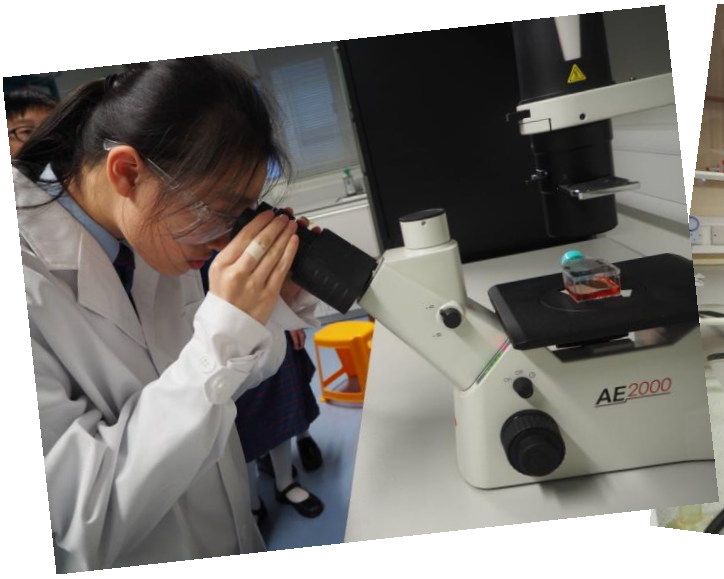
JBBC24

Potato: A Potential Therapeutic Drug Against Leukemia?

對抗白血，薯神百寶？

Written Report

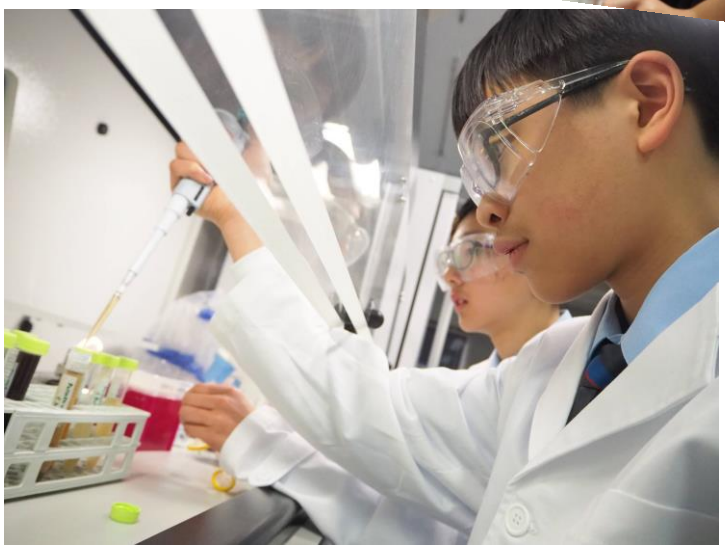


Group members:

Chan Ching Tung
Wong Wai Ching
Yu Wing Ching
Stanley Keanu Leong
Wong Ching Lam Kyle

Teacher Supervisor:

Mr. Ho Tik Shun



1. Aim and Objectives

Aim

- To find out if potato juices can act against leukemia

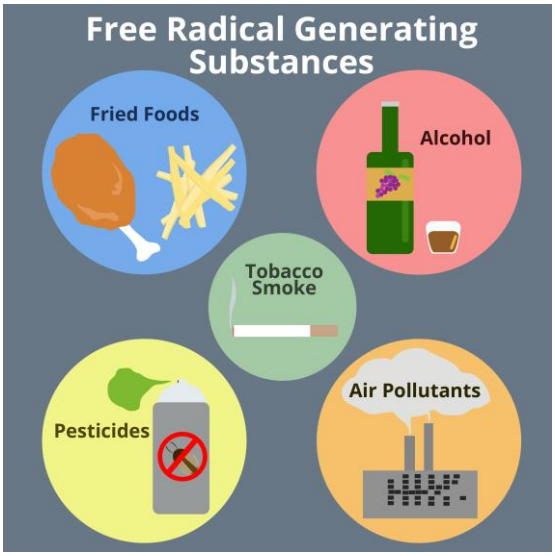
Objectives

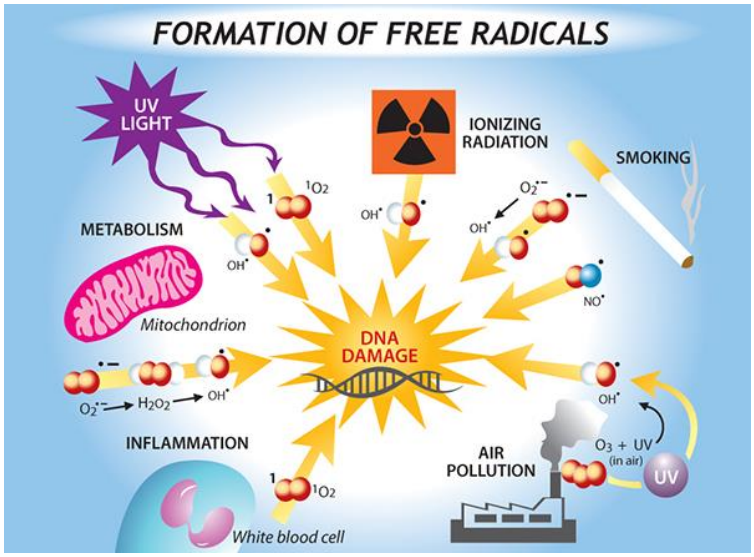
- To compare total free-radical scavenging ability of different potato juices by Briggs-Rauscher Reaction (Clock Reaction)
- To test whether potato juices (both polar and non-polar constituents) can cause cell inhibition and killing action on HL-60 leukemia cells

2. Introduction

What are antioxidants?

Antioxidants are molecules present in cells that prevent oxidative reactions by donating an electron to the free radicals without becoming destabilized themselves. An imbalance between oxidants and antioxidants is the underlying basis of oxidative stress. Oxidative stress leads to many pathophysiological conditions in the body. Some of these include neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, gene mutations and even cancers.[1 – 2]



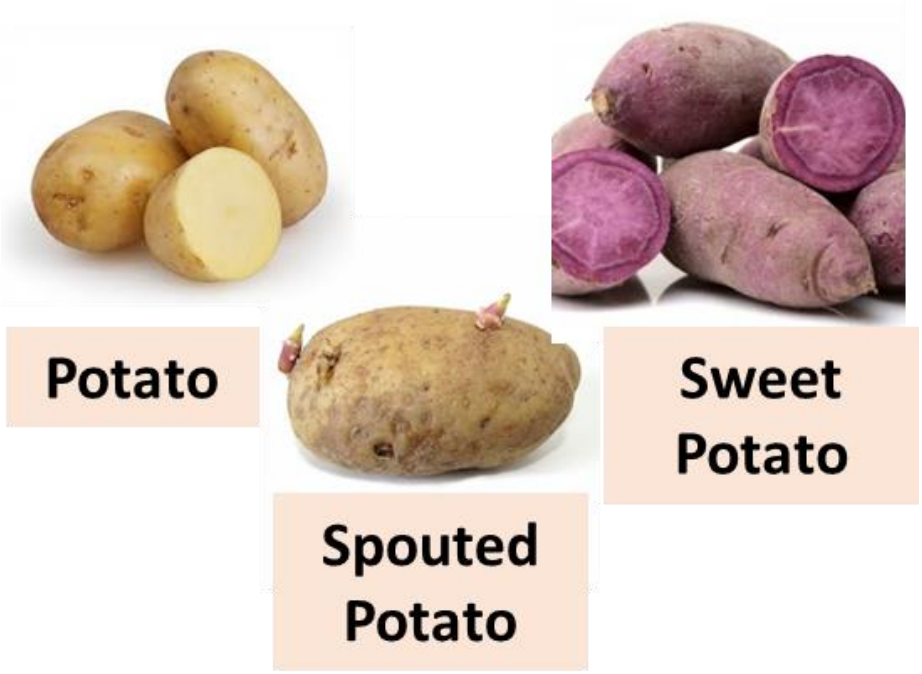


Examples of antioxidants in food

The following is a list of different kinds of antioxidants and foods that are high in each[3].

- Allium sulphur compounds: Leeks, onions, garlic
- Anthocyanins: Eggplant, grapes, berries
- Beta carotene: Pumpkin, mangoes, apricots, carrots, spinach, parsley
- Catechins: Red wine, tea
- Copper: Seafood, lean meat, milk, nuts, legumes
- Cryptoxanthins: Red peppers, pumpkin, mangoes
- Flavonoids: Tea, green tea, red wine, citrus fruits, onion, apples
- Indoles: Cruciferous vegetables such as broccoli, cabbage, cauliflower
- Lignans: Sesame seeds, bran, whole grains, vegetables
- Lutein: Corn, leafy greens (such as spinach)
- Lycopene: Tomatoes, pink grapefruit, watermelon
- Manganese: Seafood, lean meat, milk, nuts
- Polyphenols: Thyme, oregano
- Selenium: Seafood, offal, lean meat, whole grains
- Vitamin C: Oranges, berries, kiwi fruit, mangoes, broccoli, spinach, peppers
- Vitamin E: Vegetable oils, nuts, avocados, seeds, whole grains
- Zinc: Seafood, lean meat, milk, nuts
- Zoochemicals: Red meat, offal, fish

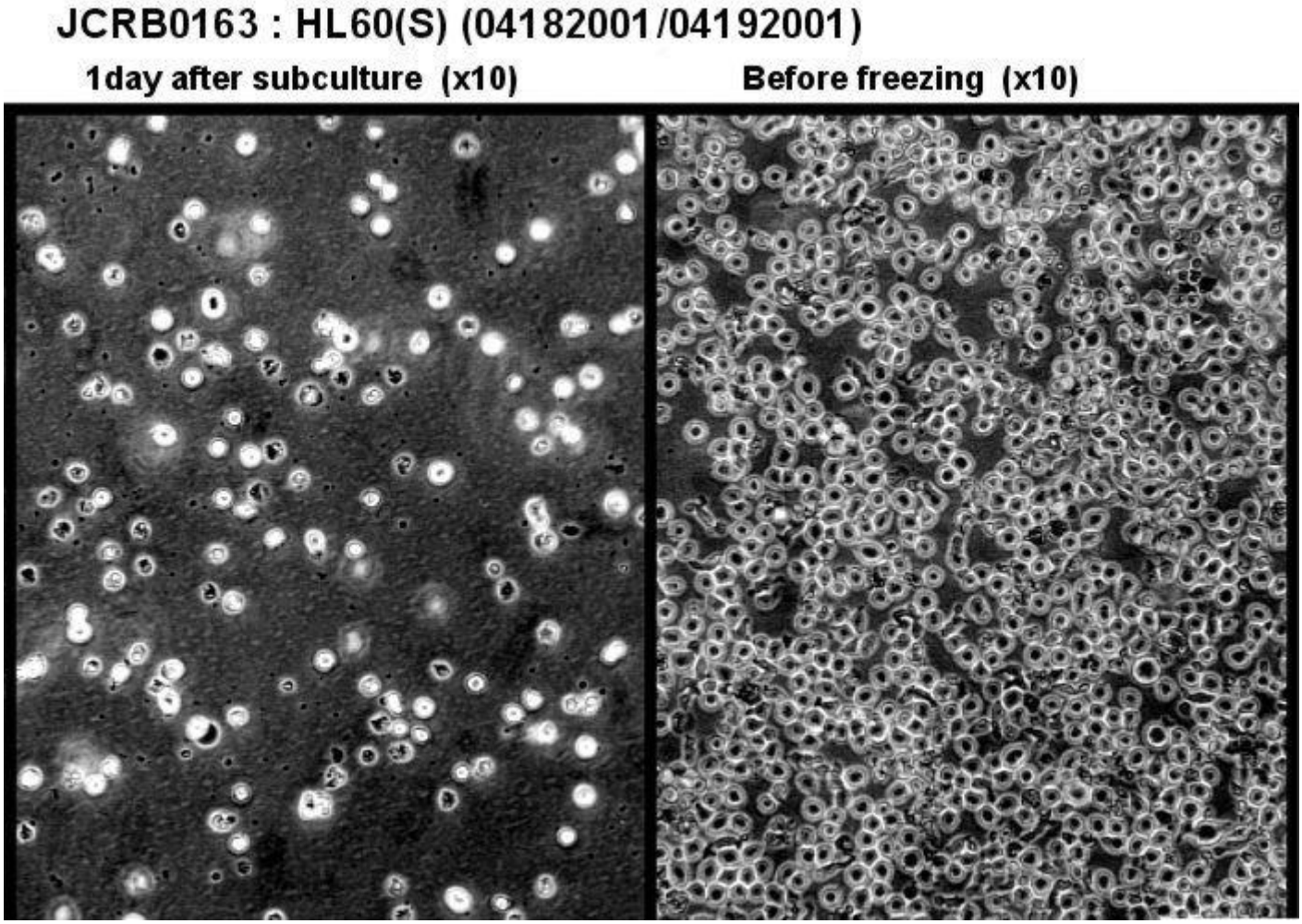
Can potato act against cancers?



Scientific studies had shown that potatoes had a lot of potential health effects on humans, including anti-obesity, anti-aging and improving mental conditions[4]. In recent years, the potential anticancer properties of raw potato juices on various cancer cell types was discovered, namely oesopharyngeal cancer, gastrointestinal cancer, lung cancer, stomach cancer and liver cancer[5,6].

Potato has the effect of suppressing cancer growth. This was gradually proven by scientific experiments. Professor Kagamin from the Hygiene study in the Akita University Medical Department, successfully extracted a suppressive material, a pro-variation substance from potatoes. The significant effect of such material was affirmed through experiments conducted on mice. The Fresh Potato Juice could not only treat cancer but also cure many people who suffer from hypertension, heart diseases, diabetes and kidney disease[7].

Human promyelocytic leukemia cells, HL-60 cells



HL-60 is a promyelocytic cell line derived by S.J. Collins, *et al*. Peripheral blood leukocytes were obtained by leukopheresis from a 36-year-old Caucasian female with acute promyelocytic leukemia[8]. HL-60 cells spontaneously differentiate and differentiation can be stimulated by butyrate, hypoxanthine, phorbol myristic acid (PMA, TPA), dimethylsulfoxide (DMSO, 1% to 1.5%), actinomycin D, and retinoic acid. The cells exhibit phagocytic activity and responsiveness to chemotactic stimuli. The line is positive for myc oncogene expression. The base medium for this cell line is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 20%.

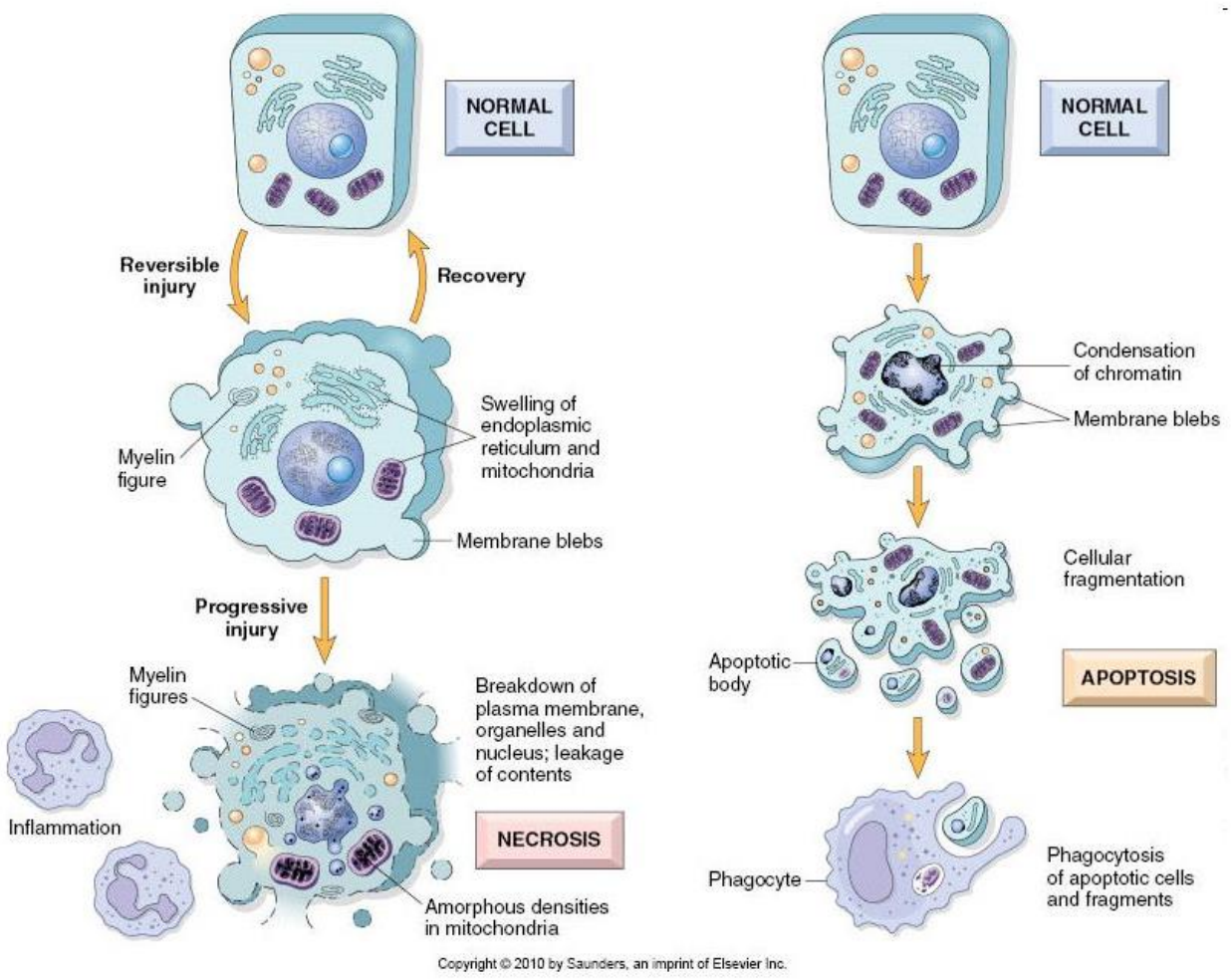
Study on Cell Growth Inhibition: Apoptosis and Necrosis

Whereas apoptosis is a form of cell death that is generally triggered by normal, healthy processes in the body, necrosis is cell death that is triggered by external factors or disease, such as trauma or infection. Apoptosis, which can also occur as a defense mechanism during healing processes, is almost always normal and beneficial to an organism, while necrosis is always abnormal and harmful. Though necrosis is being researched as a possible form of programmed cell death (that is, a sometimes natural process), it is considered an "unprogrammed" (unnatural) cell death process at this time. As a usually healthy form of a cell's life cycle, apoptosis rarely demands any form of medical treatment, but untreated necrosis can lead to serious injury or even death[9].

Both apoptosis and necrosis can be seen as part of a spectrum of shared biochemical events that both result in some form of cellular death.

Apoptosis, or programmed cell death (PCD), causes cells to shrink, develop blebs (bubble-like spots) on the cell membrane, undergo degradation of genetic and protein materials in the nucleus, and have their mitochondria break down, thus releasing cytochrome. The fragments are each wrapped in their own membrane, with other chemicals (such as ATP and UTP) released freely. These chemicals lead macrophages — cell-eating bodies — to find and eliminate the dead cells and their fragments. This "eat me" message is triggered by a phospholipid normally inert in a cell's membrane, and the macrophages in turn release cytokines that inhibit inflammatory responses.

In contrast, necrotic cells swell or may form vacuoles on their surface, with interior structures either distending or shrinking rapidly, destroying the cell's processes and chemical structures. The unregulated release of cytochrome and the cell membrane's phospholipid (called phosphatidylserine) causes immediate reactions in surrounding tissues, leading to swelling (inflammation) and edema; it also often triggers other cell deaths through apoptosis. Unlike apoptosis, necrotic cells are not targeted by macrophages for cleaning of their cellular debris, so the effects of the cell rupture can spread quickly and throughout the body for long periods of time[10].



	APOPTOSIS	NECROSIS
NATURAL	YES	NO
EFFECTS	BENEFICIAL	DETRIMENTAL
	Physiological or pathological	Always pathological
	Single cells	Sheets of cells
	Energy dependent	Energy independent
	Cell shrinkage	Cell swelling
	Membrane integrity maintained	Membrane integrity lost

3. Methodology and Procedure

Briggs-Rauscher Reaction (Clock Reaction) was the theory basis for testing samples' ability to scavenge free radical. The project was followed by comparing total free-radical scavenging ability of different potato juices. Positive control of Vitamin C solution and negative control of water were also included. Our group also investigated if there was synergy in antioxidizing action amongst boiled and raw juices from normal potatoes, sprouted potatoes and sweet potatoes. **[Part A]**

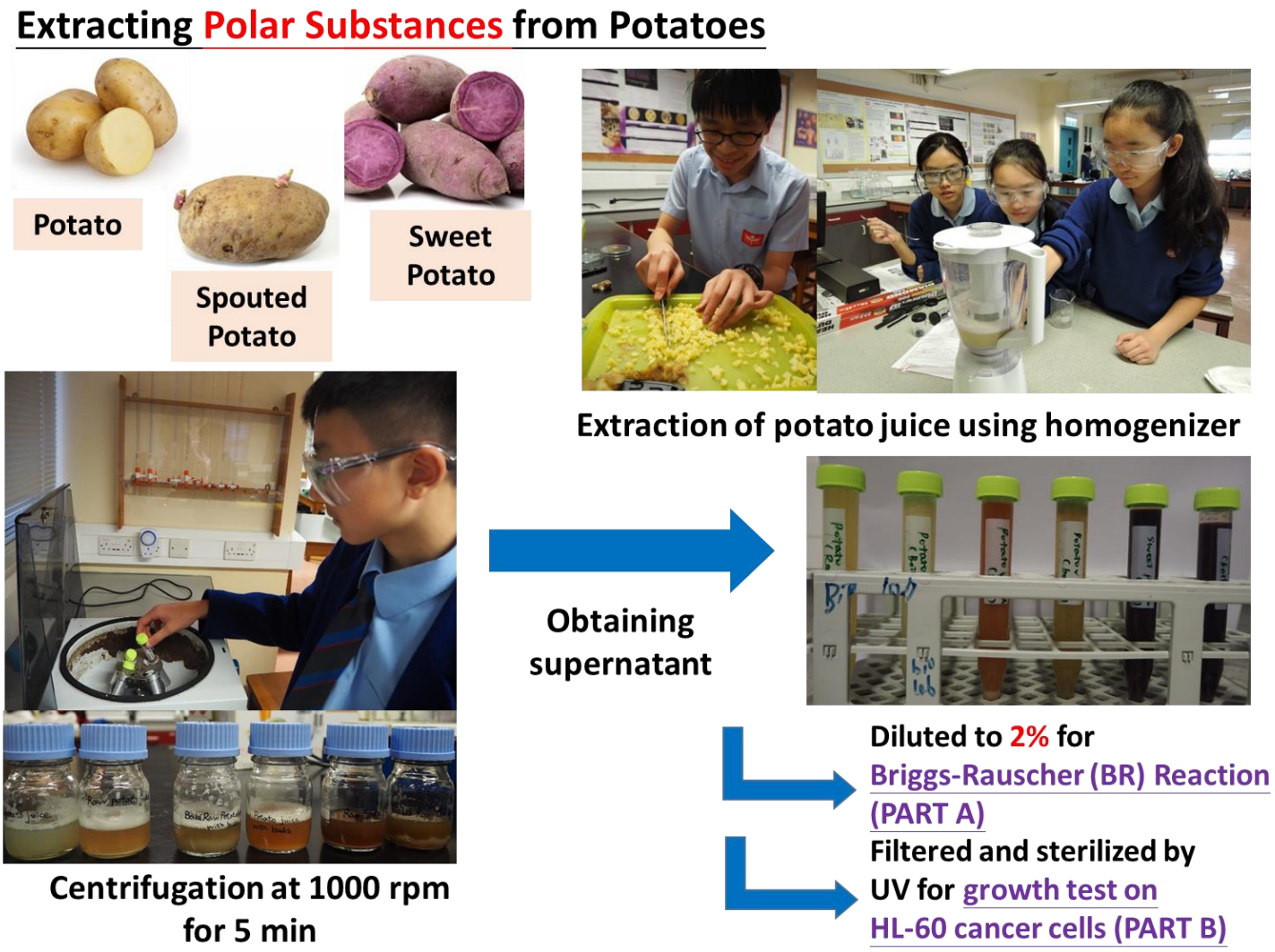
Then, our group tried to administer minute concentration of potato juices (aqueous extract) to Human promyelocytic leukemia cells, HL-60 cells in culture flasks for 24 hours, and its anticancer properties were verified through morphological studies. The cell density, their clustering patterns, and the integrity of their cell membranes were investigated by putting the culture flasks of control and treated groups under the inverted microscope stationed in Cell Biotechnology Research Centre of our school. **[Part B1]**

The experiment proceeded by administering minute concentration of potato juices (aqueous extract) to HL-60 cells seated in 96-well flat-bottomed plates for 24 to 48 hours. Trypan blue stain was used to stain the dead cells which selective permeability of the cell membrane were lost. The observation was done through light microscope. The cell density of dead and viable cells was estimated using a haemocytometer. The cell inhibitory effect and killing effect on HL-60 cells by potato juices, both polar and non-polar constituents, could be studied in more details. **[Part B2 &B3]**

Extracting Polar Substances from Potatoes

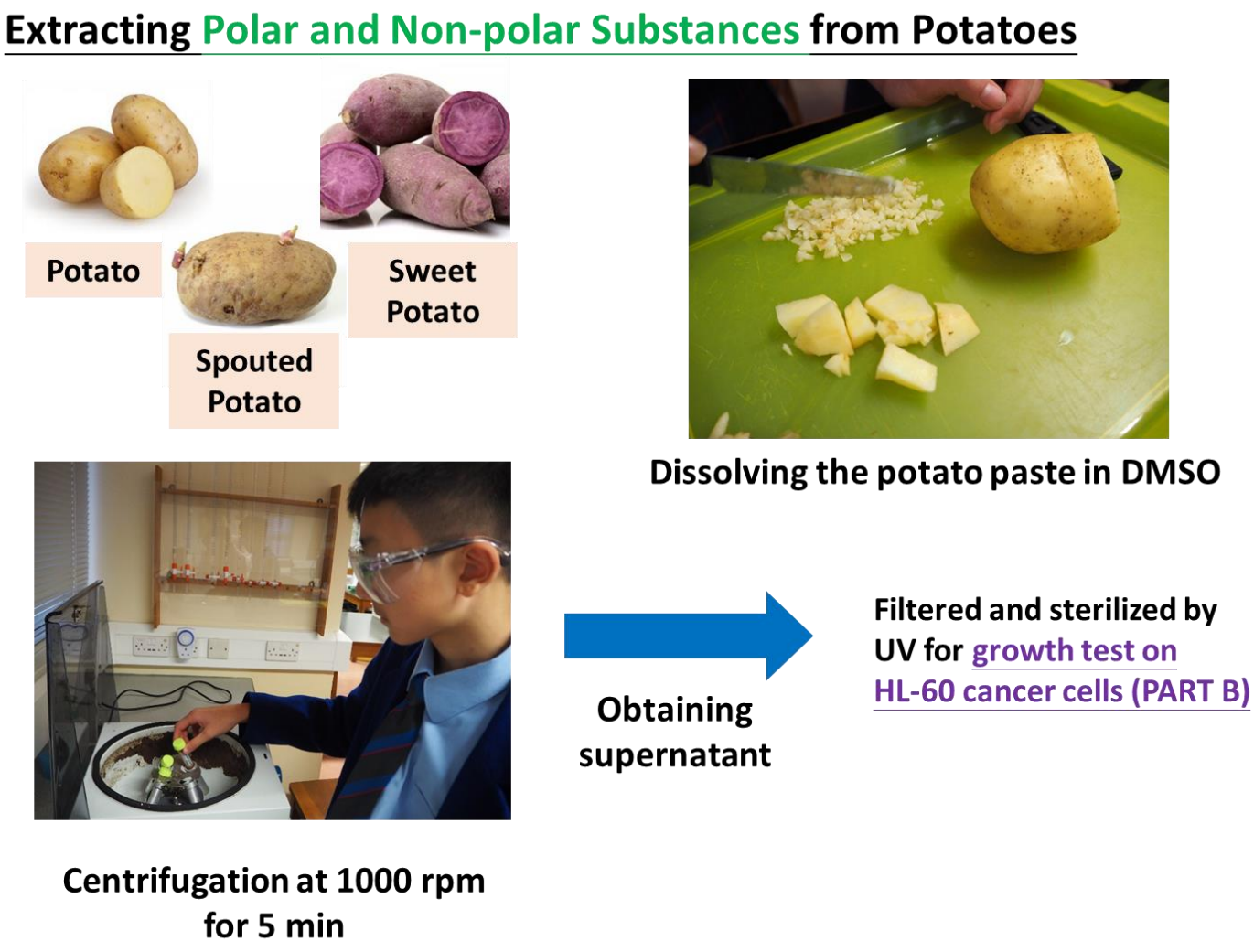
To extract potato juices, the potato pieces were ground into workable pieces by knife, followed by homogenizer and small volume of water to extract aqueous potato juices. The potatoes used included normal potatoes, sprouted potatoes and sweet potatoes. Their boiled counterparts were also prepared by boiling them on electronic hot plates for 10 minutes, so that there were 6 potato samples altogether.

To remove the flesh, the extracts were centrifuged at the low speed of 1000 rpm for 5 minutes. The supernatant of the potato juices was then collected. The supernatant was diluted to 2% for Briggs-Rauscher (BR) Reaction as in Part A, as well as to be filtered and sterilized by UB for growth test on HL-60 cancer cells in Part B of the project.



Extracting Polar and Non-Polar Substances from Potatoes

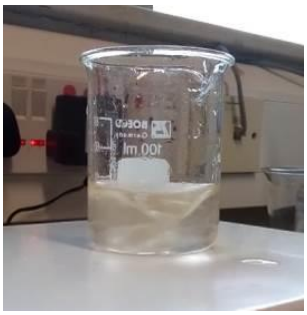
Some active ingredients might be organic phytochemicals, and thereby, dimethyl sulphoxide (DMSO), a universal solvent was used to extract both polar and non-polar components from potato samples, to investigate if these components have anti-cancer properties. Potato was first chopped into paste, then was dissolved in DMSO for 2 hours. The extraction procedure was followed by centrifugation at 1000 rpm for 5 minutes to obtain the supernatant. The supernatant was then filtered, sterilized and diluted to appropriate concentrations (i.e. 0.5 mg/ml and 1 mg/ml) for growth test on HL-60 cancer cells. To avoid toxicity effect on the cell line by DMSO, the concentrations applied to cell culture were as low as 0.1 and 0.2%, where the DMSO was found toxic to HL-60 cells at 2%.



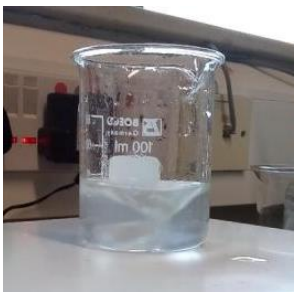
(A) Determining Antioxidant Activity of Potato Juices

– The Briggs-Rauscher (BR) Reaction

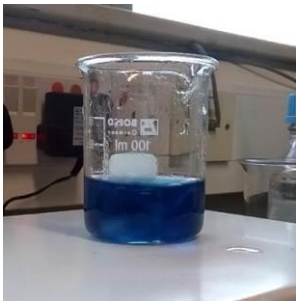
There is a variety of *in-vitro c*hemical methods used to determine the antioxidant activity of products and ingredients in food, for example, **ORAC**, **TRAP**, **TEAC** and **DPPH** etc. However, all these methods require advanced laboratory facilities and skills which could not be supported by a secondary school. In order to bring the assay to secondary school level, **Briggs-Rauscher reaction** which only needs some common reagents could be applied in order to compare the antioxidant levels in a range of different samples.



(a) Amber (Radical process)



(b) Colourless (Radical process)



(c) Blue (Non-radical process)

Fig. B A series of colour change in the Briggs-Rauscher reaction

Briggs-Rauscher reaction is an oscillating clock reaction in which a mixture of chemicals containing Iodine, Iodide ion and Iodate ion, goes through a sequence of colour changes which repeats periodically. There is switch between a radical process and a non-radical process. During the radical process, the concentration of the intermediate HIO is maintained higher than that of the intermediate I^-, the mixture remains **amber** due to the production of I_2 in the process. At the same time, radicals are consumed. I_2 further undergoes chemical reaction to produce I^- , then most of the I_2 is consumed and the mixture turns **colourless**. Accumulation of I^- stops the radical process and switches the reaction to the non-radical process. Concentration of I^- now exceeds the concentration of HIO and combines with I_2 to form a **blue** complex with starch. When the concentration of I^- becomes sufficiently low, radical process starts and the reaction cycle repeats.

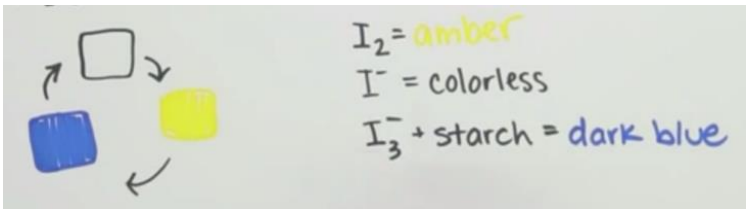
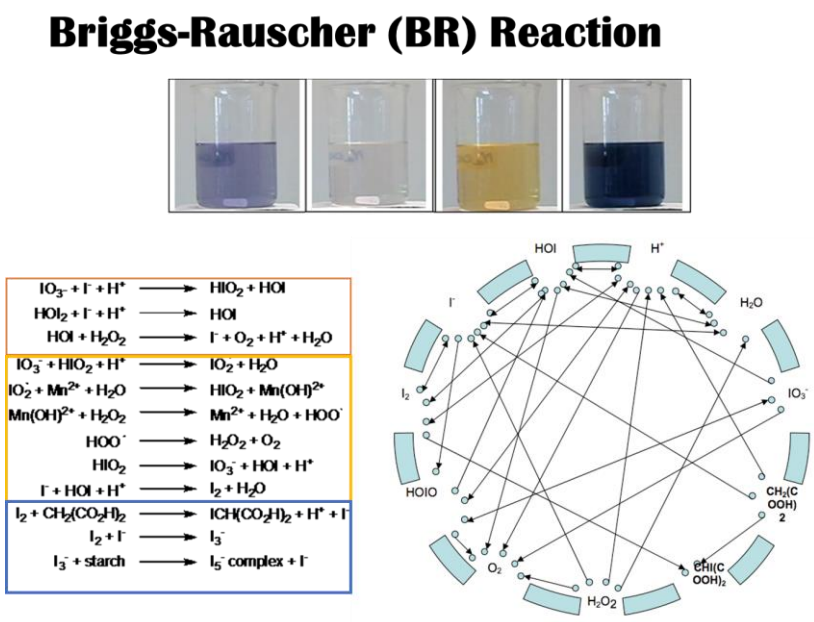
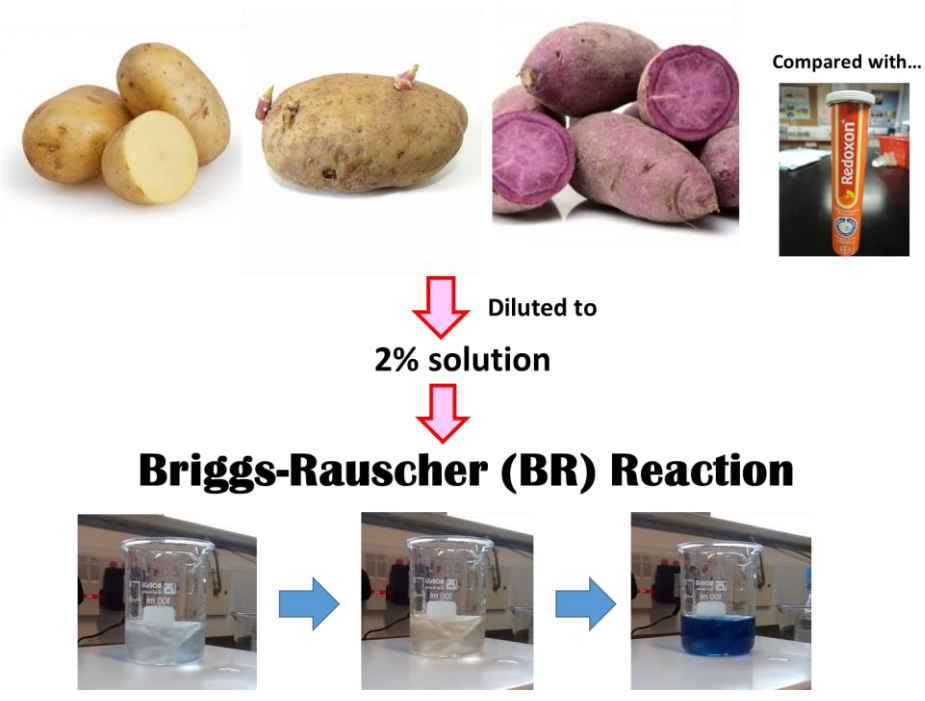


Fig. A Cycle of colour change in the Briggs-Rauscher reaction

Antioxidant test - Briggs-Rauscher (BR) reaction / Clock Reaction



In our investigation, Briggs-Rauscher reaction was performed to evaluate the level of antioxidant in different potato juices. All samples were further diluted to 2% of the original and then added into the mixture during the third round of the reaction cycle (after the second blue phase). Radicals were removed by any antioxidant and thus it took a longer time to produce enough amount to trigger the next radical process and continue the oscillating reaction. Therefore, the longer the time interval between the second and the third blue phases, the greater the antioxidant capacity the sample has. Experiments were performed 4 times for accuracy of the results.

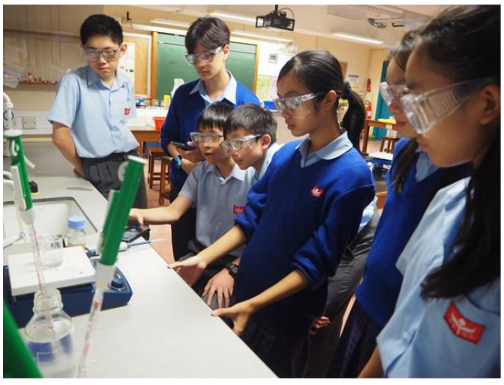


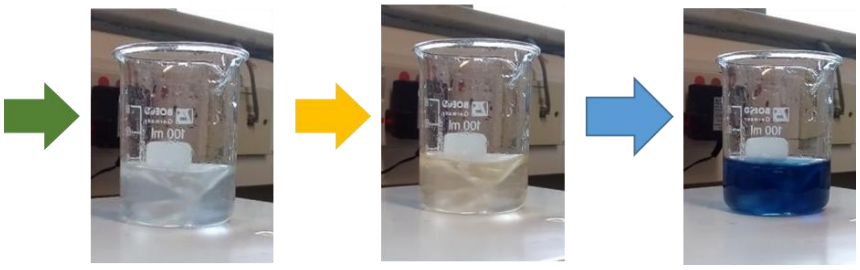
Antioxidant Test – The Briggs-Rauscher reaction experiment

1. In a 100 ml beaker containing a magnetic stirrer, 5.00 ml of 0.20 M potassium iodate, 5.00 ml 0.15 M malonic acid and 0.50 ml 2M sulphuric acid were added by using a pipette.
2. The mixture was started stirring. 5.00 ml 4 M hydrogen peroxide was added by using pipette. The colour change was waited.
3. When the amber solution turns blue for the second time, 1 ml of 2% potato extract was added and a stopwatch was started at the same time.
4. The stop watch was stopped once the third blue phase appeared. Time interval between the second and the third blue phase was recorded.
5. The experiment was repeated with other samples.

0.5 ml potato juice sample was added when the second blue phase appeared.

Briggs-Rauscher (BR) Reaction





Our project team paying attention to determine the time of sharp colour change in Clock Reaction

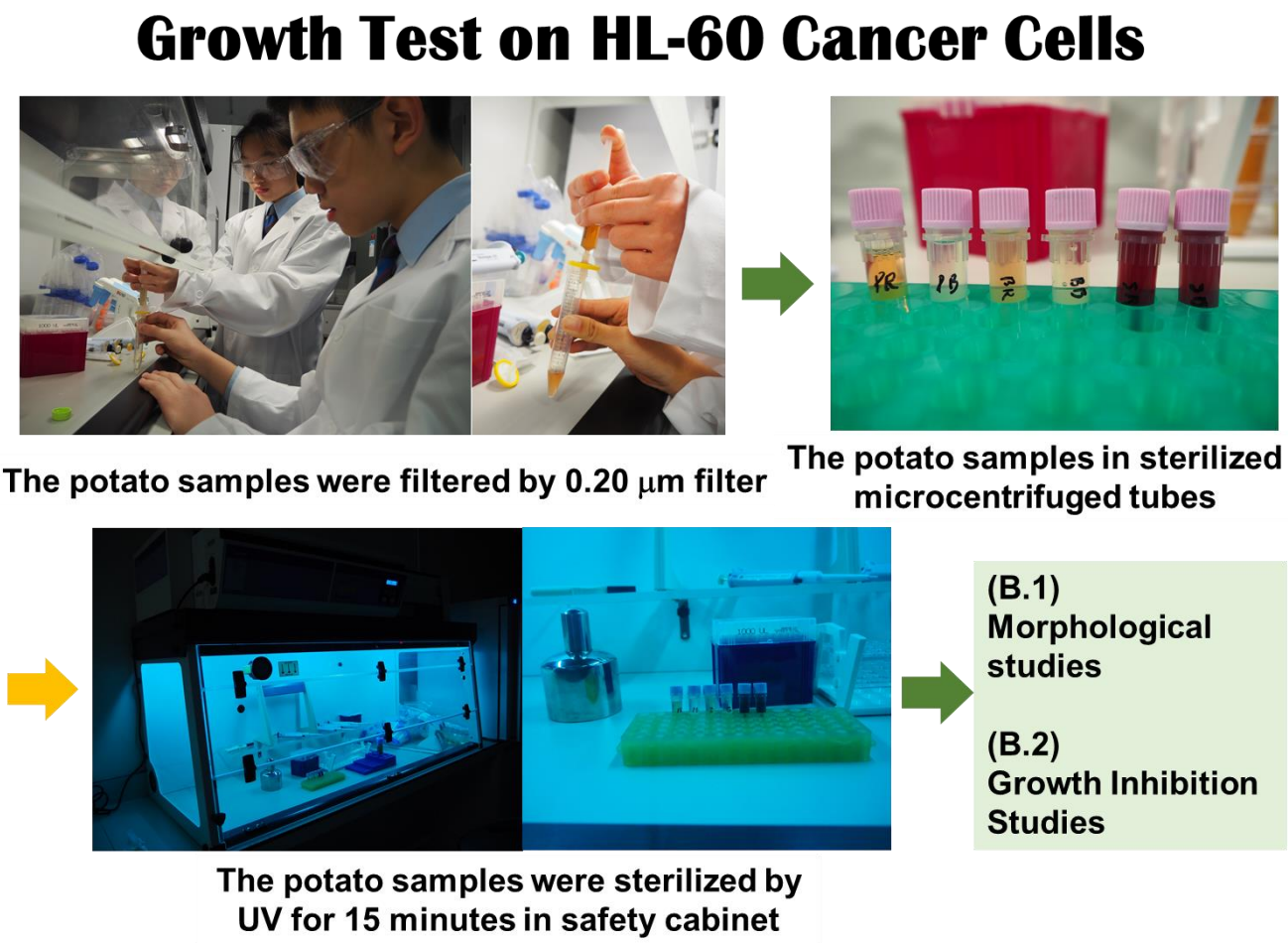
After the 2nd turning of blue colour of the solution, the solution turning amber again

When the solution turns to blue the third time, the stop watch was stopped immediately.

(B) Growth Test of Potato Juices on HL-60 Cancer Cells

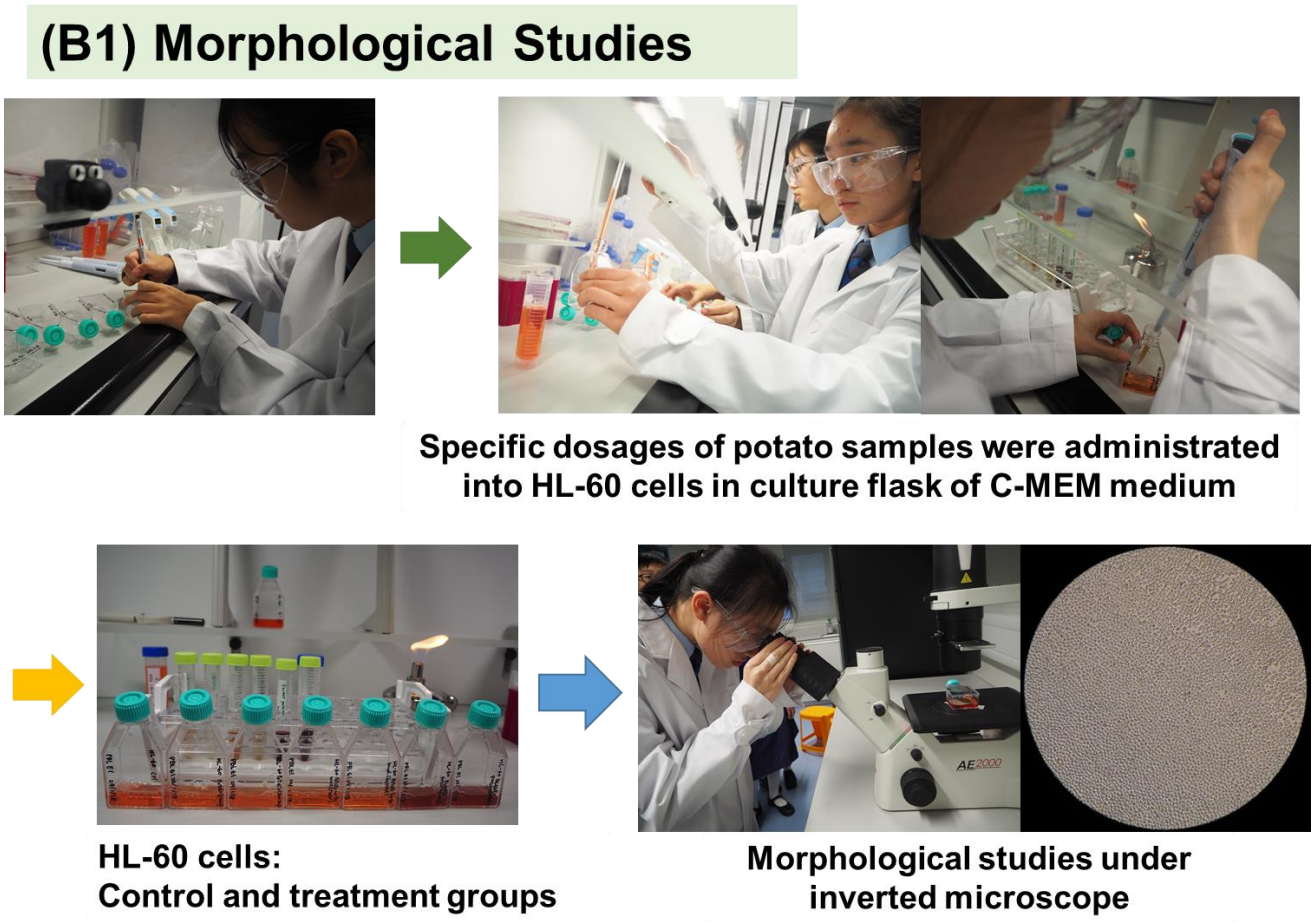
Sample Preparation

1. Potato juices were first filtered gradually by using sterilized syringe and a 0.20 μm filter in a biosafety cabinet. All the procedures performed by aseptic technique to minimize sample contamination and cross-contamination on the HL-60 cancer cells in the later steps.
2. The samples were then transferred into sealed microcentrifuged tubes.
3. The samples were further sterilized by UV in biosafety cabinet for 15 minutes.
4. The samples could then be used for evaluating anti-cancer properties through morphological studies [Part B1] and growth inhibition studies [Part B2].



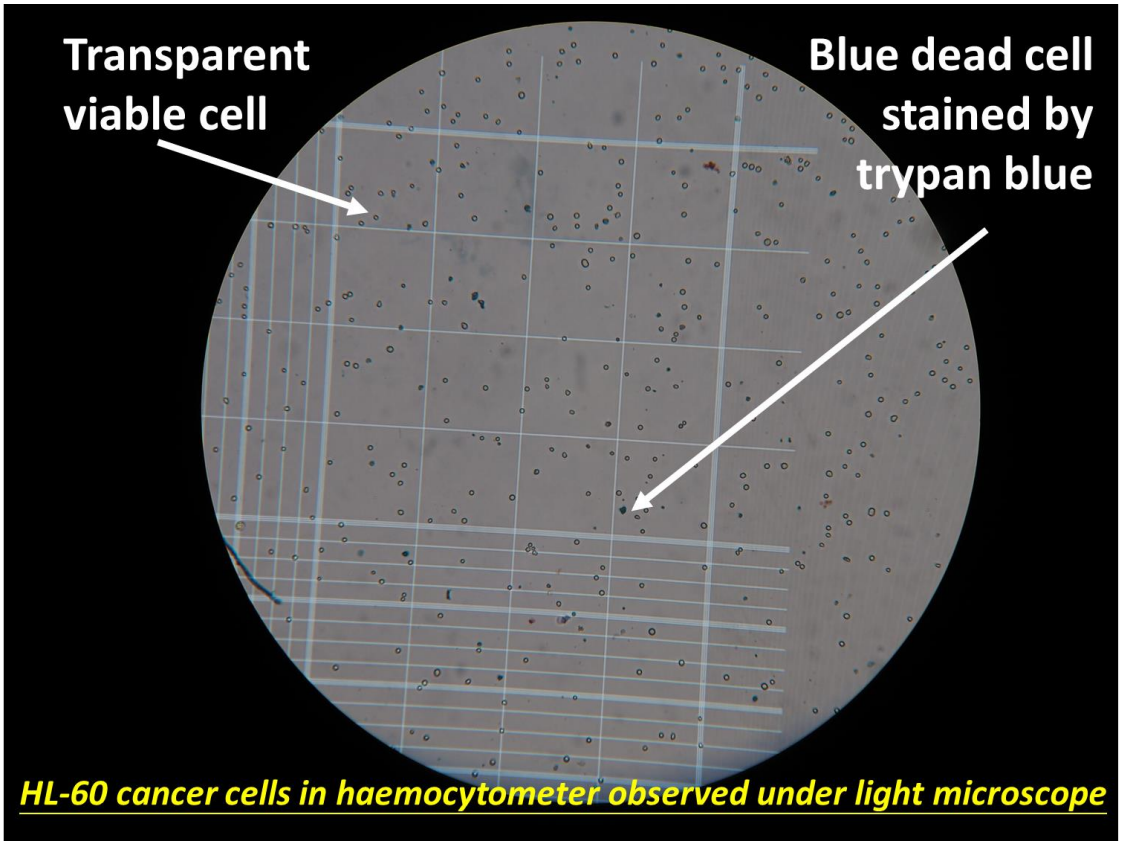
B.1 Evaluating Anticancer Properties of Potato Juices – Morphological Studies

1. The potato juices (500 mg/ml, as prepared using homogenizer) were diluted to 5 mg/ml using C-MEM culture medium.
2. 2 ml of 5 mg/ml of potato samples was administrated into HL-60 cells in culture flask of 8 ml C-MEM medium, such that the administering concentration was 1 mg/ml.
3. The culture flasks were put into an incubator, equilibrated at 37°C and 5% atmospheric carbon dioxide concentration, for 24 hours.
4. The cell density, their clustering patterns, and the integrity of their cell membranes were investigated by putting the culture flasks of control and treated groups under the inverted microscope stationed in Cell Biotechnology Research Centre of our school.

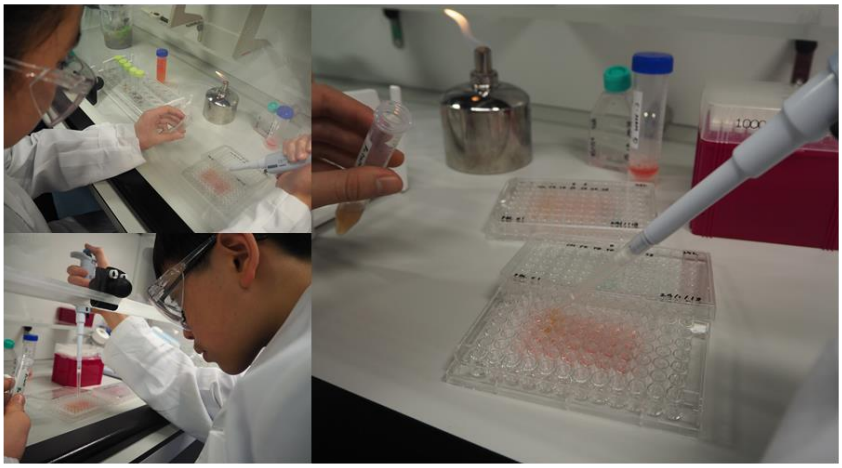


B.2 Evaluating Anticancer Properties of Potato Juices – Growth Inihibition Studies

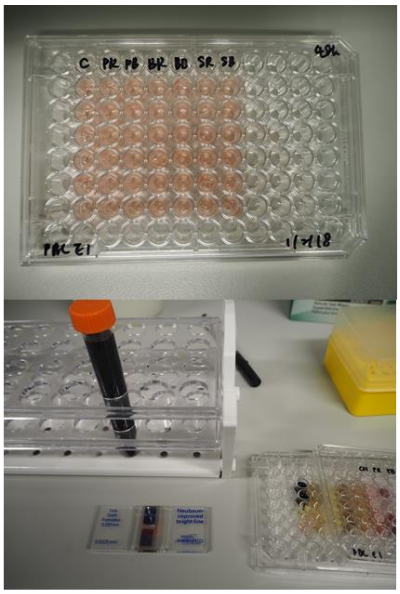
1. The potato juices (500 mg/ml, as prepared using homogenizer) were diluted to 5 mg/ml using C-MEM culture medium.
2. 10 μl and 20 μl of 5 mg/ml of potato samples were administrated into corresponding partitions of a 96-well plate seated 1 day prior with 90 μl and 80 μl of HL-60 cells (at 1 x 10^6 cells/ml) in C-MEM medium, to make up the administering concentrations as 0.5 mg/ml and 1 mg/ml.
3. The 96-well plate was put into an incubator, equilibrated at 37°C and 5% atmospheric carbon dioxide concentration, for 24 hours and 48 hours.
4. 50 μl of Trypan Blue stain was added into each partition of the 96-well plate to spot out dead cells from viable ones using a haemocytometer. Dead cells, with the lost selective permeability of cell membrane, would be stained blue, while the viable ones remained transparent and shiny as observed under the light microscope. The cell density of dead and viable HL-60 cells was then estimated.



(B2) Growth Inhibition Studies



Specific dosages of potato samples were administrated into HL-60 cells seated in 96-well plate



Trypan Blue stain added to spot out dead cells from viable ones

4. Results and Data Analysis

(A) Determining Antioxidant Activity of Potato Juices – The Briggs-Rauscher Reaction

Relative antioxidizing power of potato juices

	1st Trial (s)	2nd Trial (s)	3rd Trial (s)	4th Trial (s)	Average (s)
Water	12.20	13.52	13.53	13.46	**13.18**
Vitamin C (4g/L)	23.10	20.10	20.20	18.50	**20.48**
Potato (raw)	80.93	92.59	86.04	86.80	**86.59**
Potato (boiled)	92.00	97.33	104.90	114.33	**102.14**
Potato with bud (raw)	89.80	94.76	69.30	189.20	**110.77**
Potato with bud (boiled)	128.87	201.10	221.20	195.80	**186.74**
Sweet potato (raw)	301.50	348.87	434.77	358.59	**360.93**
Sweet potato (boiled)	558.10	442.80	472.20	490.30	**490.85**

	Average (s)	SD	T-test (with water)		T-test (with Vit C)		T-test (raw VS boiled)	
Water	**13.18**	**0.65**						
Vitamin C (4g/L)	**20.48**	**1.92**	0.0026621342	**				
Potato (raw)	**86.59**	**4.77**	0.0000586973	***	0.00000009	***		
Potato (boiled)	**102.14**	**9.70**	0.0003369146	***	0.00001114	***	0.040611239	*
Potato with bud (raw)	**110.77**	**53.44**	0.0354240892	*	0.01196887	*		
Potato with bud (boiled)	**186.74**	**40.10**	0.0032383278	**	0.00041765	***	0.066696023	*
Sweet potato (raw)	**360.93**	**55.18**	0.0010757018	**	0.00008868	***		
Sweet potato (boiled)	**490.85**	**48.92**	0.0002927657	***	0.00001504	***	0.012763096	*

Table 1 Relative antioxidizing power of various types of potato juices

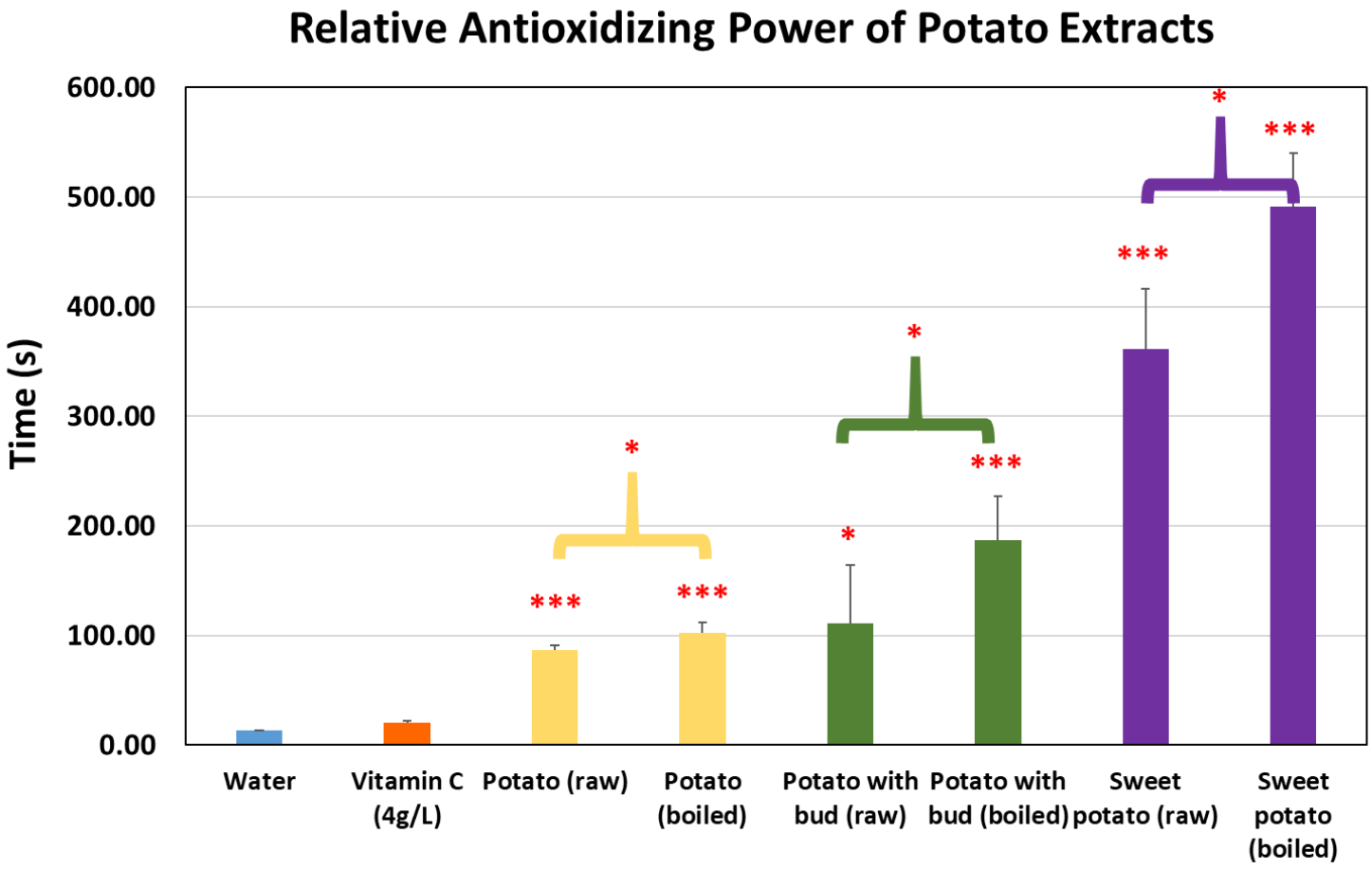


Figure 1 Relative antioxidizing power of various potato juices with the corresponding interval time in Clock Reaction presented in bar chart
(Student's *t*-test Analysis: $n = 4$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Briggs-Rauscher Reaction (Clock Reaction) is the theory basis for testing samples' ability to scavenge free radical. As mentioned in the previous session, the longer the time interval between the second and the third blue phases, the greater the antioxidant capacity the sample has.

Our project compared the relative antioxidizing power of different potato juices by Clock Reaction, since we would like to know which potato juice can scavenge free radicals in human bodies better, so that it can help prevent mutagenesis of normal cells to develop as cancer cells. All potato

juices were freshly extracted before the experiment, so did the reagents needed in the Clock Reaction. The standard Vitamin C solution (Redoxon C) was used as the reference. Distilled water was used as the negative control.

Figure 1 showed that all potato samples have significantly higher relative antioxidizing powers than vitamin C, as revealed by extremely low p-values (***$p < 0.001$) of Student's t-test in Table 1. Raw potato juice has higher antioxidizing power than vitamin C by 4.23 folds while raw sprouted potato juice has even higher antioxidizing power by 5.41 folds. The relative antioxidizing power is the highest for raw sweet potatoes, with 17.62 folds of that of Vitamin C.

	Average (s)	**Fold of relative antioxidizing power than the vitamin C**
Water	**13.18**	
Vitamin C (4g/L)	**20.48**	**/**
Potato (raw)	**86.59**	**4.23**
Potato (boiled)	**102.14**	**4.99**
Potato with bud (raw)	**110.77**	**5.41**
Potato with bud (boiled)	**186.74**	**9.12**
Sweet potato (raw)	**360.93**	**17.62**
Sweet potato (boiled)	**490.85**	**23.97**

When the juices were boiled by electronic hot plate for 10 minutes, the antioxidizing power even increased further (statistically analysis shown in Table 1). Boiled sweet potato juice got the highest antioxidizing power, with average time of 490.85 seconds in Clock Reaction, and 23.97 fold higher in free scavenging ability than that of the Vitamin C, the well-known antioxidant. This revealed that boiling could help release antioxidizing ingredient from the potato cells, rather than denaturation as generally believed by the public. Such high scavenging ability might help protect the cancer cells against strong oxidative stress by free radicals and hydroxyl radicals generated in metabolic reactions.

(B) Growth Test of Potato Juices on HL-60 Cancer Cells

B.1 Evaluating Anticancer Properties of Potato Juices – Morphological Studies

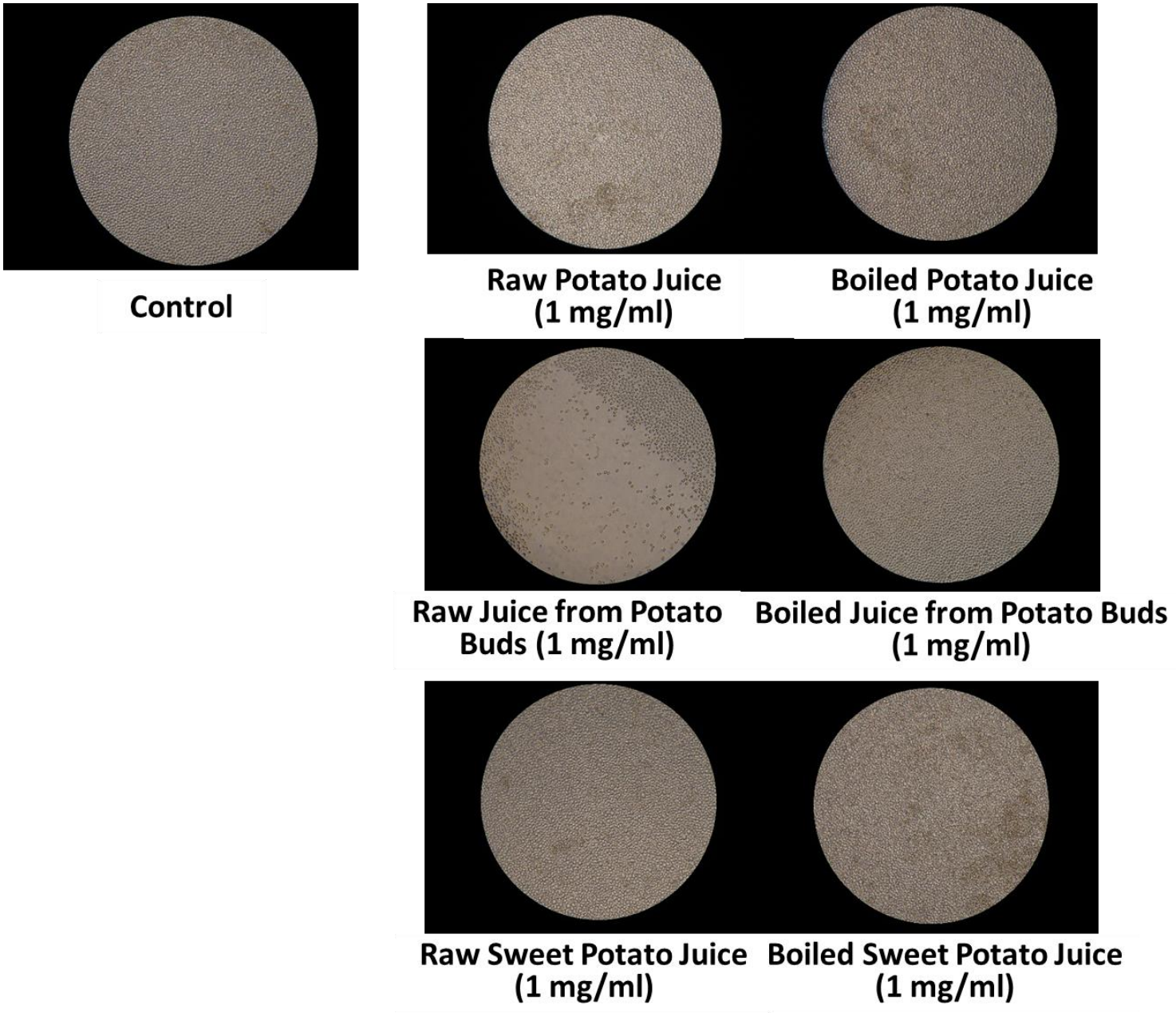


Figure 2 Morphology changes of HL-60 cancer cells when the cells were subjected to 1 mg/ml of raw potato juice, sprouted potatoes and sweet potatoes, with their boiled counterparts, as observed under inverted microscope

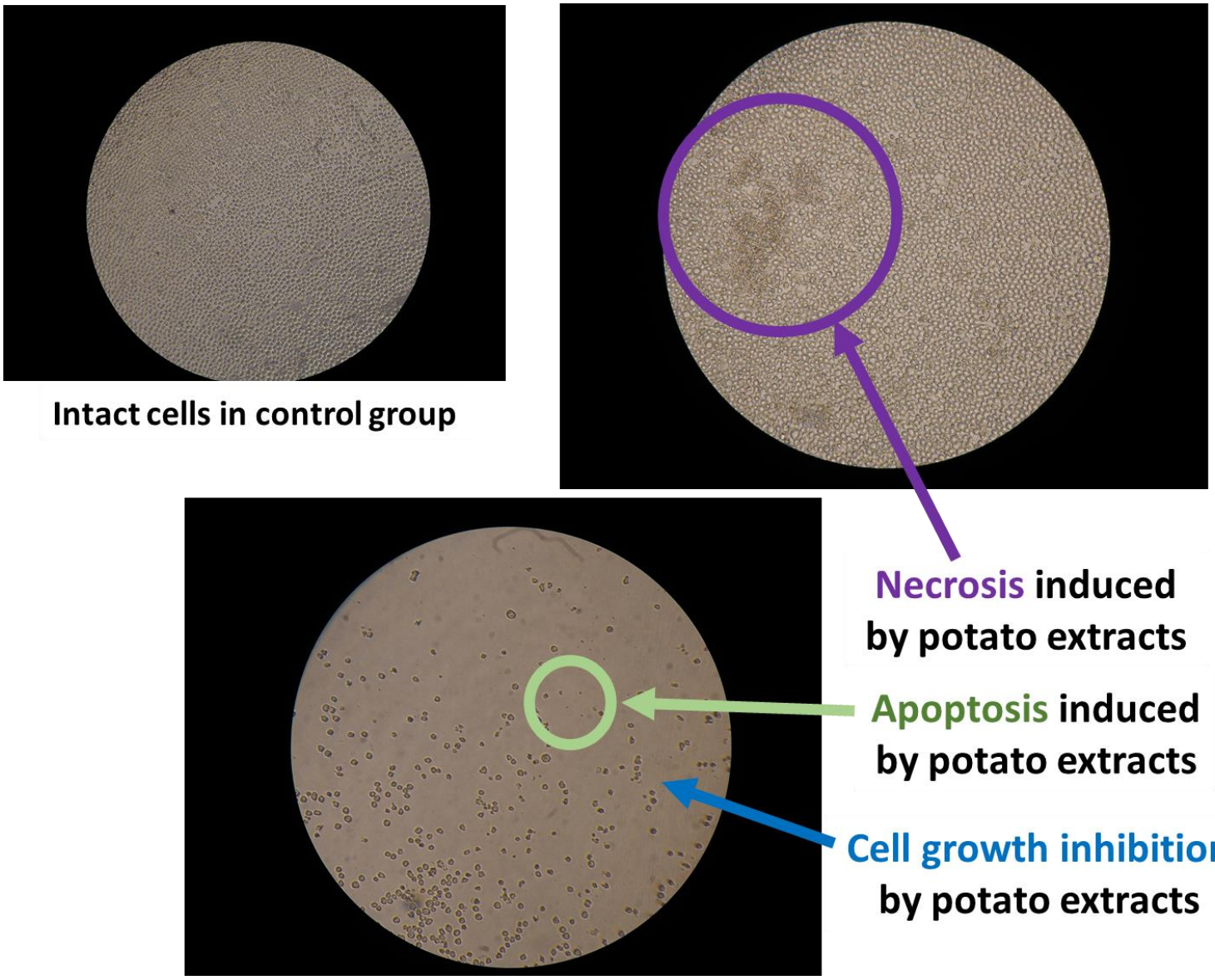


Figure 3 Morphology changes of HL-60 cancer cells when the cells were subjected to 1 mg/ml of potato juice as observed under inverted microscope

HL-60 is a promyelocytic cell line with intact and round shape as reflected by light under the inverted microscope. However, when administered 1 mg/ml of potato juice with 24 hour of incubation, it was found that the cell density significantly reduced, and the cells looked not as intact as in the control. Some cells experienced apoptosis, the programmed cell death, with apoptotic bodies in the leukemia cells, and more membrane infoldings (Figure 2 and 3). Some cells also experienced necrosis, forming vacuoles on their surface with interior structures either distending or shrinking rapidly. No matter apoptosis or necrosis, cell organelles would finally be digested by autolysis into fragments. This definitely revealed the killing ability of the samples towards HL-60 cancer cells.

B.2 Evaluating Anticancer Properties of Polar Substances in Potatoes

Growth Inhibition Test								
24 h Treatment	**1 mg/ml**							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	20	16	16	17.33		2.31		
Potato (raw)	48	50	40	46.00	165.38	5.29	0.00467	**
Potato (boiled)	28	44	44	38.67	123.08	9.24	0.04990	*
Potato with bud (raw)	43	44	45	44.00	153.85	1.00	0.00062	***
Potato with bud (boiled)	32	40	38	36.67	111.54	4.16	0.00516	**
Sweet potato (raw)	48	44	52	48.00	176.92	4.00	0.00104	**
Sweet potato (boiled)	52	36	50	46.00	165.38	8.72	0.02335	*
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	480	450	440	456.67		20.82		
Potato (raw)	405	368	396	389.67	-14.67	19.30	0.01517	*
Potato (boiled)	360	340	320	340.00	-25.55	20.00	0.00221	**
Potato with bud (raw)	405	398	376	393.00	-13.94	15.13	0.01555	*
Potato with bud (boiled)	360	384	342	362.00	-20.73	21.07	0.00521	**
Sweet potato (raw)	340	332	359	343.67	-24.74	13.87	0.00250	**
Sweet potato (boiled)	400	352	350	367.33	-19.56	28.31	0.01408	*

24 h Treatment	**0.5 mg/ml**							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	16	20	18	18.00		2.00		
Potato (raw)	36	28	40	34.67	92.59	6.11	0.03196	*
Potato (boiled)	30	36	32	32.67	81.48	3.06	0.00382	**
Potato with bud (raw)	44	44	52	46.67	159.26	4.62	0.00329	**
Potato with bud (boiled)	36	36	44	38.67	114.81	4.62	0.00781	**
Sweet potato (raw)	36	44	42	40.67	125.93	4.16	0.00400	**
Sweet potato (boiled)	44	48	36	42.67	137.04	6.11	0.01308	*
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	448	480	464	464.00		16.00		
Potato (raw)	420	424	420	421.33	-9.20	2.31	0.04135	*
Potato (boiled)	362	412	412	395.33	-14.80	28.87	0.03434	*
Potato with bud (raw)	380	372	416	389.33	-16.09	23.44	0.01380	*
Potato with bud (boiled)	392	424	428	414.67	-10.63	19.73	0.03007	*
Sweet potato (raw)	328	332	336	332.00	-28.45	4.00	0.00318	**
Sweet potato (boiled)	368	364	350	360.67	-22.27	9.45	0.00170	**

Table 2(a) Number of dead and viable HL-60 cells under 24 h treatment of potato samples (polar constituents) at 0.5 mg/ml and 1 mg/ml
(Student's *t*-test Analysis: $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

48 h Treatment	1 mg/ml							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	25	28	20	24.33		4.04		
Potato (raw)	32	44	40	38.67	58.90	6.11	0.03433	*
Potato (boiled)	40	36	40	38.67	58.90	2.31	0.01109	*
Potato with bud (raw)	44	52	56	50.67	108.22	6.11	0.00537	**
Potato with bud (boiled)	32	24	28	28.00	15.07	4.00	0.32661	
Sweet potato (raw)	48	40	44	44.00	80.82	4.00	0.00391	**
Sweet potato (boiled)	40	48	44	44.00	80.82	4.00	0.00391	**
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	452	520	440	470.67		43.14		
Potato (raw)	400	362	420	394.00	-16.29	29.46	0.07202	
Potato (boiled)	412	392	398	400.67	-14.87	10.26	0.09959	
Potato with bud (raw)	392	432	450	424.67	-9.77	29.69	0.21165	
Potato with bud (boiled)	488	516	500	501.33	6.52	14.05	0.34407	
Sweet potato (raw)	400	360	380	380.00	-19.26	20.00	0.04989	*
Sweet potato (boiled)	360	348	360	356.00	-24.36	6.93	0.04107	*

48 h Treatment	0.5 mg/ml							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	24	25	28	25.67		2.08		
Potato (raw)	60	40	48	49.33	92.21	10.07	0.05016	
Potato (boiled)	52	36	80	56.00	118.18	22.27	0.14114	
Potato with bud (raw)	56	68	40	54.67	112.99	14.05	0.06711	
Potato with bud (boiled)	28	24	44	32.00	24.68	10.58	0.40951	
Sweet potato (raw)	48	40	40	42.67	66.23	4.62	0.01247	*
Sweet potato (boiled)	42	36	32	36.67	42.86	5.03	0.04754	*
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	528	520	480	509.33		25.72		
Potato (raw)	408	436	432	425.33	-16.49	15.14	0.01378	*
Potato (boiled)	448	432	472	450.67	-11.52	20.13	0.03865	*
Potato with bud (raw)	394	424	386	401.33	-21.20	20.03	0.00545	**
Potato with bud (boiled)	484	480	520	494.67	-2.88	22.03	0.49578	
Sweet potato (raw)	360	368	380	369.33	-27.49	10.07	0.00532	**
Sweet potato (boiled)	360	340	344	348.00	-31.68	10.58	0.00345	**

Table 2(b) Number of dead and viable HL-60 cells under 48 h treatment of potato samples (polar constituents) at 0.5 mg/ml and 1 mg/ml
(Student's *t*-test Analysis: $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

To evaluate the effect of potato samples on growth of HL-60 cells, the growth inhibitory effect and killing effect were studied. Growth inhibitory effect referred to the degree of the samples to hinder the cell division process of HL-60 cells, which originally are cancerous with uncontrolled rapid cell division. On the other hand, killing effect referred to the degree of killing on HL-60 cells (maybe by means of necrosis or programmed cell death like apoptosis). To simply distinguish the two, HL-60 cells were seated in 96-well flat-bottomed plates for 24 to 48 hours. Trypan blue stain was used to stain the dead cells whose selective permeability of the cell membrane were lost. The observation was done through light microscope. The cell density of dead and viable cells was then estimated using a haemocytometer.

It was found that all potato juices (no matter raw or boiled ones) inhibit cell growth in the range of 13.94% to 25.55% at the concentration 1 mg/ml for 24 h incubation, while from 9.77% to 24.36% at the same concentration but for 48 h incubation. For study on killing effect, potato samples could increase the killing action on HL-60 cells by 2.5 times for 24 h incubation, while by 80% on average for 48 h incubation. When comparing the growth inhibitory effect and killing effect in 24 and 48 h incubation periods, HL-60 cells tended to revert the effects to a lesser degree, consistently shown by the reduced significance in statistical analysis. The growth inhibitory effect and killing actions were not prominent as potato may provide nutrients for growth which counteract the inhibitory and killing actions.

HL-60 cells also responded similarly when 0.5mg/ml potato samples were administered, with similar levels of growth inhibition and killing as compared to administering of 1 mg/ml potato samples.

The results were prominent as blended juice could give such a significant growth inhibitory and killing effects on suspension cell line HL-60 leukemia cells.

B.3 Evaluating Anticancer Properties of Non-Polar & Polar Substances in Potatoes

Growth Inhibition Test								
24 h Treatment	**1 mg/ml**							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	1	1	2	1.33		0.58		
Potato	4	6	4	4.67	250.00	1.15	0.02172	**
Potato with bud	3	4	5	4.00	200.00	1.00	0.02482	***
Sweet potato	3	4	3	3.33	150.00	0.58	0.01324	**
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	36	38	34	36.00		2.00		
Potato	24	19	23	22.00	-38.89	2.65	0.00245	**
Potato with bud	20	24	21	21.67	-39.81	2.08	0.00101	**
Sweet potato	26	22	26	24.67	-31.48	2.31	0.00323	**

24 h Treatment	**0.5 mg/ml**							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	2	1	1	1.33		0.58		
Potato	3	4	4	3.67	175.00	0.58	0.00776	*
Potato with bud	3	5	4	4.00	200.00	1.00	0.02482	**
Sweet potato	2	3	3	2.67	100.00	0.58	0.04742	**
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	35	34	40	36.33		3.21		
Potato	24	24	26	24.67	-32.11	1.15	0.01568	*
Potato with bud	26	30	27	27.67	-23.85	2.08	0.02312	*
Sweet potato	30	26	30	28.67	-21.10	2.31	0.03296	*

Table 3(a) Number of dead and viable HL-60 cells under 24 h treatment of potato samples (non-polar and polar constituents) at 0.5 mg/ml and 1 mg/ml

(Student's *t*-test Analysis: $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

48 h Treatment	1 mg/ml							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	2	3	3	2.67		0.58		
Potato	5	4	4	4.33	62.50	0.58	0.02411	*
Potato with bud	4	6	5	5.00	87.50	1.00	0.03565	*
Sweet potato	4	5	6	5.00	87.50	1.00	0.03565	*
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	50	45	49	48.00		2.65		
Potato	38	38	32	36.00	-25.00	3.46	0.01043	*
Potato with bud	29	33	35	32.33	-32.64	3.06	0.00275	**
Sweet potato	36	40	32	36.00	-25.00	4.00	0.01674	*

48 h Treatment	0.5 mg/ml							
No. of dead cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	3	3	2	2.67		0.58		
Potato	4	4	5	4.33	62.50	0.58	0.02411	*
Potato with bud	4	5	4	4.33	62.50	0.58	0.02411	*
Sweet potato	5	5	7	5.67	112.50	1.15	0.02860	*
No. of viable cells								
	1st Trial	**2nd Trial**	**3rd Trial**	**Avg**	**% change**	**SD**	**T-test**	
Control	42	40	52	44.67		6.43		
Potato	35	36	38	36.33	-18.66	1.53	0.14759	
Potato with bud	35	32	36	34.33	-23.13	2.08	0.09688	
Sweet potato	33	40	35	36.00	-19.40	3.61	0.13029	

Table 3(b) Number of dead and viable HL-60 cells under 48 h treatment of potato samples (non-polar and polar constituents) at 0.5 mg/ml and 1 mg/ml

(Student's *t*-test Analysis: $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

It was found that all potato samples in DMSO inhibit cell growth in the range of 31.48% to 39.81 at the concentration 1 mg/ml for 24 h incubation, while from 25.00% to 32.64% at the same concentration but for 48 h incubation. For study on killing effect, potato samples could increase the killing action on HL-60 cells by 2.5 times for 24 h incubation, while by 75% on average for 48 h incubation. When comparing the growth inhibitory effect and killing effect in 24 and 48 h incubation periods, HL-60 cells tended to revert the effects to a lesser degree, consistently shown by the reduced significance in statistical analysis.

The growth inhibitory effect was more prominent for potato samples extracted by DMSO than by water. This can be explained by the fact that DMSO also extracted certain organic constituents from potatoes, which were more potent substances to act against growth of HL-60 cancer cells.

HL-60 cells also responded similarly when 0.5mg/ml potato samples were administered, with similar levels of growth inhibition and killing as compared to administering of 1 mg/ml potato samples. Nevertheless, the growth inhibitory effect for 0.5mg/ml of potato samples (extracted in DMSO) was statistically insignificant.

5. Deliverables and Conclusive Remarks

To further extend our investigation, besides Clock Reaction, assay kits with higher sensitivity and accuracy could be used to evaluate the antioxidant levels[11]. On the contrary, the study can also focus on the level of Reactive Oxygen Species (R.O.S.), by using assay kit like the DCFDA-Cellular Reactive Oxygen Species Detection Assay Kit (ab113851)[12]. Since many antioxidants are organic phytochemical, it was also possible to carry similar test on organic portion of the potatoes. Of course, to obtain the organic fractions, some separating techniques would be needed.

In addition, HL-60 leukemia cells belong to suspension cell lines. Other adhesive cell lines could be cultured and tested[13]. Examples included A375 melanoma, PC-3 prostate cancer, HeLa cervical cancer cells, HepG2 hepatic cancer cells, etc. This can increase the validity of the potential growth inhibitory effect and killing effect of potato juices on cancer cell lines. To have fair comparison, corresponding normal tissue cell lines should also be included to study.

Here, we concluded the significant roles of 2% potato juices in protecting us from oxidative stress. At the low concentration of 0.5 mg/ml and 1 mg/ml, potato samples posed 20% growth inhibition to HL-60 cells and increased mortality by 2.5 folds at 24 h incubation. The growth inhibitory effect was more prominent for potato samples extracted by DMSO than by water, posing 30% growth inhibition to HL-60 cells at 24 h incubation instead. This indicated that certain organic constituents from potatoes contained more potent substances to act against growth of HL-60 cancer cells.

All in all, our studies have paved a path to elucidate the significant role of potato juices in exhibiting antioxidizing power in Clock Reaction, growth inhibitory effect and killing effect of potato samples on HL-60 promyelocytic cell line. Further investigation even enables us to unveil the mechanism of pathways of such growth inhibition and killing actions in the future.

6. References

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