The Inhibitory Effect of Spirulina Polyphenols on Ovarian Cancer Cell Lines

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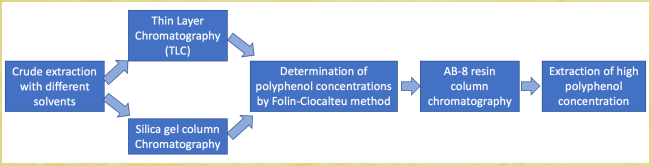
Abstract

Ovarian cancer is one of the most lethal gynecologic cancers in women worldwide. Although various targeted drugs have been developed, the 5-year survival rate remains low due to the high possibility of recurrence and resistance to chemotherapy. Plant-derived polyphenols, a subtype of antioxidants, can inhibit ovarian tumors through different mechanisms and reduce drug resistance. Research shows that spirulina, as a marine microorganism, is able to generate multiple phenolic compounds with potentially inhibitory effect on ovarian tumor cells. It is meaningful to investigate the effects of spirulina-derived polyphenols on ovarian cancer and identify possible mechanism involved. As a preliminary study, the SELF project specifically aims to better understand the characteristics of spirulina polyphenols and identify the effective ways to extract spirulina and yield polyphenols. Results show that crude extraction of spirulina using water at room temperature yields the most total phenol content. Further results indicate that other extraction methods including AB-8 resin and silica gel chromatography generate a similar level of phenol concentration as water extraction.

Method



Preliminary Results Before Summer

1. Extraction of spirulina with different solvents

Figure 1A and 1B. Spirulina extractions with different solvents exhibit multiple colors. Among them, water extraction appeared to be blue while extractions with other solvents, including acetone, ethanol, ethyl acetate, and mixtures of them, displayed green to different degrees.

2. The Thin Layer Chromatography (TLC) Results

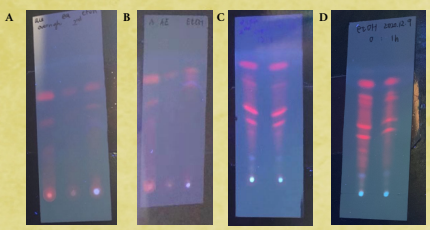


Figure 2A and 2B. TLC for acetone, ethyl acetate and ethanol extractions overnight. The eluents were hexane:ether= 3:7 (A) or 2:8 (B). Different molecules were extracted by different solvents. Figure 2C and 2D. TLC for ethyl acetate (C) and ethanol (D) extractions overnight. The eluent was composed of 1 ml hexane, 1 ml methylenechloride, and 200 µl methanol. Different molecules were extracted by different solvents.

Future Plans

Our study has confirmed that the highest total polyphenol content can be yielded by water extraction of spirulina for 6h at room temperature. We have been culturing the SKOV-3 cells, the cell line suitable for examining the inhibitory effects of spirulina polyphenols. We plan to perform CCK8 tests, flow cytometry, RT-PCR, and western-blots, in order to detect polyphenols' impacts on cell apoptosis, changes of mRNA and protein expression levels. The further research could involve applying spirulina polyphenol extract as a novel prevention or treatment for ovarian cancer. Our project is innovative in studying the interaction of spirulina extracts and ovarian cancer. Additionally, the abundance and cheapness of spirulina can hopefully contribute to a new inexpensive anti-cancer drug.

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Summer Research Results

3. Silica gel column chromatography and AB-8 resin column chromatography

Figure 3A and 3B. Eluents after silica gel column chromatography. Hexane, dichloromethane, different mixtures of hexane and dichloromethane, as well as ethyl acetate, were used as eluents. The samples were collected by colors. Each of the bands was a mixture of multiple molecules as indicated by NMR and GC/MS.

Figure 3C. Eluents after AB-8 resin column chromatography. From left to right, the eluents were original extraction, DI water, 30% ethanol, 60% ethanol, and 90% ethanol. These samples were concentrated by rotary evaporation.

4. Determination of the polyphenol concentrations by the Folin-Ciocalteu method

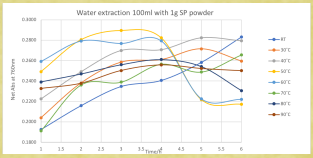


Figure 4A. The absorbance at 760nm of water extraction after folin-phenol reaction, performed by ELISA reader. Each extraction used 100ml water and 1g spirulina powder. The conditions were between 1-6 hours at room temperature-90 celsius degrees. 6h extraction at room temperature yielded highest polyphenol concentration.

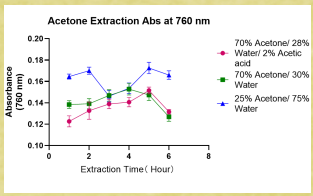


Figure 4B. The absorbance at 760nm of acetone extraction after folin-phenol reaction, performed by ELISA reader. Three concentrations of acetone were used in the spirulina extraction: 70% acetone/28% water/2% acetic acid; 70% acetone/30% water; and 25% acetone/ 75% water. Extractions were conducted from 1 to 6 hours at room temperature. All absorbance values were lower than water extractions'.

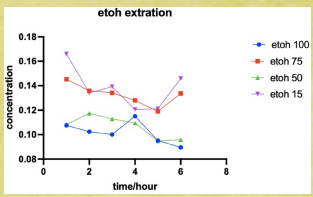
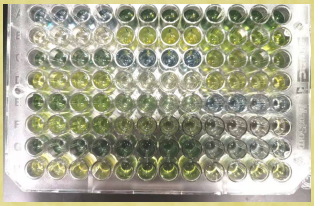


Figure 4C. The absorbance at 760nm of ethanol extraction after folin-phenol reaction, performed by ELISA reader. The concentrations of ethanol were 100%, 75%, 50%, and 15%. Extractions were conducted from 1 to 6 hours at room temperature. All absorbance values were lower than water extractions'.



Figuire 4D. The 96-well plate loaded with 15%, 50%, 75%, and 100% EtOH extraction of spirulina reacted with folin reagents.