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# Identification and Functional Evaluation of Anti-Cancer Ilmmunomodulators from Physalis peruviana (Poha)

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# Identification and Functional Evaluation of Anti-Cancer IImmunomodulators from Physalis peruviana (Poha)

Department of Biological Sciences

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## Abstract

Our Creative Inquiry team is researching the anti-tumor potential of compounds isolated from *Physalis peruviana (poha)* fruit. CellTiter 96 Aqueous Non-radiative Cell Proliferation Assays *(Promega, 2012)* were performed to screen the inhibitory activity of the compounds on human lung carcinoma A549 cells and breast cancer MDA-MB-231 cells with the corresponding non-tumorigenic epithelial cell line NL20 and MCF-10A as controls. The cells were treated with compounds at concentrations of 0µg/ml, 2µg/ml, 5µg/ml, and 20µg/ml and incubated for 24, 48, and 72 hours.

## Introduction

Native to South America, the *Physalis peruviana (poha)* plant has been widely introduced around the world particularly in Hawaii as a cultivated fruit, a medicinal plant and as an ornamental (CABI, 2015). *Poha* has been claimed to be beneficial for a number of human diseases. However, there is very limited scientific evidence to support these claims. We believe that the confirmation of anti-tumor potential of these *poha* derived compounds will provide cancer patients with more treatment options.

The samples used by this project are extracts from the plant's fruits produced by the collaboration laboratory at the University of Hawaii. The MTS assay performed determines the number of viable cells in proliferation. MTS is a substrate that can be reduced by cells into a formazan product that is soluble in tissue culture media (Promega, 2012). The amount of absorbance of the formazan product at 490nm is directly proportional to the number of living cells in culture (Cory, 1991).

## Methods

The cells are first thawed and cultured by changing the media every two days. Once enough cells are cultured, they are plated in 96-well plates with 5,000 cells per well in 200ul of media. The plated cells are treated with 5ul of *poha* samples at 2ug/mL, 5ug/mL and 20 ug/mL concentrations in triplicates. The controls include triplicates of media not treated with *poha* samples to detect contamination in the cells as well as triplicates of DMSO at 2ug/mL, 5ug/mL and 20 ug/mL concentrations which is used as a solvent for the samples. The DMSO control will serves as a baseline to determine if the *poha* sample had an effect on cell proliferation.

The MTS cell proliferation assay is performed after 24, 48, and 72 hours of incubation and the absorbance of each plate is recorded. The average absorbance of the sample triplicates are taken and compared with the DMSO controls. The fold difference of each sample at the three time points are graphed.

## Results

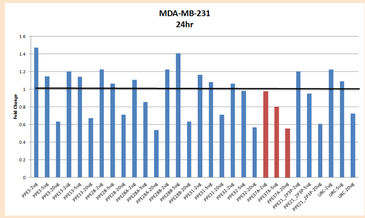


Figure 1. Breast cancer cell proliferation after 24 hours of incubation.

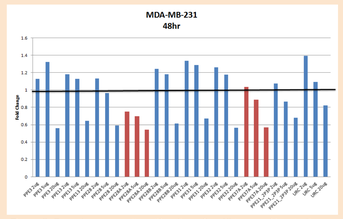


Figure 2. Breast cancer cell proliferation after 48 hours of incubation.

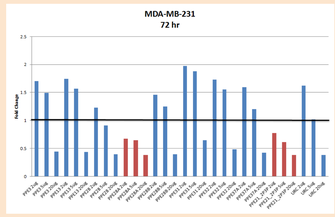


Figure 3. Breast cancer cell proliferation after 72 hours of incubation.

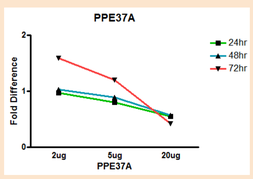


Figure 4. Breast cancer cell proliferation when treated with sample PPE37A at concentrations 2ug/ml, 5ug/ml, and 20ug/ml.

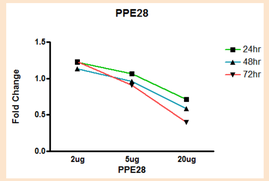


Figure 5. Breast cancer cell proliferation when treated with sample PPE28 at concentrations 2ug/ml, 5ug/ml, and 20ug/ml.

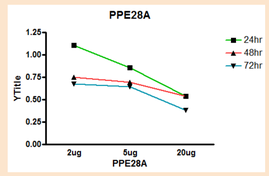


Figure 6. Breast cancer cell proliferation when treated with sample PPE28A at concentrations 2ug/ml, 5ug/ml, and 20ug/ml.

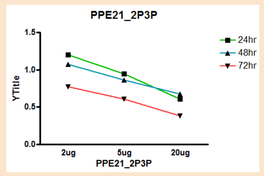


Figure 7. Breast cancer cell proliferation when treated with sample PPE21_2P3P at concentrations 2ug/ml, 5ug/ml, and 20ug/ml.

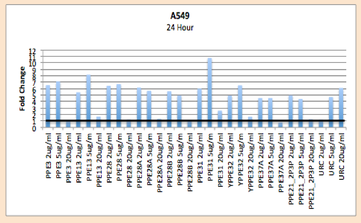


Figure 8: Lung cancer cell proliferation after 24 hours.

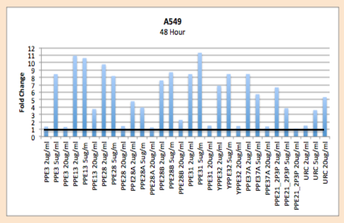


Figure 9: Lung cancer cell proliferation after 48 hours.

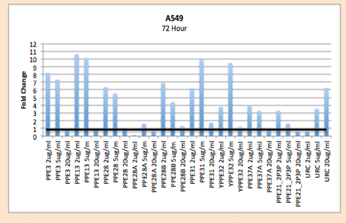


Figure 10: Lung cancer cell proliferation after 72 hours.

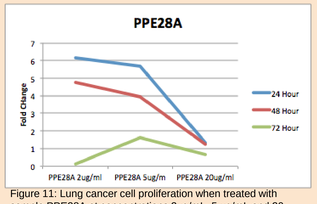


Figure 11: Lung cancer cell proliferation when treated with sample PPE28A at concentrations 2ug/mL. 5 ug/mL and 20 ug/mL.

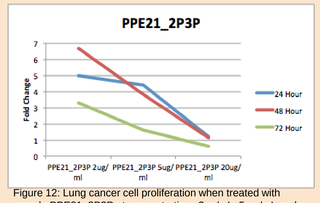


Figure 12: Lung cancer cell proliferation when treated with sample PPE21_2P3P at concentrations 2ug/mL. 5 ug/mL and 20 ug/mL.

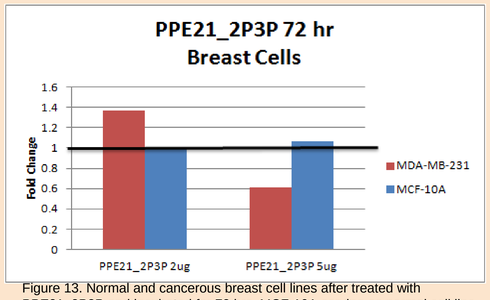


Figure 13. Normal and cancerous breast cell lines after treated with PPE21_2P3P and incubated for 72 hrs. MCF-10A serving as normal cell line control.

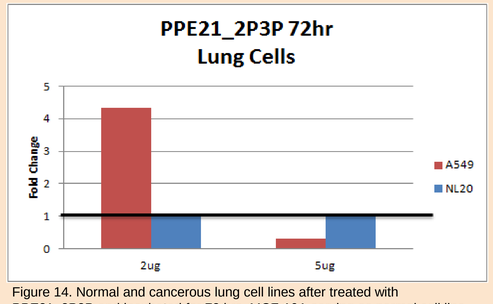


Figure 14. Normal and cancerous lung cell lines after treated with PPE21_2P3P and incubated for 72 hrs. MCF-10A serving as normal cell line control.

## Results and Discussions

The results showed a greatest reduction in cell proliferation when treated with samples PPE37A, PPE28, PPE28A and PPE21_2P3P in the MDA-MB-231 cells as seen by a fold change less than 1. In the A549 cells, PPE28A and PPE21_2P3P presented promising results. The cells showed a sample dose dependence with the greatest decrease in proliferation for the higher treatment concentration (20ug/ml). The cell proliferation also showed a dependence on incubation time displaying a greatest reduction in proliferation on average when incubated for 72 hours.

Sample PPE21_2P3P at 72 hours was found to be the most promising sample for both cell lines and the assay was repeated to compare the cancer cell lines with normal cell lines. Ideally, the samples would decrease the cancer cell proliferation but remained ineffective on the normal cell lines. This trend was observed showing a fold change of 1 in the MCF-10A and NL20 cells and a fold change below 1 in the MDA-MB-231 and A549 cells treated with samples at concentration of 5ug/ml.

## Conclusion

The sample PPE21_2P3P with a concentration of 5 ug/mL was found to be the most promising sample for both the MDA-MB-231 and A549 cell lines. A fold change under 1 was observed for both of the cell lines, indicating a decrease in cell proliferation due to the sample. The sample PPE21_2P3P was also found to not inhibit cell proliferation of the normal cell lines MCF-10A and NL20. This is because a fold change above 1 was observed for both cell lines, indicating that cell proliferation was unaffected by the addition of the sample. Future directions include another round of sample PPE21_2P3P at 72 hours to confirm results and testing of the sample PPE21_2P3P on new cell lines.

## References

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