

# **The Effects of Phytoestrogens on Breast Cancer Cells**

A Major Qualifying Project  
Submitted to the Faculty of  
WORCESTER POLYTECHNIC INSTITUTE  
in partial fulfillment of the requirements for the  
degree of Bachelor of Science

by  
Alexander Flores  
Katherine Milligan  
Michael Pettiglio  
Arno Vandebroek

Date:  
26 April 2012

Report Submitted to:

Professors Michael Buckholt and Jill Rulfs  
Worcester Polytechnic Institute

*This report represents work of WPI undergraduate students submitted to the faculty as evidence of a degree requirement. WPI routinely publishes these reports on its web site without editorial or peer review. For more information about the projects program at WPI, see <http://www.wpi.edu/Academics/Projects>*

## **Acknowledgements**

We would like to thank our advisors Michael Buckholt and Jill Rulfs for all of their guidance through this entire project. We would not have been able to do this without them. We would like to thank Abbie White for being so helpful with lab maintenance and acquiring materials that we needed. We would like to thank JoAnne Whitefleet-Smith for her help with the lyophilization machine. We would like to thank Drew Brodeur for training us in HPLC. We appreciate the access to the project lab in Goddard Laboratories.

## **Abstract**

Previous studies have shown that phytoestrogens exhibit an anti-proliferative effect on breast cancer cells. This project used HPLC to isolate components of the phytoestrogens. These components were then applied to MCF7 and T47D cell lines to determine their effect on cell proliferation. The results suggest that black cohosh has the most anti-proliferative effect.

## Table of Contents

Acknowledgements.....	ii
Abstract.....	iii
Introduction.....	1
Commercial Phytoestrogen Supplements .....	3
Cell Lines.....	4
PCNA and Caspase .....	5
PCNA.....	5
Caspase .....	5
Previous Research.....	5
Jessica Caron Thesis .....	5
Park and Patchel Paper.....	6
Methods and Materials.....	7
Cell Culture.....	7
High Performance Liquid Chromatography (HPLC).....	7
Experimental Conditions .....	8
Bradford Assay .....	9
Western blotting and Immunodetection.....	9
Results.....	9
Phytoestrogens Background.....	9
Estrogen Sensitivity .....	9
Effect of Phytoestrogens .....	11
MCF7 and T47D Cell Proliferation Assay with Whole Extracts.....	11
HPLC .....	15
Modified T47D-KBluc.....	17
Conclusions.....	19
Recommendations .....	19
Works Cited .....	21

## Introduction

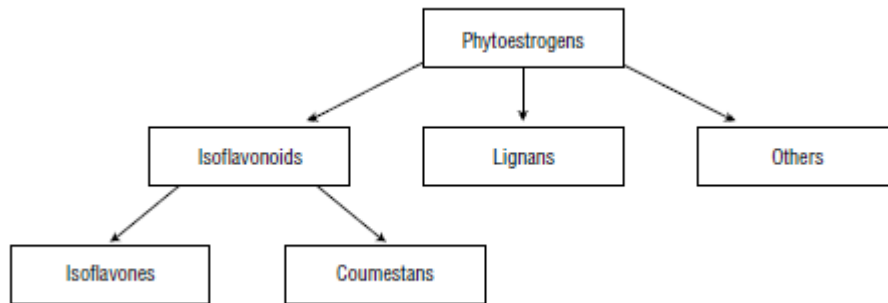
As women age, production of the hormone estrogen secreted by the ovaries decreases. Estrogen is a hormone that regulates the menstrual cycle in women. The decreasing production and circulation of estrogen in the body can cause women, usually in their late 40s or 50s, to feel some uncomfortable symptoms. This inevitable time in a women's life is referred to as menopause. During menopause, women typically feel the effects of several symptoms including, but not limited to, irregular periods, hot flashes, sleeplessness, emotional instability and incontinence (Nordqvist, 2012). Women have taken two routes to relieve these symptoms: estrogen replacement therapy and phytoestrogens.

Estrogen replacement therapy is a method of relieving symptoms of menopause. This is a treatment that includes taking estrogen with or without progestin (a synthetic form of progesterone). Progesterone is another hormone produced by the ovaries that prepares the female body for pregnancy. There are some extrinsic benefits to taking supplemental estrogen. These include the reduction in risk of disease associated with lower levels of estrogen, such as osteoporosis, coronary artery disease, and colon cancer. The negative effects of taking estrogen supplements include an increase in the risk of breast cancer, heart disease, blood clots and stroke (Mayo Clinic, 2012).

Many women choose to use hormone replacement therapy to alleviate the effects of hormone imbalance. Supplements are produced synthetically and cause other health risks. According to a study by Francine Grodstein and colleagues published in the Archives of Internal Medicine" (Grodstein *et al*, 2008) artificial estrogen replacements can increase the risk of stroke. Studies by De Prithwish and colleagues demonstrated a decreased breast cancer risk as women decreased artificial estrogen replacement therapy (DePrithwish *et al*, 2010). Beral *et al* showed an increased risk of ovarian cancer with use of estrogen supplements (Beral *et al*, 2007). Cancer is not the only risk that increases with the increased use of artificial estrogen. Romieu *et al* showed a direct correlation between artificial estrogen use and an increased risk of developing asthma (Romieu *et al.*, 2010).

Another method of reducing the symptoms of menopause is by using phytoestrogen supplements. Phytoestrogen supplements are over the counter dietary supplements targeted towards post-menopausal women. These supplements are generally plant extracts which may include many different types of phytoestrogens in them (Setchel, 2010). Phytoestrogens are a group of chemicals that are structurally similar to the hormone estrogen. While estrogen replacement therapy has been linked to breast cancer, there have been mixed results linking breast cancer to phytoestrogens. Some preliminary results suggest that phytoestrogens act similarly to estrogen and may increase the risk of breast cancer. It is suggested that phytoestrogens mimic estrogen by activating the estrogen receptor (Rice, 2006).

Phytoestrogens are found in foods such as soy, flaxseed, sprouts, and peas. There are three types of known phytoestrogens: lignans, isoflavones, and coumestans (Glazier & Bowman, 2001). Their relationship can be seen below in Figure One.



**Figure 1: Type of Phytoestrogens (Glazier & Bowman, 2001)**

The structural similarity between estradiol (the main form of estrogen) and phytoestrogens can be seen below in Figure Two.

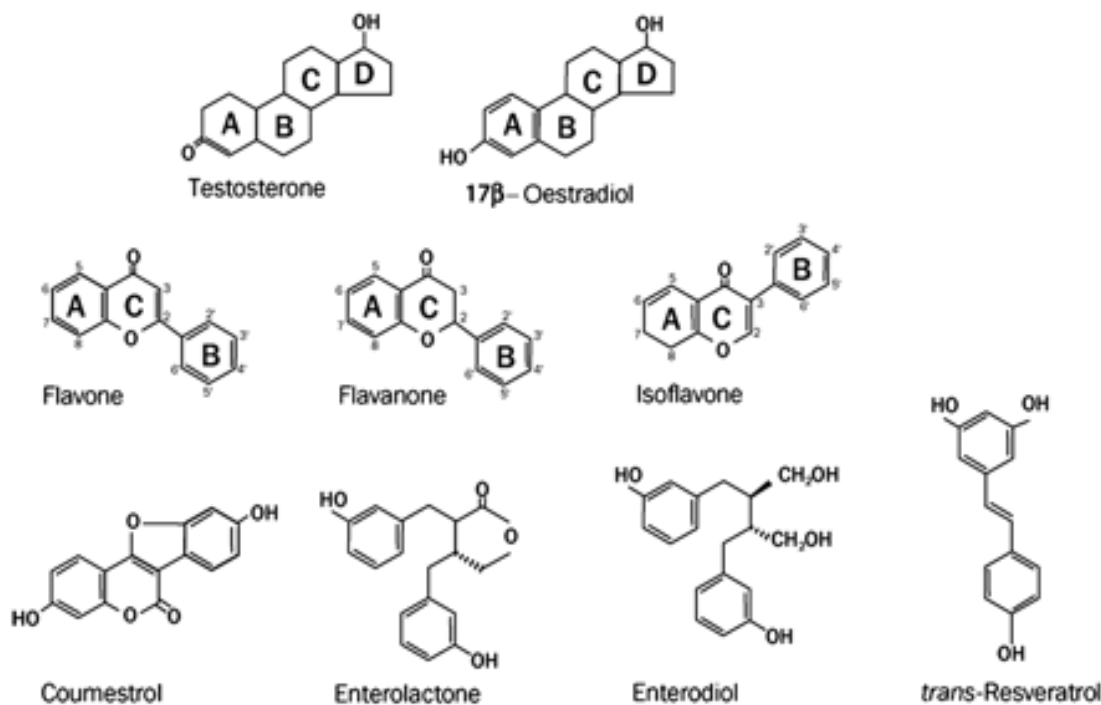


Figure 2: Chemical Structures of Testosterone, Estrogen, and the Major Classes of Phytoestrogens (Rice & Whitehead, 2006; Rice & Whitehead, 2006)

There are many different phytoestrogen supplements available; they can be taken in the form of pills, powders, and teas. Also, anecdotal evidence suggests that consuming large amounts of foods that contain these phytoestrogens, such as soy, could have an effect on menopause symptoms and possibly breast cancer cell growth. More research needs to be done to reach valid conclusions about which specific phytoestrogens affect the risk of breast cancer (Barbour, 2011) and what their mechanism of action might be .

### Commercial Phytoestrogen Supplements

In the work presented here, three commercial herbal extracts were tested for their anti-proliferative effects on breast cancer cell growth: Promensil, Black Cohosh and Natrol Soy. Other studies conducted on these and similar products have produced mixed results. A study conducted on red clover isoflavone found in Promensil, showed a reduction in breast density after 12 months (Atkinson, et al., 2004). While the phytoestrogen components of Black Cohosh remain mostly unknown, formononetin

has been identified in trace amounts in the roots and rhizomes of Black Cohosh (Al-Amier, Eyles, & Craker, 2012). Natrol soy contains a large amount of isoflavones, the most prominent one being daidzein 7- $\beta$ glucoside. Natrol soy has been shown to significantly reduce chemically induced breast cancer in animal trials (Setchell, 1998).

## Cell Lines

The two cell lines used in these studies are the MCF7 and T47D cell lines. Both cell lines originated from breast cancer tissue and are often used as models when studying the effects of compounds on estrogen sensitive cells. The MCF7 cell line was established by Dr. Herbert Soule in 1970 (Levenson & Jordan, 1997). Dr. Soule derived the cell line from a pleural effusion taken from a 69-year-old patient with metastatic breast cancer. The MCF7 cells were of particular interest due to the fact that the hormone treatment that the patient was undergoing was significantly more effective in this patient than in breast cancer patients (Levenson & Jordan, 1997). When the cells were grown *in vitro* they showed no real change in growth rate with the addition of estrogen, but when grown in an athymic mouse the proliferation rate increased. The reason for this difference is that the initial cell culture medium that the cells were grown in contained phenol red, which has a very similar chemical structure to estrogen (Levenson & Jordan, 1997). Once the phenol red was removed the cells became extremely sensitive to estrogen, making them an ideal model to use when studying the effects of estrogen on cell growth.

T47D cells differ from MCF7 cells in a few ways. Several classes of proteins are expressed differently between them. In T47D cells specific proteins involved in cell growth stimulation, anti-apoptosis mechanisms, and carcinogenesis have been shown to be more strongly expressed, while in MCF7 cells proteins involved in transcriptional and apoptotic regulation are more strongly expressed (Aka & Lin, 2011). This information is important as it means the cell lines may react differently to the phytoestrogen products. The T47D cell line also has a fluorescent variation called T47D-KBluc which was developed by transfecting normal T47D cells with a reporter construct coupled to the endogenous response elements for the estrogen receptors ER $\alpha$  and ER $\beta$  (Wilson, Bobseine, & Gray Jr, 2004). When a



compound binds to the estrogen receptor of this cell line, the luciferase reporter gene is activated. Use of this cell line allows the determination of whether or not a compound binds to the estrogen receptor of the cell.

## **PCNA and Caspase**

Two proteins were used to measure the anti-proliferative effects of the phytoestrogen supplements. These proteins are PCNA and Caspase.

### **PCNA**

Proliferating Cell Nuclear Antigen (PCNA) is a protein that assists cell growth by recruiting necessary replication factors to the DNA replication complex (Moldovan, Pfander, & Jentsch, 2007). PCNA plays a number of roles in replication, including binding DNA polymerases to the DNA, binding to DNA replication related proteins, and repairing any DNA lesions created in the S phase. Without PCNA, replication could not occur, leading to no growth of the cells (Moldovan, Pfander, & Jentsch, 2007). PCNA is often used as a proliferation marker by detecting the levels of protein in the cells to indicate the amount of proliferation.

### **Caspase**

Caspases are a class of proteases involved in the apoptosis of cells (Goodsell, 2004). Once the apoptosis signal has been received by the cell, caspases act by cleaving important proteins in the cell into small pieces, deactivating them and allowing for easier degradation (Goodsell, 2004). Caspase is used as a cell apoptosis marker by detecting the amount of caspase in the cells to indicate levels of apoptosis.

## **Previous Research**

### **Jessica Caron Thesis**

The proliferative effects of an OTC phytoestrogen extract, Promensil, and 17 $\beta$ -estradiol were tested using MCF7 cells (Caron, 2007). MCF7 cells were cultured in DMEM with 10% fetal bovine serum, 584 mg/L glutamine, and 1% non-essential amino acids. After 12 hours the cells were plated in

phenol red free media. The compounds 17 $\beta$ -estradiol, genistein or Promensil extract were added to the cells 24 hours after exposure to phenol red free media ranging in concentration from below physiologic to pharmacological. A pure genistein standard was used since previous research suggested that genistein is a major constituent of Promensil. Estradiol produced a "u-shaped" curve meaning that the highest proliferative effects were found in the lowest and highest concentrations tested,  $10^{-12}$  and  $10^{-7}$  M. Genistein produced half a "u-shaped" curve with the highest growth found at  $10^{-7}$  M concentration of genistein. Growth was measured as a percentage of the control. Finally, there was an inverse relationship between cell growth and Promensil concentration. At 100% Promensil concentration the rate of cell proliferation was at its lowest, while at 0% Promensil the rate of cell proliferation was at its highest.

The estrogenic responsiveness of MCF7 cells was then tested by comparing the results of a pharmaceutical concentration of  $10^{-7}$  M for estradiol and genistein and 100% Promensil. The data showed no increase in cell proliferation for estradiol and genistein but a significantly lower rate for the Promensil. This further supports the hypothesis that Promensil has an inhibitory effect on cell growth and that genistein is not its main active constituent.

Furthermore, a marker of apoptosis was used to determine the cause for the inhibitory effects caused by the Promensil extract. A caspase-3 immunoblot was performed and bands for procaspase-3, an inactive precursor to caspase-3, were seen in samples treated with genistein and Promensil. This suggested that phytoestrogens play some role in the upregulation of the caspase pathway. (Caron, 2007)

### **Park and Patchel Paper**

The anti-proliferative properties of methanol extracts of additional commercial phytoestrogen products were tested on MCF7 cells (Park and Patchel, 2011). Three separate phytoestrogen extracts were tested: Promensil, Black Cohosh and Natrol Soy. Cell counts were performed every 24 hours for 72 hours after exposure to phytoestrogen media. After 72 hours the cells were collected immunoblotted for the protein PCNA (proliferating cell nuclear antigen). The authors predicted that lower levels of cell proliferation would be found in the cells exposed to phytoestrogens. The cell counts showed significant anti-proliferative effects after 48 hours with Natrol soy having the least anti-proliferative effect. A second

proliferation assay was conducted testing the effects at different concentrations of extract: 2%, 1%, and 0.5%. Two percent had the lowest cell counts; Promensil showed the biggest anti-proliferative effect and Natrol Soy the smallest. Results of immunoblots showed lower levels of PCNA for MCF7 cells treated with Promensil and Black Cohosh and higher levels of cell proliferation with Natrol Soy. (Park and Patchel, 2011)

## **Methods and Materials**

### **Cell Culture**

MCF7 and T47D cells were cultured in an incubator at 37° C with 5% CO<sub>2</sub>. The MCF7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with (V/V) 10% fetal bovine serum, 0.1% insulin, and 1% Penicillin and T47D cells were grown in RPMI with (V/V) 10% fetal bovine serum, 0.2% insulin, and 1% Penicillin. For each experiment the cells were grown in 24 well plates at an initial concentration of 75000 cells per well. They were initially given 24 hours to adhere to the wells and then the media was replaced with phenol red free DMEM with 10% charcoal stripped fetal bovine serum, 0.1% insulin, and 1% Penicillin. After a 24 hour period, the cells were subjected to experimental conditions.

### **High Performance Liquid Chromatography (HPLC)**

HPLC was performed using the method described in Setchell *et al* (2001) and Jessica Caron's Thesis (2007). The sample was injected in a volume of 10 µL and a flow rate of 1 mL per minutes onto a reverse phase C18 250 X 4.6 mm column. After the injection the column was washed with 10mM ammonium acetate and 0.1% trifluoroacetic acid (TFA) for two minutes. After this period, it was eluted with a gradient of 10 mM ammonium acetate, 0.1% TFA (100-50%) and acetonitrile (0-50%) for 22 minutes. It was then held isocratic until the end of the run. Absorbance at 256 nm was measured throughout the run. Four samples were collected: a peak and finger print region of both Promensil and

methanol. The fingerprint region was collected from 1.5 to 7 minutes and the peak region was collected from 21.5 to 25 minutes.

## Experimental Conditions

After the MCF7 and T47D cells were plated and the media was switched, the cells were exposed to experimental conditions. For the estrogen sensitivity assay, the cells were treated with two different concentrations of estradiol:  $10^{-9}$  and  $10^{-8}$ . The control was the same volume of ethanol that was used to add the estradiol: 0.1%. After the assay, cell counts were performed.

The next experiment utilized extracts that were made by the Patchel and Park MQP team. These were different types of phytoestrogens with methanol. These included Promensil, Natrol Soy, and Black Cohosh. These phytoestrogens were made using an extraction method, which included being ground into a fine powder, added to methanol and refluxed using a water jacketed reflux condenser. Each phytoestrogen was filtered using 0.45 micron Whatmann syringe filter and the filtered and unfiltered extracts were added to the cells. The phytoestrogens were added at a 1% concentration (V/V) and methanol was used as a control. After the assay, pictures were taken of the cells and cell counts were performed.

The final experimental condition for MCF7 cells was testing the different fractions that were collected from HPLC. The different fractions collected were the finger print region and peak from Promensil. Methanol was run through the HPLC and was collected at the same time points to be used as a control. These fractions were lyophilized overnight in 50 mL conical tubes to concentrate them and then were brought back up in 10  $\mu$ L of methanol. The 10  $\mu$ L were added to the cells so that they were at 1% of the total volume. After the assay, pictures were taken of the cells and cell counts were performed.

## **Bradford Assay**

Cells were removed from the plates by trypsinization and pelleted by centrifugation. The cell pellets were resuspended in 50ul of PBS. Total cell protein was determined using the Bradford protein assay.

## **Western blotting and Immunodetection**

Pelleted cells were resuspended, lysed, subjected to gel electrophoresis under denaturing conditions and transferred to PVDF membranes using a semi-dry transfer apparatus (Current Protocols in Molecular Biology (John Wiley & Sons, 2012)). Immunodetection was carried out using monoclonal antibodies to PCNA and caspase-3. Briefly, following transfer, membranes were blocked using 5% non-fat dry milk and then incubated overnight with the primary antibody. After washing with TBS (Tris buffered saline: 10mM Tris, pH7.4, 150 mM NaCl) and 1% Tween-20, alkaline phosphatase secondary antibody was added. Following incubation and subsequent washing, detection was accomplished using NBT/BCIP.

## **Results**

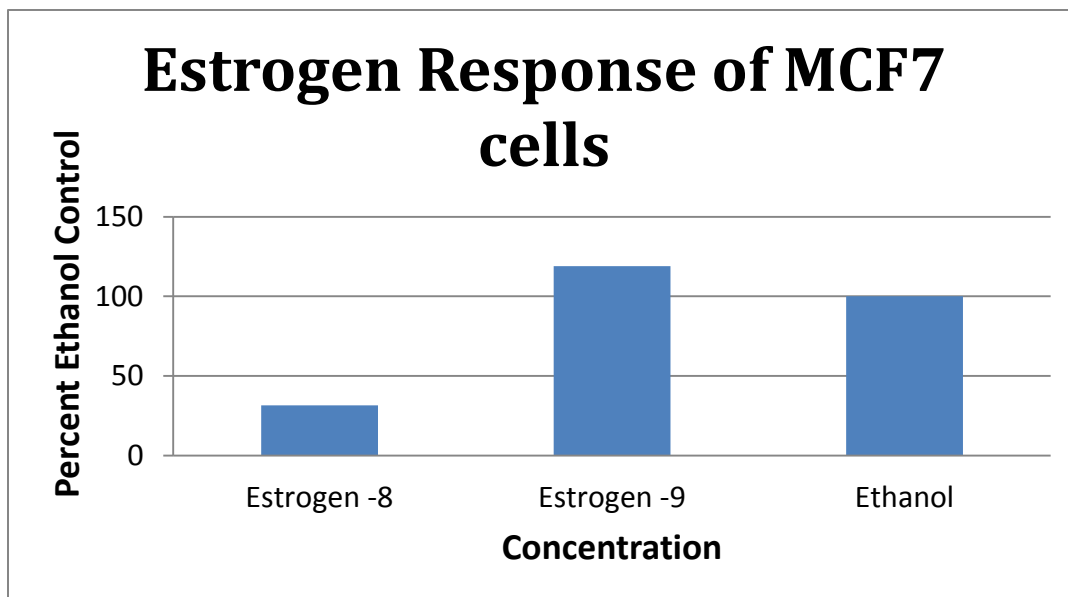
### **Phytoestrogens Background**

The three phytoestrogen used were Promensil, Black Cohosh, and Natrol Soy. The phytoestrogens, originally in pill form, were extracted and brought into to solution in 100% methanol. The extracts were produced using a reflux condenser (Patchel and Park, 2011). The phytoestrogens were still in a crude form so each was filtered using 0.45 micron Whatmann syringes. Both the filtered and unfiltered versions were used for testing on the cells.

### **Estrogen Sensitivity**

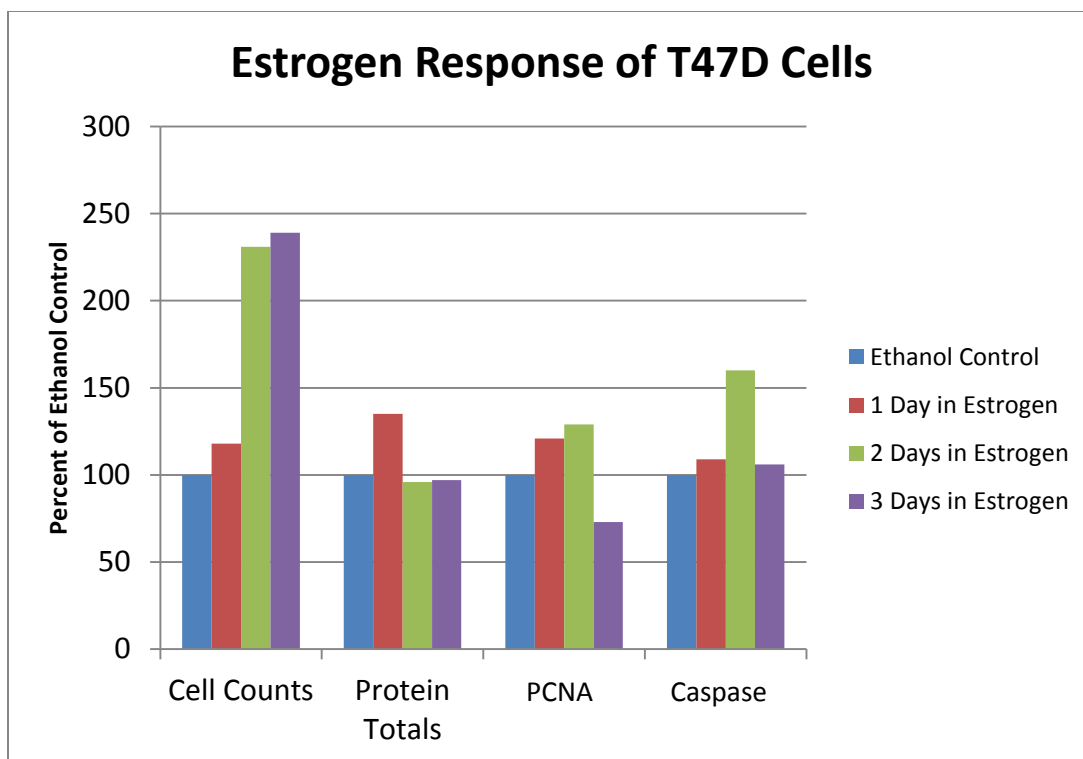
Estrogen sensitivity was tested to confirm the model system and to make sure that the estrogen concentration that was being used as a positive control is effective. The effect of estrogen on the MCF7

and T47D cell lines was tested as per the methods. Figure 4 below shows the cell counts and protein data that were performed to measure estrogen sensitivity. The measurement is a percent of the ethanol control, since the estrogen was brought into solution using ethanol. Statistical analysis was not conducted due to small sample size; data are presented as a percent of control. The cells proliferated with a concentration of  $10^{-9}$  estradiol in ethanol. Each bar represents an average of three trials.



**Figure 4: Estrogen response of MCF7 Cells:** This graph shows the cell counts that were performed to measure estrogen sensitivity. The measurement is a percent of the ethanol control. The cells proliferated with a concentration of  $10^{-9}$  estradiol in ethanol. Each bar represents an average of three trials.

For the T47D cells the estrogen sensitivity was tested in a similar manner. The T47D cells, however, were also assayed for protein content and subjected to immunodetection for caspase-3 and PCNA. This data is found in figure 5.



**Figure 5: Estrogen sensitivity of T47D cells:** Tests were run at an estrogen concentration of  $10^{-9}$ M. PCNA and caspase analysis was performed using ImageJ software on the gel pictures.

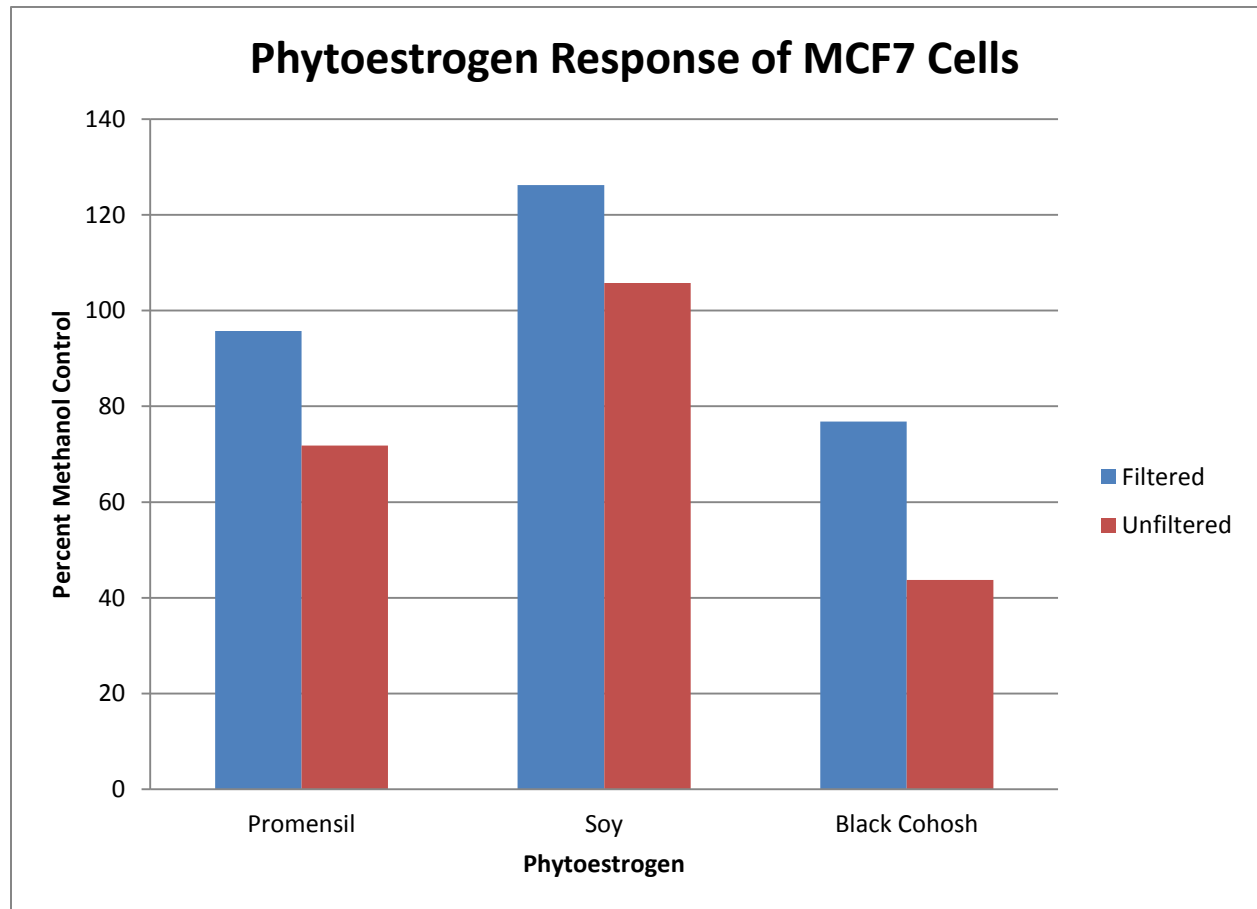
## Effect of Phytoestrogens

In the MCF7 breast cancer cell line there have been previous studies that suggest phytoestrogens play an anti-proliferative role in cell growth. In a previous MQP, specific phytoestrogens were shown to have anti-proliferative characteristics. These phytoestrogens include Promensil, Natrol Soy, and Black Cohosh.

## MCF7 and T47D Cell Proliferation Assay with Whole Extracts

The MCF7 cell proliferation assay tested the difference in effects of filtered and unfiltered phytoestrogens. This was done because the phytoestrogen extracts were filtered before being run through the HPLC. This assay was done to test if filtering affected the phytoestrogens' anti-proliferative effect. This assay tested the effect of Promensil, Natrol Soy, and Black Cohosh at a concentration of 2% in

phenol red free DMEM with 10% charcoal stripped fetal bovine serum for the MCF7 cells. The phytoestrogen response of T47D cells was done with the filtered extracts only. These assays were done with a concentration of 1% phytoestrogen extract. Total protein as well as cell counts for T47D cells, are also graphically displayed for each of the three phytoestrogens. This data is shown in figures 7, 8 and 9. The effects were measured after 72 hours in the presence of the extract. Methanol was used as a control.

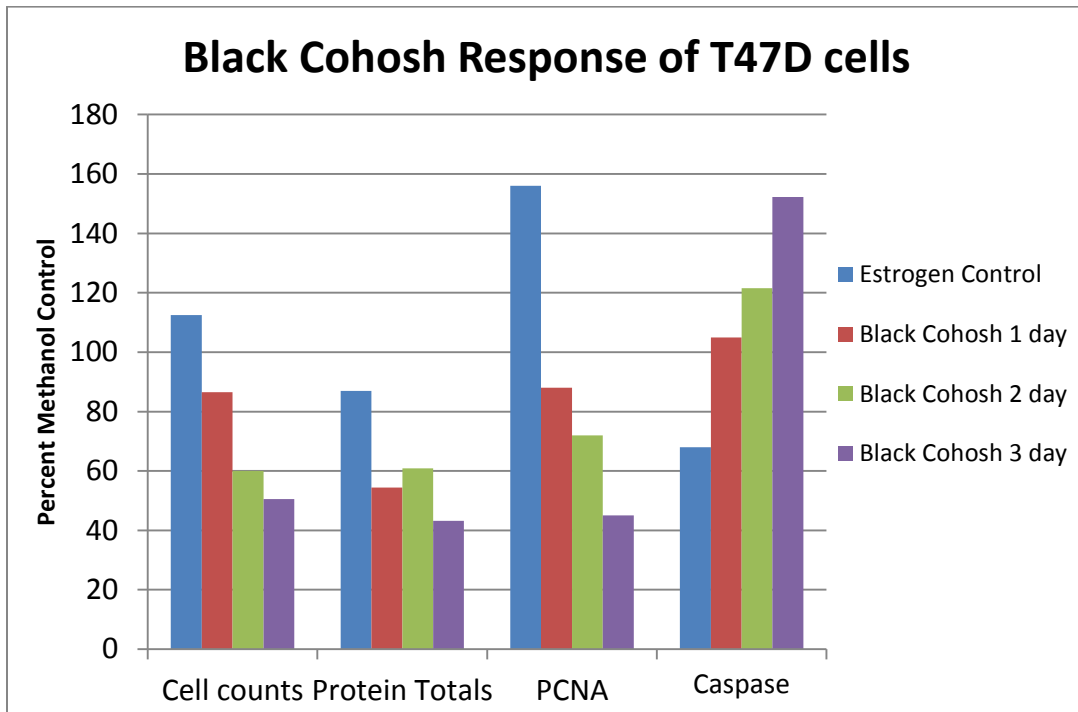


**Figure 6: Phytoestrogen response of MCF7 cells:** Two samples, one filtered and one unfiltered, for each of the phytoestrogens, Promensil, Soy, and Black Cohosh were used to examine the effect on the MCF7 cell line. The data is shown above as a percent of the methanol control. Each phytoestrogen showed a greater effect when it was not filtered.

Figure 6 shows the results of the MCF7 Proliferation assay as measured by cell counts from the wells containing the phytoestrogen extract as a percent of the methanol control. These cell counts were all performed in triplicate. After 72 hours cell counts of the MCF7 cells demonstrated an anti-proliferative effect of the unfiltered phytoestrogens by more than 10% of the ethanol control. Promensil and Black

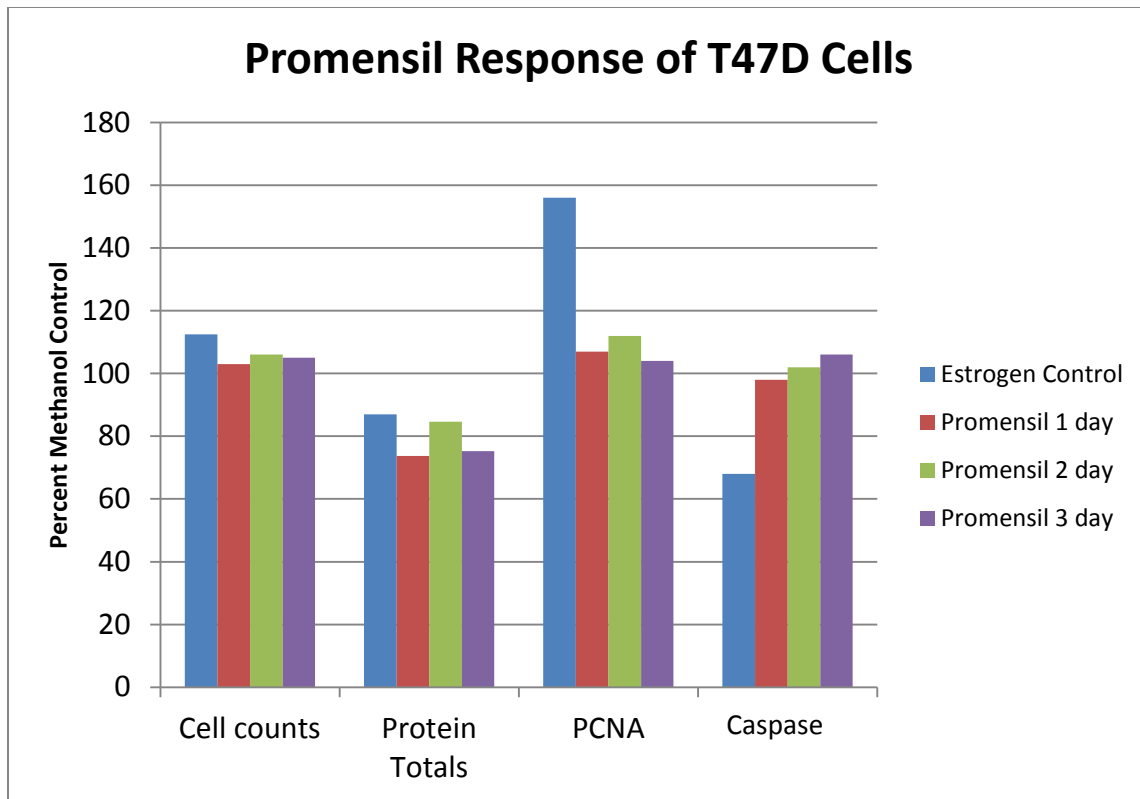


Cohosh showed greater effects than Natrol Soy, which actually showed a proliferative effect when it was filtered before applying it to the cells.



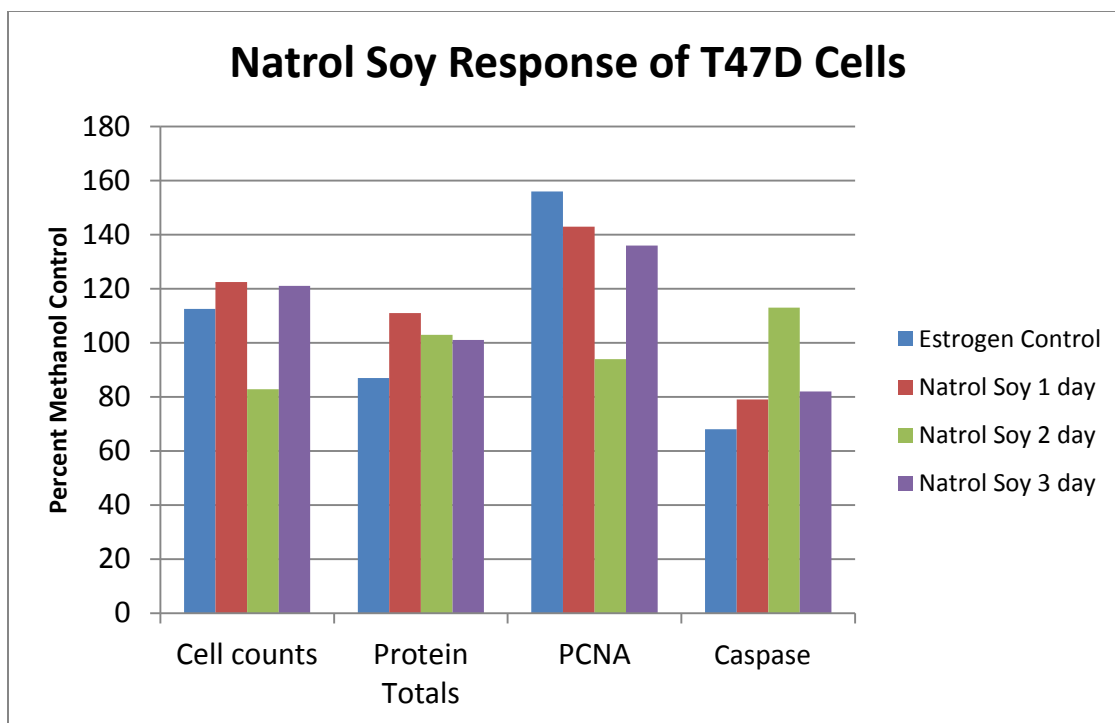
**Figure 7: Black Cohosh response of T47D cells:** Tests were run at a Black Cohosh concentration of 1% (V/V). PCNA and caspase analysis was performed using ImageJ software on the gel pictures.

Figure 7 shows the effect of Black Cohosh on the T47D cells. As the amount of time with extract added increased, the cell counts, protein totals, and PCNA levels decreased. The opposite was true for the caspase levels, which increased over time. The decreasing PCNA levels indicate less cell proliferation and the increasing caspase levels indicate a higher amount of cell death.



**Figure 8: Promensil response of T47D cells:** Tests were run at a Promensil concentration of 1% (V/V). PCNA and caspase analysis was performed using ImageJ software on the gel pictures.

Figure 8 shows the effect of Promensil on the T47D cell line. While the cell counts remained mostly stable, there is some indication of decreasing PCNA levels and increasing caspase levels which may indicate an anti-proliferative effect.



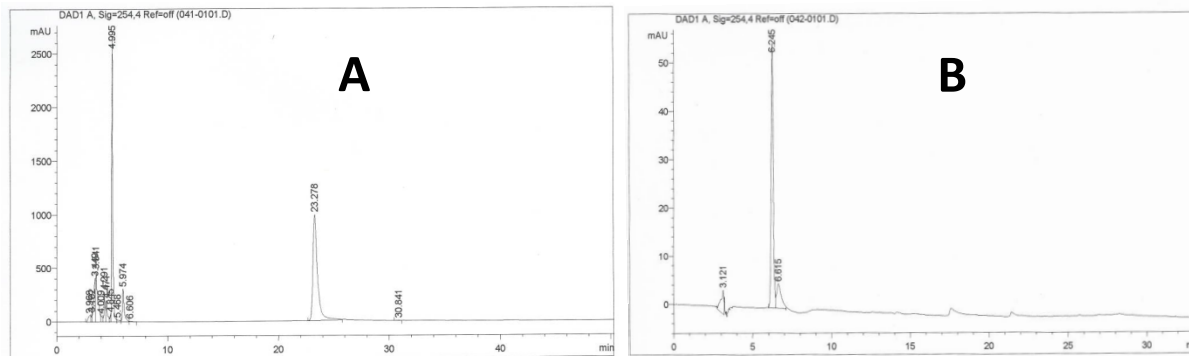
**Figure 9: Natrol Soy response of T47D cells:** Tests were run at a Natrol Soy concentration of 1% (V/V). PCNA and caspase analysis was performed using ImageJ software.

Figure 9 shows the effect of Natrol Soy on T47D cells. The day two data showing a decrease in cell counts and PCNA levels as well as an increase in caspase likely warrants further investigation. At other time points, Natrol Soy appears to have little to no anti-proliferative effect.

## HPLC

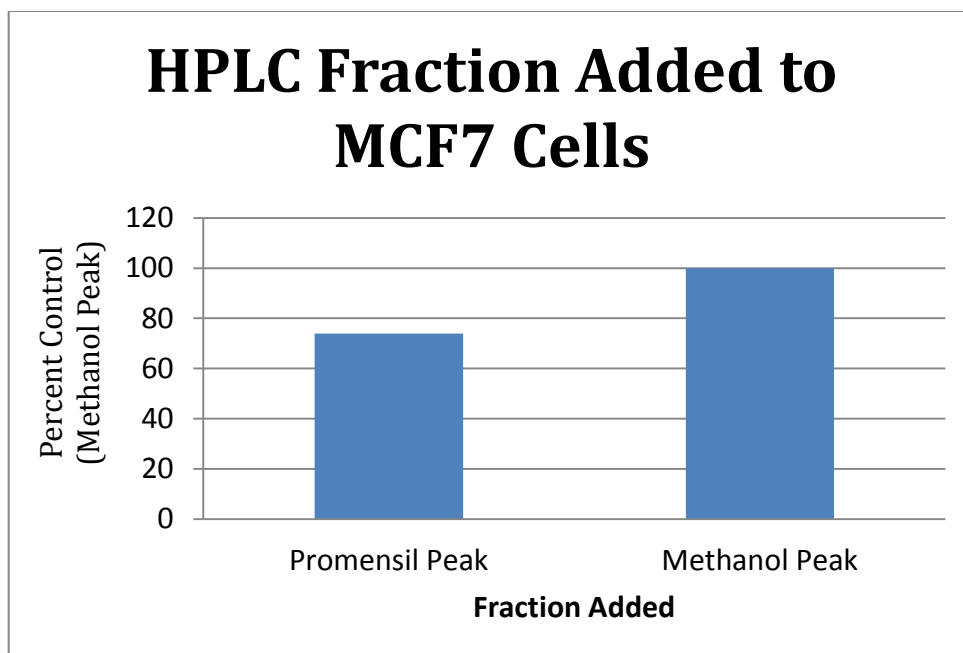
The filtered extracts of the phytoestrogens were subjected to HPLC using a C18 silica column. The HPLC was used to separate the components of the samples were separated using reverse phase chromatography to try to isolate the active fraction of the overall phytoestrogen. The samples were run through a C18 column with the solvents used were 10mM ammonium acetate, 0.1% (V/V) trifluoroacetic acid in a gradient fashion as detailed in Methods. All three of the filtered versions of the phytoestrogens were run through the HPLC machine and the chromatograms were reviewed. After the filtered Promensil was run through the HPLC there was a peak that was observed at 23 minutes. The observed peak was

collected at the time point 21.5-25 minutes to ensure that the peak was collected. As a control 100% methanol was run through the HPLC under the same conditions and collected at the same time point. These chromatograms in Figure 5 show the HPLC tracings for methanol at the top and Promensil at the bottom. The peak at 23 minutes was collected to be added to MCF7 cells. This was selected because it was the largest peak that was not in the fingerprint region. On the Promensil chromatogram, Figure 10, a fingerprint region was also observed 1.5-7 minutes and collected to test the effect on the cells as well. Methanol was collected at the same time point to be used as a control.



**Figure 10: Promensil vs. Methanol HPLC:** These chromatograms show the HPLC spectrophotometer reading of Promensil (A) and methanol (B). The peak at 23 minutes was collected to be added to MCF7 cells. This data can be seen in figure 11.

These fractions, one from Promensil and one from methanol were collected to isolate the peak of the chromatogram. Both samples were lyophilized and applied to MCF7 cells in culture as detailed in the methods. The cells were in a 24 well plate initially containing 75,000 cells per well calculated through cell counts. Figure 11 shows the cell count as a percent of control, methanol, after three days.



**Figure 11: MCF7 response to HPLC fraction:** HPLC was used to isolate a peak from Promensil in a methanol solution. The same time frame was collected from a sample of methanol as a control. These peaks were concentrated and added to the MCF7 cells.

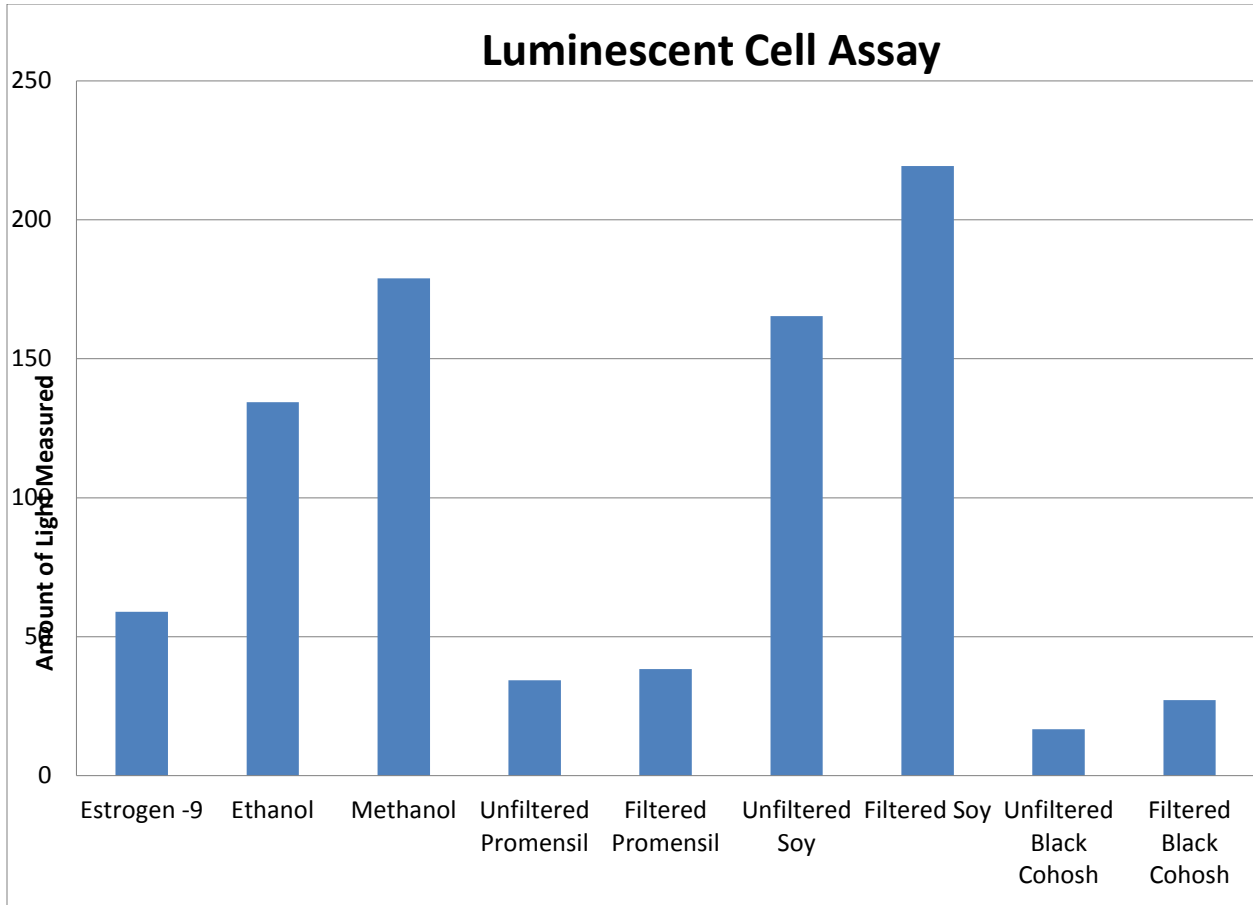
When compared to the methanol control, the peak fraction from the Promensil extract reduced the cell counts by about 25%.. This experiment was done one time with triplicate wells.

## Modified T47D-KBluc

Along with the MCF7 and T47D cell lines, a modified T47D cell line was used. The T47D-KBluc is a cell line that has been transfected with estrogen responsive (ERE) luciferase. (Pianjing , Thiantanawat, Rangkadilok, Watcharasit, Mahidol, & Satayavivad, 2011)

These cells were used in a single experiment under the same conditions as the MCF7 and T47D cell lines. They were plated on a 96 well plate and phytoestrogens were applied, along with estrogen as a positive control, ethanol as a negative control for the estrogen and methanol as a negative control for the phytoestrogens. After incubation, a luminescent cells assay was completed to determine if the phytoestrogen were acting through the estrogen receptor of the cells. The luminescent cell assay reads

and collects the amount of light emitted from each of the wells. This assay was only run once. The data collected, shown below in figure 12, was inconclusive due to the high intensity of the signal in the negative control samples, ethanol and methanol.



**Figure 12: Luminescent Cell Assay:** A fluorescent plate reader was used to collect the amount of luminescent light emitted from the cells.

This assay should be completed multiple times to create more reliable data and for conclusions to be created about whether or not the phytoestrogens are acting through the estrogen receptor.

## Conclusions

The purpose of this MQP was to validate the claim that phytoestrogens have an anti-proliferative effect on estrogen sensitive cells and to further study which specific compounds might be responsible for this effect through fractionation studies. Another objective was to determine whether or not the effects were being mediated through the estrogen receptor using the T47D KBluc cells with the ERE-luciferase reporter construct. . The anti-proliferative effects of Black Cohosh were validated on both MCF7 and T47D cell lines, as shown in figures 6 and 7. This is supported by the previous research performed with MCF7 cells (Park and Patchel, 2011). The T47D cell line showed similar results, which confirms that Black Cohosh has an anti-proliferative effect on multiple breast cancer cells lines. While there are indications that Promensil may have similar effects to Black Cohosh, the T47D data did not show enough of an effect to validate the effects of Promensil. Natrol Soy showed little to no anti-proliferative effect on either cell line and likely does not warrant any further investigation. The HPLC separation identified one major peak that appears to have an anti-proliferative effect and certainly deserves further study.

## Recommendations

There should be further experiments done to further explore the mechanism of action of phytoestrogens in reducing breast cancer cell growth. The luminescent cell assay, with T47D-KBluc cells, should be optimized so that the controls are effective. This will then show whether the phytoestrogens are acting through the estrogen receptor. If another cell line that is not estrogen sensitive is tested, then this will show whether the phytoestrogens are toxic or just having an effect on breast cancer cells. The HPLC fraction should be tested on T47D cells to further show that there is an anti-proliferative effect. Western blots should also be performed on both cell lines with the fractions added to them to measure PCNA and caspase levels. Different batches of phytoestrogen pills from the same companies should be used for HPLC in addition to

being tested on the cell lines, because different batches can have different components in them. This will be useful to compare to the HPLC runs that have already been done. Identification of additional components in the extracts and determination of their effects on cell growth may reveal specific phytoestrogens that have specific anti-proliferative effects. This may be an important way to allow women to relieve the symptoms of menopause without increasing their risk of breast cancer.



## Works Cited

- Aka, J. A., & Lin, S.-X. (2011, October 24). *Comparison of Functional Proteomic Analyses of Human Breast Cancer Cell Lines T47D and MCF7*. Retrieved April 24, 2012, from Plos One: <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0031532>
- Al-Amier, H., Eyles, S. J., & Craker, L. (2012). Evaluation of Extraction Methods for Isolation and. *Journal of Medicinally Active Plants*.
- Atkinson, C., Warren, R., Sala, E., Dowsett, M., Dunning, A., Healey, C., et al. (2004). Red-clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial. *PubMed*.
- Beral, V., & et al. (2007). Ovarian Cancer and Hormone Replacement Therapy in the Million Women Study. *Lancet*.
- Caron, J. (2007). *Comparisons of the effects of an OTC phytoestrogen extract (Promensil) and 17 $\beta$ -estradiol on the proliferation of MCF7 cells, a neoplastic breast epithelial cell line*. Worcester, MA: Worcester Polytechnic Institute .
- De Prithwish, C., & et al. (2010). Breast Cancer Incidence and Hormone Replacement Therapy in Canada. *Journal of the National Cancer Institute*.
- Glazier, G. M., & Bowman, M. A. (2001). A Review of the Evidence for the Use of Phytoestrogens as a Replacement for Traditional Estrogen Replacement Therapy. *Archives of Internal Medicine*, 1161-1172.
- Goodsell, D. (2004, August). *Caspases*. Retrieved April 25, 2012, from Protein Data Bank: <http://www.rcsb.org/pdb/101/motm.do?momID=56>
- Hansen, P. (2001, October 30). *Use of a Hemocytometer*. Retrieved December 15, 2011, from University of Florida Department of Animal Sciences: <http://www.animal.ufl.edu/hansen/protocols/hemocytometer.htm>
- John Wiley & Sons. (2012). *Current Protocols in Molecular Biology*. Hoboken.
- Levenson, A. S., & Jordan, V. C. (1997). MCF7: The First Hormone-responsive Breast Cancer Cell Line. *AACR: Cancer Research*, 3071-3078.
- Mayo Clinic Staff. (2010, Feb. 19). *Hormone Therapy: Is It Right for You?* Retrieved April 20, 2012, from Mayo Clinic: Mayo Foundation for Medical Education and Research: <http://www.mayoclinic.com/health/hormone-therapy/WO00046>
- Moldovan, G., Pfander, B., & Jentsch, S. (2007). PCNA, the maestro of the replication fork. *Cell*, 665-679.

- Nordgyist, C. (2009, June 29). *What Is Menopause? What Are The Symptoms Of Menopause?* Retrieved April 20, 2012, from Medical News Today:  
<http://www.medicalnewstoday.com/articles/155651.php>
- Pianjing , P., Thiantanawat, A., Rangkadilok, N., Watcharasit, P., Mahidol, C., & Satayavivad, J. (2011). Estrogenic Activities of Sesame Lignans and Their Metabolites. *Journal of Agricultural and Food Chamistry Article*, 59, 212-221.
- PubMed. (1997). Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet*, 1484.
- Rice, S., & Whitehead, S. A. (2006). Phytoestrogens and breast cancer - promoters or protectors. *Endocrine Related Cancer*, 995-1015.
- Romieu, I., & et al. (2010). Postmenopausal Hormone Terapy and Asthma Onset in the E3N Cohort. *Thorax*.
- Setchel, K. e. (2010). Bioavailability of Pure Isoflavones in Healthy Humans and Analysis of Commercial Soy Isoflavone Supplements. *The Journal of Nutrition*, 1362S-1375S.
- Setchell, K. (1998). Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *The American Journal of Clinical Nutrition*, 13335-13465.
- Thermo Scientific. (2012). *Coomassie Plus (Bradford) Protein Assay*. Retrieved April 24, 2012, from Pierce Protein Research Products: <http://www.piercenet.com/browse.cfm?fldID=02020104>
- Warren, B. (2011, Oct. 20). *Phytoestrogen and Breast Cancer*. Retrieved April 20, 2012, from Cornell University: <http://envirocancer.cornell.edu/factsheet/diet/fs1.phyto.cfm>
- Whitefleet-Smith, J. L. (n.d.). *BB 3511 Nerve and Muscle Physiology 2011-12 Lab Manual*.
- Wilson, V. S., Bobseine, K., & Gray Jr, E. L. (2004). Development and Characterization of a Cell Line That Stably Expresses and Estrogen-Responsive Luciferase Reporter for the Detection of Estrogen Receptor Agonist and Antagonists. *Toxicological Sciences*, 69-77.