The Effects of Various Plant Extracts On the Viability of Prostate Cancer Cells

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ABSTRACT

Prostate cancer is the second most common type of cancer in men, after skin cancer, and one of the leading causes of death in men. Prostate cancer can be treated through a variety of ways such as cryotherapy and chemotherapy, as well as natural products (NPs). NP screens involve a library of extracts from natural sources and may not be compatible with traditional target-based cell assays, instead measured through their phenotypic response. NP-based drug research begins with biological screenings of unprocessed extracts to eventually fractionate active ingredients and study organisms' responses to them.The purpose of this investigation is to evaluate the viability of prostate cancer cells when treated with a wide range of plant extracts, which are NPs. Relative fluorescence units (RFU) and luminescence units (RLU) levels through dead and live cell assays from each well of cells treated with extracts are compared to the positive and negative controls (2% DMSO Vehicle and Digitonin). When measured, a higher luminescence emitted from treated PC-3 cells indicated more live cells, while a higher fluorescence emission indicated more dead cells. Looking at the RLU and RFU values, the research showed that 11 of 108 extracts tested followed the trends of a decreased RLU value from the live assay vehicle control and an increased RFU value from the dead assay vehicle control. The 11 extracts were also found to contain anticancer properties and the ability to slow down cancer cell growth or kill cancer cells through further research.

INTRODUCTION

Prostate cancer is the second most common type of cancer in men after skin cancer, and one of the leading causes of death in men. It is probable that 1 in every 6 men will develop this cancer in their lifetime; however, the likelihood of being diagnosed with prostate cancer grows exponentially with age, with a high risk after age 40 (Mazhar & Waxman, 2002). A prostate is a small, walnut-shaped gland part of a male's reproductive system. The role of the prostate is to make semen, and it is located in front of the rectum but below the bladder, allowing it to wrap around the urethra, which carries urine and semen out of the male's body. A man's prostate increases in size as a person ages, which can cause issues if the prostate grows disproportionately.

Prostate cancer is classified as adenocarcinoma, meaning that cancer is located in the glands that line important organs. Adenocarcinoma develops first in the glandular epithelial cells. Because the main function of these cells is to secrete materials and digestive juices, excessive and uncontrollable growth of them causes tumors ("Adenocarcinoma Cancers," 2021). Prostate cancer has a low heritability rate, and multiple environmental factors have also been shown to be associated with developing prostate cancer, such as high exposure to radiation, heavy intake of saturated fat, and low levels of dietary supplements and vitamins (Mazhar & Waxman).

Prostate cancer can be treated in a variety of ways. Doctors may advise against starting treatment for the disease immediately if they believe the cancer will not spread, a process called expectant management. Prostate biopsies and prostate-specific antigen (PSA) testing are routinely performed as part of active surveillance, which involves continuously monitoring prostate cancer and treating it only when symptoms are present (Mazhar & Waxman). A prostatectomy is a viable treatment option in which the prostate is surgically removed using robotic technology. Radiation therapy is another treatment which uses high radiation to destroy the tumor by directing the energy at the tumor with a linear accelerator (Mazhar & Waxman). Other treatments include cryotherapy–a process of freezing and destroying cancer cells using a special probe–and chemotherapy, which utilizes certain medications to reduce or eradicate cancer. Hormone therapy is another possible treatment that blocks cancer cells from receiving the hormones they need to grow as well (Centers for Disease Control and Prevention, 2019).

Natural products (NPs) are known for their structural complexity and scaffold diversity, which are variations in the products' different ring systems and frameworks. With their high molecular mass and molecular rigidity, NPs have evolved to be structurally optimized to serve particular biological functions, including endogenous defense mechanisms and interactions with their environment. Active NPs are often biologically screened from unprocessed extracts; an extract library from natural sources may not be compatible with traditional target-based cell assays, in which results are characterized by phenotypic measures of response (Atanasov et al., 2021).

Natural products are becoming increasingly used in pharmaceutical development because of the immense variety of functionally relevant secondary metabolites of microbial and plant species (Ngo et al., 2013). Metabolomics allows researchers to simultaneously study the metabolites within cells, biofluids, tissues, and organisms in response to NPs (Atanasov et al., 2021). NPs are being used for cancer treatment as many compounds found in plants have anticancer properties. This includes polyphenols, which have the ability to induce apoptosis and DNA fragmentation in cancer cells. Flavonoids, a polyphenolic compound, constitute a large number of plant secondary metabolites and show many anticancer properties. Flavonoids isolated from various plant species demonstrate high cytotoxicity on lung cancer cells and high free radical scavenging activity (Greenwell & Rahman, 2015).

The purpose of this investigation is to identify and evaluate the viability of prostate cancer cells when treated with a wide range of plant extracts, which are considered NPs.

METHODS

Cell Culturing of PC-3 Cells: PC-3 cells from the American Type Culture Collection were cultured, and a commercial plant extract library was purchased. The cells were cultured and maintained in F-12K Nutrient Mixture (Kaighn's Modification) containing L-glutamine supplemented with 10% FBS. Cultures were maintained in a humidified incubator at 37ºC in an atmosphere with 5% CO2 in air. The PC-3 cell culture flasks were removed from the incubator and, when examined under a microscope, were subconfluent. The media was aspirated out of the flasks and 5 mL of Trypsin/EDTA solution was added to rinse the cells quickly. The solution was then aspirated. 2 mL more of solution was added, and the flasks returned to the incubator for about 3-5 minutes. 5 mL of media was added to each of the flasks, and 3 mL of the subsequent mixture was added to the final cell mix of a total of 10 mL. 1 mL of the cells were removed and placed into a cell counter to document cell density and viability.

Determining Vehicle (DMSO) Concentration: 2% DMSO was chosen as the vehicle because it was a concentration that allowed for minimal cell damage and allowed the extracts to be tested at a high enough concentration to allow for a possible effect on the cell viability.

Preparation of Assays: Cell-based multi-well plate assays were created to assess various plant extracts to see the differences in viability of PC-3 cells. Live cells were measured using the CellTiter-Glo 2.0 assay measuring ATP levels, while dead cells will be measured using the CellTox Green Cytotoxicity assay measuring DNA content released by the dead cells. For the live assay, the vehicle (DMSO) is established as the positive control and the Digitonin mixture is the negative control; for the dead cell assays, the control roles were flipped. About 100 µL of the Live Assay reagent are added to one 96-well plate. For the dead assay, 20 µL of the CellTox Green Cytotoxicity reagent was added to the assay buffer, made into a homogenous solution, and 100 µL of the solution were pipetted into each well of the 96 wells of the second plate. On day 1, cells were harvested with Trypsin/EDTA solution and media without FBS. For each assay, 90 µL of the PC-3 cell mixture at a viable density was micropipetted into all wells on the 96-well plate except column 12, which contained only 90 µL/well of media. Cells were cultured overnight. On day 2, the cells were treated with control compounds and extracts prepared in growth media containing 10% FBS. On day 4 and 5, the plates were analyzed using the live or dead assay kits.

Plating Extracts: A total of 108 extracts were tested. PC-3 cells were separated into six different 96-well plates. Three plates were used for the live cell assay kit and three for the dead assay to ensure replication. A solution of 80 µL of media and 20 µL of extract per extract. 10 µL of each plant extract were plated in four adjacent wells, as a way of minimizing error, totaling 18 plant extracts per 96-well plate. Column 1 in Figure 1 contained a Digitonin mixture consisting of a final concentration of 20µg/ml, as one of the controls. The two rightmost columns contained the vehicle solution, consisting of a final concentration of 2% DMSO, as the vehicle and background control; however, Column 12 contained no cells (background control) while Column 11 contained PC-3 cells (vehicle control).

RESULTS

The effects of the plant extracts tested after treatment at 40 micrograms/ml on the PC-3 cells after culturing for 48 and 72 hours will be compared to the positive and negative controls on the assay plate. From the 108 extracts tested, eleven extracts were shown to either slow prostate cancer cell growth to enter apoptosis or kill the cells entirely. These extracts were Bunge Auriculate Root, Castor Bean, Rosa Roxburghii Root, Sweet Potato Root, Melastoma Dedocandrum Herb, Phellodendron Amurense Bark, Gastrodia Rhizome, Santalum Album, Isodon Serra, Coriander Root, Cyperus Rotundus, and Feverfew. Looking at the properties and compounds present in each extract through previously conducted research in more depth, the findings support these extracts' effects on prostate cancer cells.

48-Hour Treatment

The live assay measuring luminescence and ATP levels has a high relative luminescence unit (RLU) to evaluate cell viability and cytotoxic effects. As seen in Figure 3, the 48-hour treatment of prostate cancer cells with the live assay shows a high RLU for the Live Vehicle control, indicating it had the highest prostate cancer cell viability. Looking across the graph, ten of the eleven extracts showed a lower RLU with the live assay. Bunge Auriculate Root showed the most significant decrease in relative luminescence, resulting in less than an average of 2,000 relative luminescence units compared to the live vehicle 2% DMSO control of ~44,000; this was suspected due to its many anticancer properties. Gastrodia Rhizome was the next extract that had the most decreased RLU after Bunge Auriculate Roots with an average of 24716.25 RLUs, almost half of the positive vehicle control. Coriander Root showed an increase in RLUs in the live assay, which was assumed to increase cell growth. In addition, when looking at the dead assay, Coriander Root showed no increase in RFUs compared to the live vehicle control. It was suspected that rather than killing all the cells, Coriander Roots only slowed the growth of the prostate cancer cells to allow them to enter apoptosis, although growth did not cease in all. Therefore it had an increasing RLU but still showed a similar RFU to the live vehicle solution in the dead assay as the cells died after apoptosis. 11 of the 12 extracts were repeated from the original screening plates; however, coriander was not repeated but could be investigated through further research.

Due to dead cells exhibiting a higher fluorescence compared to living cells, Digitonin was utilized as the positive control to measure cell death. The dead cell assay was expected to have inverse results of the live cell assay, as represented in Figure 4. The relative fluorescence units (RFU) of the live vehicle DMSO was lower as seen in the graph, while the Digitonin had the highest fluorescence with an average of 1.309 x 10^7 RFUs, as it killed all of its cells and therefore the positive control. Once again, Bunge Auriculate Root extract was shown to have the highest RFU values from all of the extracts at 9278390.25 RFUs; this was expected due to its significantly lower luminescence in the live assay in comparison to the other 10 of the extracts. Additionally, Gastrodia Rhizome had the next highest RFU showing its capability of killing cells, averaging 5767448.75 RLUs.

72-Hour Treatment

As seen in Figure 5, the overall RLU for the live vehicle solution in the live assay was around 50,000 RLUs. Ten of the twelve extracts showed a significant decrease in RLU versus the vehicle. After 72 hours, the Bunge Auriculate Root extract had the least relative luminescence, at an average of 9278390.25 RLUs. Gastrodia Rhizome extract had the second lowest relative luminescence, at about 5767448.75 RLUs. As in the 48-hour treatment, the luminescence of Coriander Root and Isodon Serra extracts increased, as many of the cells entered apoptosis. As the DMSO concentration increased, the number of RLUs steadily decreased.
In the 72-hour treatment of PC-3 Cells for the dead assay, according to Figure 6, the Bunge Auriculate Root surpassed its numerical RFU value compared with the Digitonin positive control. The Bunge Root killed all of its cells, with an RFU value of around 1.1x10^7, while Digitonin remained at an RFU of approximately 1.0x10^7.

Subtracting the Background

Row 12 of all graphs contained no cells as a background for the RLU and RFU values. This accounts for extraneous variables, such as cell culture media, temperature, buffering capacity, and the luminescence and fluorescence of only the wells. This relatively small value was subtracted from the values for the rest of the wells on each plate to obtain the true RLU and RFU values.

Extract Properties

Each extract was found to contain specific possible anticancer properties as well as others that contributed to their ability to slow down prostate cancer cell growth or kill them off completely.

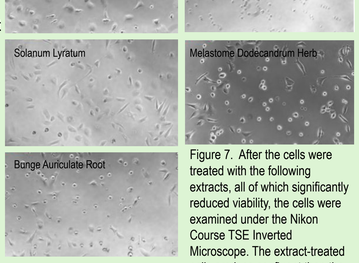

Figure 7. After the cells were treated with the following extracts, all of which significantly reduced viability, the cells were examined under the Nikon Course TSE Inverted Microscope. The extract-treated cells are less confluent than the vehicle-treated cells.

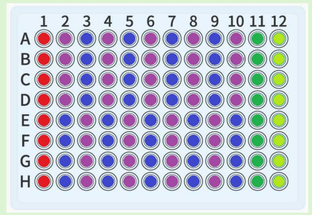

Figure 1. This is a visual representation of the 96-well plate and its contents. Column 1 contained the Digitonin control. Columns 11 and 12 contained the vehicle 2% DMSO control. Columns 2-10 contained 10 µL of various plant extracts, where one extract was plated in four wells to ensure repetition.

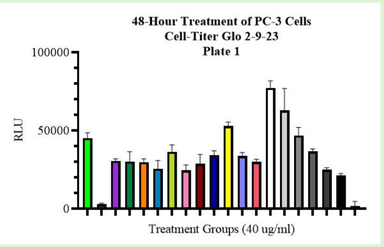

Figure 3. The Live Assay for 48-Hour Plant Extract Treatment graph. Relative Luminescence Units are being measured in this graph as an indicator of live cells present per extract.

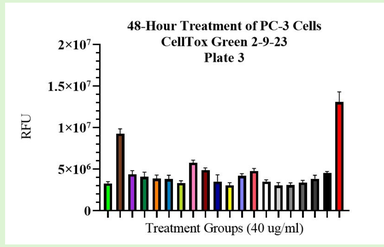

Figure 4. The Dead Assay for 48-Hour Plant Extract Treatment graph. Relative Fluorescence Units are being measured in this graph as an indicator of dead cells present per extract.

Figure 2. A key for the plant extracts and DMSO concentrations in the next graphs

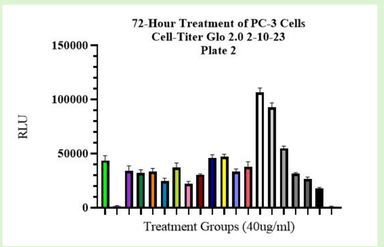

Figure 5. The Live Assay for 72-Hour Plant Extract Treatment graph. Relative Luminescence Units are being measured in this graph, and differences from the 48-hour live assay graph results are being observed.

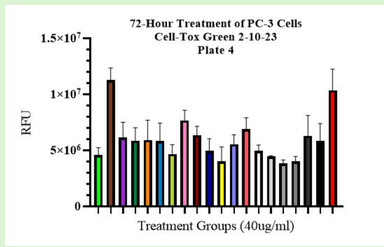

Figure 6. The Dead Assay for 72-Hour Plant Extract Treatment graph. Relative Fluorescence Units are being measured in this graph, and differences from the 48-hour dead assay graph results are being observed.

CONCLUSIONS

The research conducted determined the possibilities of utilizing plant extracts and their properties of these extracts to find potential cancer cures. Although this research did not specifically determine the cure for cancer, it helped gain insight into the overall family of plants and the specific properties of each family that could be further investigated in a research lab.

Using these same methods but conducting further steps, more analysis and conclusions can be determined in terms of the specific properties. Although a few possible extracts to use to synthesize cancer treatments were identified and observed, specific compounds with anticancer properties could be extracted from these extracts. Through thin-layer chromatography, extracts could be plated on chromatography plates. When placed in a compatible solvent, each compound would separate up the plate, allowing for its proper identification. Through further research, each compound's properties could be researched to identify its specific effects on an organism's body. Lab testing and synthesizing possible cures incorporating these compounds could be further looked into as well.

If we had more time to continue with this experiment, we would further test the effects of various plant extracts on non tumor cells. We would also test the extracts at a higher concentration than 40 micrograms/mL and different concentrations of media/serum. One possible source of error is using a relatively high concentration of DMSO to accommodate the low extract concentration. This may have resulted in greater cell death for the cells treated with the vehicle, as DMSO may have compromised the membranes.

The following compounds and properties were found in each of the extracts that showed anticancer or antitumor related abilities:

Bunge Auriculate Root: Contains a steroidal glycoside named C-21 which exerted anticancer activity through cell proliferation and stimulating apoptosis in cells. Additionally, this root possesses antitumor properties where it is able to inhibit the proliferation of cells from inducing apoptosis and regular cell linings. This shows this root's strong characteristics as seen in the above Figures 4 and 6 in killing cancer cells (Peng et al., 2011), (Norton, 2011).

Castor Bean: As the castor bean significantly decreased RLUs and increased RFUs, Ricin, a chemical in castor beans, can penetrate into the cell through its affinity to galactose sugars on the cell surface, inducing apoptosis and cell death. Ricin has been reported to possess some anticancer properties when treating various diseases (Loan et al., 2019), (Polito et al., 2019).

*Rosa Roxburghii Root: T*his root regulates signal pathways related to cancer, such as tumor necrosis factor (TNF) and mitogen-activated protein kinase (MAPK). It has anti-tumor properties and promotes apoptosis. Its fruit also contains antioxidant phytochemicals like flavonoids and terpenoids that may prevent cancer by neutralizing harmful effects of free radicals (Tang et al., 2021), (Wu et al., 2020)

Sweet Potato Root: Anthocyanins have been found to slow the growth of types of cancer cells in assays, including breast, and gastric cancers (Sugata et al., 2015)

Melastoma Dedocandrum Herb: Methanol from this herb has been found to inhibit the growth of breast cancer cell (MCF-7) lines in vitro (Roslen et al., 2014).

Phellodendron Amurense Bark: This extract has been found to reduce the progression of prostate tumors and the development of high-grade prostatic intraepithelial neoplasia in TRAMP mice (Ghosh et al., 2010), (Swanson et al., 2014)

Gastrodia Rhizome: Gastrodine, found in the rhizome of Gastrodia Elata Blume, is the primary bioactive constituent of the Gastrodia Rhizome. Gastrodin modulates neurotransmitters, regulates mitochondrial cascades, suppresses microglial activation, and has antioxidative and anti-inflammatory properties (Liu et al., 2018).

Santalum Album: α-santalol, a sesquiterpene isolated from this plant, has been found to induce cell-cycle arrest and apoptosis in melanoma cells. In PC-3 models, α-santalol inhibited angiogenesis and the growth of prostate tumors, while it had a less toxic effect on normal cells and breast cancer cells (Santha & Dwivedi, 2015).

Isodon Serra: Diterpenoids found in the plant have had known anti-tumor and anti-inflammatory effects. Nodosin, an ent-kaurene diterpenoid isolated from the plant, effectively inhibited the growth of human colorectal cancer (HCT116) cells. It inhibited overactivated transcriptional activity and induced cell cycle arrest and apoptosis afterwards (Bae et al., 2020), (Wan et al., 2017).

Coriander Root (Cilantro): Coriander extract has been tested on hepatocellular carcinoma (liver cancer) cell lines and mouse melanoma cell lines; these cells' abilities were significantly impaired by the extract. Studies show that mice fed with a diet containing coriander also possessed a smaller number of metastatic regions, suggesting that coriander may have the ability to suppress cancer cell migration and invasion (Huang et al., 2020).

Cyperus Rotundus: This extract contains ethanol which, through research, showed an anticancer effect on triple-negative breast cancer cells (TNBC). Studies found that ethanol inhibited the proliferation and induced apoptosis in TNBC in a dose-dependent manner (Wang et al., 2019).

Feverfew: This plant contains several bioactive ingredients called sesquiterpene lactones which has anti-migraine, anti-tumor, and anti-inflammatory properties. Parthenolide has been found to inhibit the growth of various breast cancer and cervical cancer cell lines (Wu et al., 2006)

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ACKNOWLEDGEMENTS

We would like to acknowledge our mentor, Mark Wolfe, principal scientist at Zoetis Inc., for assisting us and providing us with immense guidance, time, and resources to perform this research complete our project. Additionally, we would like to thank Ms. Rebecca Joyce, our research director at the Kalamazoo Area Mathematics and Science Center, for coordinating this research experience and providing connections throughout the process.