of *C. sinensis* extract (31.25 - 500 mg/mL), each cell culture was washed and prepared for MTT staining to determine the cytotoxicity of each concentration used on cell viability. Based on the absorbance reading obtained at 565 nm, the results are expressed as the percentage of cytotoxicity compared to the negative control (Figure 1).

In Figure 1, *C. sinensis* extract showed a significant dose-dependent cytotoxicity on U937 cells up to 65% at the highest concentration used ($p<0.001$). From the data, the IC_{50} value is calculated to be 170 µg/mL (Table 1). Significant cytotoxicity was also observed in the K562 cells at each concentration tested but the effect is not in a

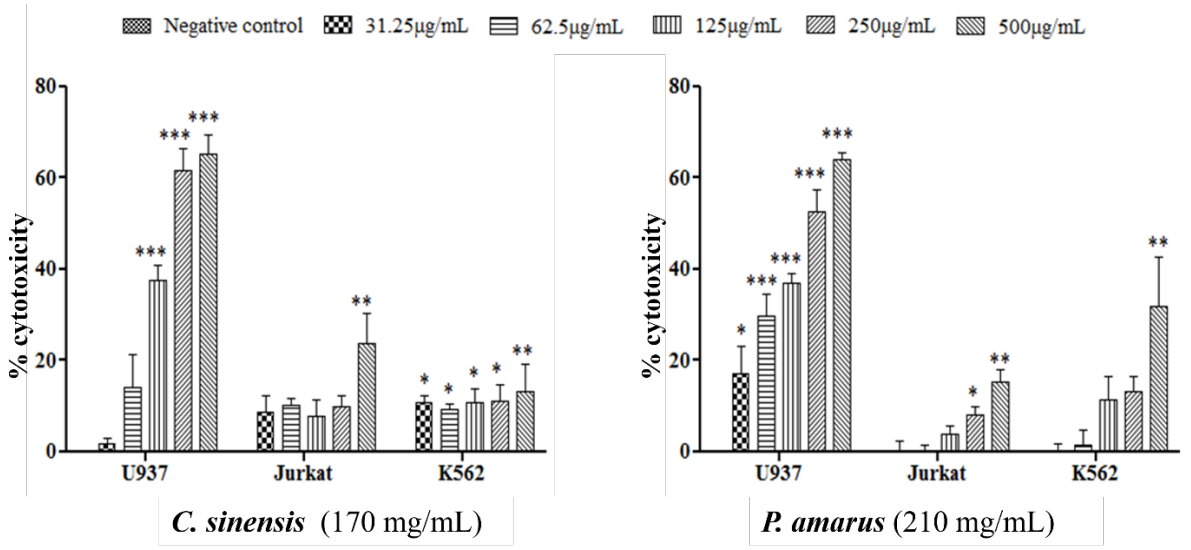

FIGURE 1. Cytotoxic effect of *C. sinensis* and *P. amarus* extracts on mononuclear cells. U937, Jurkat, and K562 cell lines were treated with *C. sinensis* and *P. amarus* extracts for 24 h before subjected to MTT assay. Data are presented as the percentage of cytotoxicity compared to negative control (vehicle). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (One-way ANOVA with Tukey's test); mean±S.E.M., n=3

dose-dependent manner and the maximum was recorded at 13% cell death. Meanwhile, up to 24% of Jurkat cells were dead at 500 µg/mL ($p<0.01$). Also in Figure 1, *P. amarus* extract showed a similar trend of significant dose-dependent cytotoxicity on U937 cells starting at the concentration of 31.25 µg/mL ($p<0.05$) until 500 µg/mL ($p<0.001$). Thus, the IC_{50} value calculated is 210 µg/mL. Interestingly, Jurkat cells were more sensitive towards *C. sinensis* extract compared to *P. amarus* extract as cell death were observed starting from 125 µg/mL, which was higher compare to the *C. sinensis* extract. K562 cell lines on the other hand were less sensitive toward *P. amarus* extract treatment as significant cytotoxicity was only able to be observed at the highest concentration, 500 µg/mL ($p<0.01$). This study showed that the sensitivity of Jurkat and K562 cells to *C. sinensis* and *P. amarus* extracts were relatively lower compared to U937 cells, because both cells did not achieve 50% cell death. Previous study of Azmi et al. (2018) that has used methanol extract of green tea showed the IC_{50} of 88±1.89, 98±1.96, and 205±2.23 µg/mL on U937, Jurkat and K-562 cells, respectively, this may be caused by influence of the toxic effect of methanol.

According to Kundu et al. (2005), treatment of HL-60 and K562 cells with extracts of green tea for 24 h showed a dose-dependent inhibition of growth as a result of cytotoxicity and suppression of cell proliferation. He also claimed that the IC_{50} values of green tea extract obtained from cytotoxicity data was clearly evident to afford significant chemotherapeutic action by imparting cytotoxicity to human leukemic cells (Kundu et al. 2005). Studies also showed that the polyphenols found in the *P. amarus* have the ability to halt cancer growth by inhibiting and killing the cancer cells (Tang et al. 2010). The polyphenols possess anti-proliferative effects associated with their natural antioxidant activity (Pettit et al. 1990).

TABLE 1. IC_{50} of *Camellia sinensis* and *Phyllanthus amarus* extracts on U937, Jurkat and K562 cells

Types of cells	Plant extracts	IC_{50} (µg/mL)
U-937	*Camellia sinensis*	170±10.39
	Phyllanthus amarus	210±6.78
Jurkat	*Camellia sinensis*	ND
	Phyllanthus amarus	
K562	*Camellia sinensis*	ND
	Phyllanthus amarus	

ND: Not determine

Figure 1 also showed that based on percent inhibition of cell viability, the U937 cells are the most sensitive to the both plant extracts with $p<0.001$ for both plant extract for concentration 62.5 to 500 µg/mL compared to negative control, followed by K-562 then Jurkat cells and the response was in a dose-dependent manner.

The sensitivity of Jurkat Clone E6-1 and K-562 cells to *C. sinensis* and *P. amarus* extracts were relatively lower compared to U-937 cells, because both cells cannot achieve 50% cell death. This finding is in accordance with the study that acute leukemia has been found to be more sensitive towards chemotherapy drugs as compared to chronic leukemia. Besides that, most chronic leukemia is often resistant to chemotherapy drugs (ACS 2015). Therefore, the same IC_{50} values obtained from U-937 cell was used in both Jurkat Clone E6-1 and K-562 cells for this work.

ANALYSIS OF APOPTOSIS INDUCTION

Using flow cytometer, cells were distinguished as viable, early apoptosis, late apoptosis and necrosis based on FITC and PI positive labelling as represented by Figure 2. The data obtained for the percentage of apoptotic and necrotic cells with and without treatment were then extrapolated as graph chart in Figure 3.

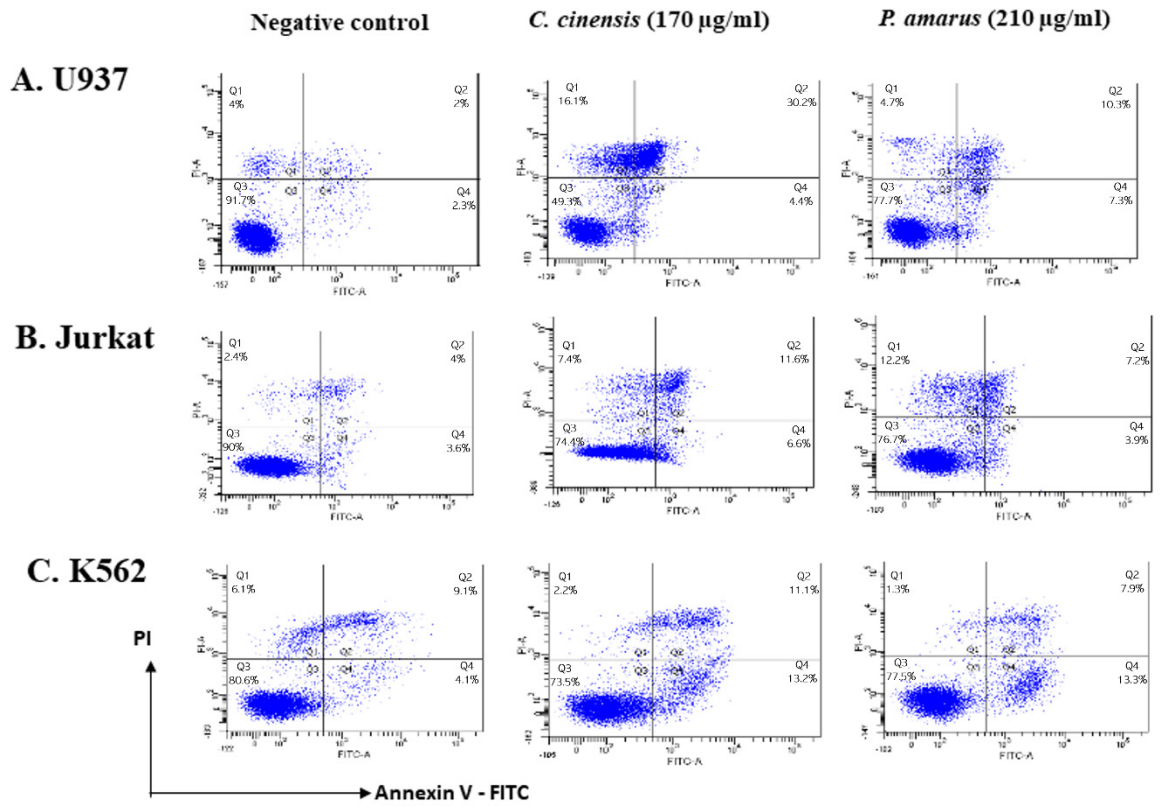

FIGURE 2. Representative flow cytometry dot plots. The figure shows viable cells (Q3), apoptotic cells (Q2+Q4) and necrotic cells (Q1) of A. U-937, B. Jurkat and C. K562 cells post 24 h incubation with *C. sinensis* extract (170 mg/mL) and *P. amarus* extract (210 mg/mL). Untreated cells as negative control

From Figure 3(A), the highest percentage of apoptotic cells ($p<0.001$) was observed in U937 cells compared to other cells with $p<0.05$ and $p<0.01$ for Jurkat and K562 cells, respectively, treated with *C. sinensis* for 24 h. This result is in accordance with our previous study which using methanol extract of *C. sinensis* showed the same pattern in inducing apoptosis, however, with higher significance such as with $p<0.001$, $p<0.01$, and $p<0.001$ for U937, Jurkat and K562 cells, respectively (Azmi et al. 2018). The methanol extract of

C. sinensis produced more significant effect compared to ethanol extract, this may be caused by the more active compounds extracted using methanol solvent or there is contribution of toxic effect from the methanol itself. *P. amarus* treatment also induce apoptotic respond in all cells but not as markedly significant as seen in *C. sinensis*. *P. amarus* treatment however led to a significant necrotic induction in Jurkat cells ($p \leq 0.05$) compared to other cells tested (Figure 3(B)). Meanwhile, both extracts were able to significantly induce similar apoptotic respond on K562 cells compared to negative control but not to the extent of causing necrosis.

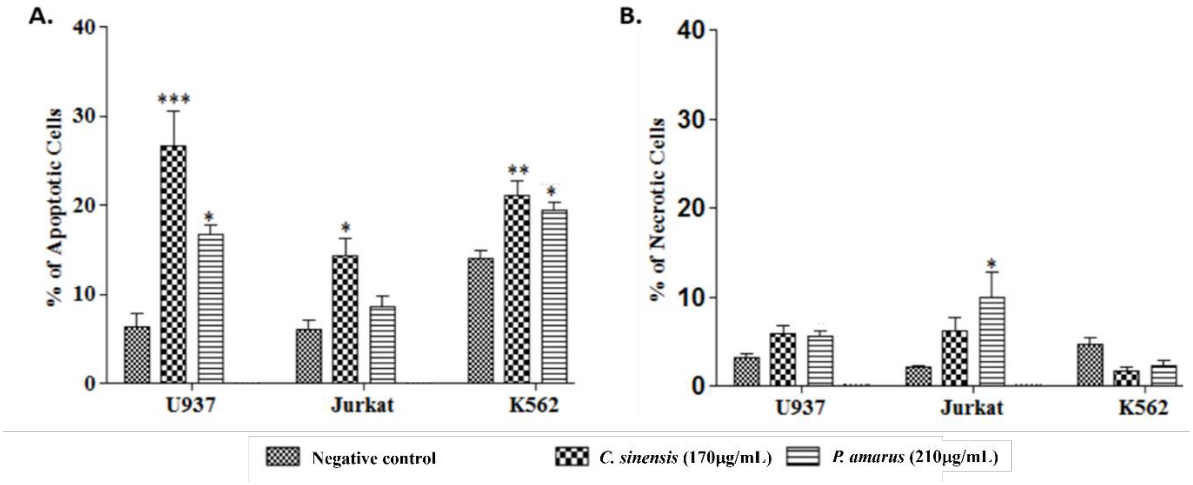

FIGURE 3. Percentage of A. Apoptotic and B. Necrotic cells of human leukemic cell lines after treated with *C. sinensis* (170 mg/mL) and *P. amarus* (210 mg/mL) extract compared to negative control containing media only. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significant difference when compared with the negative control

Previous study proved that EGCG rapidly induces apoptosis of myeloid leukemic cells via modulation of ROS production *in vitro* and inhibits tumour growth *in vivo* (Nakazato et al. 2005). Among all the tested GTPs, EGC was the most potent in accumulation of S phase cells and inhibition of the S-G_2 progression. In addition, EGC-mediated inhibition of S phase progression results in induction of apoptosis, as determined by sub-G_1 cell population, breakage of endonuclear DNA, cleavage of poly(ADP-ribose) polymerase and loss of cell viability (Smith & Dou 2001).

Based on the above result, *P. amarus* extracts significantly induced apoptosis in the U937 and K562 cell lines. The result was in accordance with research done by Harikumar et al. (2009) which proved that *P. amarus* induces the formation of apoptotic bodies in DLA cells *in vitro* evident from the morphology of the cells like plasma membrane invagination, elongation, fragmentation, and chromatin condensation. Various bioactive compounds contained in *Phyllanthus* sp. extracts have been shown to exert apoptosis-inducing effects. For instance, gallic acid prevents the metastasis of AGS and U87 cells via downregulation of Ras/P13k/AKT and Ras/MAPK signalling pathways (Singh et al. 2011). Another research reported that *P. amarus* extract induces apoptosis in human breast cancer cell line, MCF-7, by increasing the levels of intracellular ROS and decreased MMP. In addition, *P. amarus* extracts also induced expression of caspase-3 and down-regulates expression of Bcl-2 to allow apoptosis in cancer cells (Lee et al. 2011).

ANALYSIS OF CELL CYCLE ARREST

After 24 h of exposure to green tea extract, the green tea showed no significant difference in arresting cell cycle on U937, Jurkat Clone E6-1 and K652 cells when compared to the respective negative controls (Table 2). However, the exposure of *P. amarus* extract to U-937 cells has caused a high distribution of cells in S phase with a percentage of 55.71±2.06% ($p<0.001$). This result showed that *P. amarus* extract significantly arrests cell cycle at S phase

of U-937 cells when compared to negative control. Meanwhile, *P. amarus* extract significantly arrest K-562 cells at G0/G1 phase with the highest accumulation of cells observed with 56.77±4.69% ($p<0.05$) while no difference in cell cycle observed on Jurkat Clone E6-1 cells when compared to control. These results showed that when compared to negative control, *P. amarus* extract may promote cycle arrest at different phases depending on the type of cells (Table 2).

TABLE 2. Comparison of mean percentage of cells in different phases of cell cycle

Types of cells	Treatment	Percentage of cells at G0/G1 phase (%±SEM)	Percentage of cells at S phase (%±SEM)	Percentage of cells at G2/M phase (%±SEM)
U-937	Control (untreated)	46.18±1.43	43.95±0.82	9.87±1.07
	Green tea 170 µg/mL	39.26±3.61	49.79±8.41	10.94±4.81
	P. amarus 210 µg/mL	38.72±1.31	55.71±2.06 ***	5.58±3.20
Jurkat Clone E6-1	Control (untreated)	36.37±4.23	50.98±6.23	12.65±2.03
	Green tea 170 µg/mL	35.83±5.05	41.31±5.86	22.86±1.23
	P. amarus 210 µg/mL	30.08±3.90	56.11±5.78	13.82±2.97
K-562	Control (untreated)	38.99±2.43	46.84±1.95	14.18±1.68
	Green tea 170 µg/mL	52.95±5.11	37.61±4.11	9.43±1.62
	P. amarus 210 µg/mL	56.77±4.69 *	33.36±4.39	9.87±0.66

Percentage of cells treated with green tea extract and *P. amarus* extract at concentration of 170 µg/mL and 210 µg/mL respectively. All the values show the mean±SEM for 3 independent experiments (n=3). * $p<0.05$, *** $p<0.001$ significant difference when compared with the negative control

Recent studies of *P. amarus* have emphasized such as the effect suppressing in NF-κB/MAPKs/PI3K-Akt signaling pathways of *P. amarus* ethanol extract and its active compounds as phyllanthin, hypophyllanthin and niranthin (Harikrishnan et al. 2018a, 2018b, 2018c) have been performed, in addition, this plant extract also showed strong effect as anti-rheumatoid arthritis in Sprague Dawley Rats induced by type II collagen (Alam et al. 2018). Inflammation is strongly related to cancer and chronic inflammation promotes carcinogenesis by inducing proliferation, angiogenesis, metastasis and reducing the response to the immune system and chemotherapeutic agents (Zappavigna et al. 2020). Meanwhile, the extract of *P. amarus* has been found to possess a selective anti-proliferative effect on leukemia cells without showing cytotoxicity on normal cells (Huang et al. 2004). The anti-proliferative effects of *P. amarus* could be due to the presence of polyphenol compounds known as gallic acid which was being found to have anti-proliferative effects on several cancer cells (Huang et al. 2009).

The cell cycle assay was carried out to measure cellular DNA content in the human leukemic cell lines when treated with green tea and *P. amarus* extracts. Targeting the cell cycle could be an approach for anticancer agents to halt the uncontrolled proliferation of cancer cells and initiate them to undergo apoptosis (Shapiro & Harper 1999). In this study, the human leukemic cell lines were treated with 170 µg/mL of green tea extract and 210 µg/mL of *P. amarus* extract for 24 h and analyzed using PI-stained DNA content by flow cytometry. The PI that stained to DNA is directly proportional to the amount of DNA in the cells. The analysis of DNA content allows discrimination between G1, S, G2, and M phases.

There is compelling evidence that apoptotic death induced by chemopreventive agents is closely linked to perturbation of specific phase of the cell cycle (Surh et al. 1999). It was found that the effect of anti-proliferative agent on cell cycle progression appears to depend on the concentration of the compound and also on the duration of treatment (Surh et al. 1999). For example, the gradual

increase in the proportion of cells in G1/G0 phase after treatment with epigallocatechin-3-gallate, an active compound of green tea suggests that cells arrested in the G0/G1 phase. Besides that, several mechanisms have been proposed that the EGCG have been found to arrest cancer cells in the G1/G0 phase (Ahmad et al. 1997). Meanwhile, in our study, green tea extract did not show an arrest in the cell cycle of three types of leukemic cell tested, this is possible that the epigallocatechin-3-gallate in the extract was not sufficient to produce that effect.

Apart from that, *P. amarus* extract has been found to inhibit cancer growth by arresting the cancer cells at different cell cycle phase which later initiates them to undergo apoptosis. It was also claimed that the extract had been found to arrest different type of cancer cells in different phases. It was reported that *P. amarus* could induce G1 phase arrest in PC-3 cells (Tang et al. 2010). Arrested in G1 phase indicated that *P. amarus* extract might disrupt the protein synthesis that required for PC-3 cells to enter the next phase of cell cycle. When the cells are arrested at a particular phase, they lose their uncontrolled proliferation properties and the cells will be initiated to undergo apoptosis (Tang et al. 2010).

For the treatment of *P. amarus* in human leukemic cell lines, it was also showed that cell cycle arrest occurs at S phase in U-937. Generally, most of cells undergo DNA synthesis in S phase. However, due to cell cycle arrest in this phase, there was possibility that fewer cells will undergo cellular division in G2/M phase. Apart from that, cell cycle of K-562 cells that treated with *P. amarus* has been found to arrest in the G0/G1 phase. The cell cycle arrest in this phase may be response to DNA damage or deregulation of cyclin dependent kinase which is specifically demonstrated in G1 phase (Pucci et al. 2000). This showed that *P. amarus* extract may induce cell cycle arrest at different phases of cell cycle depends on the type of cells. However, *P. amarus* extract do not modulate cell cycle arrest in Jurkat Clone E6-1 cell lines at concentration tested.

Conclusion

In conclusion, both *C. sinensis* and *P. amarus* extracts are effective in inducing apoptosis in U-937, Jurkat, and K-562 cell lines. *P. amarus* extract induced cell cycle arrest in S phase for U937 cells and in G0/G1 phase for K-562 cells. Meanwhile Green tea extract did not modulate the cell cycle at concentration tested. This study suggests that *C. sinensis* and *P. amarus* contain bioactive compounds that possess potential chemopreventive activity thus could be beneficial in leukemia. More studies are needed to identify the active constituents in the extracts and to further clarify the molecular interactions between the constituents and the putative trans-signal receptors of leukemic cells that resulted in the modulation signaling pathways that are associated with apoptotic and cell death mechanism in leukemic cells.

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