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Antileukemic Activity Of Bulb And Root Of Eleutherine Americana L. Merr. On Jurkat Human Leukemia Cell Line

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Abstract

Objectives: This study was designed to evaluate the antileukemic properties of bulb and root of Eluetherine americana L. Merr. extracts on Jurkat cell line and to determine the mode of cell death of Jurkat induced by both extracts.

Methodology: Jurkat and MRC-5 cell lines were treated with desired concentrations of ethanolic extracts of bulb (EEBE) and root (EERE) of E. americana and doxorubicin (positive control). MTT assay was conducted to calculate the cell viability. The half maximal inhibitory concentrations (IC_{50}) were then calculated based on the dose-response curves. Besides, morphological changes of Jurkat were observed under inverted microscope.

Results and discussion: Bulb exhibited a significant cytotoxic activity on Jurkat (IC_{50} = 3.855 ± 0.55 µg/mL) and MRC-5 (IC_{50} = 5.906 ± 0.125 µg/mL), while root exhibited a significant cytotoxic activity on Jurkat (IC_{50} = 13.087 ± 1.799 µg/mL) but a very weak cytotoxic activity on MRC-5 (IC_{50} = 98.547 ± 2.172 µg/ml). Doxorubicin (pure compound) also exhibited a significant cytotoxic activity on Jurkat (IC_{50} = 0.043 ± 0.015 µg/mL) and MRC-5 (IC_{50} = 0.082 ± 0.009 µg/mL). All samples induced apoptosis in Jurkat at concentrations near to IC_{50} and about 10 times higher of IC_{50}. Root and doxorubicin also induced necrosis at concentration about 10 times higher of IC_{50}, but only doxorubicin prominently induced necrosis.

Conclusion: EEBE and EERE can be further studied as the alternative for leukemia treatment.

Keywords Eleutherine americana, Jurkat, MTT assay, cytotoxicity, IC_{50}, apoptosis, necrosis.

1. Introduction

Leukemia is defined as a haematological malignancy that is characterised by the uncontrolled accumulation of pathological white blood cells [1]. It starts in the bone marrow, in which the abnormally proliferating neoplastic cells diffusely replacing it [2].

In 2003, the incidence rates of lymphoid leukemia in Malaysia for both males and females were 2.8 and 1.7 per 100,000 populations respectively, among the 21, 464 cancer cases. Meanwhile, the incidence rates of myeloid leukemia in Malaysia for males and females were 3.0 and 2.7 per 100,000 populations, respectively. Leukemia is more common in males than females and diagnosed 10 times more often in adults than children [3].

Currently, chemotherapy is a major pharmacological approach in the treatment of leukemia [1], apart from radiotherapy and chemically derived drugs. Alternative treatments and therapies are also used, as treatments such chemotherapy can put patients under a lot of strain and further damage their health [4].

Plants used in traditional medicine have remained useful for a long period of time and contributed many novel compounds for preventive and curative medicine to modern science [5]. Medicinal plants and their phytoconstituents is a choice for leukemia treatment and nutraceuticals have been proven to have antileukemic activity in experimental studies [6].

Bawang Dayak (Eleutherine americana L. Merr.; synonym Eleutherine palmifolia L. Merr.) is a plant from family Iridaceae. It is widely found in Kalimantan Island, Indonesia (particularly East Kalimantan) and widely grown at 600 to 2000 m above sea level [7].

This plant has been widely used as traditional medicine. Local people of Kalimantan have been using the bulb of the plant against breast cancer and heart diseases [8]. The bulb of E. americana contains naphthoquinone group (elecanacine, eleutherine, elutherole, eleutherinone), which is known to function as anticancer as well as antioxidant [9].

Therefore, the purpose of this study was to evaluate the antileukemic properties of bulb and root of Eluetherine americana L. Merr. extracts on Jurkat cell line. In addition, the mode of cell death of Jurkat induced by each extract was determined.

2. Methodology

2.1. Plant Authentication

The whole plant of Eleutherine americana L. Merr. was sent to Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia for authentication (Voucher No.: MFI 0018/18).

2.2. Chemicals and Instruments

2.2.1. Chemicals

In sample preparation and extraction procedure, absolute ethanol from Merck Millipore, Germany was used. In cell culture techniques procedure, Jurkat and MRC-5 cell lines which were obtained from American Types Cell Collection (ATCC), USA were used. Besides, products from Life Technologies, USA such as Gibco RPMI-1640 media, Gibco fetal bovine serum (FBS), Gibco trypsin-EDTA, Gibco penicillin-streptomycin, Gibco amphotericin B and Gibco gentamicin, and products from Sigma-Aldrich, UK such as phosphate buffered saline (PBS) and dimethyl sulfoxide (DMSO) were used. Absolute ethanol, FBS and RPMI-1640 media were used in cytotoxic drug preparation and cell treatment procedure, together with Adriamycin (doxorubicin) 2 mg/ml solution for injection from Pfizer, USA. MTT from Phyto Technology Laboratories, USA was used in in vitro cytotoxicity studies procedure.

2.2.2. Instruments

In sample preparation and extraction procedure, Frontier Duo fume hood from ESCO, Singapore was used. Meanwhile, in cell culture techniques procedure, NuAire LabGard category II laminar air flow (LAF) cabinet and RS Biotech Galaxy S carbon dioxide (CO_2) humidified incubator from USA, Eppendorf Research Micropipette and Eppendorf 5804 centrifuge from Germany, and Olympus CK40 inverted light microscope from Japan were used. The LAF cabinet and incubator were used in both cytotoxic drug preparation and cell treatment procedure and in vitro cytotoxicity studies procedure. In addition, Memmert drying oven and Eppendorf Research Plus 300 µl multichannel micropipette from Germany were used in cytotoxic drug preparation and cell treatment procedure. Lastly, inverted light microscope was used in in vitro cytotoxicity studies procedure, besides Chromate ELISA reader from Awareness Technology, USA.

2.3. Sample Preparation and Extraction

The leaf was removed from the whole plant of Eleutherine americana L. Merr. The red bulbs and root of E. americana were separated before being weighed and washed with distilled water. They were then sun-dried for 3 hours and sliced thinly. Next, both bulb and root were dried in the oven at 50°C for 24 hours. Thus, the heat sensitive microorganisms and vitamins were eliminated. Subsequently, by using a small grinder at 3000 rpm, they were grounded into powder.

After both powders were ready, 50 g of each powder (bulb and root) was weighed and transferred into a 500 mL glass bottle. For the bulb powder, absolute ethanol was added until the volume reached 300 mL, while for the root powder, absolute ethanol was added until the volume reached 500 mL (as root took more space of

the bottle than bulb). After that, both bottles were shaken vigorously for a while before being left soaked for three days (72 hours). Then, both mixtures were filtered using 125 mm filter paper.

Each filtrate was collected into a beaker and the top were covered using aluminium foil and secured with parafilm. The beakers were then stored in the normal refrigerator. Again, the same powders were shaken and soaked with fresh absolute ethanol for another 3 times (until the filtrates formed become nearly transparent). All filtrates of each bulb and root were then mixed and left to be evaporated in the fume hood until concentrated.

2.4. Cell Culture Techniques

To maintain the highest sterility and prevent any contamination that may occur, all of the cell culture procedures were performed under a category II LAF cabinet.

2.4.1. Cell Lines

There were 2 cell lines used in this study; human acute T-cell leukemia cell line, Jurkat and human normal lung fibroblast cell line, MRC-5 (functions to evaluate the 'cytotoxicity selectivity' of the samples).

2.4.2. Thawing of Stock Cells

Since the thawing procedures are stressful to cells, good and correct techniques must be followed strictly besides working quickly. These are to ensure the high survival of cells after the procedures.

The cryotubes of both Jurkat and MRC-5 cell lines were retrieved from the liquid nitrogen freezer. They were thawed by being warmed within palms of both hands until the suspension melted and loose enough to be transferred into 2 separate 15 mL conical centrifuge tubes containing growth media. Next, both tubes were centrifuged at 1800 rpm for 5 minutes, and the supernatants were removed.

The cells, being in suspension form, were resuspended in 10 mL of fresh complete growth medium with 10% FBS right before being transferred into 2 separate 25 cm^2 canted tissue culture flasks. The flasks then were incubated at 37°C in a 5% CO_2 humidified incubator until confluent [10].

2.4.3. Maintenance of Cell Lines

Both cell lines were maintained using standard culture procedures. In RPMI-1640 media supplemented with 10% FBS, Jurkat cell line was grown as non-adherent culture while MRC-5 cell lines as adherent culture. Penicillin-streptomycin, amphotericin B and gentamicin were added to both cultures to prevent contamination. They were grown separately in 25 cm^2 and 75 cm^2 canted neck tissue culture flasks in a 5% CO_2 humidified incubator at 37°C until confluent. In order to make sure the cells were healthy and free from microbial contamination;

the major features of the cells were inspected using inverted light microscope. The flasks will be removed immediately from the incubator and discarded if contamination was detected.

2.4.4. Passaging / Subculturing

Once the cells reach confluence, they may stop growing due to contact inhibition. Therefore, subculturing procedures must be done to prolong the life and/or expand the number of cells. Jurkat, being a non-adherent cell, has a different subculturing procedure compared to MRC-5, which is an adherent cell.

For Jurkat cell line, firstly, all content of the flask was transferred into a 15 mL conical centrifuge tube. Next, like the thawing procedures, the tube was centrifuged at 1800 rpm for 5 minutes, the supernatant was removed, and the cells are now in suspension form. The cells were then resuspended in 1 mL of fresh serum-containing medium. After that, small proportion of the cells was transferred into new culture flasks before fresh serum-containing medium was further added.

For MRC-5 cell line, firstly, the media from the flask was discarded. Subsequently, to entirely remove the serum-containing medium (contains trypsin inhibitors) from the cell culture, the flask was washed with PBS. Trypsin-EDTA was added to the flask before it was incubated for 3 to 5 minutes in a 5% CO_2 humidified incubator at 37°C. These are to detach the cells from the wall of the flask. If the cells are hard to detach, the flask can be slightly tapped. Then, to inactivate the trypsin, fresh serum-containing medium will be added into the tryptinized cells once the cells detached from the wall (can be confirmed by using inverted light microscope). This is because trypsin is cytotoxic, so over-treatment by trypsin can cause cell death. The cells are now in suspension form. Small proportion of cells was then transferred into new culture flasks.

2.4.5. Haemocytometer Counting

Haemocytometer counting was done to determine the number of cells in a culture suspension. The same procedures were carried out for any cell line including Jurkat and MRC-5.

Firstly, a cover slip was placed over the haemocytometer counting chamber. 10 µL of cell suspension was resuspended with 10 µL of 0.4% trypan blue to stain the cells. Subsequently, 10 µL of the mixture was placed at the edge of the chamber by using a micropipette and allowed to be drawn into the chamber by capillary action. This step was done carefully so as not to over fill or under fill the chamber. Next, the chamber was

placed under inverted light microscope to count the number of cells. The number of cells was counted in 4 smaller squares (each of 1 mm^2 represents a volume of $1x10^{-4}$ mL (0.1 mm^3)).

After the average cell numbers of all 1 mm^2 areas counted was taken, the cell concentration was calculated as follows:

$$C = \frac{n}{v} \times d$$

C = Cell concentration in cells/ml

n = Average number of cells/mm^2

v = Volume counted ($1x10^{-4}$ ml)

d = Dilution factor

2.4.6. Storage / Freezing of Cells

Cells were frozen to store them for future use. The principle was to prepare cells with high density (but not confluent) and froze them slowly with a preservative. Only cells with healthy features of growth and free from contamination were selected (after being examined under inverted microscope). Due to changes that may occur during subculturing, recently thawed cells were preferred to replenish stocks, instead of the cells that have been cultured for several passages. The same procedures were carried out for any cell line including Jurkat and MRC-5.

The cells from the flask were transferred into a centrifuge tube before being spun at 1800 rpm for 5 minutes and the supernatant (culture media) was discarded. Next, PBS was added to wash the cell pellet before being recentrifuged again at 1800 rpm for 5 minutes. After the supernatant was discarded, the cell pellet was resuspended in preservative freezing medium DMSO to approximately $1x10^6$ to $1x10^7$ cells/mL. Then, 1 to 1.5 mL of the suspension was filled in 2.0 mL cryotubes with. They were kept at 4°C for 1 hour first, followed by -30°C for 3 to 4 hours, then at -80°C overnight and finally at -196°C in liquid nitrogen. In case the cells will be needed for future use within weeks, some cells were kept at only -80°C instead of -196°C [10].

2.5. Cytotoxic Drug Preparation and Cell Treatment

Empty 1.5 mL microcentrifuge tubes with lids were weighed and filled with the concentrated extracts. Microcentrifuge tubes filled with extracts were then weighed and put into heating and drying oven at 37°C for about 24 hours to eliminate ethanol residual (extraction solvent). This step was repeated until constant weight

was obtained to get exact weight of the extracts. Extracts were then dissolved in absolute ethanol to the concentration of 100 mg/mL. Subsequently, the dissolved extracts were then mixed in a 15 mL conical centrifuge tube.

After that, complete growth media (RPMI-1640) with 5% FBS was added to each of the dissolved extracts to further dissolve them to the concentration of 400 µg/ml and 10 µg/mL (starting from here, all steps were performed under a category II LAF cabinet). Since ethanol is possibly cytotoxic, its concentration should not exceed 1% of the final solution. Solutions of 400 µg/mL and 10 µg/mL became the stock solutions.

As for the positive control treatment, doxorubicin solution was used. To prepare the stock solutions of doxorubicin, complete growth media with 5% FBS was added to the solution in a 15 mL conical centrifuge tube, to the concentration of 4 µg/mL and 1 µg/mL.

For the cell treatment procedure, for each Jurkat and MRC-5, plates were prepared in triplicate. Firstly, 100 µL of cells ($1x10^4$ cells/mL) from the stock cells of Jurkat and MRC-5 that has complete growth medium containing 5% FBS, were transferred into each well of the 96-well sterile plates using multichannel micropipette (for each plate of Jurkat, only 80 wells were filled with cells and for each plate of MRC-5, only 70 wells were filled with cells, meanwhile, the rest were left empty).

Based on calculation using the standard dilution equation ($M_1V_1 = M_2V_2$, in which the final volume (V_2) must be 200 µL), specific volume of the stock solutions (V_1) of samples (bulb, root and doxorubicin) were then transferred into their respective wells (for each plate of Jurkat, 24 wells were specified for each samples and 8 wells were specified for untreated control, meanwhile, for each plate of MRC-5, 21 wells for were specified each samples and 7 wells were specified for untreated control). This was based on the desired concentrations (M_2) as shown in Table 1 and Table 2; the concentrations for stock solutions (M_1) which were prepared previously were chosen to suit the desired concentrations. Subsequently, they were dissolved with complete growth medium containing 5% FBS into the desired concentrations. This was done using serial dilution techniques. Then, all plates were incubated for 3 days (72 hours) at 37°C in a 5% CO_2 humidified incubator.

Table 1: Concentrations of Samples for Treatment on Jurkat Cell Line

Sample	**Concentration**	**Stock Solution**
Negative Control	No treatment	-
Sample 1	A 100	400 µg/mL

(Ethanolic Extract of Bulb of Eleutherine americana L. Merr.)		µg/mL	
	B	50 µg/mL	
	C	30 µg/mL	
	D	10 µg/mL	
	E	5 µg/mL	10 µg/mL
	F	1 µg/mL	
	G	0.5 µg/mL	
	H	0.1 µg/mL	
Sample 2 (Ethanolic Extract of Root of Eleutherine americana L. Merr.)	A	100 µg/mL	400 µg/mL
	B	50 µg/mL	
	C	30 µg/mL	
	D	10 µg/mL	
	E	5 µg/mL	10 µg/mL
	F	1 µg/mL	
	G	0.5 µg/mL	
	H	0.1 µg/mL	
Positive Control (Doxorubicin	A	2 µg/mL	4 µg/mL
	B	1 µg/mL	
	C	0.5	

Solution)		µg/mL	
	D	0.3 µg/mL	
	E	0.2 µg/mL	
	F	0.1 µg/mL	
	G	0.05 µg/mL	1 µg/mL
	H	0.01 µg/mL	

Table 2: Concentrations of Samples for Treatment on MRC-5 Cell Line

Sample		Concentration	Stock Solution
Negative Control		No treatment	-
Sample 1 (Ethanolic Extract of Bulb of Eleutherine americana L. Merr.)	A	200 µg/mL	400 µg/mL
	B	100 µg/mL	
	C	50 µg/mL	
	D	30 µg/mL	
	E	10 µg/mL	
	F	5 µg/mL	10 µg/mL

	G	1 µg/mL	
Sample 2 (Ethanolic Extract of Root of Eleutherine americana L. Merr.)	A	200 µg/mL	400 µg/mL
	B	100 µg/mL	
	C	50 µg/mL	
	D	30 µg/mL	
	E	10 µg/mL	
	F	5 µg/mL	10 µg/mL
	G	1 µg/mL	
Positive Control (Doxorubicin Solution)	A	2 µg/mL	4 µg/mL
	B	1 µg/mL	
	C	0.5 µg/mL	
	D	0.2 µg/mL	
	E	0.1 µg/mL	
	F	0.05 µg/mL	1 µg/mL
	G	0.01 µg/mL	

2.6. In Vitro Cytotoxicity Studies

2.6.1. MTT Assay

After 72 hours of incubation, 20 µl (5 mg/mL) of MTT was added into each well, under a category II LAF cabinet (with lights off) of the plates prepared for cell treatment and the plates were wrapped with aluminium foil (since MTT is light sensitive). They were then reincubated for another 3 to 4 hours at 37°C in 5% CO_2 incubator. Next, the supernatant was discarded carefully using micropipette and 100 µL of DMSO was added to each well. The solubilised formazan salts formed were then spectrophotometrically quantified at 570 nm wavelength by ELISA reader [11].

Viability of the treated cells was expressed as mean percent viability, in which they were compared to the untreated control. Viability was calculated as follows:

$$\% \text{ cell viability} = \frac{\text{OD treated}}{\text{OD untreated control}} \text{ x } 100\%$$

OD: Optical Density

A dose-response curve and an inhibition percentage curve were plotted for each of the samples (bulb, root, and doxorubicin) on both Jurkat and MRC-5 cell lines. From each dose-response curve, the concentration which gave 50% inhibition of cell growth (IC_{50}) was then calculated using Graphpad Prism 8 software. The IC_{50} values of the samples (bulb, root, and doxorubicin) on Jurkat were compared with each other and to that of MRC-5.

2.6.2. Morphological Changes Observation

The morphology of the treated Jurkat as well as the untreated control in each of 96-well plates was inspected after 72 hours (3 days) of incubation, under inverted light microscope.

3. Results and Discussion

3.1. MTT Assay

Half maximal inhibitory concentration (IC_{50}) is generally defined as a measure of the potency of a substance in inhibiting a specific biological or biochemical function. Commonly, it is used as a measure of antagonist drug potency. In in vitro cytotoxicity study, IC_{50} is used to determine the concentration of the tested drug or compound able to cause death to 50% of the viable cells. Thus, the degree of cytotoxic effect can be predicted. The lower the IC_{50} value, the more cytotoxic is the substance [12].

Figure 1 shows the mean and standard deviation (SD) of IC_{50} values of 3 samples (bulb, root, and doxorubicin) treated on Jurkat and MRC-5 cell lines. Overall, the IC_{50} values of all samples on Jurkat cell line were lower

than those on MRC-5 cell line. This indicated that all samples exhibited certain degree of selective toxicity towards Jurkat compared to MRC-5.

In comparing between the 2 crude extracts of the plant (bulb and root), IC_{50} values of bulb on both Jurkat and MRC-5 cell lines were lower than those of the root. This suggested that bulb exhibited higher cytotoxic activity than root towards both cells. However, by comparing the difference between IC_{50} values on Jurkat and MRC-5 cell lines, root had a very big difference (13.087 µg/mL and 98.547 µg/mL) while bulb only had a small difference (3.855 µg/mL and 5.906 µg/mL). Therefore, root was suggested to exhibit higher degree of selective toxicity than bulb towards Jurkat compared to MRC-5, thus, causing less harm to normal cells.

Meanwhile, IC_{50} values of doxorubicin on both Jurkat and MRC-5 cell lines were the lowest among the 3 samples and the difference between IC_{50} values on Jurkat and MRC-5 cell lines is very small (0.043 µg/mL and 0.082 µg/mL). These showed that doxorubicin exhibited the highest cytotoxic activity and had the least degree of selective toxicity towards Jurkat compared to MRC-5. Doxorubicin doxorubicin is a pure compound and a well-known drug used as conventional treatment for cancer including leukemia. Besides being toxic to cancer cells, conventional cytotoxic agents can also be toxic to normal cells [13].

US National Cancer Institute (NCI) plant screening program proposed that a crude extract with IC_{50} value ≤ 20 µg/mL is generally considered to have in vitro cytotoxic activity, while the proposed value for a pure compound is ≤ 4 µg/mL [14]. Based on the criteria proposed for a crude extract, bulb was indicated to have a significant cytotoxic activity on both Jurkat (IC_{50} = 3.855 ± 0.55 µg/mL) and MRC-5 (IC_{50} = 5.906 ± 0.125 µg/mL) cell lines. As for root, it was indicated to have a significant cytotoxic activity on Jurkat (IC_{50} = 13.087 ± 1.799 µg/mL) cell line but a very weak cytotoxic activity on MRC-5 (IC_{50} = 98.547 ± 2.172 µg/mL) cell line. On the other hand, doxorubicin as a pure compound was indicated to have a significant cytotoxic activity on both Jurkat (IC_{50} = 0.043 ± 0.015 µg/mL) and MRC-5 (IC_{50} = 0.082 ± 0.009 µg/mL) cell lines.

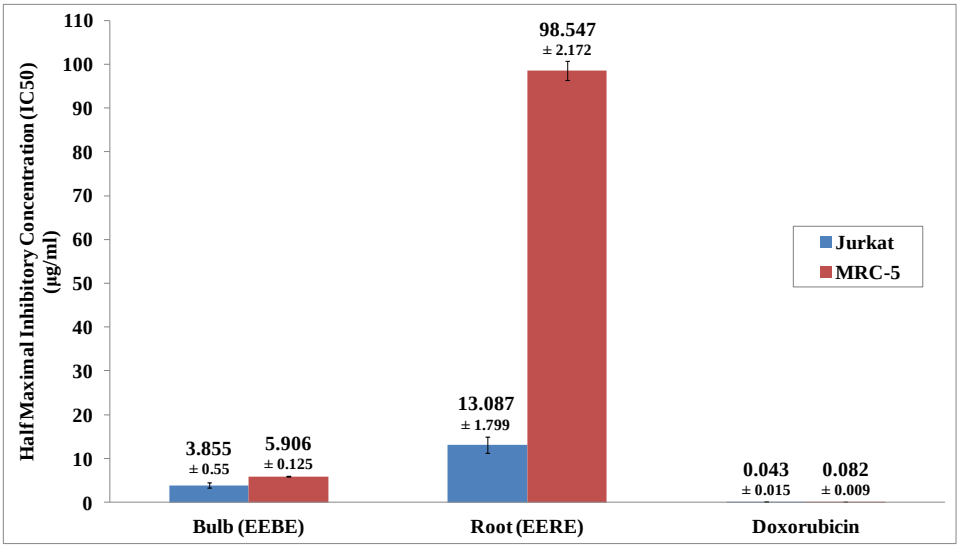


Figure 1. IC_{50} (mean ± SD) of the samples on Jurkat and MRC-5 cell lines

3.2. Morphological Changes Observation

After 72 hours of incubation, the morphology of untreated and treated Jurkat cells were observed under the light microscope. This was done to detect any changes in form, shape and structure of the treated cells when compared to the untreated cells. Subsequently, by differentiating the changes detected, the mode of cell death of Jurkat cells caused by each of the samples (bulb, root, doxorubicin) was then determined.

The main irreversible modes of cell death are apoptosis and necrosis [15]. An agent that induces cell death via apoptosis is preferred to be an anticancer drug. Apoptosis is a programmed cell death that functions to remove targeted unwanted or dead cells. The features of apoptotic cells include cell shrinkage, condensation of the cytoplasm and nucleus, aggregation of chromatin, and formation of membrane-bound vesicles known as apoptotic bodies. On the other hand, necrosis is a pathological activity, which is known to be pro-inflammatory. The features of necrotic cells include swelling of the cell, which is often accompanied by chromatin condensation. Eventually, they will experience cellular and nuclear lyses, which subsequently followed by inflammation. Thus, it is unfavourable for an anticancer agent [16].

Jurkat is normally round cell (non-adherent cell) which is derived from peripheral blood. Therefore, instead of dramatic changes in their cell shape (roundness), decrease in their cell area is expected during apoptosis [17]. Figure 2 shows that the untreated Jurkat cells remain healthy, as they were round, grew singly or in clumps.

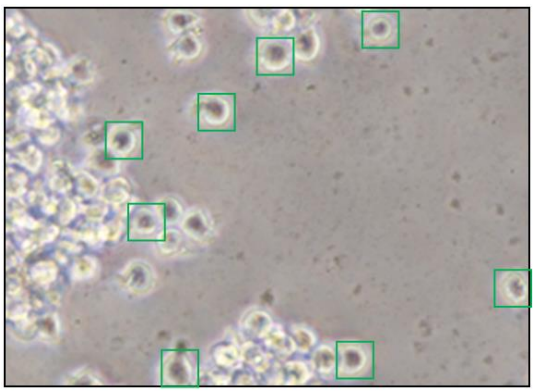


Figure 2. Untreated Jurkat (Green box: Healthy cells)

There were morphological changes detected in Jurkat cells treated with all samples. Specifically, the effect of each sample on Jurkat cells, at the concentration near to IC_{50} value and 10 times higher of it were compared.

Based on Figure 3 to 8, Jurkat cells treated with 5 µg/mL and 50 µg/mL of bulb (IC_{50} = 3.855 ± 0.55 µg/mL), 10 µg/mL and 100 µg/mL of root (IC_{50} = 13.087 ± 1.799 µg/mL), 0.05 µg/mL and 0.5 µg/mL of doxorubicin (IC_{50} = 0.043 ± 0.015 µg/mL) were showing the features of apoptotic cells (cell shrinkage and nuclear condensation). This suggested that all samples induced apoptosis in Jurkat cells at both concentrations, with a smaller number of apoptotic bodies detected at the concentration near to IC_{50} value.

In addition, features of necrotic cells (cell swelling and membrane rupture) were also detected in Jurkat cells treated with 100 µg/mL of root and 0.5 µg/mL of doxorubicin. This suggested that at the concentration about 10 times higher of IC_{50} value, root and doxorubicin also induced necrosis in Jurkat cells. Only a few necrotic cells were detected among Jurkat cells treated with 100 µg/mL of root, while more necrotic cells were detected among those treated with 0.5 µg/mL of doxorubicin, when compared to the number of apoptotic cells detected. Thus, only doxorubicin was indicated to prominently induce necrosis in Jurkat cells, while root prominently induced apoptosis.

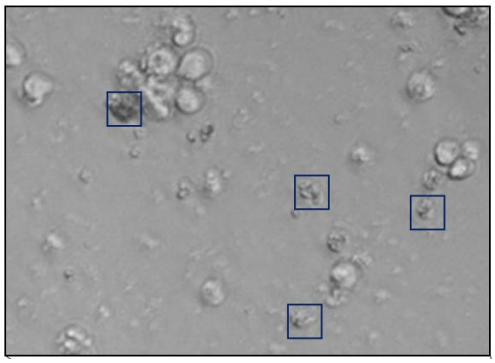


Figure 3. Jurkat treated with 5 µg/mL EEBE. (Blue box: Apoptotic cells)

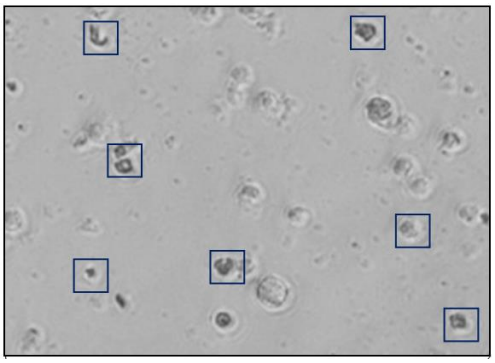
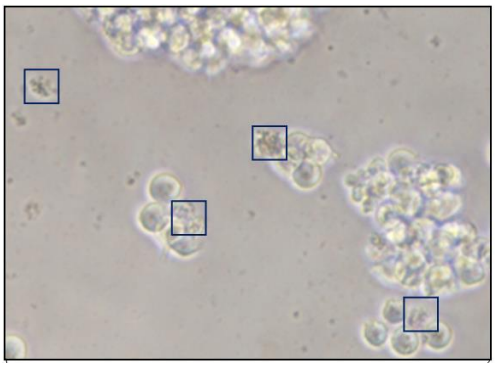


Figure 4. Jurkat treated with 50 µg/mL EEBE. (Blue box: Apoptotic cells)



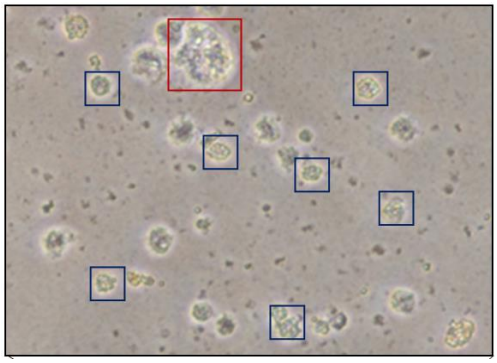


Figure 5. Jurkat treated with 10 µg/mL EERE. (Blue box: Apoptotic cells)

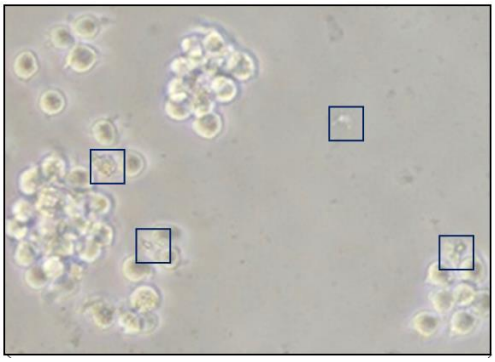


Figure 6. Jurkat treated with 100 µg/mL EERE. (Blue box: Apoptotic cells, red box: Necrotic cells)

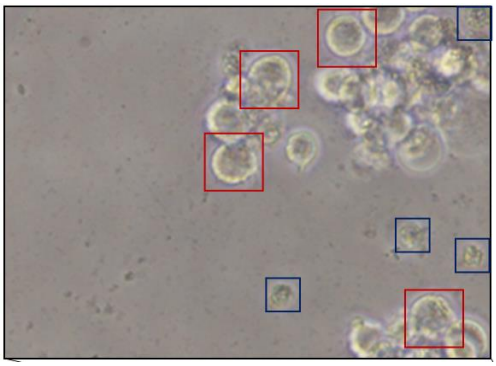


Figure 7. Jurkat treated with 0.05 µg/mL doxorubicin. (Blue box: Apoptotic cells)

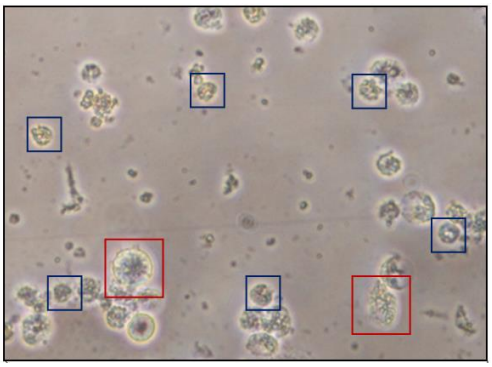


Figure 8. Jurkat treated with 0.5 µg/mL doxorubicin. (Blue box: Apoptotic cells, red box: Necrotic cells)

Figure 9. Jurkat treated with 2 µg/mL doxorubicin. (Blue box: Apoptotic cells, red box: Necrotic cells)

However, based on Figure 9, more apoptotic cells were detected compared to necrotic cells. This suggested that, at much higher concentration (2 µg/mL), doxorubicin prominently induced apoptosis instead of necrosis in Jurkat cells. This matches the finding of a study, which reported that the effects exhibited by most anti-tumour drugs (including doxorubicin) on tumour cells were mainly via the induction of apoptosis, but relatively low concentrations were shown to prominently induce necrosis instead of apoptosis [18].

4. Conclusion

Ethanolic extract of bulb (EEBE) and root (EERE) of Eleutherine americana L. Merr. were shown to exhibit a significant in vitro antiproliferative potential on Jurkat cell line. Comparing between these crude extracts, bulb was suggested to have better antileukemic activity as it had lower IC_{50} value than root.

However, bulb was suggested to exhibit a significant cytotoxic activity on MRC-5 cell line too, indicating it to be toxic to normal fibroblast cells. Similarly, doxorubicin (pure compound) which was used as positive control had a significant cytotoxic activity on both Jurkat and MRC-5 cell lines. On the other hand, root only exhibited a very weak cytotoxic effect on MRC-5 cell line. Thus, root was said to exhibit higher degree of selective toxicity towards Jurkat compared to MRC-5, suggesting it to cause less harm towards normal cells.

An agent that induces cell death via apoptosis is preferred to be an anticancer drug compared to necrosis (as it can stimulate inflammation). Overall, at the concentration near to IC_{50} value and about 10 times higher of IC_{50} value, all samples were suggested to induce apoptosis. In addition, at the concentration about 10 times higher of IC_{50} value, root and doxorubicin were also suggested to induce necrosis in Jurkat cells, but only doxorubicin prominently induced necrosis, while root prominently induced apoptosis. However, at much higher concentration, doxorubicin was suggested to prominently induce apoptosis, instead of necrosis in Jurkat cells.

Both ethanolic extract of bulb (EEBE) and root (EERE) of Eleutherine americana L. Merr. can be further studied as the alternative for leukemia treatment, apart from the currently available conventional therapeutic modalities.

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