

Hong Kong Student Science Project Competition 2022

Template of Extended Abstract (Investigation)

(Word Limit: 1,000 words, Pages: 2 pages only)

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Project Title: The anti-inflammatory effect of colorectal cancers (HT-29) with *Dimocarpus longan* (龍眼), *Zingiber officinale* (生薑), *Crataegus pinnatifida* (山楂) and *Rheum rhabarbarum* (大黃)

Project Type: Investigation

To our best knowledge and after thorough literature research, as at 30 / 06 / 2022, there are / are no* similar works.

I. Background

Our group aims to investigate whether the four type of Chinese Medicine: *Crataegus pinnatifida* (山楂), *Dimocarpus longan* (龍眼), *Rheum rhabarbarum* (大黃) and *Zingiber officinale* (生薑) can successfully kill colorectal cancer cells (HT-29) or prevent metastases at 0.25mg/ml, 0.5 mg/ml, 0.75mg/ml, 1mg/ml concentration. These four types of Chinese Medicines were useful in treating breast cancer and cervical cancer. Yet, no research is found to have used these four types of medicines for treating colorectal cancer, which is the second commonest cancer in Hong Kong.

II. Objectives

1. To investigate whether *Crataegus pinnatifida* (山楂), *Dimocarpus longan* (龍眼), *Rheum rhabarbarum* (大黃) and *Zingiber officinale* (生薑) are useful to have anti-proliferative effects HT-29.
2. To compare the migration rate after the addition of different medicines.
3. To compare which concentration and which Chinese Medicines can effectively suppress HT-29.

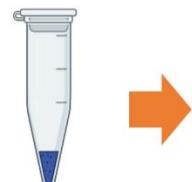
III. Hypothesis

Hypotheses for testing:

1. The four types of Chinese medicines can have inhibitory effects on the growth of HT-29, or even have killing effects on HT-29 by inducing apoptosis (in low concentration) or necrosis (in high concentration).
2. The Migration assay can be reference for proofing whether the Chinese medicines can prevent metastasis by measuring the distance changes in 48 hours interval.
3. The cell culture in the culture flask that are put in the incubator can more of less mimic the environment for growing HT-29 in animal/human body in the colons.

IV. Methodology

1. Pre-test: Cell counting for fair test

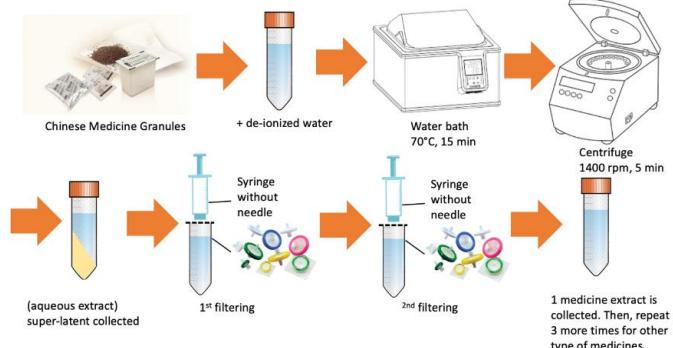


Cell suspension with Trypan Blue stain

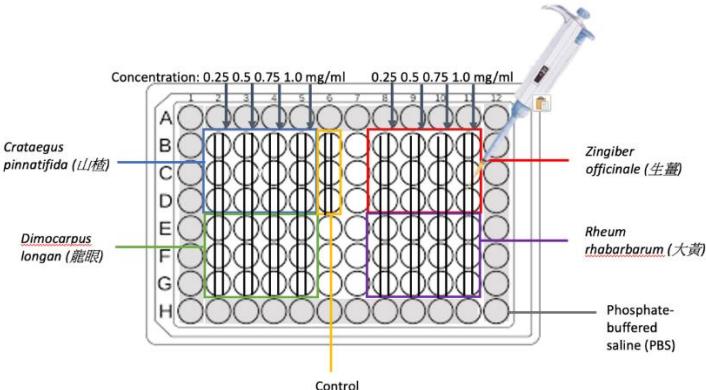


Hemacytometer
Cell counting under the microscope

2. Chinese medicine extraction:



Session 1 (MTT Assay) and Session 2 (Migration Assay)



1. One-way ANOVA

It is used in MTT Assay for calculating the variations and the accuracy for our results in the graphs. It is a famous statistical method that helps to understand the relationship between the four types of medicines and the ability to kill or suppress the growth of HT-29.

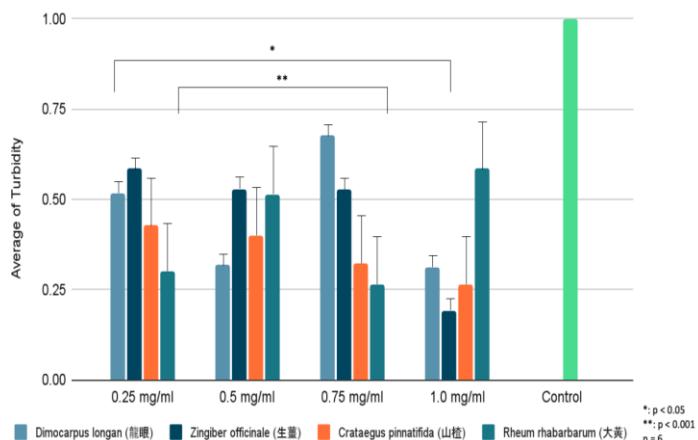
2. MotiConnect

It is useful in migration assay. This is an application that can be used in iPad or phone for having real time image with the microscope. This helped us to accurately measuring the distance changes under the microscope.

V. Results

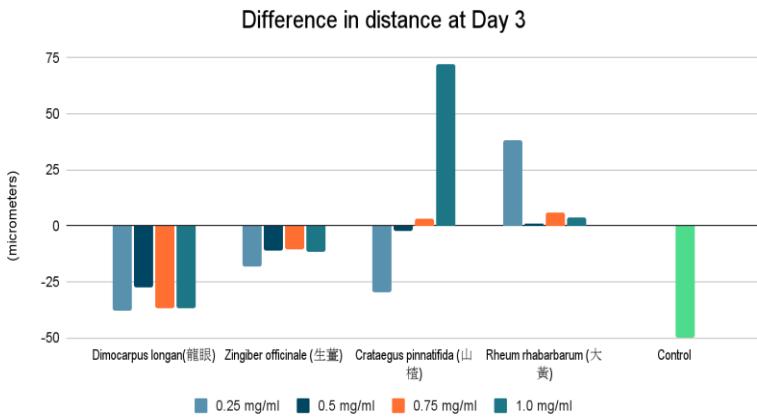
MTT Results

A table described the HT-29 viability in MTT after the treatment of 4 Chinese medicines



Zingiber officinale (生薑) was the best among the four Chinese Medicines. It has different chemical constituents (An Aldehyde, Gingerol, Shogaol, and Paradol) inhibited the proliferation of HT-29 cells with an increasing concentration (Banerjee, Mullick, 2011) induced a higher rate of apoptosis in colorectal cancer cells at increasing concentration (Abdullah, Abidin, Murad, Makpol, Ngah, Yusof, 2010).

Migration Assay:



Rheum rhabarbarum (大黃) was the most effective to inhibit the growth of HT-29 in all concentrations has a mild yet powerful and effective laxative that empties the intestines and cleanses the bowels thoroughly

Crataegus pinnatifida (山楂) was the second most significant medicines among the four types of medicines. It showed that 1.0 mg/ml concentration was the most significant and had good antioxidant activities a good source of natural antioxidants

Limitation:

1. Time limit- due to the pandemic, the time for us to actually carried out experiments were only 3 months.
2. Western Blot could not be done to vary out hypothesized pathway for HT-29.

VI. Conclusion

The most effective Chinese medicine among the four types of Chinese medicine was *Crataegus pinnatifida* (山楂). It has acetone extract that can induce the among of p-21, caspase-9 and caspase-3 which are all enzymes that induce apoptosis in HT-29. Other medicines also have shown to induce apoptosis. Yet, if we could test the presence of caspase-9 and caspase-3 using Western Blot, we can further verify our findings.