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Isolation and Characterization of Active Elderberry Fractions that Inhibit Melanoma Growth in vitro and in vivo

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ISOLATION AND CHARACTERIZATION OF ACTIVE ELDERBERRY FRACTIONS THAT INHIBIT MELANOMA GROWTH IN VITRO AND IN VIVO

A Thesis

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Dedicated to my parents, Pam and Walt Okihiro, for their unwavering love and support.
I could not have done this without you.

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ABSTRACT

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The incidence rates of melanoma continue to rise annually despite recent progression in cancer treatments. Cancer is the most prevalent amongst elderly individuals, where immunosenescence has compromised some immune function, and therefore decreased certain tumor detection abilities. Current tumor removal strategies include radiation, chemotherapy and surgical excision: treatments that aim to lower cancer cells, but may also affect normal cells in the process. In the case of chemotherapy, which targets and kills rapidly dividing cells, many immune cells are lowered as a side effect, leaving many patients immune-suppressed and more susceptible to infection. There is a need for naturopathic treatments capable of decreasing tumor cell proliferation without compromising the body's normal immune function. Extracts from elderberry (*Sambucus nigra*) may be able to satisfy this need. Previous reports suggest that phytochemicals, such as the ones present in elderberry, may stimulate the immune response by secretion of cytokines, provide antioxidant protection to prevent cellular damage, and inhibit tumor growth directly.

Our primary goal was to separate the active components of elderberry and assess their inhibitory effects on the growth of multiple cancerous and transformed cell lines, as well as characterize their effects on stimulation of T lymphocyte proliferation and IL-2 secretion *in vitro*. Murine melanoma model experiments were also performed with crude elderberry and elderberry fractions to analyze the tumor-suppressive activity of elderberry treatments *in vivo.* Spleen cell proliferation and *in vivo* experiments were also performed with different aged groups of mice to uncover the tumor –inhibiting and immune-inducing effects of elderberry and active elderberry fractions on aged mice. Active elderberry fractions were then preliminarily identified.

All separated elderberry fractions were able to significantly suppress the growth of B16-F10 murine melanoma and SH-SY5Y human neuroblastoma cells *in vitro*. Several separated fractions also inhibited growth of a human melanoma cell line, MeWo, and a transformed non-cancerous line, CHO-K1. When incubated with concanavalin A (Con A, a known mitogen) and spleen cells from a middle aged and old mouse, separated fractions of elderberry did not increase proliferation above the positive control (cells incubated with con A only) , however, they induced a larger proliferation response in the older mouse spleen cells. Three active fractions induced secretion of IL-2 from spleen cells above the positive control. In general, mice induced to produce tumors developed smaller, localized tumors when treated with crude elderberry compared to mice treated with water, whose tumors were larger and metastatic. The active elderberry fractions were too potent to be successfully implemented in an *in vivo* experiment, and need to be diluted for future mouse model experiments. Of the four primary anthocyanins in elderberry, cyanidin 3-sambubioside and cyanidin 3-glucoside were identified as the

major tumor-inhibiting, immune-inducing components in different active fractions separated from elderberry.

The positive benefits of active fractions on tumor suppression and potentially on modulation of immune-inducing mechanisms provide further support for the use of bioactive phytochemicals in preventative cancer treatment.

INTRODUCTION

Melanoma

Cancer is a disease characterized by the uncontrolled growth and decreased regulation of cell function, resulting in abnormal and atypical cell behavior. Often, these abnormal cells invade adjacent tissues or even migrate to other organs in a process known as metastasis, which can greatly increase the severity of the disease. In 2010, it was predicted that 1,500 individuals in the United States would die each day from cancerous diseases. Currently, cancer claims the second highest number of lives in America each year, exceeded only by heart disease. However, the trend in comparably developed countries, such as England and Canada, suggest that cancer will soon overtake heart disease as the number one cause of fatalities in the United States. The lifetime risk of developing cancer is slightly less than 50% in men, and slightly above 1 in 3 in women. Cancer is most often diagnosed in individuals who are middle aged or older. Approximately 77% of cancers are found in individuals aged 55 or older (American Cancer Society, 2013). The natural process of aging brings about changes to the immune system known as immunosenescence: a catch-all term describing the general decline of immune efficiency that correlates with maturation, which makes elderly people an at-risk population for many illnesses, including cancer. Genetically, cancer arises from DNA mutations that cause malfunction in important cell growth, division and modulation

processes. Skin, being the body's largest organ, has a high incidence rate of cancer. Basal cell and squamous cell cancers (nonmelanoma cancers) make up the majority of skin cancer diagnoses; an estimated 3.5 million cases were diagnosed in the United States in 2006. Basal cell and squamous cell cancers are often highly curable and do not account for many cancer fatalities, especially when detected in early stages of the disease. Melanoma (or cutaneous melanoma, or malignant melanoma) is far less common, however it is the skin cancer type that claims the most lives compared to all other forms of skin cancer. It is estimated that melanoma causes 71-80% of skin cancer deaths (Brozyna et al., 2007). Melanoma begins in the melanin pigment forming cells (melanocytes) found in the deepest layer of the epidermis. The normal function of melanocytes is to increase melanin production in a protective response against ultra violet (UV) radiation. Melanin is also responsible for skin color phenotype. Faulty repair of DNA damage in melanocytes ultimately leads to aberrant cell function, which can lead to transformation of cells into cancerous ones. According to the American Cancer Society, incidence rates of melanoma have been increasing over the past 30 years and mortality due to melanoma increases approximately 0.4% annually (Linos et. al, 2009). In 2000, the lifetime risk of developing malignant melanoma was 1 in 74, and the trend has been steadily increasing since 1935 (Rigel & Carucci, 2000) despite advances in cancer treatments and declining incidence rates of other cancers.

Etiology of Melanoma Development

Certain induction agents, such as chemical carcinogens, viruses, and radiation, have been shown to increase cancer risk. The most well known risk for melanoma

development is chronic UV radiation exposure. Substantial buildup of reactive oxygen species (ROS) consequent of UV radiation can cause oxidative stress in active melanocytes, increasing creation of free radicals capable of damaging DNA and increasing the likelihood of a DNA-repair error mutation, which may lead to aberrant cell function. The DNA mutations consequent of UV exposure in spontaneous melanoma cases can cause mutations in the important cell cycle tumor suppressor protein p53 and proteins from the Cyclin-dependent kinase inhibitor 2A gene (CDKN2A), a gene important in familial melanoma and some sporadic melanoma cases as well. The p53 protein, encoded by the TP53 gene on the short arm of chromosome 17, has a variety of normal functions, including initiation of apoptosis (programmed cell death) and induction of DNA repair in genetically damaged cells. It is one of the key proteins in a cell that maintains genomic stability. In melanoma, abnormal p53 function causes the melanocyte to proceed through a cell cycle 'check-point', even if DNA has been damaged, resulting in survival of the genetically abnormal cell. The CDKN2A gene (Cyclin-dependent kinase inhibitor 2A) is located on the short arm of chromosome 9 and has two reading frames, producing proteins $p16^{INK4}$ and p14ARF. Both proteins function to inhibit cyclin-dependent kinases, enzymes that promote cellular growth and progression within the cell cycle by binding to their respective cyclin counterparts. $P16^{INK4}$ inhibits cyclin-dependent kinase 4 by causing a conformational change to the kinases' active site, thus decreasing its phosphorylating ability and overall function. P14ARF functions by inhibiting murine double minute (MDM2), a negative regulator of p53, thus freeing p53 to maintain cellular integrity of the melanocyte (Brozyna et al., 2007). Therefore mutation in the CDKN2A gene increases the risk of improper cell cycle regulation that may lead to cancer.

Melanoma can also be the result of genetic disposition. Approximately 10% of melanoma cases are familial (Tung, 2011). A rare genetic disorder (autosomal recessive) called xeroderma pigmentosum (XP) affects an individual's ability to repair damaged DNA consequent of UV exposure, greatly increasing lifetime likelihood of melanoma development. Approximately 90% of XP patients have mutations in p53, which greatly increases the risk of melanoma, as well as other skin cancers (Brozyna, et al., 2007). Many familial melanoma cases involve mutations in p16, the tumor suppressor gene essential in cell-cycle arrest encoded by the CDKN2A gene.

Over 50% of melanomas can be traced back to a DNA mutation in the V-raf murine sarcoma virus oncogene homolog B1 (BRAF) gene, located on the long arm of chromosome 7 in humans. A common mutation in this gene protein is the point mutation of valine to glutamine at amino acid codon 600 ($BRAF^{V600E}$). This mutation has been found in other cancers (such as papillary thyroid carcinomas); however it is the most common in melanoma. In normal melanocytes, BRAF functions as a modulator of the Mek/Erk pathway. The Mek/Erk pathway begins when a ligand binds a cellular receptor, activating Ras protein, which creates a cascade of phosphorylation events, ultimately leading to increased cellular proliferation. The point mutation in BRAF causes continuous kinase activation (phosphorylation at the active site) which results in Ras-independent activation of the Mek/Erk pathway with the end result being continuous cell division and avoidance of apoptosis (Brozyna et al., 2007). It is currently unclear whether or not UV exposure directly contributes to the acquisition of BRAF mutations, as a correlation between the two is uncommon.

Individuals with a certain skin phenotype have an increased lifetime risk for melanoma development. Individuals with fair skin (less melanin), possession of multiple nevi and freckles, and individuals prone to sunburning are more likely to develop melanoma. Increased age is also a factor in assessing lifetime risk of melanoma. Alterations to the immune system consequent of immunosenescence in elderly individuals may decrease immunosurveillance against cancerous cells, which contributes to tumor development. Immunosuppressed individuals, such as recent organ transplant patients, also exhibit increased risk of cancers including melanoma, suggesting that a functional immune system may play a vital role in suppressing the onset of cancer (Kubica & Brewer, 2012).

Components of the Immune System

The immune system is a highly adaptable, dynamic network of cells and molecules specialized in identifying and eliminating foreign pathogens from the body. There are two systems of immunity that function together to provide this protection. *Innate immunity* is the body's first line of defense consisting of physical, chemical, and cellular barriers present before the onset of infection, such as skin, mucosal membrane enzymes, and stomach acidity. Beyond these barriers are a number of nonspecific leukocyte host cells ready to engulf, neutralize and kill any foreign invaders who breach the immunological barriers, such as macrophages, neutrophils, dendritic cells and natural killer (NK) cells. Certain molecular patterns on invaders may activate the nonspecific complement system, which promotes opsonization of foreign agents and recruitment of phagocytic cells by inflammation (Goldsby et al., 2003). Generation of cytokines such as

interferon, tumor necrosis factor and interleukin is also the result of innate immunity. Cytokines are proteins or glycoproteins that bind to specific membrane receptors and induce a cascade of events via signal-transduction, ultimately resulting in gene expression alteration in specific cells. Cytokines secreted by injured cells or nonspecific leukocytes act in an antigen-nonspecific manner to promote cell proliferation and differentiation, regulate inflammation, and influence *adaptive immunity*, the second, and more specific, line of immune defense (Goldsby et al., 2003).

Adaptive immunity serves as a specific responsive against foreign pathogens that are able to evade the innate immunity mechanisms, and is therefore activated after onset of the infection. Whereas innate immunity recognizes large-scale molecular patterns on foreign invaders, adaptive immunity recognizes small immunologically active substances on pathogens known as “antigens”. B lymphocytes (white blood cells that mature in the bone marrow) and T lymphocytes (white blood cells that mature in the thymus) are the main cellular components of adaptive immunity. B lymphocytes play a large role in humoral immunity: immunity by non-cellular substances, such as antibodies, present in humours (body fluids). Antibodies are able to activate complement, promote antigen phagocytosis and induce death in antibody-bound foreign target cells. When an antigen binds the surface antibody of a naïve B lymphocyte, the cell undergoes clonal expansion and differentiation into effector plasma cells (which secrete antibodies) and long-lived memory cells. Following a secondary response to an identical or similar antigen, memory cells secrete high affinity antibodies to bind and clear the antigen quickly and effectively.

T lymphocytes are the major cell type involved in attacking cancer cells and play a large role in cell-mediated immunity. They function by interacting with other host cells

by binding antigenic peptides and major histocompatibility complex (MHC) presented by designated antigen-presented cells (APCs), which have phagocytized the foreign pathogen and displayed it on their surface. During maturation in the thymus, T lymphocytes are permitted to survive only if their T-cell receptor (TCR) can recognize self-major histocompatibility complex (MHC) and react to it at an appropriate affinity (Goldsby et al., 2003). If recognition of self-MHC is too strong, the T lymphocyte will be killed. Presence of MHC is necessary for a T lymphocyte to be activated. Two important subsets of mature T lymphocytes are CD4+ T-helper cells (T_H) and CD8+ T-cytotoxic cells (T_C). T_H cells bind MHC class II, which is frequently found on the surface of dendrites, macrophages and other specialized phagocytic APCs. Once bound, the T_H cell induces multiple intracellular signals, including release of IL-2, which causes autocrine and paracrine proliferation of T_H cells, which ultimately leads to differentiation into effector, regulatory, and memory T_H cells, secretion of multiple other cytokines and recruitment of helpful B lymphocytes and T_C cells. T_C cells bind MHC class I, which is present on all nucleated cells, and is able to directly kill a target cell expressing a foreign antigen, such as a host cell infected by a virus. The T_C cell killing process can occur by many mechanisms, including receptor-ligand binding of the FAS death receptor molecules which induces programmed steps to cell death and exocytosis of perforin, granzymes, and other lytic proteins leading to necrosis or apoptosis of the target cell by activation of caspases (Groscurth & Filgueira, 1998). Once activated, T_C cells also proliferate and differentiate in response to cytokine signals to increase the immune response. The major difficulty in attacking cancerous cells is that, although they function abnormally, they are self-cells.

Tumor Suppressive Function of the Immune System

Of the multiple components of the immune system, the most important functional component is the systems' ability to provide self-nonself discrimination, effectively distinguishing the body's own cells from foreign agents and altered host cells, therefore being especially important in detection and elimination of cancerous cells. Evidence of tumor immunology is rapidly accumulating; however the basic concepts and mechanisms are continuously being debated and revised (Weinberg, 2007). Cancer cells may possess two different types of antigen, differentiating them from host cells. The first is a tumor-specific transplantation antigen (TSTA), which is an antigen unique to the tumor cell due to a genetic event such as a mutation. T_C cells can recognize these novel proteins if present in conjunction with MHC class I and are able to mount a cell-mediated immune response and effectively kill the tumor cell. The second tumor antigen type is a tumor-associated transplantation antigen (TATA) and occurs more frequently than TSTAs. TATAs are not unique to the tumor cell; rather they are proteins expressed by normal cells, but at much higher levels in tumor cells, which can also be identified and targeted by T_C cells. Nonspecific immune system components such as macrophages, NK cells and cytokines also play a prominent role in the immune response to tumors. Both activated macrophages and NK cells are able to act against tumor cells in an MHC-independent mechanism, and are observed to surround tumor cells and mediate antibody-dependent cell-mediated cytotoxicity (Goldsby et al., 2003). NK cells in particular are important in recognizing cancer cells that are only slightly aberrant, sometimes by lacking only a single MHC-I allele, and can induce cytotoxicity rapidly due to their constitutively expressed lytic machinery (Zamai et al., 2007). IL-2 is a cytokine of particular interest for

cancer immunotherapy due to its ability to cause proliferation, recruitment, activation and differentiation of several key immunological cells, including tumor-specific T_C cells, capable of recognizing tumor cells. The presence of tumor-specific antibodies and T lymphocytes, as well as abundance of macrophages, in patients with tumors also confirms the immune systems active response to aberrant cells. Therefore, it is convincing that both innate and adaptive immunity components are important in maintaining cell integrity and suppressing tumor cell growth.

Despite the vast array of protective mechanisms contributed by the immune system against tumors, some cancer cells use strategies of immunoevasion to remain undetected. If continuous expression of a TATA or TSTA is not essential to neoplastic growth, the simplest way for a cancer cell to avoid immune detection is to stop displaying the antigen. Antigen-negative variants of the cancer cell would therefore be more successful in evading immune reactivity (Weinberg, 2007). If continuous expression of tumor-antigens is required for tumor proliferation, cancer cells are able to escape immune response by other mechanisms, such as down-regulation of MHC class I, increased resistance to caspase and Fas-ligand (FASL) mediated apoptosis, and secretion of immunosuppressive chemokines such as CCL22, which increases the number of T-regulatory cells, a line of T-lymphocytes that directly inhibit or kill T_C and T_H cells, (Weinberg, 2007). Melanoma cells in particular demonstrate many ways to evade suppression by the immune system. By secreting large amounts of transforming growth factor-beta (TGF-β), melanoma cells are able to transform $CD4^+CD25^-$ T cells into T-regulatory cells (Liu et al., 2007). The $BRAF^{V600E}$ mutation seen in the majority of melanoma tumors causes secretion of immunosuppressive vascular endothelial growth

factor (VEGF), IL-10 and IL-6, as well as a decreased production of inflammatory cytokines IL-12 and tumor necrosis factor-alpha (TNF-α) (Sumimoto et al., 2006).

Current Treatments of Melanoma

Current treatments available for melanoma include surgical excision of cancerous tumors, radiation therapy and chemotherapy, all invasive procedures that affect both cancerous and noncancerous cells. Surgical excision remains the primary treatment for melanoma tumor removal. During this procedure, the cancerous mass and surrounding healthy tissue, and in some cases adjacent lymph nodes, are removed from the body, often leading to scarring and possible bleeding or infection around the excision site. This treatment does not guarantee permanent removal of the cancer, as local recurrence and distant metastasis are both possible following surgical treatment. Therefore, surgical excision is most effective before the cancerous cells have metastasized to other locations. Radiation therapy of melanoma uses high doses of radiation to reduce tumor size, which often leads to damage of normal surrounding cells, causing nausea and potential abdominal region problems. Another treatment for melanoma that can be administered primarily, or in conjunction with other treatments, is chemotherapy. Chemotherapy in the form of anticancer drugs, such as Dacarbazine, act by targeting DNA replication in rapidly proliferating cells. The toxicity of anticancer drugs cause apoptosis of cancerous cells, but may also kill healthy cells. Undesirable side effects of chemotherapy include, but are not limited to, fatigue, vomiting, stomatitis and diarrhea in the patient. Recently, two new drugs have been FDA approved for use to treat melanoma in conjunction with chemotherapy. The first is Zelboraf (vemurafenib), a small molecular inhibitor of the

active site of BRAF protein that contains the V600E mutation, a mutation commonly seen in melanoma patients. Zelboraf binds the active site with greater affinity than ATP, which inhibits the kinase's ability to phosphorylate other substrates in the Mek/Erk pathway (Davis & Schlessinger, 2012). Zelboraf has also shown inhibitory activity on BRAF mutations that are V600K substitutions, a mutation that occurs in 5% of BRAF mutations (Chapman, 2012). The second drug recently FDA-approved for the treatment of metastatic melanoma is Ipilimumab (Yervoy), an anti-CTLA-4 monoclonal antibody. CTLA-4 stands for cytotoxic T lymphocyte antigen 4, and it is a negative modulator of T-cell response, which can decrease the immune response to a tumor. By blocking this antigen, which is present on T-cells, antitumor activity of T lymphocytes is not inhibited by cancer cell mechanisms (Weber, 2007). Adverse effects of these new drugs include fatigue, rash, inflamed intestines (colitis) and a higher rate of serious side effects in patients versus treatment with radiation and chemotherapy alone. Using monoclonal antibodies to bind target cells and stimulate a cytotoxic reaction is not uncommon in the discussion of cancer therapies; however there are limits to their effectiveness. Some TSTAs cannot be purified for monoclonal antibody preparation, and if they can be, it is likely that the monoclonal antibody would target only syngeneic tumor cells with high affinity.

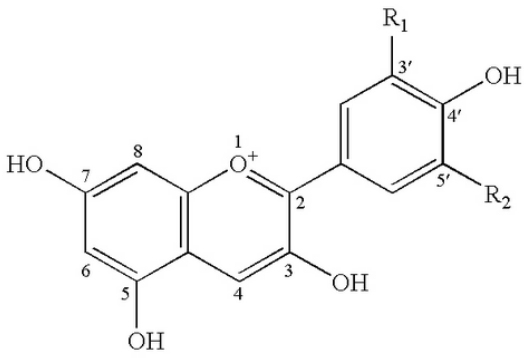
Bioactive Components of Berries

The use of bioactive food components in naturopathic therapies is a growing field of interest. Utilization of extracts from dark-pigmented berries, such as chokeberry, blueberry, bilberry and elderberry, is one of the areas receiving considerable attention due

to their diverse positive health benefits, global availability and attainability. For example, recent studies link increased consumption active berry extracts with decreased neuronal and behavioral changes related to aging, decreased risk of hypertension and cardiovascular disease, and increased protection against viral and bacterial infection (Joseph et al., 2009) (Zafra-Stone et al., 2007). Several berry extracts also show promise as a preventative strategy against human cancer cell growth, angiogenesis and metastasis (Zafra-Stone et al., 2007) (Matchett et al., 2005). One of the most important physiological functions of active berry extracts is their antioxidant activity. Cells can incur oxidative stress from environmental pollutants as well as through cellular processes, potentially causing oxidative damage to DNA, lipids, and proteins (Trachootham et al., 2009). Reactive oxygen species (ROS) activate essential components of the immune system by stimulating release of cytokines and mediating the inflammatory response; therefore, a small quantity of ROS is necessary in sustaining functional biological systems. Moderate increases in ROS are capable of modulating cell growth and cell differentiation by influencing important cell growth signal transduction pathways, which can play a role in eliminating foreign pathogens (Trachootham et al., 2009). Certain cellular antioxidants, such as superoxide dismutase (SOD) function to maintain a homeostasis of ROS. Excessive increases in ROS may offset the biological equilibrium and cause permanent damage to important cellular molecules, including DNA, which may cause apoptosis of the cell, or a mutation that leads to cell transformation. The latter may be a precursor event to the onset of cancer.

Over the past 20 years, anticancer researchers have been studying minimally invasive cancer treatments to address the severity of the many current treatment side

effects. A proposed treatment in the forefront is utilization of dark-pigmented berry extracts that are able to selectively target and suppress cancer cell growth (Katsube et al., 2003). The anticancer potential of dark-pigmented berries comes from the phytochemical properties of their extracts, most of which are phenolic components (Seeram, 2008). Flavanoids, including classes such as flavanols, flavonols and anthocyanins, act via multiple cellular mechanisms to modulate events associated with cancer cell development (Kanadaswami et al., 2005). Anthocyanins in particular have been the sub group of flavonoids most widely studied for their suppression of human cancer cell growth. Proposed mechanisms of cancer cell growth inhibition by anthocyanins include initiating apoptotic pathways, initiating cell cycle arrest, and inhibiting expression of tumor-associated enzymes in human cancer cells (Seeram, 2008). There are hundreds of different anthocyanins distributed throughout the plant kingdom, with the most abundant being cyanidin, delphinidin, malvidin, petunidin, peonidin and pelargonidin derivatives. Anthocyanins are water-soluble pigments responsible for the bright coloring of berries, ranging from orange and scarlet to blue and purple. All anthocyanins share the same basic 3-ring molecular skeleton (Figure 1), and are commonly glycosylated at position C-3. Naturally occurring anthocyanins are always glycosylated at position C-3. Glycosylation at position C-5 also occurs, and glycosylation at position C-7 is rare. The aglycone of an anthocyanin is referred to as an anthocyanidin.



Aglycone	R_1 Substitution	R_2 Substitution
Cyanidin	-OH	-H
Delphinidin	-OH	-OH
Malvidin	- OCH_3	- OCH_3
Petunidin	-OCH_3	-OH
Peonidin	-OCH_3	-H
Pelargonidin	-H	-H

Figure 1. The structure of common anthocyanidins.

In many *in vitro* and recently *in vivo* studies, it has been found that certain anthocyanins exhibit significant anticancer benefits, and are proposed to function by affecting a broad range of cellular mechanisms. Anthocyanins block cancer cell proliferation during various stages of the cell cycle, effecting important regulator proteins such as p53 and cyclin A, and have also been shown to induce apoptosis in cancerous cells via caspase activation or modulation of FAS and FASL expression on cancer cells (Wang & Stoner, 2008). Some anthocyanins are able to inhibit aberrant increases of

nuclear factor-kappa B (NF-κB) and cyclooxygenase-2 (COX-2), two proteins commonly up-regulated in cancers (Wang & Stoner, 2008). There have also been reports suggesting that anthocyanins are capable of decreasing metastatic risk and angiogenesis of cancer cells by inhibiting extracellular matrix degradation by matrix metalloproteinases (MMPs) and VEGF receptor and ligand expression on endothelial cells (Wang & Stoner, 2008). The antioxidant nature of anthocyanins is extremely important as a chemopreventative strategy to reduce risk of cancer, and it is also suggested that anthocyanins can induce ROS-mediated mitochondrial caspase-independent pathways, leading to tumor cell death (Wang & Stoner, 2008). The multiple number of anticancer benefits resultant of anthocyanin treatment and the increasing body of evidence supporting their activity warrants further study in this discipline.

European Black Elder (*Sambucus nigra)*

Sambucus is a genus consisting of approximately 20 different fruit-bearing small trees and shrubs, with European black elder (*Sambucus nigra* L) being the most common species of elder, a flowering plant in the Adoxaceae family, used in complementary and alternative medicine (Atkinson & Atkinson, 2002). Although native to Europe, Africa and Asia, European black elder has become widespread and commercially grown in the United States, found in the form of syrups, wines and jams. The elder tree native to North America (*Sambucus canadensis*) is closely related to the European black elder; however the medicinal benefits of this particular species are not yet well-defined. Future mention of ‘elderberry’ in this thesis should be assumed to be the berry product of European black elder unless otherwise notified.

Elderberry has been used previously in traditional and folk medicine for the treatment of multiple viral infections, including the common cold (acute viral nasopharyngitis), influenza, and herpes virus. Recent studies confirm the benefits of elderberry as an antiviral. In a 2009 randomized, double-blind, placebo-controlled pilot clinical study, it was concluded that elderberry extract given in the form of a slow-dissolve lozenge was able to significantly relieve multiple influenza symptoms within 24 hours of treatment compared to the placebo (Kong, 2009). In the same year, flavonoids isolated from elderberry proved to inhibit Human influenza A (H1N1) infection *in vitro* by binding directly to H1N1 virions, effectively blocking their ability to bind and enter host cells (Roschek et al., 2009). Multiple current studies now support the use of elderberry extracts for treatment of not only flu-like symptoms, but for treatment and prevention of diabetes, cardiovascular disease, open wounds, and cancers. Studies indicate that active components present in elderberry are capable of stimulating and modulating immune response, as well as providing anti-oxidant protection against cellular damage (Roxas & Jurenka, 2007). Sambucol, an elderberry extract product, has been shown to increase production of inflammatory cytokines IL-1b, TNF-α, IL-6 and IL-8, thereby stimulating a number of immune effector cells (Barak et al., 2001). Elderberry anthocyanins can be incorporated into vascular endothelial cells and exhibit significant oxidative protection against a number of oxidative stressors, thereby maintaining cellular integrity and preventing DNA mutation (Youdim et al., 2000). The benefits previously reported of elderberry extracts, and in particular of elderberry anthocyanins, are promising as a naturopathic treatment for many infections and diseases.

The anticancer potential of certain anthocyanins *in vitro* is now widely accepted (Wang & Stoner, 2008); however, anticancer studies of specific elderberry anthocyanins *in vitro* and *in vivo* have been limited. Elderberry contains four primary anthocyanins: cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside, all of which are 100% nonacylated with minimal glycosylation (Youdim et al., 2000) (Jing et al., 2008). It was found that nonacylated monoglycosylated anthocyanins have a great effect on inhibition of colon cancer cell growth *in vitro* (Jing et al., 2008). Cyanidin 3-glucoside in particular has a strong inhibitory effect on the cellular growth of metastatic breast cancer *in vivo* (Chen et al., 2005). It has also recently been shown that the glycosylated structures of anthocyanins in elderberry can be absorbed in humans, despite previous claims that anthocyanin form is changed prior to absorption (Cao et al., 2001)(Milbury et al., 2002). One study showed that certain elderberry fractions demonstrate inhibition of COX-2 (anticancer function) and induction of quinone reductase (antioxidant function) *in vitro*. Based on these findings, it is reasonable to assume that elderberry anthocyanins may be able to prevent the onset of cancer by antioxidant activity, and also modulate a variety of cellular mechanisms capable of inducing cancer cell death.

Aim

The goal of this research endeavor is to obtain evidence that further supports the use of natural berry extracts in a therapeutic manner to help treat and prevent incidence of melanoma. This research aims to separate and identify the individual components of elderberry by column chromatography and examine their effects on human melanoma

tumor cell growth *in vitro*. Active elderberry fractions will be pooled and assessed for suppressive activity against many cell lines, including human and murine melanoma lines, a human neuroblastoma line, void of caspase-8 (a pro-apoptotic protein), and a non-cancerous transformed cell line derived from the ovary of a Chinese hamster. Additionally, pooled active column fractions will be evaluated for stimulatory activity of spleen cells from young and old mice, as well as induction of cytokine IL-2. Pooled active fractions that demonstrate increased immune response in elderly (immunosenescent) mice and significant tumor suppressive ability will be used in a murine melanoma model to evaluate tumor suppressive ability *in vivo*. Final identification of active fraction components will be achieved through high-performance liquid chromatography (HPLC). Proper identification of melanoma-suppressing, immune-inducing elderberry fractions may lead to diet-based strategies for natural prevention and suppression of melanoma.

METHODS

Elderberry

The elderberry preparation used for experimentation was 13% standardized *Sambucus nigra* elderberry powder, generously provided by Artemis International, Inc. (Fort Wayne, IN). To prepare the powder, pure elderberry was concentrated by a physical process without solvent, then spray dried onto an excipient (Artemis International, Inc., 2005). Anthocyanin and polyphenol contents were expressed as a minimum 13g/100g and 17g-29g/100g, respectively (Artemis International, Inc., 2005). When not in use, the elderberry powder was stored in a cool, dry area to avoid moisture absorption and to maintain the integrity of the chemicals. 10 mg/mL and 1mg/mL crude elderberry stocks were prepared, filter-sterilized using a 0.2µm Nalgene filter, and stored at 4^{o}C until use.

Extraction of Elderberry Samples

Gravity column chromatography was used to separate the components of *Sambucus nigra* elderberry powder. In this procedure, elderberry powder components are eluted through a solid polyamide stationary phase by a series of liquid mobile phases. Polarity of the components, polarity of the stationary phase, polarity of the mobile phases and hydrostatic pressure due to gravity are the driving forces behind the elution. It is well established that phenolic compounds, such as the ones present in berries, can be well

resolved using polyvinylpyrrolidone (PVP) as the stationary phase and varying concentrations of water-methanol mobile phases for gradient elution (Strack & Mansell, 1975). The retention times for the different components are generally determined by the difference in between the polarity of the stationary phase and mobile phases. By running mobile phases in order of decreasing polarity by gradient elution, strongly retained (late-eluting) components will elute from the column at a reasonable retention time.

The protocol used for gravity column chromatography was taken from Rizvi (2012); an adaptation from the protocol first published by Strack & Mansell (1975). A 250-mL Kimex® chromatography column (Internal Diameter ~2.5 cm) was used for the first extraction. Following addition of a sterile cotton ball (Diameter ~2.5 cm) placed at the base of the column, 25 g of dry PVP was added to fill the column to an approximate height of 18.4 cm. Suitable elderberry solute for extraction was prepared by adding 2 g 13% standardized elderberry powder to 4 mL of 0.01*N* hydrochloric acid (HCl). The elderberry powder-HCl preparation was added to the column on top of the stationary phase. Gradient elution was carried out using mobile phases of sterile deionized water, methanol, and a consistent amount of 0.01 *N* HCL (Figure 2a). The first solvent was 100% sterile deionized water with 15 mL 0.01 *N* HCL, and 10 other mobile phases were added sequentially in order of decreasing polarity after the preceding mobile phase was eluted. See Appendix for a complete list of mobile phases.

14.5 mL increments of elderberry samples were sequentially collected from the column into 15 mL sterile vials (Figure 2) and stored at -18^{o}C to preserve elutant chemical integrity. After collection of elutants was complete, the majority of the mobile phase solvent was evaporated from each individual samples by Buchi rotary evaporator

(Figure 2b). The samples were concentrated down to 1-1.5 mL volumes on medium water bath heat (40-50^{o}C) and collected into autoclaved 1.5 mL microcentrifuge tubes. To fully eliminate the presence of all methanol solvent, the elderberry samples in 1.5 mL microcentrifuge tubes were evaporated dry using a vacuum centrifuge on medium (36^{o}C) heat (Figure 2c). Each sample was first covered with parafilm, and small holes were poked through the parafilm using a sterile needle to allow evaporated solvent escape. Once fractions were completely dry, they were re-dissolved in 0.5 mL filter-sterile phosphate buffered saline (PBS), agitated until homogenous, and stored at -18^{o}C until further use.

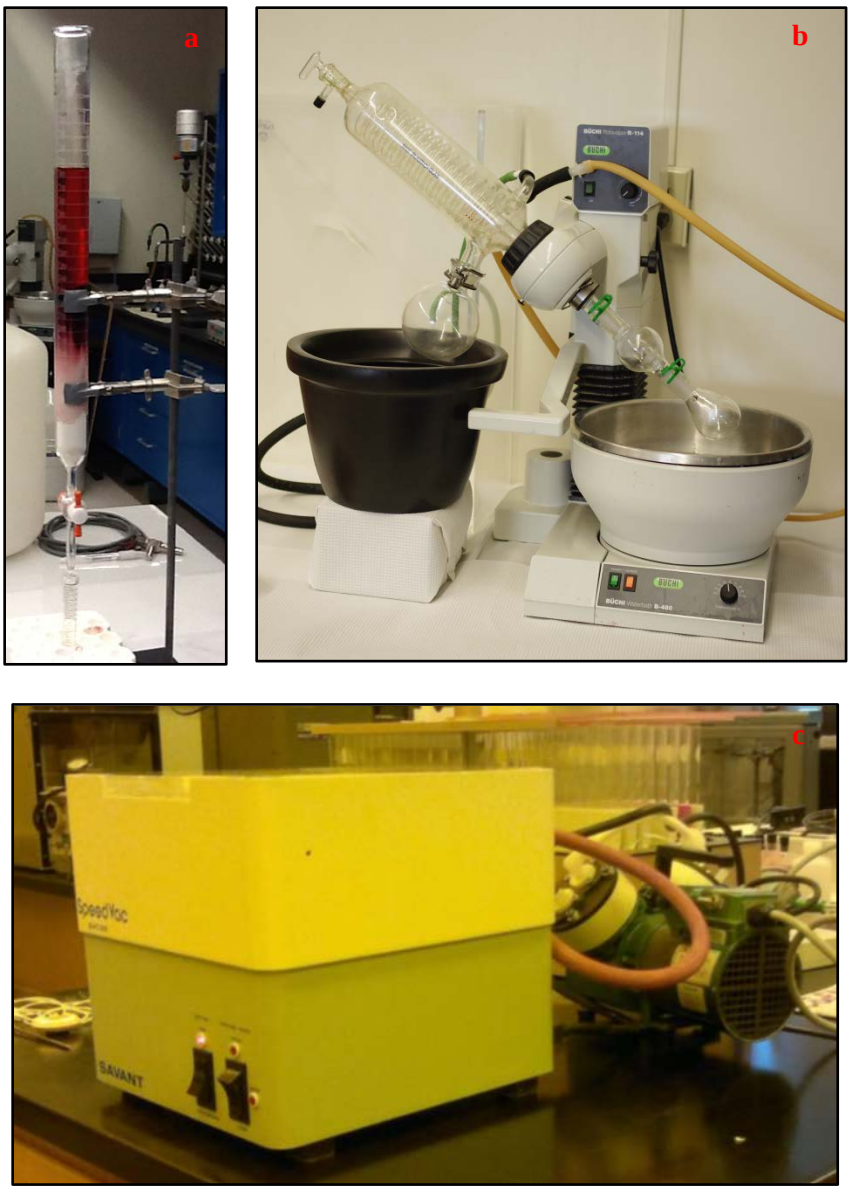


Figure 2. Separation and solvent removal techniques. (a) Column chromatography, (b) rotary evaporation and (c) vacuum centrifugation.

Two additional 250-mL columns (Internal Diameter ~2.5 cm) were purchased from C-Tech Glassware (New Jersey; Item #: CL-0009-016) to collect large quantities of elderberry samples for analysis. Following the same gravity column chromatography protocol previously described, 23 additional columns were run using the 250-mL

Kimax® chromatography column and the two 250-mL chromatography columns from C-Tech Glassware. The PVP stationary phase reached an approximate height of 16.2 cm in the C-Tech Glassware columns. Column elution for Columns #3-24 was ended after completion of the 20% Water/80% Methanol mobile phase, because samples of interest for future experiments elute before addition of the 10% Water/90% Methanol and 0% Water/100% Methanol mobile phases. 14.5 mL increments of elderberry samples were sequentially collected from the columns into 15 mL sterile vials and stored at -18^{o}C to preserve chemical integrity. After collection of elutants was complete, the mobile phase solvent was evaporated from each individual samples by Buchi rotary evaporator on medium water bath heat (40-50^{o}C). The fractions were evaporated dry in 100-mL round bottom flasks, re-dissolved in 0.5 mL PBS, and collected into autoclaved 1.5 mL microcentrifuge tubes, which were stored at -18^{o}C until further use. This method of completely removing methanol from the samples can be validated by Escribano-Bailón et al. who also used a combination of column chromatography and rotary evaporation to achieve organic solvent-free samples for further analysis (2006).

Cell Lines

Multiple cells lines were used over the course of this study. The human melanoma line, MeWo, used to measure individual sample tumor suppressive ability was obtained from Dr. Robert Visalli (IPFW, Department of Biology, IN) and was maintained in Eagle minimum essential media (MEM) supplemented with 10% fetal bovine serum (FBA) and 1% NEAA nutrient mixture. MeWo cells were kept in 75 cm^3 flasks in a 7.5% CO_2 incubator at a constant temperature of 37.5^{o}C, and were sub-cultured every 3-4 days

based on confluence. The murine melanoma line, B16-F10, was obtained from ATCC (Manassas, VA; #CRL-6475) and selected for its' well-established use in murine melanoma models. B16-F10 cells are syngeneic to C57BL/6J mice. The B16-F10 cells were maintained in RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, and were sub-cultured every 2-3 days based on confluence. The B16-F10 lines were also used to determine tumor suppressive ability of elderberry fractions, and were kept in 75 cm^3 flasks in a 7.5% CO_2 incubator at a constant temperature of 37.5^oC. Chinese hamster ovary (CHO-K1) cells, a commonly cell line derived from a line of transformed cells taken from a Chinese hamster ovary, were obtained from ATCC (Manassas, VA; #CCL-61) for use in a noncancerous, transformed cell proliferation assay with active elderberry fractions. The CHO-K1 cells were maintained in RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, incubated in 75 cm^3 flasks at 37.5^oC and 7.5% CO_2, and were sub-cultured every 1-2 days based on confluence. To determine whether active fractions also suppressed tumor growth of another cancer line, SH-SY5Y neuroblastoma cells were generously provided by Dr. Robert Ross (Fordham University, Department of Neurobiology, NY) for use in this assay. SH-SY5Y cells lack caspase-8, and are therefore resistant to apoptosis induction through caspase-8 pathways. The SH-SY5Y cells were maintained in RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, incubated in 75 cm^3 flasks at 37.5^oC and 7.5% CO_2, and were sub-cultured every 4-5 days based on confluence. To assess immunological response induced by active fractions, spleen cells were obtained from BALB/c male mice or C57BL/6J male mice. The C57BL/6J mouse strain was used when comparing the

immunological response of young and old mice. When it was possible, having two different cell lines in the CO_2 incubator was avoided to reduce the risk of cross-contamination. If two or more cell lines were being used in different experiments simultaneously, the flasks were kept on different levels of the CO_2 incubator. When cell lines were not in use, they were appropriately prepared for cryopreservation in 1.5 mL cryovials, and were quickly transferred into a liquid nitrogen tank after 1-2 days of slow cooling at -80^{o}C. Biocidal ZF^{TM} disinfectant and AquaClean microbiocidal additive for heating bath fluids from ConTaFree Liquids was generously provided by Dr. Shree Dhawale (IPFW, Department of Biology, IN) for cleaning of the CO_2 incubator.

Preparation of Spleen Cells

BALB/c male mice or C57BL/6J male mice were used to prepare spleen cells for proliferative assays. Mice were sacrificed through cervical dislocation, and laid ventral side up in a dissecting pan. A few drops of 70% ethanol were used to wet the fur and eliminate interference during dissection. Using heat-sterilized scissors and heat-sterilized forceps, a small left paramedical incision was made in the abdominal region to expose the parietal peritoneum. A second careful incision through the parietal peritoneum exposes the abdominal organs contained within the mesenteries, including the spleen. The spleen was dissected out of the abdominal cavity with heat-sterilized forceps, and any additional visceral peritoneum and fatty tissue was removed. The spleen was placed into a sterile petri dish on top of a heat-sterilized mesh screen. 1 mL of RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin was pipetted over the spleen into the petri dish. Using the plunger from a 1 mL syringe, the spleen was

gently massaged into the mesh screen, creating a single cell suspension. A 26 gauge needle was used to collect the cell suspension. The spleen cells were immediately used in experimental assays.

Cell Harvesting and Liquid Scintillation Counting

Cell harvesting was accomplished with a Brandel (Model# M-24) cell harvester (Figure 3a). Due to their adherent ability, tumor cells and transformed cells in 96-well tissue culture plates were treated with 2 µL trypsin EDTA, 1X 5 minutes prior to cell harvest, to release the cells from the plate and increase accuracy of cell counting. Preliminary studies with MeWo cells and trypsin EDTA, 1X identified 2 µL as the optimal volume for increasing the release of cancer cells (Appendix, Figure B1). Trypsin EDTA, 1X was not added prior to spleen cell harvesting. To harvest, the cells were washed 25-30 times with PBS and precipitated onto Whatman™ filter paper sheets with 10% trichloroacetic acid (TCA). After drying (approximately 30 minutes after harvesting) the filter paper discs were placed into individual 6 mL vials, filled with 3 mL Ecolume™ scintillation cocktail, and were tightly capped. Tritiated thymidine is not strong enough to be counted by a Geiger counter. Addition of scintillation cocktail, which contains fluors, allows the radioisotope-containing nuclei to be counted by scintillation counter in counts per minute (CPM). A Beckman Coulter™ LS 6500 Multi-Purpose Scintillation Counter was used for all cell counting assays (Figure 3b).

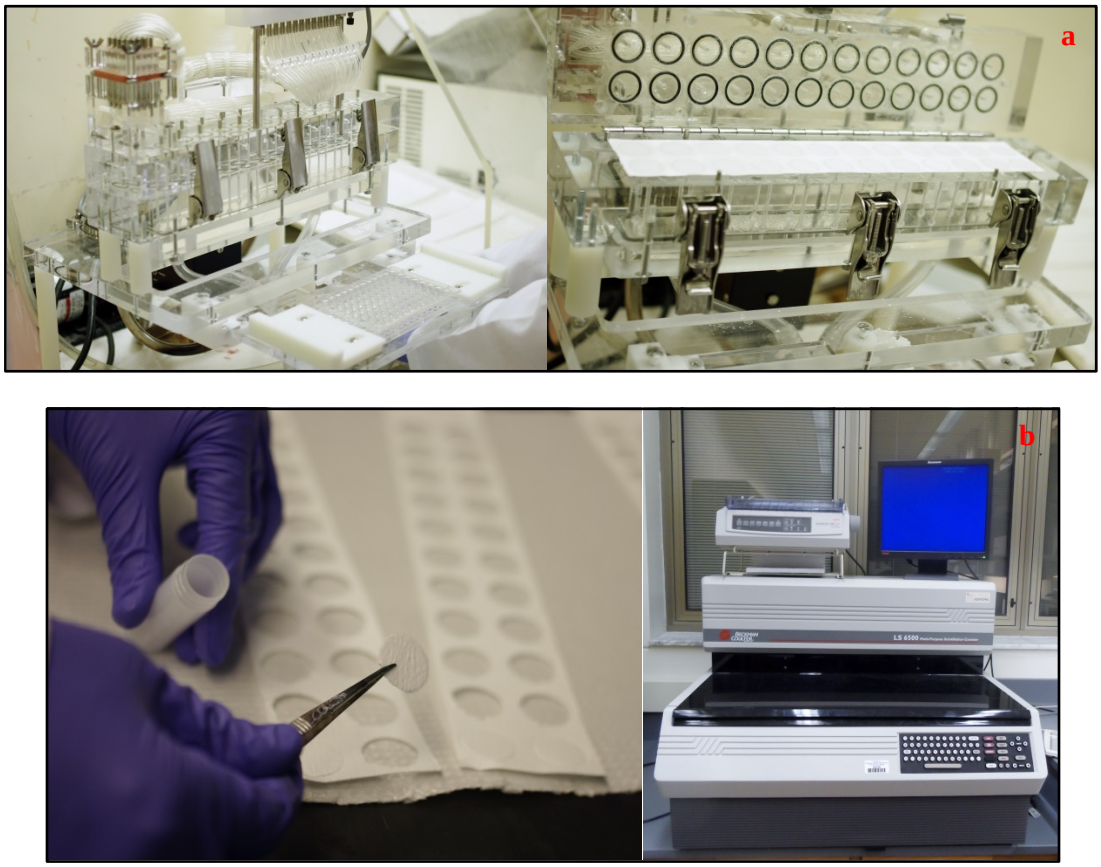


Figure 3. Cell harvesting and counting techniques. (a) Cell harvester and (b) Whatman™ filter paper collection and scintillation counter.

Mouse Habitat Conditions and Handling

BALB/c male mice and C57BL/6J mice were housed in cages and fed a dry pellet diet and water *ad* libitum. A 12-hour light/dark cycle was maintained for the duration of all experiments. Certain C57BL/6J mice exude social dominance by removing pelage and whiskers from other mice that share the same habitat in a behavior known as barbering. Any C57BL/6J mice seen barbering or fighting other mice were placed in their own separate cage, to eliminate possible speculation that physical pelage abnormalities was caused by experimental treatment. Prior to any experimentation, mice were acclimatized

for no less than three weeks in this environment. When working with the mice, hands were washed before and after handling, and gloves and a lab coat were worn at all times for sanitary purposes.

Tumor Cell Proliferation Assays with Separated Elderberry Samples

To assess the tumor suppressing ability of elderberry samples from Column #1, an assay was set up to measure percent proliferation of MeWo cells following elderberry treatment in a 72-hour thymidine uptake assay *in vitro*. MeWo cells were collected from culture flasks, counted using a hemocytometer, and diluted with Eagle MEM X media supplemented with 10% FBS and 1% NEAA to a final concentration of 5×10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL MeWo cell dilution and 100 µL additional Eagle MEM X media supplemented with 10%FBS and 1% NEAA, bringing the final cell concentration of each well to 5×10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, crude 1mg/mL elderberry, or elderberry fraction was added to the appropriate wells and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. This radioisotope is incorporated into DNA replication events of dividing cellular nuclei. At 72 hours, the cells were harvested and counted. This experiment was repeated three times validate results.

To assess the tumor-suppressing ability of the individual fractions collected from columns #2-24, a B16-F10 cell proliferation assay was performed for the fractions of each column. Since the separated fractions of interest were between the approximate

range of sample 45-90, only samples 45-90 were used in the column #23 and column #24 B16-F10 cell proliferation assays. B16-F10 cells were diluted with RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 5×10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL B16-F10 cell dilution and 100 µL additional RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 5×10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry 10 µL of separated elderberry fraction was added to the appropriate plate wells and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. This experiment was performed once for each column.

Pooling of Assumed Active Elderberry Samples

Individual elderberry samples from Column #1 that showed similar MeWo cell proliferation inhibition were pooled together in equal concentrations to make pooled fractions.

Three pooled fractions from column #1 were chosen as active pooled fractions of interest for future analysis, due to their high tumor-suppressive activity and stimulation of immune response. Using the results from the MeWo cell proliferation assay with separated fractions from column #1 as a guide, separated fractions from column 2-24 were appropriately pooled with neighboring active fractions to create pools with the same

activity pattern as the pooled fractions from column #1. The pools from column #2-24 that matched the activity of the pools of interest from column #1 were stored at -18^{o}C until further use. The recreated pooled fractions 16, 24, and 29 were renamed P16, P24, and P29 respectively to avoid confusion with the terms “pool 16”, “pool 24”, and “pool 29”, which refer exclusively to the fractions pooled from column #1.

MeWo Cell Proliferation Assay with Pooled Elderberry Fractions

To assess the tumor suppressing ability of pooled fractions from Column #1, an assay was set up to measure percent proliferation of MeWo cells following pooled elderberry treatment in a 72-hour thymidine uptake assay *in vitro*. MeWo cells were diluted with Eagle MEM X media supplemented with 10%FBS and 1% NEAA to a final concentration of 5 x 10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL MeWo cell dilution and 100 µL additional Eagle MEM X media supplemented with 10%FBS and 1% NEAA, bringing the final cell concentration of each well to 5 x 10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, 10 µL of crude 1mg/mL elderberry, or 10 µL of pooled elderberry fraction was added to the appropriate plate wells and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. This experiment was repeated twice.

B16-F10 Cell Proliferation Assay with Pooled Elderberry Fractions

Similar to the MeWo cell proliferation assay with pooled elderberry fractions from Column #1, this assay implements the same method using B16-F10 melanoma cells as the target cells. B16-F10 cells were diluted with RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 5 x 10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL B16-F10 cell dilution and 100 µL additional RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 5 x 10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, 10 µL of crude 1mg/mL elderberry, or 10 µL of pooled elderberry fraction was added to the appropriate plate wells and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. The experiment was performed three times for validation.

SH-SY5Y Cell Proliferation Assay with Pooled Elderberry Fractions

Similar to the B16-F10 cell proliferation assay with pooled elderberry fractions from Column #1, this assay implements the same method using SH-SY5Y neuroblastoma cells as the target cells. SH-SY5Y cells were diluted with RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 5 x 10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL B16-F10 cell dilution and 100 µL

additional RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 5 x 10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, 10 µL of crude 1mg/mL elderberry, or 10 µL of pooled elderberry fraction was added to the appropriate plate wells and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. The experiment was performed three times for validation.

CHO-K1 Cell Proliferation Assay with Pooled Elderberry Fractions

This assay implements the same method as the SH-SY5Y neuroblastoma and B16-F10 melanoma cell proliferation assay with pooled fractions using CHO-K1 cells as the target cells to determine the effect of pooled fractions from Column #1 on the growth of a noncancerous transformed cell line. CHO-K1 cells were diluted with RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 5 x 10^5 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL B16-F10 cell dilution and 100 µL additional RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 5 x 10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, 10 µL of crude 1mg/mL elderberry, or 10 µL of pooled elderberry fraction was added to the appropriate plate wells in triplicate and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated

thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. The experiment was performed three times.

Middle Aged Mouse Spleen Cell Proliferation Assay with Pooled Elderberry Fractions

To assess the immune-inducing effects of the pooled elderberry fractions, a spleen cell proliferation assay was performed. Spleen cells were incubated with and without concanavalin A (Con A), a known mitogen to induce proliferation of T lymphocytes. Rizvi (2012) showed that Con A addition was essential to demonstrate the immune-inducing effect of elderberry fractions. Optimal Con A concentration, 2.5 µL of 50 µg/mL Con A (equivalent to 0.125 µg/mL Con A), was determined in a preliminary study using the spleen from a BALB/c male mouse (Appendix, Figure B2). Spleen cells for this assay were prepared from a C57BL/6J male mouse (11 months old), and diluted with RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 10×10^6 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL spleen cell dilution and 100 µL additional RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 1×10^6 cells/200 µL supplemented media. 2.5 µL of 50µg/mL con A was added to all wells, except for control wells, which remained spleen cells in supplemented media only. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL pooled elderberry fractions were added to wells containing con A in duplicate, and were incubated for another 24 hours. The control and spleen cells + con A only wells were plated in triplicate. At 48

hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted.

Young and Old Mouse Comparative Spleen Cell Proliferation Assay with Pooled Elderberry Fractions

To compare the immunological effect of pooled elderberry fractions on spleen cell proliferation from a young and old mouse, a comparative spleen cell proliferation assay was performed. A young mouse (5 months old) and an old mouse (retired breeder, 18 months old) were sacrificed, and their spleens were appropriately prepared and diluted with RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 10×10^6 cells/mL. In two MICROTEST^{TM} 96-well tissue culture plates, each experimental well was filled with 100 µL of either young or old spleen cell dilution and 100 µL additional RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 1×10^6 cells/200 µL supplemented media. 2.5 µL of 50µg/mL Con A was added to all wells, except for control wells. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, 10 µL of crude 10mg/mL elderberry, 10 µL of crude 1mg/mL elderberry, or 10 µL of pooled elderberry fractions were added to wells containing Con A in duplicate, for both young and old spleen cell plates, and were incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted.

Modulation of Cytokine IL-2 by Pooled Elderberry Fractions

To assess the production of the immune modulating cytokine IL-2 by spleen cells treated with pooled fractions, a colorimetric assay was used. A Quantikine® ELISA (enzyme-linked immunosorbant assay) kit for mouse IL-2 was obtained from R&D Systems (Minneapolis, MN). This assay employs the quantitative sandwich enzyme immunoassay technique, where a polyclonal antibody specific for mouse IL-2 is immobilized in each well of a 96-well microtiter plate. To prepare the samples, a C57BL/6J mouse (4 months old) was sacrificed and a single cell suspension of spleen cells was obtained and diluted to 10×10^6 cells/mL. In 12-well costar® microtiter plates, 600 µL RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin and 400 µL of spleen cell dilution was added to each well. Excluding the negative control well and well designated for Con A only (+12.5 µl 50µg/mL Con A), each well received an addition of 50 µl pooled fraction and 12.5 µl 50µg/mL Con A. The 12-well microtiter plates were incubated for 24 hours at 37.5°C and 7.5% CO_2. Following incubation, the contents of each well were gently centrifuged for 5 minutes and the supernatant was removed for use in the assay. The assay was performed by the manufacturer's instructions and treatments were plated in duplicate. A Packard SpectraCount™ was used to determine the optical density (O.D.) for each well at 405 nm, 490 nm and 570 nm wavelengths. Subtraction of the 570 nm reading from the average of the 405 nm and 490 nm readings allows for correction of optical imperfections present the microtiter plate.

Old Mouse *in vivo* Murine Melanoma Assay with Crude Elderberry

To assess the tumor-suppressive and immune-inducing activity of crude 10mg/mL elderberry in old (senescent) mice, an *in vivo* murine melanoma model was used. Six C57BL/6J male retired breeders (20 months old) were randomly grouped into a water treatment (control) group and a 10mg/mL elderberry treatment group. Each mouse was weighed on day 1, day 36 (successful tumor cell injection) and prior to sacrifice. For 36 consecutive days, each mouse received a daily i.p. injection of either 0.5 mL filter-sterilized deionized water or 0.5 mL filter-sterilized crude 10mg/mL using a sterile 26 gauge needle. On day 36, each mouse received a 0.1 mL subcutaneous (s.c.) injection of 1×10^6 cells/mL B16-F10 cells. Therefore, each mouse received 1×10^5 B16-F10 cells. All mice were given daily i.p. injection of either 0.5 mL filter-sterilized deionized water or 0.5 mL filter-sterilized crude 10mg/mL for an additional 7 days (day 37-43), then were left undisturbed for an additional 7 days (day 44-50), and were sacrificed via cervical dislocation after tumors were formed, but were not burdensome to the mice. Additionally, two old mice were given daily i.p. injection of 0.5 mL filter-sterilized crude 10mg/mL elderberry for 36 days, were not given a s.c. injection of B16-F10 cells, and were sacrificed via cervical dislocation. Initial and final weights were recorded.

Tumor weight was recorded and tumor size was measured using calipers for all tumor-bearing mice. Spleens from all mice were dissected and prepared for a spleen cell proliferation assay. Spleen cells were counted using a hemocytometer, and diluted with RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 10×10^6 cells/mL. In MICROTEST^{TM} 96-well tissue culture plates, experimental wells were filled with 100 µL of spleen cell dilution

from each mouse and 100 µL additional RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 1 x 10^6 cells/200 µL supplemented media. Either 0 µL (control), 2.5 µL or 5 µL of a stock 50µg/mL Con A were added in triplicate for each mouse cell dilution. After 48 hours of incubation at 37.5^{o}C and 7.5% CO_2, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted.

Young Mouse *in vivo* Murine Melanoma Assay with Crude Elderberry

To assess the tumor preventative ability of crude 10mg/mL elderberry in young mice, an *in vivo* murine melanoma model was used. Nine C57BL/6J male young mice (1.5 months old) were randomly grouped into a water treatment (control) group, a 10mg/mL elderberry treatment group, and a 10mg/mL group (no-cancer challenge). Each mouse was weighed on day 1, day 21 (tumor cell injection) and prior to sacrifice. From day 1 to day 7, each mouse received a daily i.p. injection of either 0.5 mL filter-sterilized deionized water or 0.5 mL filter-sterilized crude 10mg/mL using a 26 gauge sterile needle. On day 7, each mouse received 0.1 mL s.c. injection of 1 x 10^5 cells/mL B16-F10 cells to the right flank. Therefore, each mouse received 1 x10^4 B16-F10 cells. All mice were given daily i.p. injection of either 0.5 mL filter-sterilized deionized water or 0.5 mL filter-sterilized crude 10mg/mL for an additional 7 days (day 8-14), then were left undisturbed for an additional 7 days (day 15-21). On day 21, mice were re-injected with 0.1 mL s.c. injection of 1 x 10^6 cells/mL B16-F10 cells. Therefore, each mouse received 1 x10^5 B16-F10 cells. The reason for re-injection was due to the lack of tumor cell

observation in all mice. All mice were given daily i.p. injection of either 0.5 mL filter-sterilized deionized water or 0.5 mL filter-sterilized crude 10mg/mL for an additional 7 days (day 21-27), then were left undisturbed for an additional 5-6 days (day 28-33), and mice were sacrificed via cervical dislocation after tumors were formed, but were not burdensome to the mice.

Tumor weight was recorded and tumor size was measured using calipers for all tumor-bearing mice. Spleens from all mice were dissected and prepared for a spleen cell proliferation assay. Spleen cells were counted using a hemocytometer, and diluted with RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 10×10^6 cells/mL. In MICROTEST^{TM} 96-well tissue culture plates, experimental wells were filled with 100 µL of spleen cell dilution from each mouse and 100 µL additional RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 1×10^6 cells/200 µL supplemented media. Either 0 µL (control), 2.5 µL or 5 µL of 50µg/mL Con A were added in triplicate for each mouse cell dilution. After 48 hours of incubation at 37.5^{o}C and 7.5% CO_2, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted.

Middle Aged Mouse *in vivo* Murine Melanoma Assay with Active Pooled Elderberry Fractions

To assess the tumor suppressive ability of active pooled fractions of interest *in vivo*, a murine melanoma model was used. Twenty C57BL/6J male mice (6 months old)

were randomly grouped into a water treatment (control) group, a 10mg/mL elderberry treatment group, a P16 group, a P24 group, and a P29 group. Each mouse was weighed on day 1. From day 1 to day 7, each mouse received a daily i.p. injection of either 0.5 mL filter-sterilized deionized water, 0.5 mL filter-sterilized crude 10mg/mL elderberry, or 0.5 mL filter-sterilized pooled fraction using a 26 gauge sterile needle. On day 7, each mouse received 0.1 mL s.c. injection of 1 x 10^6 cells/mL B16-F10 cells to the right flank. Therefore, each mouse received 1 x10^5 B16-F10 cells. All mice were given daily 0.5 mL i.p. injections of their respective treatment for an additional 7 days (day 8-14), then were left undisturbed for an additional 7 days (day 15-21). Throughout the experiment, mortalities and behavioral cues, such as weakness, from the pooled fraction treated mice suggested that the pooled fractions were too potent and too concentrated to be used in the assay. Remaining mice were sacrificed via cervical dislocation and the experiment was ended prematurely.

Preliminary Identification of Active Pooled Elderberry Fractions by Heat-Induced Denaturation in a B16-F10 Cell Proliferation Assay

Proteins can be denatured and rendered inactive or less active by changes in temperature and pH. To determine if the tumor suppressing ability in active elderberry fractions was due to a protein, certain active elderberry fractions were subject to heat treatment prior to B16-F10 cell addition *in vitro*. B16-F10 cells were diluted with RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 5 x 105 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL B16-F10 cell dilution and 100 µL

additional RPMI-1640 media supplemented with 10% FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 5 x 10^4 cells/200 µL supplemented media. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, crude 10mg/mL elderberry, crude 1mg/mL elderberry, and select active pooled elderberry fractions were heated at 100^{o}C for 5 minutes, and then were allowed to cool back to room temperature. 10 µL of heated and non-heated crude 10mg/mL elderberry, 10 µL of heated and non-heated crude 1mg/mL elderberry, and 10 µL of heated and non-heated pooled elderberry fraction were added to the appropriate plate wells in triplicate and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. The experiment was performed twice.

Preliminary Identification of Active Pooled Elderberry Fractions by Heat-Induced Denaturation in a Spleen Cell Proliferation Assay

To determine if the immune inducing ability in active elderberry fractions was due to a protein, certain active elderberry fractions were subject to heat treatment prior to spleen cell addition *in vitro*. Spleen cells for this assay were prepared from a C57BL/6J male mouse (16 months old), and diluted with RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin to a final concentration of 10 x 10^6 cells/mL. In a MICROTEST^{TM} 96-well tissue culture plate, each experimental well was filled with 100 µL spleen cell dilution and 100 µL additional RPMI-1640 media supplemented with 10%FBS and 1% Penicillin-Streptomycin-Amphotericin, bringing the final cell concentration of each well to 1 x 10^6 cells/200 µL supplemented media. 2.5 µL

of 50µg/mL Con A was added to all wells, except for control wells. After 24 hours of incubation at 37.5^{o}C and 7.5% CO_2, crude 10mg/mL elderberry, crude 1mg/mL elderberry, and select active pooled elderberry fractions were heated at 100^{o}C for 5 minutes, and then were allowed to cool back to room temperature. 10 µL of heated and non-heated crude 10mg/mL elderberry, 10 µL of heated and non-heated crude 1mg/mL elderberry, and 10 µL of heated and non-heated pooled elderberry fraction were added to the appropriate plate wells in triplicate and incubated for another 24 hours. At 48 hours, each well was given 1 mCi/mL tritiated thymidine and incubated for an additional 24 hours. At 72 hours, the cells were harvested and counted. This experiment was performed twice.

Identification of Active Pooled Elderberry Fractions by Reversed-Phase High-Performance Liquid Chromatography (HPLC)

The 4 major anthocyanins in *Sambucus nigra* were separated by reversed-phase HPLC using a Hewlett-Packard 1050 Model pump system (Hewlett-Packard, Palo Alto, CA) with a photo-diode array detector (model 1040A) at 520 nm. The system was equipped with a 4.6 x 250 mm 5 µm C_8 column. Anthocyanins were eluted using a gradient elution sequence adapted from von Baer et al. (2008). Pooled fractions of interest were also eluted on this column to determine the primary anthocyanin(s) in the sample. The column was set to a flow rate of 0.8 mL/min and a 20 µL injection of 1 mg/mL filter-sterilized crude elderberry was used for the elution. Milli Q water, formic acid and HPLC-grade acetonitrile were used to prepare the solvents for elution. The solvents for the elution were (A) water/formic acid/acetonitrile 87:10:3 (v/v/v) and (B)

water/formic acid/acetonitrile 40:10:50 (v/v/v). The gradient starts with 6% solvent B for 1 minute, a linear gradient from 6% solvent B to 30% solvent B at 16 minutes, a linear gradient from 30% B to 50% B at 31 minutes, then back down to 6% B at 32 minutes. This gradient is followed by a 10 minute post run at 6% solvent B. The total time of the elution was 42 minutes.

Pooled fractions P16, P24, and P29 were diluted 1:50 and eluted based on the HPLC protocol developed for the crude elderberry elution.

RESULTS

Extraction of Elderberry Samples

Elderberry extraction of Column #1 by column chromatography yielded 111 individual 14.5 mL solvent-containing samples. As the mobile phase decreased in polarity, the collection rate of eluting samples also decreased, eluting off of the column at a slower pace. The first mobile phase run through the column (100% water/0% methanol + 0.01*N* HCl) resulted in the fastest collection time, with samples being eluted at a rate of 0.37 mL/min. The final mobile phase run through the column (0% water/100% methanol + 0.01*N* HCl) yielded the slowest fraction collection time at a rate of 0.12 mL/min (Table 1). Also, as the mobile phase decreased in polarity throughout the column elution, the colors of the samples changed in color and increased in color intensity. Collected samples in solvent varied from a pale yellow color to a dark and vibrant red. Following removal of solvent by rotary evaporation and vacuum centrifugation, the samples were re-dissolved in 0.5 mL PBS, an aqueous based solvent that does not affect cell proliferation (Rizvi, 2012). Samples eluted with increased methanol solvent were evaporated to 1-1.5mL by rotary evaporation quicker than samples eluted with high water solvent concentration (Table 2). The final sample colors ranged from pastel yellow to dark purple, including oranges, pinks, and reds in between.

Table 1. The collection times for elutants resolved by column chromatography using prepared standard elderberry powder (Column #1).

Mobile Phase Composition	Fraction Numbers	Collection Rate (mL/min)
100% water/0% Methanol	1-5	0.37
90% water/10% Methanol	6-16	0.33
80% water/20% Methanol	17-27	0.31
70% water/30% Methanol	28-38	0.28
60% water/40% Methanol	39-49	0.25
50% water/50% Methanol	50-60	0.21
40% water/60% Methanol	61-70	0.20
30% water/70% Methanol	71-80	0.18
20% water/80% Methanol	81-91	0.17
10% water/90% Methanol	92-101	0.14
0% water/100% Methanol	102-111	0.12

Table 2. The evaporation times for elutants resolved from column chromatography to 1-1.5 mL by rotary evaporator (Column #1).

Mobile Phase Composition	Fraction Numbers	Evaporation Time to 1-1.5 mL (minutes)
100% water/0% Methanol	1-5	21.50
90% water/10% Methanol	6-16	20.50
80% water/20% Methanol	17-27	19.00
70% water/30% Methanol	28-38	18.25
60% water/40% Methanol	39-49	16.00
50% water/50% Methanol	50-60	15.50
40% water/60% Methanol	61-70	15.00
30% water/70% Methanol	71-80	13.00
20% water/80% Methanol	81-91	12.25
10% water/90% Methanol	92-101	10.50
0% water/100% Methanol	102-111	9.25

Column #2 yielded 115 individual 14.5 mL solvent-containing samples. Identical to Column #1, mobile phases with higher concentrations of methanol (less polar) decreased the collection rate of solvent-containing samples. The first mobile phase run

through the column (100% water/0% methanol + 0.01*N* HCl) resulted in the fastest collection time, with samples being eluted at a rate of 0.39 mL/min. The final mobile phase run through the column (0% water/100% methanol + 0.01*N* HCl) yielded the slowest sample collection time at a rate of 0.13 mL/min (Table 3). The colors of the samples changed in color and increased in color intensity with decreasing polarity of mobile phases, similar to Column #1. Samples were eluted and completely dried using a rotary evaporator, and evaporation times increased as solvent methanol concentration increased (Table 4). The final sample colors followed the same range as those eluted and evaporated from Column #1.

Table 3. The collection times for elutants resolved by column chromatography using prepared standard elderberry powder (Column #2).

Mobile Phase Composition	**Fraction Numbers**	**Collection Rate (mL/min)**
100% water/0% Methanol	1-6	0.39
90% water/10% Methanol	7-17	0.36
80% water/20% Methanol	18-28	0.34
70% water/30% Methanol	29-39	0.31
60% water/40% Methanol	40-50	0.28
50% water/50% Methanol	51-61	0.27
40% water/60% Methanol	62-72	0.24
30% water/70% Methanol	73-83	0.21
20% water/80% Methanol	84-94	0.17
10% water/90% Methanol	95-105	0.13
0% water/100% Methanol	106-115	0.13

Table 4. The complete evaporation times for elutants resolved from column chromatography by rotary evaporator (Column #2).

Mobile Phase Composition	**Fraction Numbers**	**Complete Evaporation Time (minutes)**
100% water/0% Methanol	1-6	36.50
90% water/10% Methanol	7-17	34.25
80% water/20% Methanol	18-28	31.00
70% water/30% Methanol	29-39	28.50
60% water/40% Methanol	40-50	28.00
50% water/50% Methanol	51-61	25.25
40% water/60% Methanol	62-72	22.00
30% water/70% Methanol	73-83	19.00
20% water/80% Methanol	84-94	17.00
10% water/90% Methanol	95-105	14.50
0% water/100% Methanol	106-115	12.75

Columns #3-24 were eluted following the same column chromatography protocol, and resulted in similar sample collection rates and evaporation times as Column #1 and Column #2. Columns #3-24 were concluded after addition of the 20% water/80% methanol + 0.01*N* HCl, because individual samples beyond elution by these mobile phases were not of interest for future study. Between 87 and 93 individual solvent-containing samples were collected from each column. The collection rates and evaporation times for Columns #3-24 were averaged and recorded in Table 5 and 6.

Table 5. The average collection times for elutants resolved by column chromatography using prepared standard elderberry powder (Column #3-24).

Mobile Phase Composition	Collection Rate (mL/min)
100% water/0% Methanol	0.46
90% water/10% Methanol	0.41
80% water/20% Methanol	0.38
70% water/30% Methanol	0.35
60% water/40% Methanol	0.33
50% water/50% Methanol	0.31
40% water/60% Methanol	0.30
30% water/70% Methanol	0.30
20% water/80% Methanol	0.28
10% water/90% Methanol	0.25
0% water/100% Methanol	0.24

Table 6. The average complete evaporation times for elutants resolved from column chromatography by rotary evaporator (Column #3-24).

Mobile Phase Composition	Complete Evaporation Time (minutes)
100% water/0% Methanol	40.50
90% water/10% Methanol	39.25
80% water/20% Methanol	36.50
70% water/30% Methanol	33.25
60% water/40% Methanol	29.00
50% water/50% Methanol	25.25
40% water/60% Methanol	21.00
30% water/70% Methanol	18.50
20% water/80% Methanol	16.75
10% water/90% Methanol	14.00
0% water/100% Methanol	13.75

Tumor Cell Proliferation Assays with Separated Elderberry Samples

The MeWo cell proliferation assay with the samples isolated from column chromatography, rotary evaporation and vacuum centrifugation demonstrated that

individual components isolated from Column #1 have tumor-suppressive effects. The assay was repeated three times and the average of the trials revealed that all of the samples suppressed tumor growth by some degree. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of control cells at 100%. When 10 µL additions were used, 102 of the 111 (91.9%) individual samples decreased proliferation of the MeWo cell line by 40% or more compared to the untreated control. 88 of the 111 individual fractions (79.2%) inhibited MeWo growth by 60% or more. The 10 mg/mL and 1 mg/mL crude elderberry treatments decreased MeWo cell proliferation by 41.8% and 8.3%, respectively from the control (Figure 4).

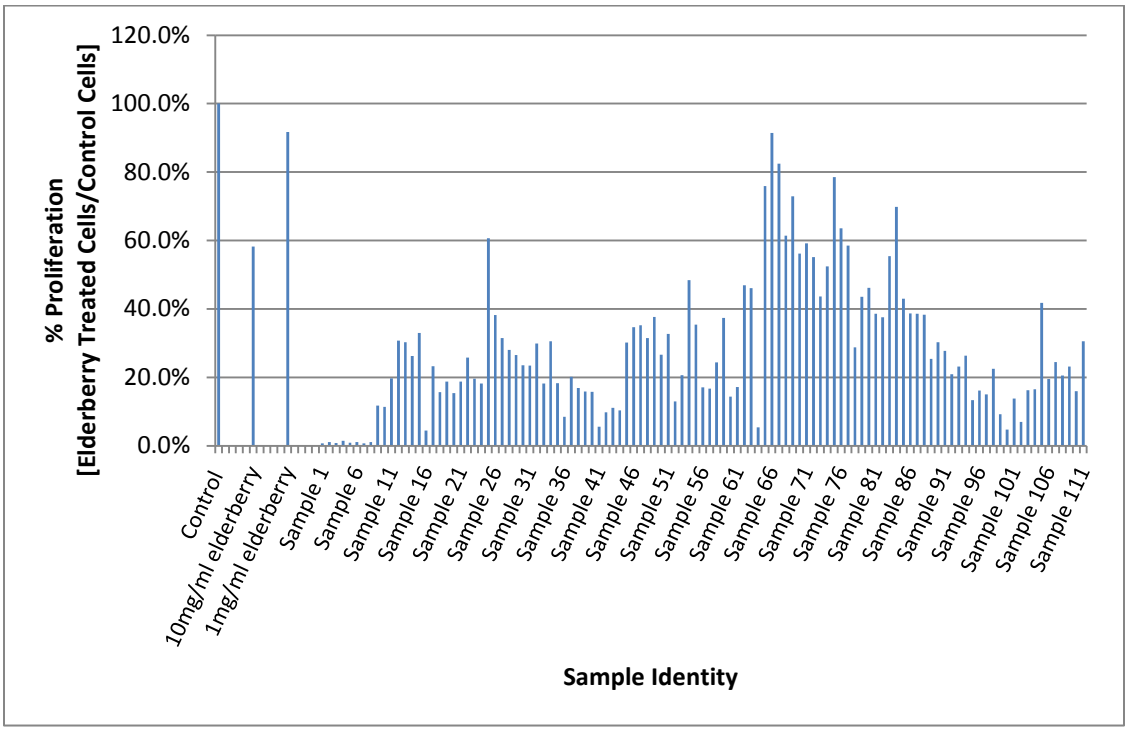


Figure 4. MeWo cell suppression by individually separated elderberry samples (n=3).

A B16-F10 murine melanoma cell proliferation assay was performed for the separated samples from Columns #2-24. Appendix Tables A1-A23 show the results for the B16-F10 cell proliferation assays. Each assay produced a similar tumor cell suppression profile, facilitating subsequent pooling of active samples into pooled fractions. The B16-F10 cell proliferation assays for Column #23 and #24 start at sample 45 because it was unnecessary to plate the preceding samples to develop pool 16, pool 24 and pool 29.

Pooling of Assumed Active Elderberry Samples

Based on the results obtained from the MeWo cell proliferation assay with separated fractions from Column #1, neighboring fractions with similar tumor-suppressive ability were pooled generating 39 pooled fractions (Appendix, Table A24). Assumed active fractions from Columns #2-24 were pooled together to recreate pooled fractions of interest from Column #1 (Pool 16, 24 and 29). A list of the individual fractions combined to recreate pooled fractions of interest for each column can be found in the appendix (Appendix, Table A25).

MeWo Cell Proliferation Assay with Pooled Elderberry Fractions

This assay was performed to assess the tumor-suppressive activity of Column #1 pooled fractions on MeWo cell growth. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of control cells at 100%. 20 of the 39 pooled fractions decreased MeWo cell proliferation by 40% or more. The 10 mg/mL and 1 mg/mL crude elderberry

treatments decreased MeWo cell proliferation by 42.5% and 37.6%, respectively from the control (Figure 5). This assay was performed twice.

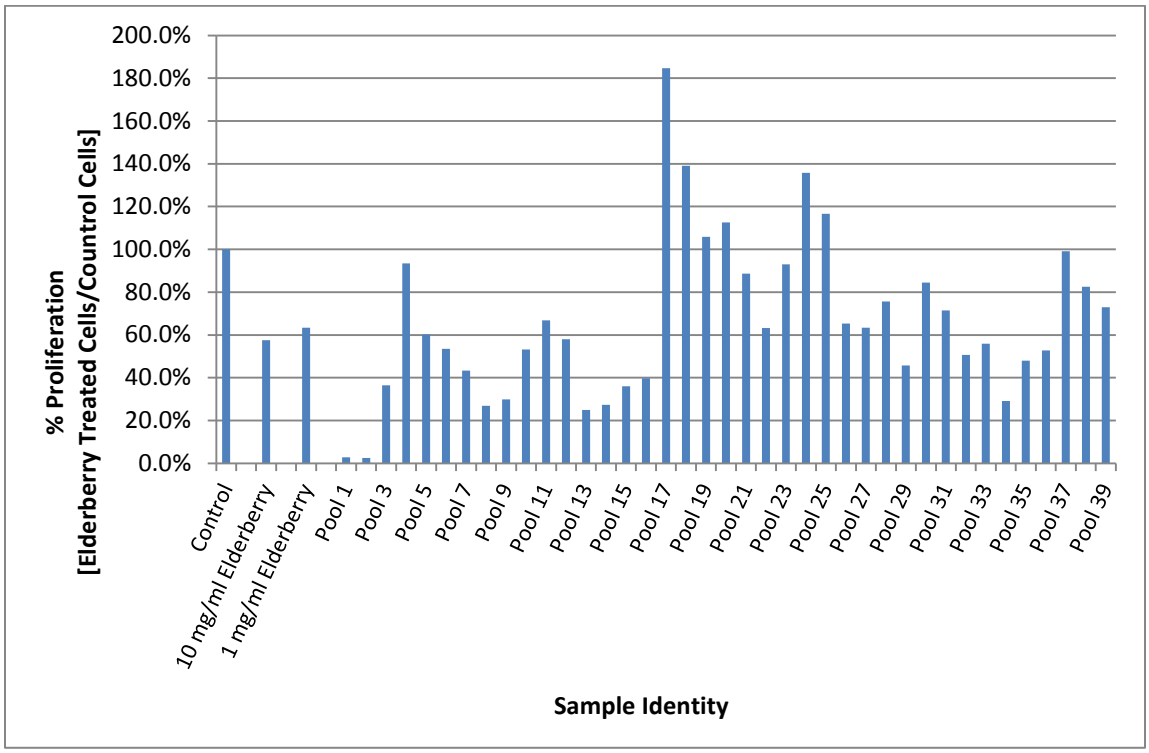


Figure 5. MeWo cell suppression by pooled elderberry fractions (n=2).

B16-F10 Cell Proliferation Assay with Pooled Elderberry Fractions

Similar to the MeWo cell proliferation assay with the Column #1 pooled fractions, this assay aimed to assess the tumor-suppressive activity of Column #1 pooled fractions on B16-F10 murine melanoma cells. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of control cells at 100%. All 39 pooled fractions decreased B16-F10 cell proliferation by 40% or more (Figure 6). The assay was repeated three

times and the average of the trials revealed that the tumor-suppressive ability of the pooled fractions was significantly different than the control. The 10 mg/mL crude elderberry treatment significantly suppressed B16-F10 cell proliferation by 51.9% and the 1 mg/mL crude elderberry treatment decreased B16-F10 cell proliferation by 19.1%, although not significantly different from the control. Column #1 pooled fractions on average inhibit the growth of B16-F10 cells more compared to MeWo cells.

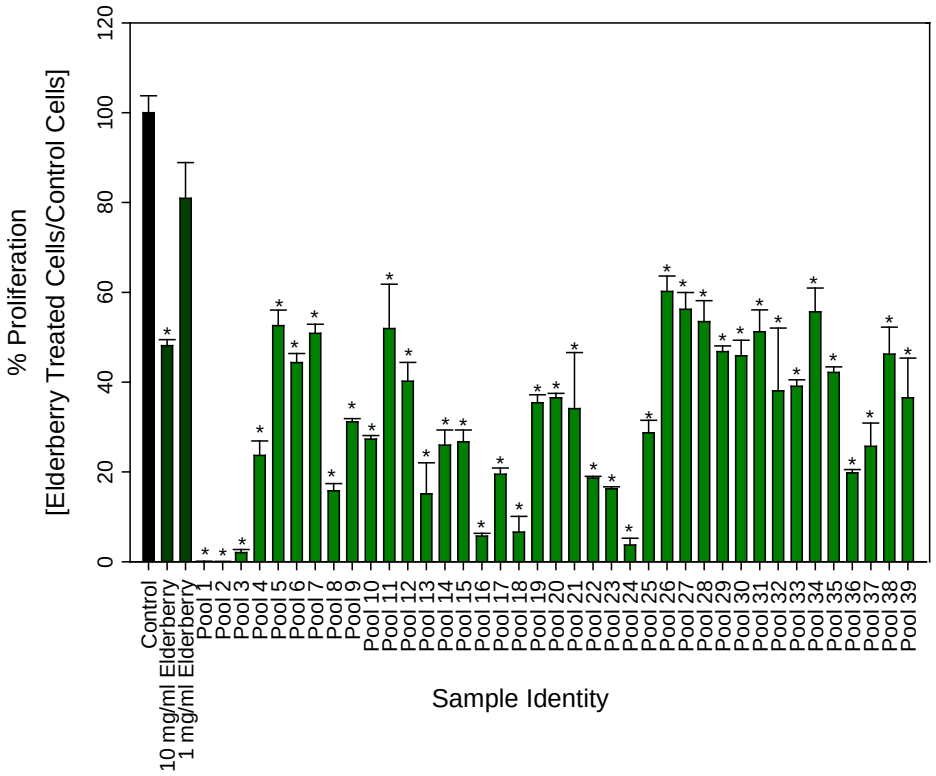


Figure 6. B16-F10 cell suppression by pooled elderberry fractions. Asterisks represent a significant proliferative difference vs. control cell growth ($p<0.05$) (n=3).

SH-SY5Y Cell Proliferation Assay with Pooled Elderberry Fractions

To assess the ubiquity of pooled fraction tumor-suppressing activity, a tumor cell proliferation assay was performed to assess the tumor-inhibiting ability of Column #1 pooled fractions on SH-SY5Y human neuroblastoma cells. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of control cells at 100%. All 39 pooled fractions decreased SH-SY5Y cell proliferation by 20% or more (Figure 7). The assay was repeated three times and the average of the trials revealed that the tumor-suppressive ability of the pooled fractions was significantly different than the control. The 10 mg/mL crude elderberry treatment significantly suppressed SH-SY5Y cell proliferation by 27.3% and the 1 mg/mL crude elderberry treatment decreased SH-SY5Y cell proliferation by 13.7%, although not significant from the control.

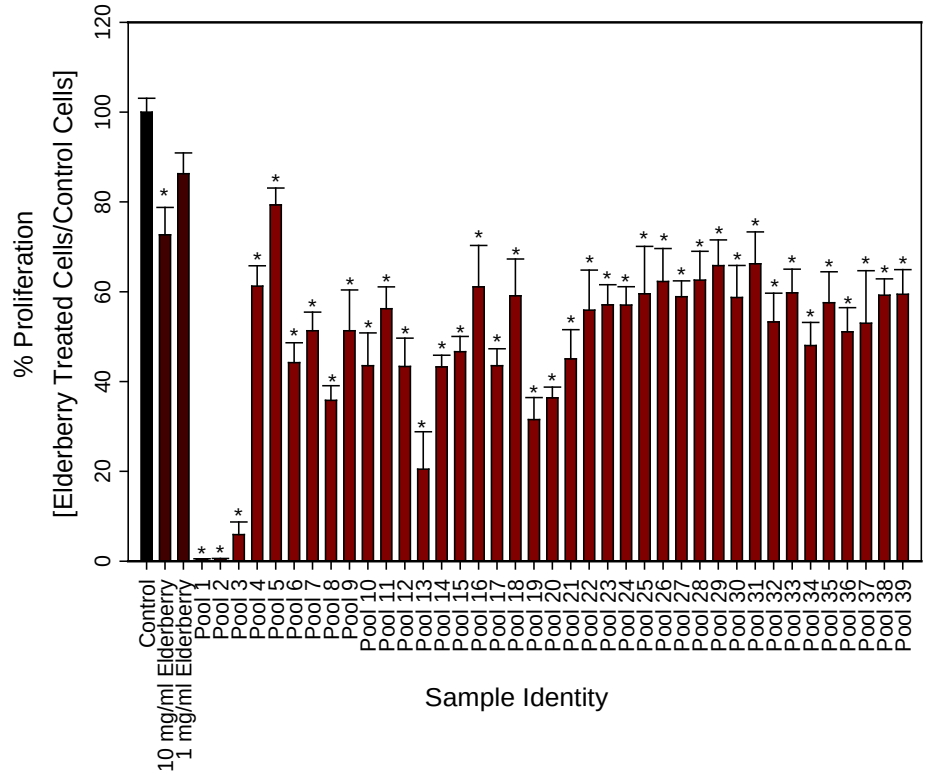


Figure 7. SH-SY5Y cell suppression by pooled elderberry fractions. Asterisks represent a significant proliferative difference vs. control cell growth ($p<0.05$) (n=3).

CHO-K1 Cell Proliferation Assay with Pooled Elderberry Fractions

To assess the growth suppressing activity of Column #1 pooled fractions on a noncancerous cell line, a cell proliferation assay was performed on CHO-K1 cells, a line of transformed noncancerous cells derived from the ovary of a Chinese hamster. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of control cells at 100%. The assay was repeated three times and the average of the trials was graphed (Figure 8). 25 of the 39 pooled fractions decreased CHO-K1 cell proliferation by a statistically significant

percent. Pooled fractions 4, 5, 12, 18, 19, 25, 26, 27, 31, 32, 33, 38 and 39 did not decrease CHO-K1 cell proliferation significantly. Both the 10 mg/mL crude elderberry treatment and the 1 mg/mL crude elderberry treatment did not significantly decrease CHO-K1 cell proliferation significantly compared to the control cells, whereas the 10 mg/mL crude elderberry did significantly suppress both B16-F10 and SH-SY5Y cells. In general, Column #1 pooled fractions that were exceptionally inhibitory in the B16-F10 and SH-SY5Y cell proliferation assays also decreased cell proliferation of CHO-K1 cells.

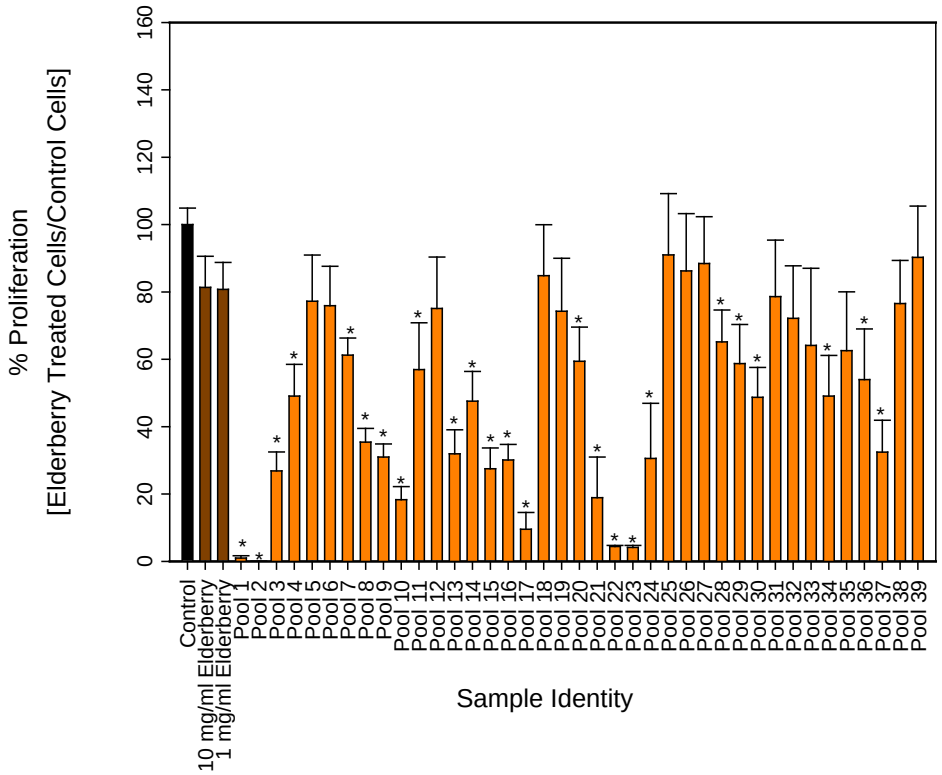


Figure 8. CHO-K1 cell suppression by pooled elderberry fractions. Asterisks represent a significant proliferative difference vs. control cell growth ($p<0.05$) (n=3).

Middle Aged Mouse Spleen Cell Proliferation Assay with Pooled Elderberry Fractions

Column #1 pooled fractions were plated with spleen cells and Con A. Spleen cells plated with Con A in the absence of pooled fractions was designated as the positive control. The Column #1 pooled elderberry fractions incubated with con A did not induce spleen cell proliferation greater than the positive control when using 10 µL treatments. When compared amongst other pooled fractions, certain pooled fractions incubated with con A elicited great stimulation of spleen cells than others (Table 4). Pools 14, 16, 17, 19 and 20 incubated with Con A were the top five pooled fractions that increased spleen cell proliferation compared to the other pooled fractions, but not more than the positive control. Each treatment was repeated in duplicate.

Table 7. Average middle aged mouse spleen cell proliferation elicited by pooled fractions incubated with Con A.

Sample Identity	CPM (Average)	Sample Identity	CPM (Average)
Control (resting)	166	20 + 2.5 µl Con A	5785
2.5 µl Con A	14151	21 + 2.5 µl Con A	2974
1 + 2.5 µl Con A	100	22 + 2.5 µl Con A	1892
2 + 2.5 µl Con A	138	23 + 2.5 µl Con A	807
3 + 2.5 µl Con A	141	24 + 2.5 µl Con A	2845
4 + 2.5 µl Con A	72	25 + 2.5 µl Con A	1513
5 + 2.5 µl Con A	885	26 + 2.5 µl Con A	71.5
6 + 2.5 µl Con A	1374	27 + 2.5 µl Con A	1307
7 + 2.5 µl Con A	1667	28 + 2.5 µl Con A	1882
8 + 2.5 µl Con A	94	29 + 2.5 µl Con A	1644
9 + 2.5 µl Con A	1370	30 + 2.5 µl Con A	1381
10 + 2.5 µl Con A	442	31 + 2.5 µl Con A	1467
11 + 2.5 µl Con A	895	32 + 2.5 µl Con A	262
12 + 2.5 µl Con A	1753	33 + 2.5 µl Con A	137
13 + 2.5 µl Con A	329	34 + 2.5 µl Con A	103
14 + 2.5 µl Con A	4274	35 + 2.5 µl Con A	78
15 + 2.5 µl Con A	1309	36 + 2.5 µl Con A	96
16 + 2.5 µl Con A	5050	37 + 2.5 µl Con A	54
17 + 2.5 µl Con A	5731	38 + 2.5 µl Con A	62
18 + 2.5 µl Con A	2601	39 + 2.5 µl Con A	64
19 + 2.5 µl Con A	5804		

Young and Old Mouse Comparative Spleen Cell Proliferation Assay with Pooled Elderberry Fractions

Column #1 pooled fractions were plated with young and old mouse spleen cells and Con A. The percent proliferation of elderberry treated cells was assessed based on the growth of the positive control cells (addition of 2.5 µL Con A) at 100%. The pooled elderberry fractions incubated with Con A did not induce spleen cell proliferation greater than the positive control when using 10 µL treatments in either the young nor senescent

mouse spleen cells. Addition of crude elderberry treatments (10 mg/mL and 1 mg/mL) with Con A elicited a stronger proliferative response in young mouse spleen cells than in senescent mouse spleen cells. Addition of pooled fractions with Con A elicited a stronger proliferative response in senescent spleen cells compared to young mouse spleen cells for all 39 pooled fractions, and the range of relative increase was between 3% and 53% (Figure 9). Each treatment was repeated in duplicate.

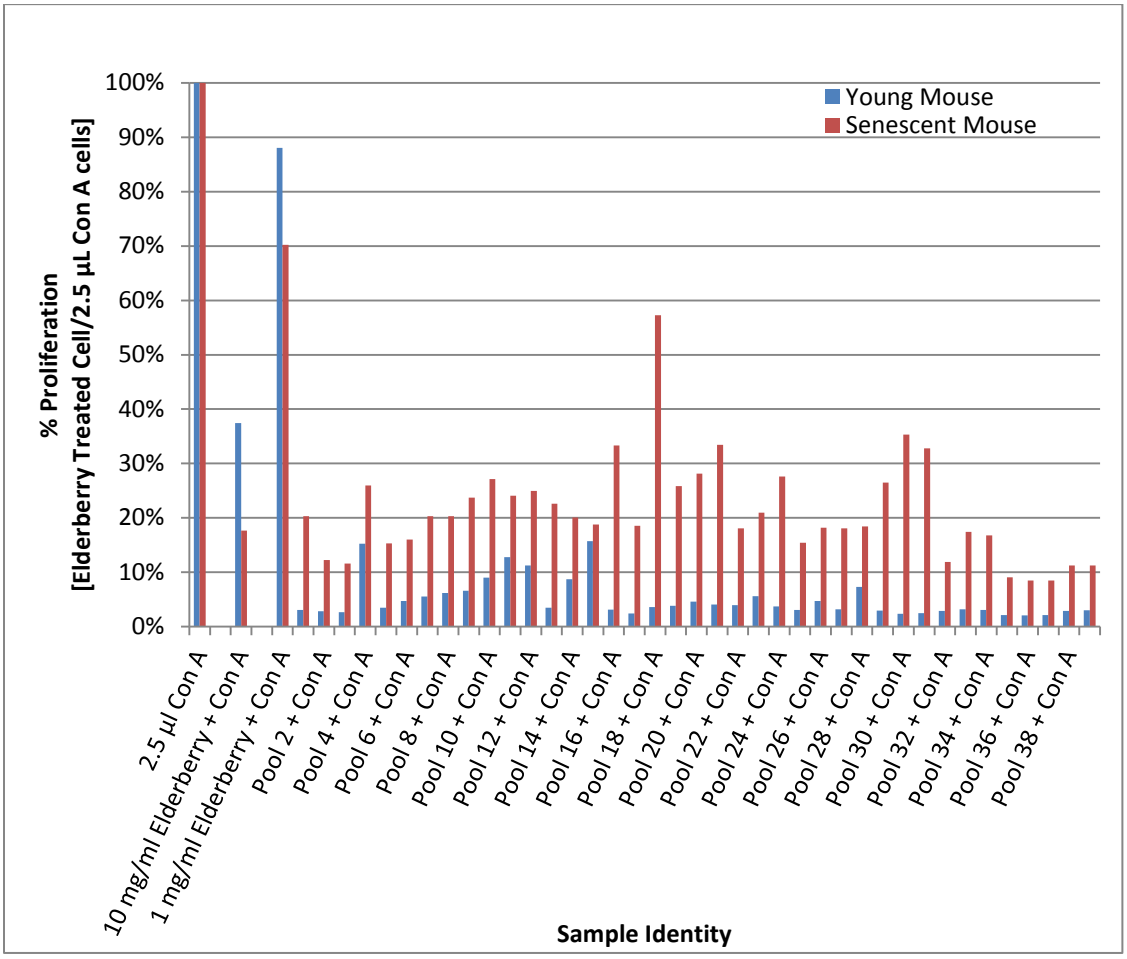


Figure 9. Senescent and young mouse spleen cell growth stimulation by pooled elderberry fractions.

Modulation of Cytokine IL-2 by Pooled Elderberry Fractions

The spleen obtained for this assay was from a young 4 month old mouse. Standard optical densities (O.D.) measuring IL-2 protein concentration obtained from the ELISA were averaged and plotted to generate a linear standard curve for IL-2 protein concentration (Appendix, Figure B3). Average O.D. readings of pooled fraction treated wells were converted into IL-2 concentrations based upon the linear formula generated from the IL-2 standard curve ($R^2 = 0.9967$). Three column #1 pooled fractions incubated with Con A increased IL-2 concentration greater than the positive control. Pool 16, Pool 26, and Pool 30 treated wells generated IL-2 concentrations of 269.86 pg/mL, 431.13 pg/mL, and 270.50 pg/mL, respectively. The spleen cells incubated with 10 mg/mL elderberry + Con A stimulated IL-2 secretion greater that the spleen cells incubated with 1 mg/mL elderberry + Con A (Figure 10).

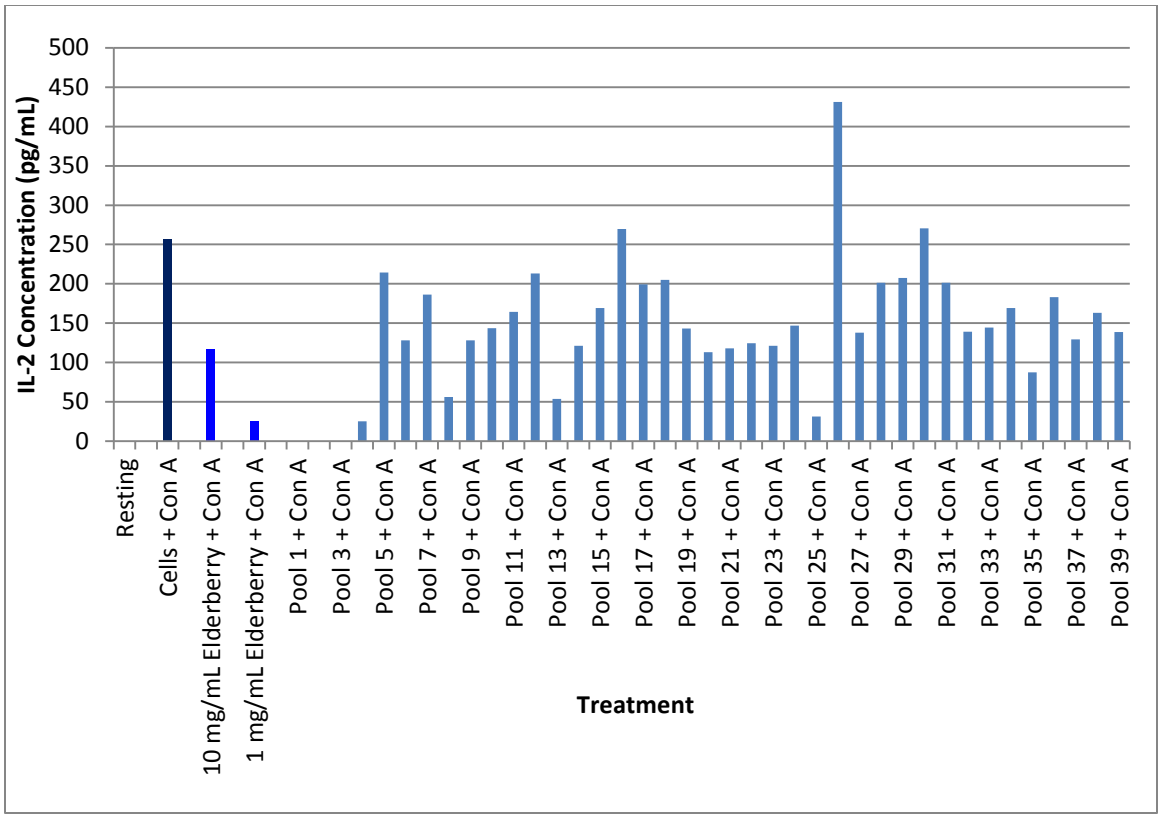


Figure 10. Stimulation of IL-2 secretion from spleen cells treated with pooled elderberry fractions.

Old Mouse *in vivo* Murine Melanoma Assay with Crude Elderberry

All mice were weighed at the start of the experiment, prior to s.c. injection of B16-F10 murine melanoma cells (or on the equivalent day) and prior to sacrifice. Over the course of the experiment the weight of each mouse did not change significantly. Senescent mouse weight averages for each group were not significantly different between each weighing day. Also, senescent mouse weight averages for all groups were not significantly different from each other (Figure 11).

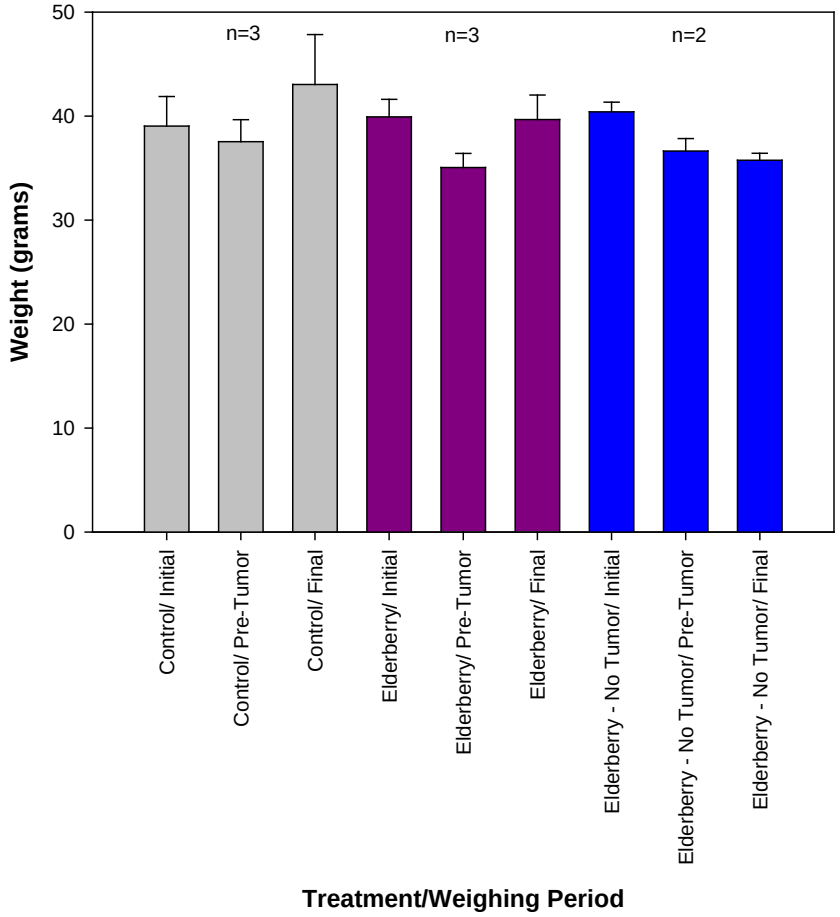


Figure 11. Body weight results for old tumor-bearing mice given control and elderberry treatments. Senescent mice were weighed on day 1 (initial), day 36 (pre-tumor) and day 50 (final).

Two senescent control mice and two senescent 10 mg/mL elderberry treated mice who were injected with B16-F10 cells, developed visible tumors which were removed after the mice were sacrificed. Elderberry treated mice developed smaller tumors in weight and volume compared to the control mice, although not significantly different. The average weight and tumor volume of the senescent control mice was 2.48g and 5.56

cm^3, respectively. The average weight and tumor volume of the senescent elderberry treated mice was 1.67g and 2.16 cm^3, respectively (Table 8).

Table 8. Average tumor weight and volume for all tumor-bearing mice in the old mouse *in vivo* murine model assay with crude elderberry.

Treatment Group	**Tumor Weight (g)**	**Tumor Volume (cm^3)**	**Average Tumor Weight (g)**	**Average Tumor Volume (cm^3)**
Control 1	3.63	8.42	2.48 ± 1.15	5.56 ± 2.86
Control 2	1.33	2.7		
Elderberry 1	1.85	2.25	1.67 ± 0.18	2.16 ± 0.09
Elderberry 2	1.48	2.07		

The spleens from all senescent mice were removed and prepared for a spleen cell proliferation assay with different concentrations of a known mitogen, Con A. The CPM measurements were averaged together amongst treatment groups and percent proliferation was determined based on the growth of resting (un-stimulated) control cells at 100%. For all treatment groups, there was a dose dependent increase in percent proliferation with increasing concentrations of mitogen up to 0.25 µg/mL Con A. There was no significant difference in percent proliferation induced by mitogen between all groups for senescent mice (Table 9).

Table 9. Senescent mouse spleen cell stimulation by Con A in the old mouse *in vivo* murine model assay with crude elderberry.

Senescent Mouse Treatment	Cell Treatment	CPM	% Proliferation
Control (n=3)	Resting	1823	100%
	0.125 µg/mL Con A	4153	265%
	0.25 µg/mL Con A	10534	816%
Elderberry (n=3)	Resting	3295	100%
	0.125 µg/mL Con A	4301	174%
	0.25 µg/mL Con A	7674	726%
Elderberry-No Tumor (n=2)	Resting	448	100%
	0.125 µg/mL Con A	646	149%
	0.25 µg/mL Con A	2262	510%

Young Mouse *in vivo* Murine Melanoma Assay with Crude Elderberry

All mice were weighed at the start of the experiment, prior to tumor injection (or on the equivalent day) and prior to sacrifice. 13 days following s.c. 1 x 10^4 B16-F10 cell injection to control and crude elderberry treated mice, no tumors were present. Mice were re-injected with 1 x 10^5 B16-F10 cells on the same flank to induce tumor growth. Injections of treatments (0.5 mL of water and 10 mg/mL elderberry) were continued 7 days after the re-injection of cancer cells. 12 days following the re-injection of cancer cells, a control mouse succumbed to cancer. The tumor was removed for weighing and measuring. All other experimental mice were sacrificed the following day and tumors were removed for weighing and measuring. Spleens were also removed and prepared for a spleen cell proliferation assay. Over the course of the experiment the weight of each young mouse gained weight, which is to be expected in young mice still growing. Mouse weight averages for the elderberry treated and elderberry – no tumor group were not significantly different between each weighing day. Between the pre-tumor injection

weighing period and the final weighing period, the average control mouse weights were significantly different. Average control mouse weights increased significantly from 24.3 grams to 27.0 grams between these two weighing periods (Figure 12).

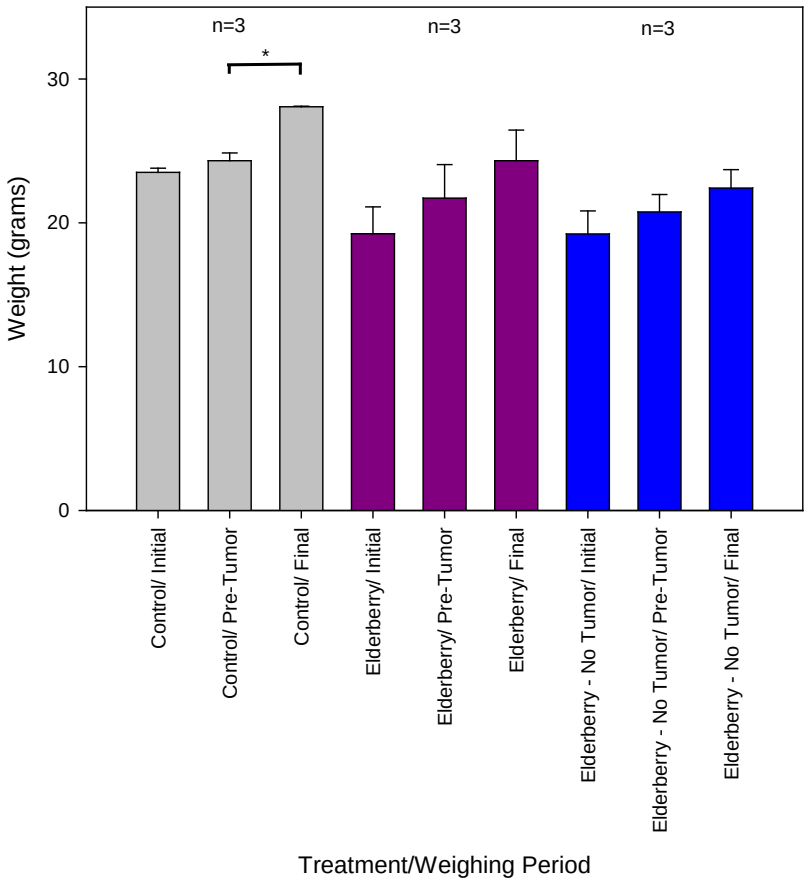


Figure 12. Body weight results for young tumor-bearing mice given control and elderberry treatments. Young mice were weighed on day 1 (initial), day 36 (pre-tumor) and day 50 (final). Asterisks represent a significant difference between two treatment/weighing period groups.

All six mice injected with B16-F10 cells developed tumors, which were removed after the mice were sacrificed. Two of the three control mouse tumors metastasized into

the peritoneal cavity, whereas all elderberry treated mouse tumors remained local. Elderberry treated tumor-bearing mice developed smaller tumors in weight and volume compared to the control mice, although not significantly different. The average weight and tumor volume of the control mice was 2.88g and 5.33 cm^3, respectively. The average weight and tumor volume of the elderberry treated tumor-bearing mice was 0.54g and 0.57 cm^3, respectively (Table 10, Figure 13).

Table 10. Average tumor weight and volume for all tumor-bearing mice in the young mouse *in vivo* murine model assay with crude elderberry.

Treatment Group	**Tumor Weight (g)**	**Tumor Volume (cm^3)**	**Average Tumor Weight (g)**	**Average Tumor Volume (cm^3)**
Control 1	0.02	0.02	2.88 ± 1.46	5.33 ± 2.66
Control 2	4.83	8.18		
Control 3	3.78	7.79		
Elderberry 1	1.42	1.53	0.54 ± 0.44	0.57 ± 0.48
Elderberry 2	0.14	0.17		
Elderberry 3	0.05	0.02		

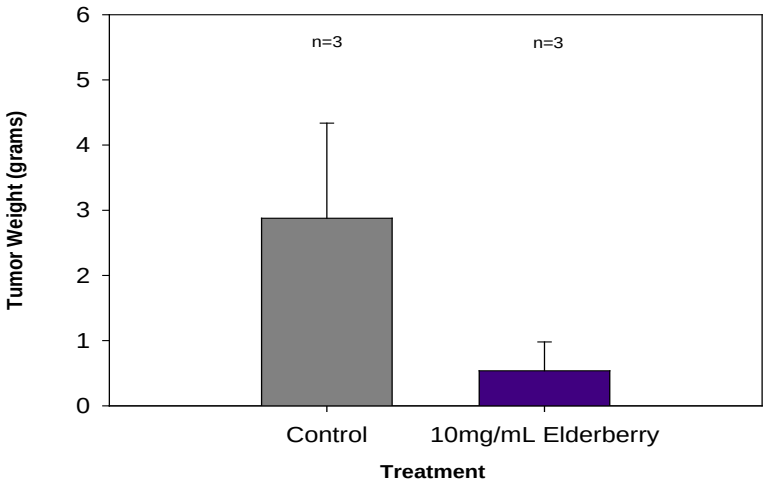


Figure 13. Tumor weight results for young tumor-bearing mice given control and elderberry treatments (n=3).

The spleens from all young mice were removed and prepared for a spleen cell proliferation assay with different concentrations of a known mitogen, Con A. The CPM measurements were averaged together amongst treatment groups and percent proliferation was determined based on the growth of resting (un-stimulated) control cells at 100%. For all treatment groups, there was a dose dependent increase in percent proliferation with increasing concentrations of mitogen up to 0.25 µg/mL Con A. There was no significant difference in percent proliferation induced by the different concentrations of mitogen between the groups (Table 11).

Table 11. Young mouse spleen cell stimulation by Con A in the young mouse *in vivo* murine model assay with crude elderberry.

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Young Mouse Treatment	**Cell Treatment**	**CPM**	**% Proliferation**
Control (n=3)	Resting	1823	100%
	0.125 µg/mL Con A	4153	1023%
	0.25 µg/mL Con A	10534	2371%
Elderberry (n=3)	Resting	3295	100%
	0.125 µg/mL Con A	4301	458%
	0.25 µg/mL Con A	7674	834%
Elderberry-No Tumor (n=3)	Resting	448	100%
	0.125 µg/mL Con A	646	548%
	0.25 µg/mL Con A	2262	2209%

Middle Aged Mouse *in vivo* Murine Melanoma Assay with Active Pooled Elderberry Fractions

All mice were weighed at the start of the experiment, prior to s.c. injection of B16-F10 murine melanoma cells (or on the equivalent day) and prior to sacrifice. Mice from the 10 mg/mL crude elderberry treatment group started to show delayed reaction

time and weakness prior to tumor cell injection. Examination of the crude elderberry extract revealed a possible fungal contamination despite filter-sterilization. Mice from P16, P24, and P29 treatment groups also exhibited behavioral and physical changes post-tumor cell injection, and were sacrificed prior to the end of the experiment.

Preliminary Identification of Active Pooled Elderberry Fractions by Heat-Induced Denaturation in a B16-F10 Cell Proliferation Assay

After examining the effect of pooled elderberry fractions on human and murine melanoma proliferation *in vitro*, as well as effect on T lymphocyte proliferation with optimal Con A *in vitro*, we identified Column #1 pooled fractions that were considered "active" in both tumor cell suppression and immune induction. Pool 7, 14, 16, and 29 were chosen as active fractions in both tumor suppressive ability and immune modulating activity, and were subjected to boiling to denature the majority of the protein components of the pooled fractions. CPM measurements from the scintillation counter were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of the control cells at 100%. All treatments, excluding the 1 mg/mL crude unheated elderberry treatment, decreased B16-F10 cell proliferation significantly from the control. When the 10 mg/mL crude elderberry treatment was heated to induce denaturing of proteins, the proliferation of B16-F10 cells decreased significantly from 76.5% to 55.7%. The 1 mg/mL crude elderberry treatment also decreased proliferation of B16-F10 cells after heating from 90.1% proliferation to 77.7% proliferation, but the difference was not significant. Of the four chosen pooled fractions of interest, pool 14, pool 16 and pool 29 showed a greater suppression of B16-F10 growth after being heated. Two of the Column

#1 pooled fractions decreased B16-F10 cell proliferation significantly after heating. When the pool 16 treatment was heated, the proliferation of B16-F10 cells decreased significantly from 29.6% to 12.7%. When the pool 29 treatment was heated, the proliferation of B16-F10 cells decreased significantly from 51.0% to 18.8% (Figure 14). Treatments were plated in triplicate and the experiment was repeated twice.

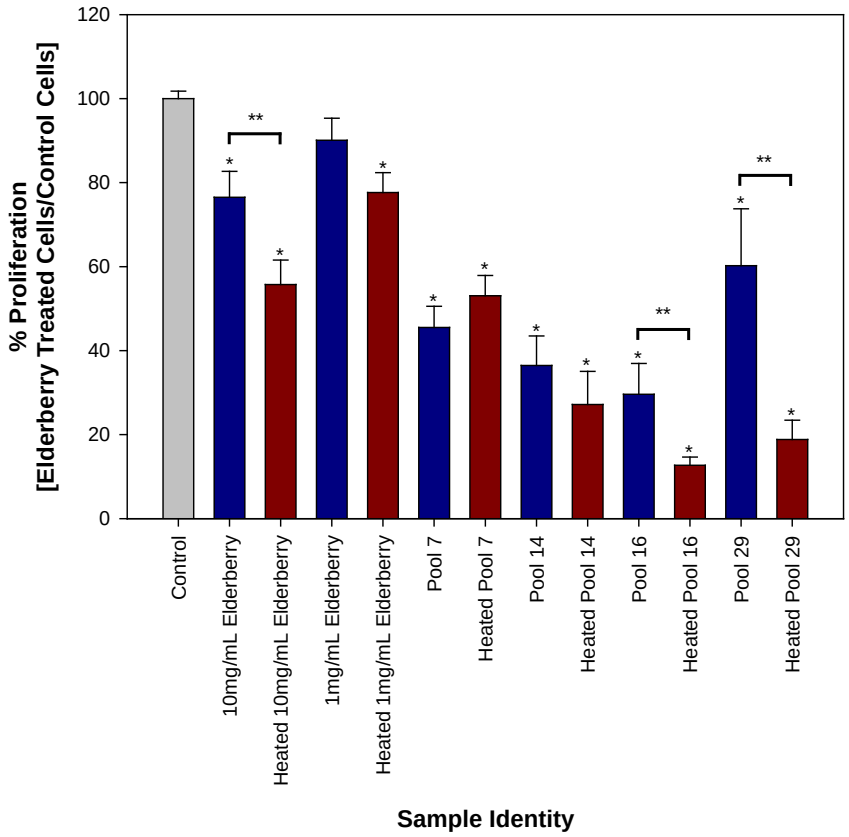

Figure 14. B16-F10 cell suppression by heated active pooled elderberry fractions. Single asterisks represent a significant proliferative difference vs. the control. Double asterisks represent a significant proliferative difference vs. the same elderberry fraction when heated ($p<0.05$) ($n=2$).

Preliminary Identification of Active Pooled Elderberry Fractions by Heat-Induced Denaturation in a Spleen Cell Proliferation Assay

Heated treatments of Pool 7, 14, 16, and 29 were also used in a spleen cell proliferation assay to determine the effect of heated pooled fractions on senescent mouse spleen cell growth. CPM counts were analyzed, and the percent proliferation of elderberry treated cells was assessed based on the growth of the positive control cells (addition of 2.5 µL optimal Con A) at 100%. All treatments, with the exception of the 1 mg/mL unheated crude elderberry treatment and the unheated pool 16 treatment, decreased spleen cell proliferation significantly from the positive control. When heated, the 10mg/mL and 1 mg/mL crude elderberry treatments did not elicit significant spleen cell proliferation differences compared to their respective unheated treatments. Of all the pooled fractions investigated, only heated pooled fraction 16 differed significantly from its unheated treatment. Heating pooled fraction 16 resulted in a decrease in spleen cell proliferation from 95.1% to 32.5% (Figure 12). Treatments were plated in triplicate and the experiment was repeated twice.

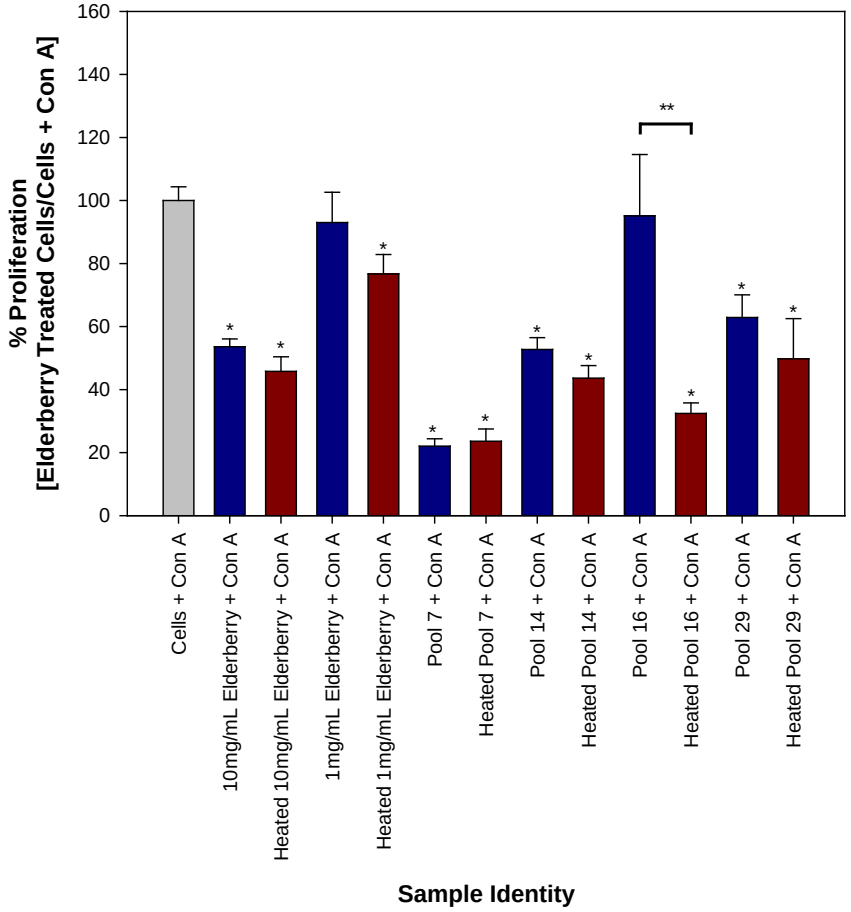

Figure 15. Senescent mouse spleen cell proliferation by heated active pooled elderberry fractions. Single asterisks represent a significant proliferative difference vs. the control. Double asterisks represent a significant proliferative difference vs. the same elderberry fraction when heated ($p<0.05$) (n=2).

Identification of Active Pooled Elderberry Fractions by Reversed-Phase High-Performance Liquid Chromatography (HPLC)

The anthocyanin peaks of elderberry were eluted in the following order: cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside (Figure 16). Comparison to the nearly identical crude elderberry chromatogram reported by Youdim et al. (2000) validated the peak identities.

Comparing the pooled fractions chromatogram peaks for P16, P24, and P29 to the crude elderberry chromatogram, it was revealed that P16 contained 85% cyanidin 3-sambubioside (Figure 17). Pool 24 contained a combination of 7% cyanidin 3-sambubioside-5-glucoside, 18% cyanidin 3,5-diglucoside, 42% cyanidin 3-sambubioside, and 31% cyanidin 3-glucoside (Figure 18). P29 contained 83% cyanidin 3-glucoside (Figure 19).

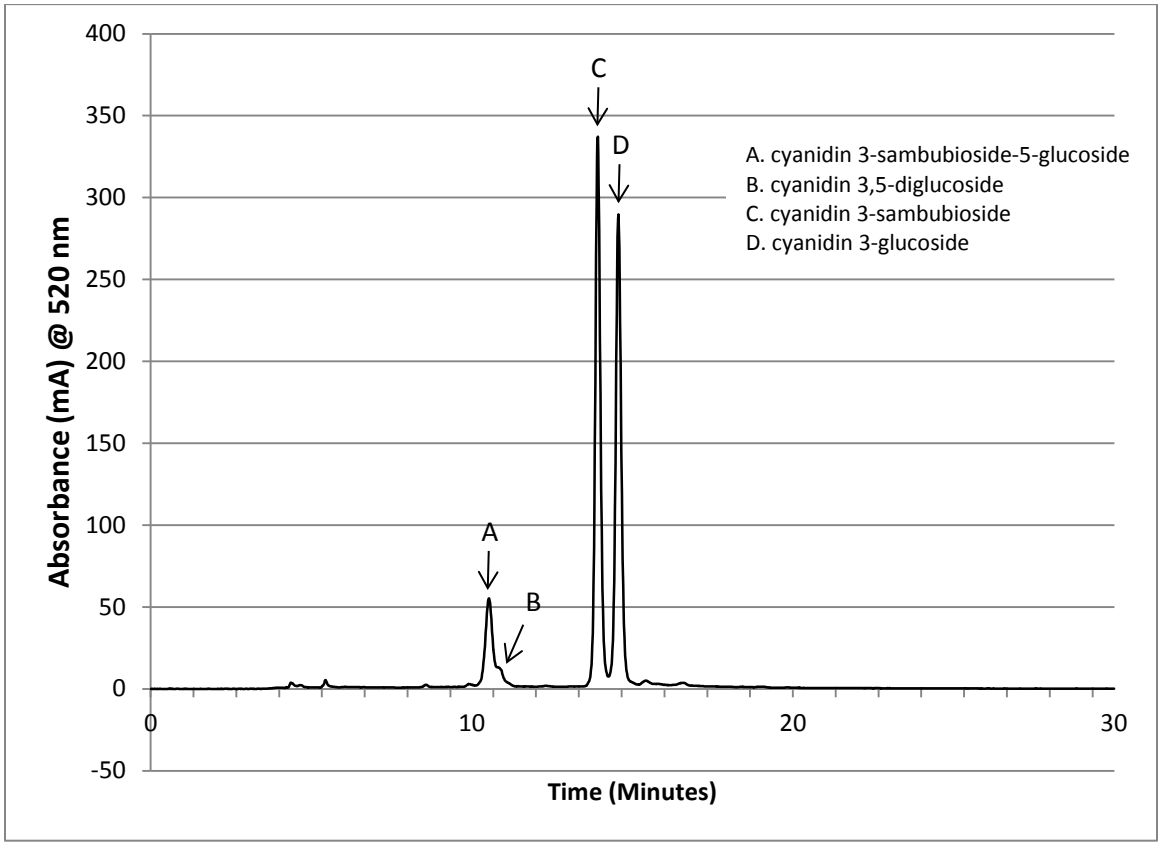

Figure 16. HPLC chromatogram of crude elderberry anthocyanins measured at 520 nm.

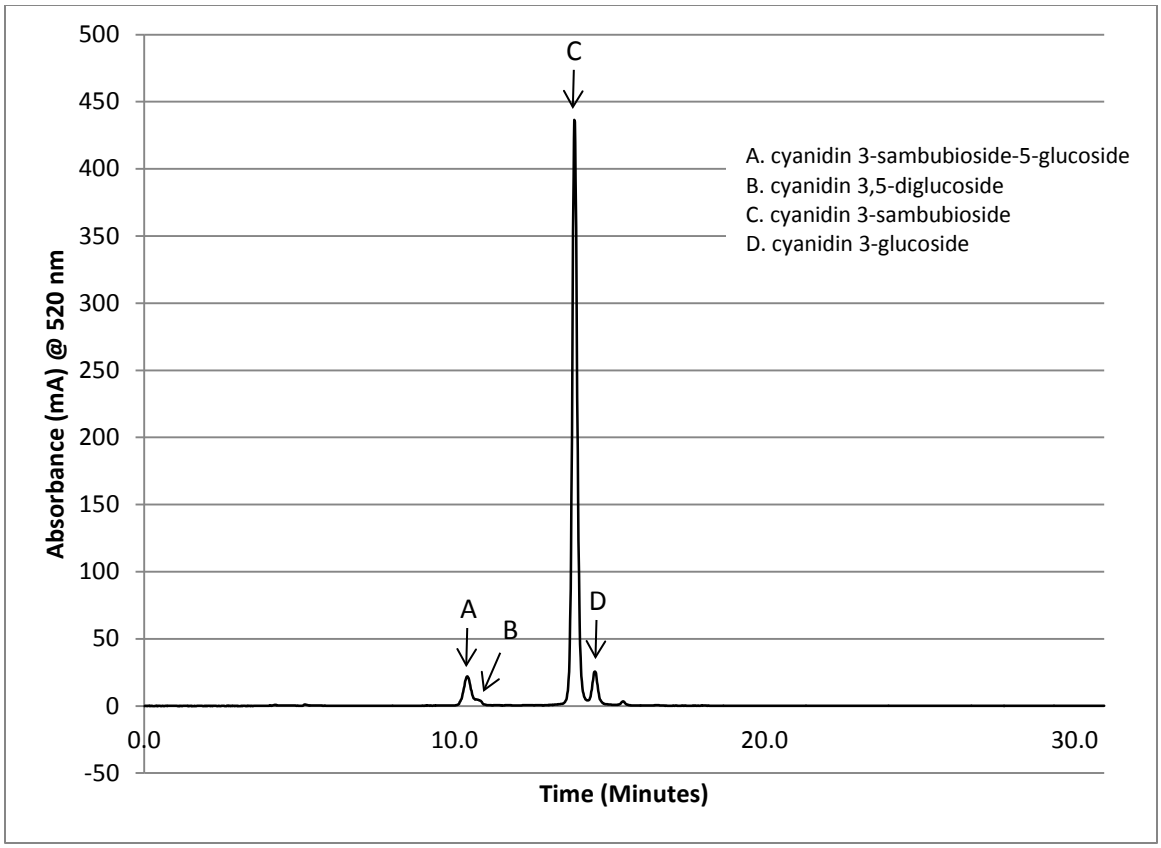

Figure 17. HPLC chromatogram of 1:50 diluted pooled fraction P16 anthocyanins measured at 520 nm.

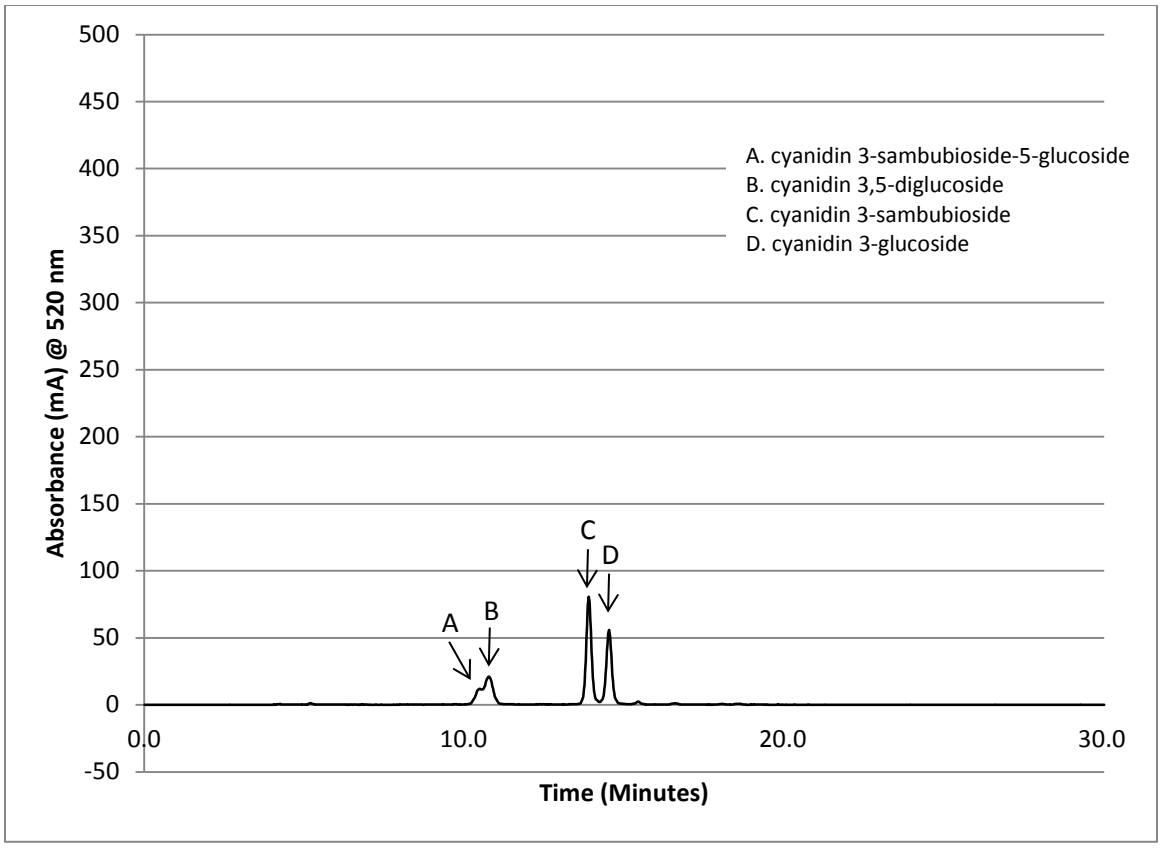

Figure 18. HPLC chromatogram of 1:50 diluted pooled fraction P24 anthocyanins measured at 520 nm.

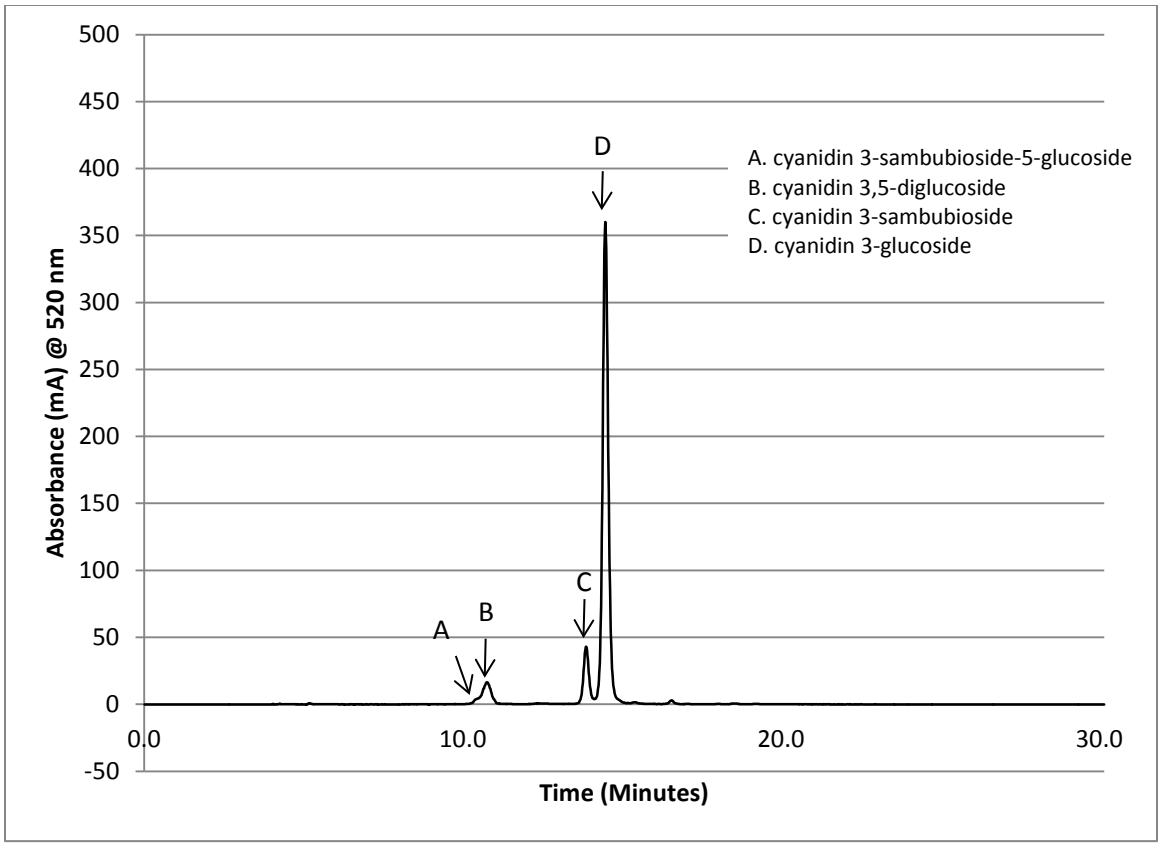

Figure 19. HPLC chromatogram of 1:50 diluted pooled fraction P29 anthocyanins measured at 520 nm.

DISCUSSION

The use of bioactive foods as naturopathic treatments for many diseases and health conditions is a growing field of interest. Phytochemicals from dark-pigmented berries have been the focus of many studies that examine natural therapeutic modulators of cardiac disease, neurological disease, viral infection, and cancers. The pathways and proteins affected by berry extracts that lead to their beneficial activity are still not well understood. It was the objective of this research to identify the active components of elderberry capable of inhibiting melanoma cell proliferation *in vitro* and *in vivo* and to determine the effect of active elderberry components on T lymphocyte proliferation and IL-2 concentration. The immune system plays an important role in tumor cell detection and removal. By inhibiting tumor cell growth directly and by increasing the immune response against cancerous cells by natural means, elderberry extracts could be a bioactive food of interest in the fight against cancer and other diseases. Also, this research aimed to chemically identify the active components of elderberry capable of eliciting this 'dual-edged' sword effect to further contribute to the growing field of alternative and complementary medicinal studies.

Crude elderberry extracts and multiple pooled elderberry fractions exhibited tumor suppressive ability in both human and murine melanoma cell lines. The B16-F10 cell proliferation assay demonstrated that all pooled elderberry fractions, as well as the 10

mg/mL crude elderberry sample, were able to significantly decrease melanoma cell growth *in vitro* compared to the control. Over half of the pooled elderberry fractions decreased B16-F10 cell proliferation more than the 10 mg/mL crude elderberry sample. These results suggest some of the separated components of elderberry are capable of inhibiting melanoma growth to a greater extent than crude elderberry treatments. All pooled fractions also significantly decreased human neuroblastoma cell proliferation, demonstrating the ubiquitous activity of the pooled fractions, suggesting that elderberry extracts may be useful in directly suppressing the growth of multiple cancers. Once again, the 10 mg/mL crude elderberry sample also significantly decreased SH-SY5Y cell proliferation, but there were multiple pooled fractions that decreased SH-SY5Y cell proliferation to a greater degree.

Interestingly, when crude and pooled elderberry fractions were added to CHO-K1 noncancerous transformed cells, only 25 of the 39 pooled fractions decreased CHO-K1 cells significantly, and both the 10 mg/mL and 1 mg/mL crude elderberry treatments did not significantly suppress transformed cell growth. Many of the pooled fractions that significantly decreased both B16-F10 and SH-SY5Y cell proliferation did not significantly suppress CHO-K1 cell growth *in vitro*, suggesting that the pooled fractions may have a more selective killing effect on cancerous cell lines compared to transformed, noncancerous cell lines.

Elderly people are at a higher risk for illnesses, such as cancer, due to immunosenescence. Extracts from dark-pigmented berries may also have immune-boosting benefits, which may indirectly lead to cancer cell suppression by augmenting proliferation of T lymphocytes and secretion of important cytokines, including IL-2.

Spleen cells from a mouse (11 months old) incubated with pooled fractions and Con A did not increase spleen cell proliferation greater than spleen cells incubated with Con A only, suggesting that 10 µL treatments may not be the optimal concentration of pooled fraction to increase spleen cell proliferation in this *in vitro* assay. When spleen cells from a young mouse (5 mounts old) and a senescent, retired breeder mouse (18 months old) were incubated with pooled fractions and Con A were compared, the results suggest that the same concentration of pooled sample increases spleen cell proliferation in senescent mice more than in young mice. The percent increase of senescent, retired breeder spleen cell proliferation compared to young mouse spleen cell proliferation ranged from a 3-53% increase. These results demonstrate that pooled elderberry fractions may be able to induce T lymphocyte proliferation in elderly individuals, who are more susceptible to disease, compared to younger individuals, who are more likely to have maintained functional immunity. Three pooled fractions incubated with Con A elicited a stronger IL-2 response from mouse spleen cells compared to spleen cells incubated with Con A only *in vitro*, suggesting that these pooled fractions may act in an additive manner with Con A to affect the growth and differentiation of T lymphocytes. It is important to note that the three pooled fractions that increased IL-2 secretion also increased the proliferation of senescent, retired breeder mouse spleen cells between 13-33% when compared to young mouse spleen cell proliferation.

To examine the tumor-suppressive ability of crude elderberry and certain pooled elderberry fractions *in vivo*, randomly sorted groups of mice were given either sterile water i.p. injections or 10 mg/mL crude elderberry or pooled elderberry i.p. injections. The initial and final weights of the mice were not significantly different from each other

across all treatment groups, suggesting that injection of elderberry samples does not have an adverse effect on mouse weight and health. On average, the tumors dissected from tumor-bearing senescent control mice were larger than the tumors dissected from tumor-bearing senescent elderberry treated mice. Young mice were used in a second *in vivo* experiment and the tumors from tumor-bearing young control mice were on average larger than those dissected from tumor-bearing young mice given crude elderberry treatments. The increase in body weight of control mice pre-tumor injection and before sacrifice was significant, supporting the quantitative analysis of control mouse tumor weights. Also, 67% of young control mouse tumors metastasized into the peritoneal cavity, whereas all crude elderberry treatment mouse tumors remained local, suggesting that crude elderberry treatment may also decrease risk of tumor metastasis.

In the murine *in vivo* experiment using pooled fractions P16, P24 and P29, mice treated with pooled fractions died before the end of the experiment, suggesting that the pooled fractions collection directly from the columns were too concentrated to be used in 0.5 mL injection volumes. This result supports the idea that the pooled fractions from the column have more potent activity compared to the crude 10 mg/mL elderberry samples, and a diluted version of the pooled fractions should be used in future murine melanoma models.

Preliminary identification of active components in pooled fractions was accomplished by heating pooled fractions to denature any proteins found within the pooled fractions. It was hypothesized that any active proteins would lose their function after heating, and would not suppress B16-F10 murine melanoma proliferation significantly. All treatments regardless of heating with the exception of the 1 mg/mL

crude elderberry non-heated sample significantly decreased proliferation of B16-F10 cells *in vitro*, suggesting that the active component in these samples was not a protein. The 1 mg/mL crude elderberry sample was not expected to result in significant B16-F10 cell proliferation based on previous studies. Interestingly, heating the crude 10 mg/mL elderberry sample, as well as heating pooled fractions 16 and 29, further decreased the proliferation of B16-F10 cells significantly compared to their respective non-heated samples. Yue and Xu reported that heating anthocyanins extracted from bilberry, a similar dark-pigmented berry to elderberry, resulted in increased free radical scavenging ability (2008). Heating anthocyanins can cleave the sugar moiety from the compound, producing the corresponding anthocyanidin, which are also much more effective in tumor killing compared to their respective anthocyanin (Jing, 2006). Another possible explanation for the increased tumor-inhibiting activity of heated pooled fractions is the presence of proteins that inhibit anthocyanins. When the pooled fractions are heated, these inhibitors of anthocyanins would be denatured. It was then hypothesized that the tumor suppressive and immune-increasing activity of the pooled fractions 16 and 29 could be due to anthocyanins present in elderberry.

When the same heated pooled fractions were applied to mouse spleen cells in a proliferation assay, the non-heated crude 1 mg/mL elderberry treatment and the non-heated pool 16 treatment were the only treatments that did not significantly decrease spleen cell proliferation. These results suggest that the potency of the elderberry treatments increases upon heating, likely for the same reason tumor suppressive ability is increased. It is noted that the pool 16 non-heated treatment did not significantly affect

spleen cell proliferation, and validates further studies for the use of certain pooled fractions as immunotherapeutic agents to combat disease.

To further identify the chemical identity of the active pooled elderberry fractions, a crude elderberry sample was first analyzed by HPLC to generate a chromatogram of the four primary anthocyanins present in elderberry. P16, P24, and P29 were diluted and analyzed using the same gradient elution as the crude elderberry analysis through HPLC. P16 contained 85% cyanidin 3-sambubioside (the third peak eluted from the crude elderberry sample), and P29 contained 83% cyanidin 3-glucoside (the fourth peak eluted from the crude elderberry sample). These results support the hypothesis that many active components responsible for tumor suppression and immune cell modulation are anthocyanins in elderberry, and validate the need for further study regarding the medicinal properties of these naturally derived phytochemicals. There is still much to learn concerning the signal pathways affected in response to anthocyanin treatment, and an understanding of these interactions may lead to the identification of novel naturopathic treatments to prevent the onset of cancer and other diseases.

Future directions for this research include identifying anti-metastatic activity of pooled elderberry fractions, characterization of additional immunological and antioxidant mechanisms affected by pooled elderberry fractions, and performing additional analytical chemistry methods to confirm with certainty the presence of anthocyanins in the pooled fractions, as well as additional factors in the pooled fractions. The severity of many cancers increases dramatically after metastatic events; therefore utilization of natural factors able to suppress metastatic events may lower the risk of fatal cancer development. Identification of metastasis-suppressing pooled fractions can be achieved by analyzing

suppression of matrix metalloproteinases (MMP), proteinases whose expression is overexpressed in cancerous cells and assist in degrading the extracellular matrix, facilitating invasion of cancerous cells into adjacent tissues. Matchett et al. were able to show that flavonoids from blueberries possessed the ability to inhibit matrix metalloproteinases in human prostate cancer cells (2005).

Our studies have shown that pooled elderberry fractions directly prepared by column chromatography may be too concentrated to elicit a positive spleen cell proliferation response. Spleen cell proliferation assays utilizing dilution of the pooled elderberry fractions should be formed to determine an optimal concentration for spleen cell proliferation. There are many other immunological factors that may be affected by pooled elderberry fractions that have not yet been investigated. Studies involving other berry phytochemicals show promise in protecting cellular integrity by inhibiting VEGF receptor-2 phosphorylation (Lamy et al., 2006), and inhibiting the pro-survival function of NF-κB (Hafeez, et al., 2008). There is also a need for the examination of the disease-preventative mechanisms implemented by pooled elderberry fractions and elderberry anthocyanins. One article by Youdim et al. shows evidence that elderberry anthocyanins may be useful to prevent DNA damage by protecting against oxidative stress (2000). Further analysis of the antioxidant activity of elderberry anthocyanins may further support the use of berry extracts as a preventative strategy against the onset of multiple diseases, including cancer.

To be certain of the exact anthocyanins present in elderberry, nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) can be performed to identify functional groups present in the phytochemicals' structure by analyzing chemical shifts and

spectrum peaks, respectively. Mass spectroscopy (MS) may also be useful in differentiating anthocyanins and other phytochemicals based upon differing molecular weights. Acquisition of pure anthocyanin standards may also be used under identical HPLC gradient elution conditions, and the peaks from the consequent chromatogram could be compared to the peaks generated from pooled fraction chromatograms to validate the identity of the assumed anthocyanins.

The results of this thesis work characterize the direct tumor killing activity of pooled elderberry fractions, touch on the immune inducing effects of the pooled elderberry fractions on spleen cell proliferation in senescent mice and on IL-2 secretion, and identify the major component of active pooled elderberry fractions as anthocyanins. Specifically, cyanidin 3-sambubioside and cyanidin 3-glucoside were the anthocyanins with the most beneficial activity concerning modulation of melanoma cancer cells. Further study using the fractions separated from elderberry may validate their use in immunotherapy and natural chemotherapy, as well as support their use as a disease-preventative, naturopathic treatment for melanoma.

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APPENDICES

Appendix A: Tables

Table A1. Results for B16-F10 cell proliferation assay with Column #2 individual samples.

Sample Identity	CPM	% Proliferation (Control = 100%)	Eluting Mobile Phase
Average Control	41389	100.0%	N/A
Average 10 mg/mL Elderberry	17683	42.7%	N/A
Average 1 mg/mL Elderberry	27227	65.8%	N/A
Column #2 Sample 1	52	0.1%	0% MeOH / 100% H_2O
Column #2 Sample 2	40	0.1%	0% MeOH / 100% H_2O
Column #2 Sample 3	49	0.1%	0% MeOH / 100% H_2O
Column #2 Sample 4	68	0.2%	0% MeOH / 100% H_2O
Column #2 Sample 5	67	0.2%	0% MeOH / 100% H_2O
Column #2 Sample 6	49	0.1%	Transition
Column #2 Sample 7	70	0.2%	10% MeOH / 90% H_2O
Column #2 Sample 8	56	0.1%	10% MeOH / 90% H_2O
Column #2 Sample 9	198	0.5%	10% MeOH / 90% H_2O
Column #2 Sample 10	11626	28.1%	10% MeOH / 90% H_2O
Column #2 Sample 11	21671	52.4%	10% MeOH / 90% H_2O
Column #2 Sample 12	43429	104.9%	10% MeOH / 90% H_2O
Column #2 Sample 13	57995	140.1%	10% MeOH / 90% H_2O
Column #2 Sample 14	53455	129.2%	10% MeOH / 90% H_2O
Column #2 Sample 15	46866	113.2%	10% MeOH / 90% H_2O
Column #2 Sample 16	38163	92.2%	10% MeOH / 90% H_2O
Column #2 Sample 17	38668	93.4%	Transition
Column #2 Sample 18	42985	103.9%	20% MeOH / 80% H_2O
Column #2 Sample 19	37350	90.2%	20% MeOH / 80% H_2O
Column #2 Sample 20	34672	83.8%	20% MeOH / 80% H_2O
Column #2 Sample 21	36727	88.7%	20% MeOH / 80% H_2O
Column #2 Sample 22	38948	94.1%	20% MeOH / 80% H_2O
Column #2 Sample 23	22393	54.1%	20% MeOH / 80% H_2O
Column #2 Sample 24	18475	44.6%	20% MeOH / 80% H_2O
Column #2 Sample 25	19836	47.9%	20% MeOH / 80% H_2O
Column #2 Sample 26	16295	39.4%	20% MeOH / 80% H_2O
Column #2 Sample 27	16069	38.8%	20% MeOH / 80% H_2O
Column #2 Sample 28	16769	40.5%	transition

Table A1, continued.

Column #2 Sample 29	13401	32.4%	30% MeOH / 70% H_2O
Column #2 Sample 30	15988	38.6%	30% MeOH / 70% H_2O
Column #2 Sample 31	19102	46.2%	30% MeOH / 70% H_2O
Column #2 Sample 32	34146	82.5%	30% MeOH / 70% H_2O
Column #2 Sample 33	40363	97.5%	30% MeOH / 70% H_2O
Column #2 Sample 34	34518	83.4%	30% MeOH / 70% H_2O
Column #2 Sample 35	32376	78.2%	30% MeOH / 70% H_2O
Column #2 Sample 36	27712	67.0%	30% MeOH / 70% H_2O
Column #2 Sample 37	24175	58.4%	30% MeOH / 70% H_2O
Column #2 Sample 38	29357	70.9%	30% MeOH / 70% H_2O
Column #2 Sample 39	21396	51.7%	transition
Column #2 Sample 40	24130	58.3%	40% MeOH / 60% H_2O
Column #2 Sample 41	25978	62.8%	40% MeOH / 60% H_2O
Column #2 Sample 42	6265	15.1%	40% MeOH / 60% H_2O
Column #2 Sample 43	8005	19.3%	40% MeOH / 60% H_2O
Column #2 Sample 44	4613	11.1%	40% MeOH / 60% H_2O
Column #2 Sample 45	3327	8.0%	40% MeOH / 60% H_2O
Column #2 Sample 46	3737	9.0%	40% MeOH / 60% H_2O
Column #2 Sample 47	3587	8.7%	40% MeOH / 60% H_2O
Column #2 Sample 48	4811	11.6%	40% MeOH / 60% H_2O
Column #2 Sample 49	1611	3.9%	40% MeOH / 60% H_2O
Column #2 Sample 50	3636	8.8%	transition
Column #2 Sample 51	4212	10.2%	50% MeOH / 50% H_2O
Column #2 Sample 52	7977	19.3%	50% MeOH / 50% H_2O
Column #2 Sample 53	14912	36.0%	50% MeOH / 50% H_2O
Column #2 Sample 54	15212	36.8%	50% MeOH / 50% H_2O
Column #2 Sample 55	10483	25.3%	50% MeOH / 50% H_2O
Column #2 Sample 56	4370	10.6%	50% MeOH / 50% H_2O
Column #2 Sample 57	19505	47.1%	50% MeOH / 50% H_2O
Column #2 Sample 58	8314	20.1%	50% MeOH / 50% H_2O
Column #2 Sample 59	7547	18.2%	50% MeOH / 50% H_2O
Column #2 Sample 60	5615	13.6%	50% MeOH / 50% H_2O
Column #2 Sample 61	13976	33.8%	transition

Table A1, continued.

Column #2 Sample 62	6791	16.4%	60% MeOH / 40% H_2O
Column #2 Sample 63	9372	22.6%	60% MeOH / 40% H_2O
Column #2 Sample 64	8700	21.0%	60% MeOH / 40% H_2O
Column #2 Sample 65	5255	12.7%	60% MeOH / 40% H_2O
Column #2 Sample 66	5581	13.5%	60% MeOH / 40% H_2O
Column #2 Sample 67	5857	14.2%	60% MeOH / 40% H_2O
Column #2 Sample 68	6547	15.8%	60% MeOH / 40% H_2O
Column #2 Sample 69	3245	7.8%	60% MeOH / 40% H_2O
Column #2 Sample 70	4334	10.5%	60% MeOH / 40% H_2O
Column #2 Sample 71	8620	20.8%	60% MeOH / 40% H_2O
Column #2 Sample 72	9938	24.0%	transition
Column #2 Sample 73	7389	17.9%	70% MeOH / 30% H_2O
Column #2 Sample 74	7646	18.5%	70% MeOH / 30% H_2O
Column #2 Sample 75	2927	7.1%	70% MeOH / 30% H_2O
Column #2 Sample 76	10150	24.5%	70% MeOH / 30% H_2O
Column #2 Sample 77	11414	27.6%	70% MeOH / 30% H_2O
Column #2 Sample 78	9093	22.0%	70% MeOH / 30% H_2O
Column #2 Sample 79	5901	14.3%	70% MeOH / 30% H_2O
Column #2 Sample 80	7182	17.4%	70% MeOH / 30% H_2O
Column #2 Sample 81	10759	26.0%	70% MeOH / 30% H_2O
Column #2 Sample 82	20702	50.0%	70% MeOH / 30% H_2O
Column #2 Sample 83	7671	18.5%	transition
Column #2 Sample 84	9516	23.0%	80% MeOH / 20% H_2O
Column #2 Sample 85	8466	20.5%	80% MeOH / 20% H_2O
Column #2 Sample 86	9052	21.9%	80% MeOH / 20% H_2O
Column #2 Sample 87	9220	22.3%	80% MeOH / 20% H_2O
Column #2 Sample 88	9585	23.2%	80% MeOH / 20% H_2O
Column #2 Sample 89	9996	24.2%	80% MeOH / 20% H_2O
Column #2 Sample 90	7352	17.8%	80% MeOH / 20% H_2O
Column #2 Sample 91	8670	20.9%	80% MeOH / 20% H_2O
Column #2 Sample 92	7188	17.4%	80% MeOH / 20% H_2O
Column #2 Sample 93	8284	20.0%	80% MeOH / 20% H_2O
Column #2 Sample 94	7498	18.1%	transition

Table A1, continued.

Column #2 Sample 95	6104	14.7%	90% MeOH / 10% H_2O
Column #2 Sample 96	6360	15.4%	90% MeOH / 10% H_2O
Column #2 Sample 97	5324	12.9%	90% MeOH / 10% H_2O
Column #2 Sample 98	6652	16.1%	90% MeOH / 10% H_2O
Column #2 Sample 99	5639	13.6%	90% MeOH / 10% H_2O
Column #2 Sample 100	6683	16.1%	90% MeOH / 10% H_2O
Column #2 Sample 101	6656	16.1%	90% MeOH / 10% H_2O
Column #2 Sample 102	2847	6.9%	90% MeOH / 10% H_2O
Column #2 Sample 103	6500	15.7%	90% MeOH / 10% H_2O
Column #2 Sample 104	7488	18.1%	90% MeOH / 10% H_2O
Column #2 Sample 105	6958	16.8%	transition
Column #2 Sample 106	6970	16.8%	100% MeOH / 0% H_2O
Column #2 Sample 107	4894	11.8%	100% MeOH / 0% H_2O
Column #2 Sample 108	7735	18.7%	100% MeOH / 0% H_2O
Column #2 Sample 109	5634	13.6%	100% MeOH / 0% H_2O
Column #2 Sample 110	1370	3.3%	100% MeOH / 0% H_2O
Column #2 Sample 111	5136	12.4%	100% MeOH / 0% H_2O
Column #2 Sample 112	7442	18.0%	100% MeOH / 0% H_2O
Column #2 Sample 113	9571	23.1%	100% MeOH / 0% H_2O
Column #2 Sample 114	16465	39.8%	100% MeOH / 0% H_2O
Column #2 Sample 115	15239	36.8%	100% MeOH / 0% H_2O

Table A2. Results for B16-F10 cell proliferation assay with Column #3 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	48855	100.0%	N/A
Average 10 mg/mL Elderberry	31877	65.2%	N/A
Column #3 Sample 1	100	0.2%	0% MeOH / 100% H_2O
Column #3 Sample 2	69	0.1%	0% MeOH / 100% H_2O
Column #3 Sample 3	85	0.2%	0% MeOH / 100% H_2O
Column #3 Sample 4	65	0.1%	0% MeOH / 100% H_2O
Column #3 Sample 5	61	0.1%	transition

Table A2, continued.

Column #3 Sample 6	52	0.1%	10% MeOH / 90% H_2O
Column #3 Sample 7	54	0.1%	10% MeOH / 90% H_2O
Column #3 Sample 8	64	0.1%	10% MeOH / 90% H_2O
Column #3 Sample 9	244	0.5%	10% MeOH / 90% H_2O
Column #3 Sample 10	69	0.1%	10% MeOH / 90% H_2O
Column #3 Sample 11	43	0.1%	10% MeOH / 90% H_2O
Column #3 Sample 12	13649	27.9%	10% MeOH / 90% H_2O
Column #3 Sample 13	2674	5.5%	10% MeOH / 90% H_2O
Column #3 Sample 14	11562	23.7%	10% MeOH / 90% H_2O
Column #3 Sample 15	9050	18.5%	10% MeOH / 90% H_2O
Column #3 Sample 16	8006	16.4%	transition
Column #3 Sample 17	11162	22.8%	20% MeOH / 80% H_2O
Column #3 Sample 18	1659	3.4%	20% MeOH / 80% H_2O
Column #3 Sample 19	2280	4.7%	20% MeOH / 80% H_2O
Column #3 Sample 20	14223	29.1%	20% MeOH / 80% H_2O
Column #3 Sample 21	12928	26.5%	20% MeOH / 80% H_2O
Column #3 Sample 22	16241	33.2%	20% MeOH / 80% H_2O
Column #3 Sample 23	11254	23.0%	20% MeOH / 80% H_2O
Column #3 Sample 24	11069	22.7%	20% MeOH / 80% H_2O
Column #3 Sample 25	5619	11.5%	20% MeOH / 80% H_2O
Column #3 Sample 26	3316	6.8%	20% MeOH / 80% H_2O
Column #3 Sample 27	294	0.6%	transition
Column #3 Sample 28	7317	15.0%	30% MeOH / 70% H_2O
Column #3 Sample 29	8691	17.8%	30% MeOH / 70% H_2O
Column #3 Sample 30	86	0.2%	30% MeOH / 70% H_2O
Column #3 Sample 31	8667	17.7%	30% MeOH / 70% H_2O
Column #3 Sample 32	9341	19.1%	30% MeOH / 70% H_2O
Column #3 Sample 33	7536	15.4%	30% MeOH / 70% H_2O
Column #3 Sample 34	8465	17.3%	30% MeOH / 70% H_2O
Column #3 Sample 35	15733	32.2%	30% MeOH / 70% H_2O
Column #3 Sample 36	13755	28.2%	30% MeOH / 70% H_2O
Column #3 Sample 37	16576	33.9%	30% MeOH / 70% H_2O
Column #3 Sample 38	18992	38.9%	transition

Table A2, continued.

Column #3 Sample 39	19144	39.2%	40% MeOH / 60% H_2O
Column #3 Sample 40	19914	40.8%	40% MeOH / 60% H_2O
Column #3 Sample 41	21953	44.9%	40% MeOH / 60% H_2O
Column #3 Sample 42	20312	41.6%	40% MeOH / 60% H_2O
Column #3 Sample 43	17788	36.4%	40% MeOH / 60% H_2O
Column #3 Sample 44	14397	29.5%	40% MeOH / 60% H_2O
Column #3 Sample 45	5547	11.4%	40% MeOH / 60% H_2O
Column #3 Sample 46	6240	12.8%	40% MeOH / 60% H_2O
Column #3 Sample 47	7212	14.8%	40% MeOH / 60% H_2O
Column #3 Sample 48	9224	18.9%	40% MeOH / 60% H_2O
Column #3 Sample 49	3917	8.0%	transition
Column #3 Sample 50	7479	15.3%	50% MeOH / 50% H_2O
Column #3 Sample 51	6723	13.8%	50% MeOH / 50% H_2O
Column #3 Sample 52	7130	14.6%	50% MeOH / 50% H_2O
Column #3 Sample 53	2007	4.1%	50% MeOH / 50% H_2O
Column #3 Sample 54	4960	10.2%	50% MeOH / 50% H_2O
Column #3 Sample 55	15970	32.7%	50% MeOH / 50% H_2O
Column #3 Sample 56	11037	22.6%	50% MeOH / 50% H_2O
Column #3 Sample 57	11154	22.8%	50% MeOH / 50% H_2O
Column #3 Sample 58	9427	19.3%	50% MeOH / 50% H_2O
Column #3 Sample 59	15286	31.3%	50% MeOH / 50% H_2O
Column #3 Sample 60	9966	20.4%	transition
Column #3 Sample 61	7618	15.6%	60% MeOH / 40% H_2O
Column #3 Sample 62	7290	14.9%	60% MeOH / 40% H_2O
Column #3 Sample 63	7004	14.3%	60% MeOH / 40% H_2O
Column #3 Sample 64	8185	16.8%	60% MeOH / 40% H_2O
Column #3 Sample 65	1425	2.9%	60% MeOH / 40% H_2O
Column #3 Sample 66	5005	10.2%	60% MeOH / 40% H_2O
Column #3 Sample 67	5655	11.6%	60% MeOH / 40% H_2O
Column #3 Sample 68	63	0.1%	60% MeOH / 40% H_2O
Column #3 Sample 69	1906	3.9%	60% MeOH / 40% H_2O
Column #3 Sample 70	5717	11.7%	60% MeOH / 40% H_2O
Column #3 Sample 71	3692	7.6%	transition

Table A2, continued.

Column #3 Sample 72	2402	4.9%	70% MeOH / 30% H_2O
Column #3 Sample 73	3349	6.9%	70% MeOH / 30% H_2O
Column #3 Sample 74	3425	7.0%	70% MeOH / 30% H_2O
Column #3 Sample 75	13975	28.6%	70% MeOH / 30% H_2O
Column #3 Sample 76	5787	11.8%	70% MeOH / 30% H_2O
Column #3 Sample 77	7543	15.4%	70% MeOH / 30% H_2O
Column #3 Sample 78	9988	20.4%	70% MeOH / 30% H_2O
Column #3 Sample 79	7923	16.2%	70% MeOH / 30% H_2O
Column #3 Sample 80	9032	18.5%	70% MeOH / 30% H_2O
Column #3 Sample 81	16001	32.8%	70% MeOH / 30% H_2O
Column #3 Sample 82	9316	19.1%	transition
Column #3 Sample 83	11196	22.9%	80% MeOH / 20% H_2O
Column #3 Sample 84	646	1.3%	80% MeOH / 20% H_2O
Column #3 Sample 85	13387	27.4%	80% MeOH / 20% H_2O
Column #3 Sample 86	9133	18.7%	80% MeOH / 20% H_2O
Column #3 Sample 87	9703	19.9%	80% MeOH / 20% H_2O
Column #3 Sample 88	13082	26.8%	80% MeOH / 20% H_2O
Column #3 Sample 89	11094	22.7%	80% MeOH / 20% H_2O
Column #3 Sample 90	11020	22.6%	80% MeOH / 20% H_2O
Column #3 Sample 91	12612	25.8%	80% MeOH / 20% H_2O

Table A3. Results for B16-F10 cell proliferation assay with Column #4 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	36128	100.0%	N/A
Average 10 mg/mL Elderberry	12873	35.6%	N/A
Column #4 Sample 1	75	0.2%	0% MeOH / 100% H_2O
Column #4 Sample 2	70	0.2%	0% MeOH / 100% H_2O
Column #4 Sample 3	68	0.2%	0% MeOH / 100% H_2O
Column #4 Sample 4	75	0.2%	0% MeOH / 100% H_2O
Column #4 Sample 5	42	0.1%	0% MeOH / 100% H_2O
Column #4 Sample 6	35	0.1%	0% MeOH / 100% H_2O

Table A3, continued.

Column #4 Sample 7	52	0.1%	transition
Column #4 Sample 8	51	0.1%	10% MeOH / 90% H_2O
Column #4 Sample 9	67	0.2%	10% MeOH / 90% H_2O
Column #4 Sample 10	54	0.1%	10% MeOH / 90% H_2O
Column #4 Sample 11	58	0.2%	10% MeOH / 90% H_2O
Column #4 Sample 12	37236	103.1%	10% MeOH / 90% H_2O
Column #4 Sample 13	2564	7.1%	10% MeOH / 90% H_2O
Column #4 Sample 14	440	1.2%	10% MeOH / 90% H_2O
Column #4 Sample 15	660	1.8%	10% MeOH / 90% H_2O
Column #4 Sample 16	98	0.3%	10% MeOH / 90% H_2O
Column #4 Sample 17	35767	99.0%	10% MeOH / 90% H_2O
Column #4 Sample 18	206	0.6%	transition
Column #4 Sample 19	2019	5.6%	20% MeOH / 80% H_2O
Column #4 Sample 20	7177	19.9%	20% MeOH / 80% H_2O
Column #4 Sample 21	11739	32.5%	20% MeOH / 80% H_2O
Column #4 Sample 22	14218	39.4%	20% MeOH / 80% H_2O
Column #4 Sample 23	4583	12.7%	20% MeOH / 80% H_2O
Column #4 Sample 24	2460	6.8%	20% MeOH / 80% H_2O
Column #4 Sample 25	18601	51.5%	20% MeOH / 80% H_2O
Column #4 Sample 26	23554	65.2%	20% MeOH / 80% H_2O
Column #4 Sample 27	21861	60.5%	20% MeOH / 80% H_2O
Column #4 Sample 28	13477	37.3%	20% MeOH / 80% H_2O
Column #4 Sample 29	16261	45.0%	transition
Column #4 Sample 30	16639	46.1%	30% MeOH / 70% H_2O
Column #4 Sample 31	11518	31.9%	30% MeOH / 70% H_2O
Column #4 Sample 32	10965	30.4%	30% MeOH / 70% H_2O
Column #4 Sample 33	10781	29.8%	30% MeOH / 70% H_2O
Column #4 Sample 34	1584	4.4%	30% MeOH / 70% H_2O
Column #4 Sample 35	6532	18.1%	30% MeOH / 70% H_2O
Column #4 Sample 36	28360	78.5%	30% MeOH / 70% H_2O
Column #4 Sample 37	25265	69.9%	30% MeOH / 70% H_2O
Column #4 Sample 38	10496	29.1%	30% MeOH / 70% H_2O
Column #4 Sample 39	21896	60.6%	30% MeOH / 70% H_2O

Table A3, continued.

Column #4 Sample 40	27133	75.1%	transition
Column #4 Sample 41	23016	63.7%	40% MeOH / 60% H_2O
Column #4 Sample 42	21787	60.3%	40% MeOH / 60% H_2O
Column #4 Sample 43	29564	81.8%	40% MeOH / 60% H_2O
Column #4 Sample 44	22981	63.6%	40% MeOH / 60% H_2O
Column #4 Sample 45	40393	111.8%	40% MeOH / 60% H_2O
Column #4 Sample 46	33031	91.4%	40% MeOH / 60% H_2O
Column #4 Sample 47	29565	81.8%	40% MeOH / 60% H_2O
Column #4 Sample 48	30023	83.1%	40% MeOH / 60% H_2O
Column #4 Sample 49	22881	63.3%	40% MeOH / 60% H_2O
Column #4 Sample 50	22783	63.1%	40% MeOH / 60% H_2O
Column #4 Sample 51	18265	50.6%	transition
Column #4 Sample 52	19646	54.4%	50% MeOH / 50% H_2O
Column #4 Sample 53	19862	55.0%	50% MeOH / 50% H_2O
Column #4 Sample 54	16143	44.7%	50% MeOH / 50% H_2O
Column #4 Sample 55	213	0.6%	50% MeOH / 50% H_2O
Column #4 Sample 56	3524	9.8%	50% MeOH / 50% H_2O
Column #4 Sample 57	87	0.2%	50% MeOH / 50% H_2O
Column #4 Sample 58	13566	37.5%	50% MeOH / 50% H_2O
Column #4 Sample 59	15813	43.8%	50% MeOH / 50% H_2O
Column #4 Sample 60	20294	56.2%	50% MeOH / 50% H_2O
Column #4 Sample 61	23564	65.2%	transition
Column #4 Sample 62	21322	59.0%	60% MeOH / 40% H_2O
Column #4 Sample 63	21500	59.5%	60% MeOH / 40% H_2O
Column #4 Sample 64	14928	41.3%	60% MeOH / 40% H_2O
Column #4 Sample 65	17772	49.2%	60% MeOH / 40% H_2O
Column #4 Sample 66	14859	41.1%	60% MeOH / 40% H_2O
Column #4 Sample 67	17263	47.8%	60% MeOH / 40% H_2O
Column #4 Sample 68	16293	45.1%	60% MeOH / 40% H_2O
Column #4 Sample 69	14354	39.7%	60% MeOH / 40% H_2O
Column #4 Sample 70	13414	37.1%	60% MeOH / 40% H_2O
Column #4 Sample 71	12798	35.4%	60% MeOH / 40% H_2O
Column #4 Sample 72	7566	20.9%	transition

Table A3, continued.

Column #4 Sample 73	1323	3.7%	70% MeOH / 30% H_2O
Column #4 Sample 74	1091	3.0%	70% MeOH / 30% H_2O
Column #4 Sample 75	11731	32.5%	70% MeOH / 30% H_2O
Column #4 Sample 76	14640	40.5%	70% MeOH / 30% H_2O
Column #4 Sample 77	15418	42.7%	70% MeOH / 30% H_2O
Column #4 Sample 78	19787	54.8%	70% MeOH / 30% H_2O
Column #4 Sample 79	23174	64.1%	70% MeOH / 30% H_2O
Column #4 Sample 80	18114	50.1%	70% MeOH / 30% H_2O
Column #4 Sample 81	24605	68.1%	70% MeOH / 30% H_2O
Column #4 Sample 82	13562	37.5%	70% MeOH / 30% H_2O
Column #4 Sample 83	23604	65.3%	transition
Column #4 Sample 84	17434	48.3%	80% MeOH / 20% H_2O
Column #4 Sample 85	15350	42.5%	80% MeOH / 20% H_2O
Column #4 Sample 86	11827	32.7%	80% MeOH / 20% H_2O
Column #4 Sample 87	16919	46.8%	80% MeOH / 20% H_2O
Column #4 Sample 88	14823	41.0%	80% MeOH / 20% H_2O
Column #4 Sample 89	23519	65.1%	80% MeOH / 20% H_2O
Column #4 Sample 90	22277	61.7%	80% MeOH / 20% H_2O
Column #4 Sample 91	14378	39.8%	80% MeOH / 20% H_2O
Column #4 Sample 92	24157	66.9%	80% MeOH / 20% H_2O
Column #4 Sample 93	32431	89.8%	80% MeOH / 20% H_2O

Table A4. Results for B16-F10 cell proliferation assay with Column #5 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	48315	100.0%	N/A
Average 10 mg/mL Elderberry	33912	70.2%	N/A
Column #5 Sample 1	77	0.2%	0% MeOH / 100% H_2O
Column #5 Sample 2	48	0.1%	0% MeOH / 100% H_2O
Column #5 Sample 3	82	0.2%	0% MeOH / 100% H_2O
Column #5 Sample 4	80	0.2%	0% MeOH / 100% H_2O
Column #5 Sample 5	44	0.1%	0% MeOH / 100% H_2O

Table A4, continued.

Column #5 Sample 6	46	0.1%	transition
Column #5 Sample 7	32	0.1%	10% MeOH / 90% H_2O
Column #5 Sample 8	53	0.1%	10% MeOH / 90% H_2O
Column #5 Sample 9	76	0.2%	10% MeOH / 90% H_2O
Column #5 Sample 10	147	0.3%	10% MeOH / 90% H_2O
Column #5 Sample 11	27986	57.9%	10% MeOH / 90% H_2O
Column #5 Sample 12	180	0.4%	10% MeOH / 90% H_2O
Column #5 Sample 13	117	0.2%	10% MeOH / 90% H_2O
Column #5 Sample 14	7681	15.9%	10% MeOH / 90% H_2O
Column #5 Sample 15	722	1.5%	10% MeOH / 90% H_2O
Column #5 Sample 16	720	1.5%	10% MeOH / 90% H_2O
Column #5 Sample 17	1246	2.6%	transition
Column #5 Sample 18	10105	20.9%	20% MeOH / 80% H_2O
Column #5 Sample 19	17666	36.6%	20% MeOH / 80% H_2O
Column #5 Sample 20	22182	45.9%	20% MeOH / 80% H_2O
Column #5 Sample 21	22374	46.3%	20% MeOH / 80% H_2O
Column #5 Sample 22	16252	33.6%	20% MeOH / 80% H_2O
Column #5 Sample 23	18966	39.3%	20% MeOH / 80% H_2O
Column #5 Sample 24	7072	14.6%	20% MeOH / 80% H_2O
Column #5 Sample 25	14584	30.2%	20% MeOH / 80% H_2O
Column #5 Sample 26	16278	33.7%	20% MeOH / 80% H_2O
Column #5 Sample 27	14841	30.7%	20% MeOH / 80% H_2O
Column #5 Sample 28	22224	46.0%	transition
Column #5 Sample 29	14674	30.4%	30% MeOH / 70% H_2O
Column #5 Sample 30	617	1.3%	30% MeOH / 70% H_2O
Column #5 Sample 31	1631	3.4%	30% MeOH / 70% H_2O
Column #5 Sample 32	8683	18.0%	30% MeOH / 70% H_2O
Column #5 Sample 33	12052	24.9%	30% MeOH / 70% H_2O
Column #5 Sample 34	1749	3.6%	30% MeOH / 70% H_2O
Column #5 Sample 35	35387	73.2%	30% MeOH / 70% H_2O
Column #5 Sample 36	25290	52.3%	30% MeOH / 70% H_2O
Column #5 Sample 37	24141	50.0%	30% MeOH / 70% H_2O
Column #5 Sample 38	23159	47.9%	30% MeOH / 70% H_2O

Table A4, continued.

Column #5 Sample 39	29120	60.3%	transition
Column #5 Sample 40	31903	66.0%	40% MeOH / 60% H_2O
Column #5 Sample 41	30018	62.1%	40% MeOH / 60% H_2O
Column #5 Sample 42	313	0.6%	40% MeOH / 60% H_2O
Column #5 Sample 43	13872	28.7%	40% MeOH / 60% H_2O
Column #5 Sample 44	6725	13.9%	40% MeOH / 60% H_2O
Column #5 Sample 45	20648	42.7%	40% MeOH / 60% H_2O
Column #5 Sample 46	17246	35.7%	40% MeOH / 60% H_2O
Column #5 Sample 47	11460	23.7%	40% MeOH / 60% H_2O
Column #5 Sample 48	17664	36.6%	40% MeOH / 60% H_2O
Column #5 Sample 49	17138	35.5%	40% MeOH / 60% H_2O
Column #5 Sample 50	13097	27.1%	transition
Column #5 Sample 51	9958	20.6%	50% MeOH / 50% H_2O
Column #5 Sample 52	7149	14.8%	50% MeOH / 50% H_2O
Column #5 Sample 53	3593	7.4%	50% MeOH / 50% H_2O
Column #5 Sample 54	352	0.7%	50% MeOH / 50% H_2O
Column #5 Sample 55	17534	36.3%	50% MeOH / 50% H_2O
Column #5 Sample 56	22791	47.2%	50% MeOH / 50% H_2O
Column #5 Sample 57	222	0.5%	50% MeOH / 50% H_2O
Column #5 Sample 58	16631	34.4%	50% MeOH / 50% H_2O
Column #5 Sample 59	26417	54.7%	50% MeOH / 50% H_2O
Column #5 Sample 60	19664	40.7%	50% MeOH / 50% H_2O
Column #5 Sample 61	17836	36.9%	transition
Column #5 Sample 62	16880	34.9%	60% MeOH / 40% H_2O
Column #5 Sample 63	3543	7.3%	60% MeOH / 40% H_2O
Column #5 Sample 64	13138	27.2%	60% MeOH / 40% H_2O
Column #5 Sample 65	8554	17.7%	60% MeOH / 40% H_2O
Column #5 Sample 66	54	0.1%	60% MeOH / 40% H_2O
Column #5 Sample 67	8662	17.9%	60% MeOH / 40% H_2O
Column #5 Sample 68	7012	14.5%	60% MeOH / 40% H_2O
Column #5 Sample 69	4733	9.8%	60% MeOH / 40% H_2O
Column #5 Sample 70	4136	8.6%	60% MeOH / 40% H_2O
Column #5 Sample 71	7241	15.0%	60% MeOH / 40% H_2O

Table A4, continued.

Column #5 Sample 72	3198	6.6%	transition
Column #5 Sample 73	2076	4.3%	70% MeOH / 30% H_2O
Column #5 Sample 74	2774	5.7%	70% MeOH / 30% H_2O
Column #5 Sample 75	16474	34.1%	70% MeOH / 30% H_2O
Column #5 Sample 76	2828	5.9%	70% MeOH / 30% H_2O
Column #5 Sample 77	10486	21.7%	70% MeOH / 30% H_2O
Column #5 Sample 78	5097	10.5%	70% MeOH / 30% H_2O
Column #5 Sample 79	4028	8.3%	70% MeOH / 30% H_2O
Column #5 Sample 80	9730	20.1%	70% MeOH / 30% H_2O
Column #5 Sample 81	1890	3.9%	70% MeOH / 30% H_2O
Column #5 Sample 82	6088	12.6%	70% MeOH / 30% H_2O
Column #5 Sample 83	491	1.0%	transition
Column #5 Sample 84	7037	14.6%	80% MeOH / 20% H_2O
Column #5 Sample 85	1057	2.2%	80% MeOH / 20% H_2O
Column #5 Sample 86	1480	3.1%	80% MeOH / 20% H_2O
Column #5 Sample 87	97	0.2%	80% MeOH / 20% H_2O
Column #5 Sample 88	860	1.8%	80% MeOH / 20% H_2O
Column #5 Sample 89	479	1.0%	80% MeOH / 20% H_2O
Column #5 Sample 90	512	1.1%	80% MeOH / 20% H_2O
Column #5 Sample 91	1145	2.4%	80% MeOH / 20% H_2O
Column #5 Sample 92	261	0.5%	80% MeOH / 20% H_2O

Table A5. Results for B16-F10 cell proliferation assay with Column #6 individual samples.

Sample Identity	**CPM**	**%Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	47472	100.0%	N/A
Average 10 mg/mL Elderberry	26053	54.9%	N/A
Column #6 Sample 1	66	0.1%	0% MeOH / 100% H_2O
Column #6 Sample 2	73	0.2%	0% MeOH / 100% H_2O
Column #6 Sample 3	60	0.1%	0% MeOH / 100% H_2O
Column #6 Sample 4	60	0.1%	0% MeOH / 100% H_2O

Table A5, continued.

Column #6 Sample 5	61	0.1%	0% MeOH / 100% H_2O
Column #6 Sample 6	57	0.1%	transition
Column #6 Sample 7	61	0.1%	10% MeOH / 90% H_2O
Column #6 Sample 8	73	0.2%	10% MeOH / 90% H_2O
Column #6 Sample 9	112	0.2%	10% MeOH / 90% H_2O
Column #6 Sample 10	1004	2.1%	10% MeOH / 90% H_2O
Column #6 Sample 11	64	0.1%	10% MeOH / 90% H_2O
Column #6 Sample 12	63	0.1%	10% MeOH / 90% H_2O
Column #6 Sample 13	5621	11.8%	10% MeOH / 90% H_2O
Column #6 Sample 14	6056	12.8%	10% MeOH / 90% H_2O
Column #6 Sample 15	6394	13.5%	10% MeOH / 90% H_2O
Column #6 Sample 16	8497	17.9%	10% MeOH / 90% H_2O
Column #6 Sample 17	856	1.8%	transition
Column #6 Sample 18	17027	35.9%	20% MeOH / 80% H_2O
Column #6 Sample 19	12150	25.6%	20% MeOH / 80% H_2O
Column #6 Sample 20	13565	28.6%	20% MeOH / 80% H_2O
Column #6 Sample 21	11891	25.0%	20% MeOH / 80% H_2O
Column #6 Sample 22	3724	7.8%	20% MeOH / 80% H_2O
Column #6 Sample 23	1235	2.6%	20% MeOH / 80% H_2O
Column #6 Sample 24	3363	7.1%	20% MeOH / 80% H_2O
Column #6 Sample 25	1424	3.0%	20% MeOH / 80% H_2O
Column #6 Sample 26	95	0.2%	20% MeOH / 80% H_2O
Column #6 Sample 27	12295	25.9%	20% MeOH / 80% H_2O
Column #6 Sample 28	9620	20.3%	transition
Column #6 Sample 29	10880	22.9%	30% MeOH / 70% H_2O
Column #6 Sample 30	10115	21.3%	30% MeOH / 70% H_2O
Column #6 Sample 31	846	1.8%	30% MeOH / 70% H_2O
Column #6 Sample 32	7110	15.0%	30% MeOH / 70% H_2O
Column #6 Sample 33	513	1.1%	30% MeOH / 70% H_2O
Column #6 Sample 34	1434	3.0%	30% MeOH / 70% H_2O
Column #6 Sample 35	12870	27.1%	30% MeOH / 70% H_2O
Column #6 Sample 36	16047	33.8%	30% MeOH / 70% H_2O
Column #6 Sample 37	15847	33.4%	30% MeOH / 70% H_2O

Table A5, continued.

Column #6 Sample 38	94	0.2%	30% MeOH / 70% H_2O
Column #6 Sample 39	157	0.3%	transition
Column #6 Sample 40	13930	29.3%	40% MeOH / 60% H_2O
Column #6 Sample 41	18414	38.8%	40% MeOH / 60% H_2O
Column #6 Sample 42	12110	25.5%	40% MeOH / 60% H_2O
Column #6 Sample 43	6395	13.5%	40% MeOH / 60% H_2O
Column #6 Sample 44	794	1.7%	40% MeOH / 60% H_2O
Column #6 Sample 45	4639	9.8%	40% MeOH / 60% H_2O
Column #6 Sample 46	410	0.9%	40% MeOH / 60% H_2O
Column #6 Sample 47	3623	7.6%	40% MeOH / 60% H_2O
Column #6 Sample 48	1198	2.5%	40% MeOH / 60% H_2O
Column #6 Sample 49	2306	4.9%	40% MeOH / 60% H_2O
Column #6 Sample 50	821	1.7%	transition
Column #6 Sample 51	1162	2.4%	50% MeOH / 50% H_2O
Column #6 Sample 52	267	0.6%	50% MeOH / 50% H_2O
Column #6 Sample 53	712	1.5%	50% MeOH / 50% H_2O
Column #6 Sample 54	631	1.3%	50% MeOH / 50% H_2O
Column #6 Sample 55	13835	29.1%	50% MeOH / 50% H_2O
Column #6 Sample 56	6754	14.2%	50% MeOH / 50% H_2O
Column #6 Sample 57	5390	11.4%	50% MeOH / 50% H_2O
Column #6 Sample 58	5701	12.0%	50% MeOH / 50% H_2O
Column #6 Sample 59	7093	14.9%	50% MeOH / 50% H_2O
Column #6 Sample 60	5289	11.1%	50% MeOH / 50% H_2O
Column #6 Sample 61	9456	19.9%	transition
Column #6 Sample 62	3998	8.4%	60% MeOH / 40% H_2O
Column #6 Sample 63	7868	16.6%	60% MeOH / 40% H_2O
Column #6 Sample 64	5036	10.6%	60% MeOH / 40% H_2O
Column #6 Sample 65	3215	6.8%	60% MeOH / 40% H_2O
Column #6 Sample 66	3260	6.9%	60% MeOH / 40% H_2O
Column #6 Sample 67	3374	7.1%	60% MeOH / 40% H_2O
Column #6 Sample 68	2709	5.7%	60% MeOH / 40% H_2O
Column #6 Sample 69	2491	5.2%	60% MeOH / 40% H_2O
Column #6 Sample 70	1261	2.7%	60% MeOH / 40% H_2O

Table A5, continued.

Column #6 Sample 71	1843	3.9%	60% MeOH / 40% H_2O
Column #6 Sample 72	825	1.7%	transition
Column #6 Sample 73	758	1.6%	70% MeOH / 30% H_2O
Column #6 Sample 74	857	1.8%	70% MeOH / 30% H_2O
Column #6 Sample 75	5002	10.5%	70% MeOH / 30% H_2O
Column #6 Sample 76	9578	20.2%	70% MeOH / 30% H_2O
Column #6 Sample 77	9248	19.5%	70% MeOH / 30% H_2O
Column #6 Sample 78	12537	26.4%	70% MeOH / 30% H_2O
Column #6 Sample 79	2830	6.0%	70% MeOH / 30% H_2O
Column #6 Sample 80	8937	18.8%	70% MeOH / 30% H_2O
Column #6 Sample 81	10465	22.0%	70% MeOH / 30% H_2O
Column #6 Sample 82	14247	30.0%	70% MeOH / 30% H_2O
Column #6 Sample 83	11608	24.5%	transition
Column #6 Sample 84	4704	9.9%	80% MeOH / 20% H_2O
Column #6 Sample 85	6945	14.6%	80% MeOH / 20% H_2O
Column #6 Sample 86	500	1.1%	80% MeOH / 20% H_2O
Column #6 Sample 87	92	0.2%	80% MeOH / 20% H_2O
Column #6 Sample 88	56	0.1%	80% MeOH / 20% H_2O
Column #6 Sample 89	70	0.1%	80% MeOH / 20% H_2O

Table A6. Results for B16-F10 cell proliferation assay with Column #7 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	40183	100.0%	N/A
Average 10 mg/mL Elderberry	24796	61.7%	N/A
Column #7 Sample 1	105	0.3%	0% MeOH / 100% H_2O
Column #7 Sample 2	67	0.2%	0% MeOH / 100% H_2O
Column #7 Sample 3	64	0.2%	0% MeOH / 100% H_2O
Column #7 Sample 4	41	0.1%	0% MeOH / 100% H_2O
Column #7 Sample 5	62	0.2%	0% MeOH / 100% H_2O
Column #7 Sample 6	76	0.2%	transition
Column #7 Sample 7	86	0.2%	10% MeOH / 90% H_2O

Table A6, continued.

Column #7 Sample 8	86	0.2%	10% MeOH / 90% H_2O
Column #7 Sample 9	80	0.2%	10% MeOH / 90% H_2O
Column #7 Sample 10	66	0.2%	10% MeOH / 90% H_2O
Column #7 Sample 11	6346	15.8%	10% MeOH / 90% H_2O
Column #7 Sample 12	4230	10.5%	10% MeOH / 90% H_2O
Column #7 Sample 13	41	0.1%	10% MeOH / 90% H_2O
Column #7 Sample 14	94	0.2%	10% MeOH / 90% H_2O
Column #7 Sample 15	194	0.5%	10% MeOH / 90% H_2O
Column #7 Sample 16	1093	2.7%	10% MeOH / 90% H_2O
Column #7 Sample 17	122	0.3%	transition
Column #7 Sample 18	140	0.3%	20% MeOH / 80% H_2O
Column #7 Sample 19	91	0.2%	20% MeOH / 80% H_2O
Column #7 Sample 20	364	0.9%	20% MeOH / 80% H_2O
Column #7 Sample 21	8303	20.7%	20% MeOH / 80% H_2O
Column #7 Sample 22	5913	14.7%	20% MeOH / 80% H_2O
Column #7 Sample 23	345	0.9%	20% MeOH / 80% H_2O
Column #7 Sample 24	259	0.6%	20% MeOH / 80% H_2O
Column #7 Sample 25	9712	24.2%	20% MeOH / 80% H_2O
Column #7 Sample 26	9018	22.4%	20% MeOH / 80% H_2O
Column #7 Sample 27	12454	31.0%	20% MeOH / 80% H_2O
Column #7 Sample 28	9379	23.3%	transition
Column #7 Sample 29	6238	15.5%	30% MeOH / 70% H_2O
Column #7 Sample 30	6522	16.2%	30% MeOH / 70% H_2O
Column #7 Sample 31	8729	21.7%	30% MeOH / 70% H_2O
Column #7 Sample 32	6311	15.7%	30% MeOH / 70% H_2O
Column #7 Sample 33	9834	24.5%	30% MeOH / 70% H_2O
Column #7 Sample 34	657	1.6%	30% MeOH / 70% H_2O
Column #7 Sample 35	16776	41.7%	30% MeOH / 70% H_2O
Column #7 Sample 36	8854	22.0%	30% MeOH / 70% H_2O
Column #7 Sample 37	18773	46.7%	30% MeOH / 70% H_2O
Column #7 Sample 38	17193	42.8%	30% MeOH / 70% H_2O
Column #7 Sample 39	13997	34.8%	transition
Column #7 Sample 40	9963	24.8%	40% MeOH / 60% H_2O

Table A6, continued.

Column #7 Sample 41	7878	19.6%	40% MeOH / 60% H_2O
Column #7 Sample 42	7285	18.1%	40% MeOH / 60% H_2O
Column #7 Sample 43	6543	16.3%	40% MeOH / 60% H_2O
Column #7 Sample 44	1636	4.1%	40% MeOH / 60% H_2O
Column #7 Sample 45	9208	22.9%	40% MeOH / 60% H_2O
Column #7 Sample 46	6973	17.4%	40% MeOH / 60% H_2O
Column #7 Sample 47	4294	10.7%	40% MeOH / 60% H_2O
Column #7 Sample 48	4379	10.9%	40% MeOH / 60% H_2O
Column #7 Sample 49	8550	21.3%	40% MeOH / 60% H_2O
Column #7 Sample 50	1563	3.9%	transition
Column #7 Sample 51	1212	3.0%	50% MeOH / 50% H_2O
Column #7 Sample 52	192	0.5%	50% MeOH / 50% H_2O
Column #7 Sample 53	231	0.6%	50% MeOH / 50% H_2O
Column #7 Sample 54	264	0.7%	50% MeOH / 50% H_2O
Column #7 Sample 55	14907	37.1%	50% MeOH / 50% H_2O
Column #7 Sample 56	18244	45.4%	50% MeOH / 50% H_2O
Column #7 Sample 57	6622	16.5%	50% MeOH / 50% H_2O
Column #7 Sample 58	12692	31.6%	50% MeOH / 50% H_2O
Column #7 Sample 59	7532	18.7%	50% MeOH / 50% H_2O
Column #7 Sample 60	7555	18.8%	50% MeOH / 50% H_2O
Column #7 Sample 61	10246	25.5%	transition
Column #7 Sample 62	8713	21.7%	60% MeOH / 40% H_2O
Column #7 Sample 63	8026	20.0%	60% MeOH / 40% H_2O
Column #7 Sample 64	2439	6.1%	60% MeOH / 40% H_2O
Column #7 Sample 65	1746	4.3%	60% MeOH / 40% H_2O
Column #7 Sample 66	1322	3.3%	60% MeOH / 40% H_2O
Column #7 Sample 67	1598	4.0%	60% MeOH / 40% H_2O
Column #7 Sample 68	1913	4.8%	60% MeOH / 40% H_2O
Column #7 Sample 69	2064	5.1%	60% MeOH / 40% H_2O
Column #7 Sample 70	1393	3.5%	60% MeOH / 40% H_2O
Column #7 Sample 71	774	1.9%	60% MeOH / 40% H_2O
Column #7 Sample 72	819	2.0%	transition
Column #7 Sample 73	196	0.5%	70% MeOH / 30% H_2O

Table A6, continued.

Column #7 Sample 74	211	0.5%	70% MeOH / 30% H_2O
Column #7 Sample 75	6947	17.3%	70% MeOH / 30% H_2O
Column #7 Sample 76	3517	8.8%	70% MeOH / 30% H_2O
Column #7 Sample 77	4743	11.8%	70% MeOH / 30% H_2O
Column #7 Sample 78	2097	5.2%	70% MeOH / 30% H_2O
Column #7 Sample 79	4789	11.9%	70% MeOH / 30% H_2O
Column #7 Sample 80	5104	12.7%	70% MeOH / 30% H_2O
Column #7 Sample 81	9064	22.6%	70% MeOH / 30% H_2O
Column #7 Sample 82	2909	7.2%	transition
Column #7 Sample 83	164	0.4%	80% MeOH / 20% H_2O
Column #7 Sample 84	1369	3.4%	80% MeOH / 20% H_2O
Column #7 Sample 85	119	0.3%	80% MeOH / 20% H_2O
Column #7 Sample 86	774	1.9%	80% MeOH / 20% H_2O
Column #7 Sample 87	91	0.2%	80% MeOH / 20% H_2O
Column #7 Sample 88	552	1.4%	80% MeOH / 20% H_2O
Column #7 Sample 89	341	0.8%	80% MeOH / 20% H_2O
Column #7 Sample 90	340	0.8%	80% MeOH / 20% H_2O
Column #7 Sample 91	1105	2.7%	80% MeOH / 20% H_2O
Column #7 Sample 92	1133	2.8%	80% MeOH / 20% H_2O

Table A7. Results for B16-F10 cell proliferation assay with Column #8 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	31548	100.0%	N/A
Average 10 mg/mL Elderberry	16893	53.5%	N/A
Column #8 Sample 1	82	0.3%	0% MeOH / 100% H_2O
Column #8 Sample 2	93	0.3%	0% MeOH / 100% H_2O
Column #8 Sample 3	72	0.2%	0% MeOH / 100% H_2O
Column #8 Sample 4	67	0.2%	0% MeOH / 100% H_2O
Column #8 Sample 5	56	0.2%	0% MeOH / 100% H_2O
Column #8 Sample 6	42	0.1%	transition
Column #8 Sample 7	57	0.2%	10% MeOH / 90% H_2O

Table A7, continued.

Column #8 Sample 8	60	0.2%	10% MeOH / 90% H_2O
Column #8 Sample 9	62	0.2%	10% MeOH / 90% H_2O
Column #8 Sample 10	59	0.2%	10% MeOH / 90% H_2O
Column #8 Sample 11	10780	34.2%	10% MeOH / 90% H_2O
Column #8 Sample 12	86	0.3%	10% MeOH / 90% H_2O
Column #8 Sample 13	112	0.4%	10% MeOH / 90% H_2O
Column #8 Sample 14	209	0.7%	10% MeOH / 90% H_2O
Column #8 Sample 15	122	0.4%	10% MeOH / 90% H_2O
Column #8 Sample 16	8297	26.3%	10% MeOH / 90% H_2O
Column #8 Sample 17	64	0.2%	transition
Column #8 Sample 18	75	0.2%	20% MeOH / 80% H_2O
Column #8 Sample 19	513	1.6%	20% MeOH / 80% H_2O
Column #8 Sample 20	285	0.9%	20% MeOH / 80% H_2O
Column #8 Sample 21	6506	20.6%	20% MeOH / 80% H_2O
Column #8 Sample 22	10621	33.7%	20% MeOH / 80% H_2O
Column #8 Sample 23	6602	20.9%	20% MeOH / 80% H_2O
Column #8 Sample 24	6756	21.4%	20% MeOH / 80% H_2O
Column #8 Sample 25	1427	4.5%	20% MeOH / 80% H_2O
Column #8 Sample 26	1715	5.4%	20% MeOH / 80% H_2O
Column #8 Sample 27	3562	11.3%	20% MeOH / 80% H_2O
Column #8 Sample 28	3451	10.9%	transition
Column #8 Sample 29	3772	12.0%	30% MeOH / 70% H_2O
Column #8 Sample 30	4678	14.8%	30% MeOH / 70% H_2O
Column #8 Sample 31	4789	15.2%	30% MeOH / 70% H_2O
Column #8 Sample 32	3225	10.2%	30% MeOH / 70% H_2O
Column #8 Sample 33	4015	12.7%	30% MeOH / 70% H_2O
Column #8 Sample 34	4753	15.1%	30% MeOH / 70% H_2O
Column #8 Sample 35	16805	53.3%	30% MeOH / 70% H_2O
Column #8 Sample 36	14262	45.2%	30% MeOH / 70% H_2O
Column #8 Sample 37	15158	48.0%	30% MeOH / 70% H_2O
Column #8 Sample 38	17160	54.4%	30% MeOH / 70% H_2O
Column #8 Sample 39	15760	50.0%	transition
Column #8 Sample 40	13234	41.9%	40% MeOH / 60% H_2O

Table A7, continued.

Column #8 Sample 41	14901	47.2%	40% MeOH / 60% H_2O
Column #8 Sample 42	11558	36.6%	40% MeOH / 60% H_2O
Column #8 Sample 43	10756	34.1%	40% MeOH / 60% H_2O
Column #8 Sample 44	9748	30.9%	40% MeOH / 60% H_2O
Column #8 Sample 45	10146	32.2%	40% MeOH / 60% H_2O
Column #8 Sample 46	10084	32.0%	40% MeOH / 60% H_2O
Column #8 Sample 47	8061	25.6%	40% MeOH / 60% H_2O
Column #8 Sample 48	10571	33.5%	40% MeOH / 60% H_2O
Column #8 Sample 49	8850	28.1%	40% MeOH / 60% H_2O
Column #8 Sample 50	6621	21.0%	transition
Column #8 Sample 51	6529	20.7%	50% MeOH / 50% H_2O
Column #8 Sample 52	1046	3.3%	50% MeOH / 50% H_2O
Column #8 Sample 53	1013	3.2%	50% MeOH / 50% H_2O
Column #8 Sample 54	2294	7.3%	50% MeOH / 50% H_2O
Column #8 Sample 55	23891	75.7%	50% MeOH / 50% H_2O
Column #8 Sample 56	16343	51.8%	50% MeOH / 50% H_2O
Column #8 Sample 57	17584	55.7%	50% MeOH / 50% H_2O
Column #8 Sample 58	11952	37.9%	50% MeOH / 50% H_2O
Column #8 Sample 59	13380	42.4%	50% MeOH / 50% H_2O
Column #8 Sample 60	19642	62.3%	50% MeOH / 50% H_2O
Column #8 Sample 61	14145	44.8%	transition
Column #8 Sample 62	19813	62.8%	60% MeOH / 40% H_2O
Column #8 Sample 63	15190	48.1%	60% MeOH / 40% H_2O
Column #8 Sample 64	15397	48.8%	60% MeOH / 40% H_2O
Column #8 Sample 65	5104	16.2%	60% MeOH / 40% H_2O
Column #8 Sample 66	2505	7.9%	60% MeOH / 40% H_2O
Column #8 Sample 67	2395	7.6%	60% MeOH / 40% H_2O
Column #8 Sample 68	2718	8.6%	60% MeOH / 40% H_2O
Column #8 Sample 69	2726	8.6%	60% MeOH / 40% H_2O
Column #8 Sample 70	2483	7.9%	60% MeOH / 40% H_2O
Column #8 Sample 71	2554	8.1%	60% MeOH / 40% H_2O
Column #8 Sample 72	3150	10.0%	transition
Column #8 Sample 73	766	2.4%	70% MeOH / 30% H_2O

Table A7, continued.

Column #8 Sample 74	1755	5.6%	70% MeOH / 30% H_2O
Column #8 Sample 75	9966	31.6%	70% MeOH / 30% H_2O
Column #8 Sample 76	8614	27.3%	70% MeOH / 30% H_2O
Column #8 Sample 77	8903	28.2%	70% MeOH / 30% H_2O
Column #8 Sample 78	9225	29.2%	70% MeOH / 30% H_2O
Column #8 Sample 79	9279	29.4%	70% MeOH / 30% H_2O
Column #8 Sample 80	7845	24.9%	70% MeOH / 30% H_2O
Column #8 Sample 81	9762	30.9%	70% MeOH / 30% H_2O
Column #8 Sample 82	12822	40.6%	70% MeOH / 30% H_2O
Column #8 Sample 83	10701	33.9%	transition
Column #8 Sample 84	8158	25.9%	80% MeOH / 20% H_2O
Column #8 Sample 85	6923	21.9%	80% MeOH / 20% H_2O
Column #8 Sample 86	9859	31.3%	80% MeOH / 20% H_2O
Column #8 Sample 87	9299	29.5%	80% MeOH / 20% H_2O
Column #8 Sample 88	5860	18.6%	80% MeOH / 20% H_2O
Column #8 Sample 89	7870	24.9%	80% MeOH / 20% H_2O
Column #8 Sample 90	6489	20.6%	80% MeOH / 20% H_2O
Column #8 Sample 91	4234	13.4%	80% MeOH / 20% H_2O
Column #8 Sample 92	5095	16.1%	80% MeOH / 20% H_2O
Column #8 Sample 93	3843	12.2%	80% MeOH / 20% H_2O

Table A8. Results for B16-F10 cell proliferation assay with Column #9 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	92120	100.0%	N/A
Average 10 mg/mL Elderberry	71605	77.7%	N/A
Column #9 Sample 1	90	0.1%	0% MeOH / 100% H_2O
Column #9 Sample 2	133	0.1%	0% MeOH / 100% H_2O
Column #9 Sample 3	50	0.1%	0% MeOH / 100% H_2O
Column #9 Sample 4	57	0.1%	0% MeOH / 100% H_2O
Column #9 Sample 5	51	0.1%	0% MeOH / 100% H_2O

Table A8, continued.

Column #9 Sample 6	47	0.1%	transition
Column #9 Sample 7	43	0.0%	10% MeOH / 90% H_2O
Column #9 Sample 8	65	0.1%	10% MeOH / 90% H_2O
Column #9 Sample 9	76	0.1%	10% MeOH / 90% H_2O
Column #9 Sample 10	146	0.2%	10% MeOH / 90% H_2O
Column #9 Sample 11	602	0.7%	10% MeOH / 90% H_2O
Column #9 Sample 12	23397	25.4%	10% MeOH / 90% H_2O
Column #9 Sample 13	23488	25.5%	10% MeOH / 90% H_2O
Column #9 Sample 14	31276	34.0%	10% MeOH / 90% H_2O
Column #9 Sample 15	60029	65.2%	10% MeOH / 90% H_2O
Column #9 Sample 16	58216	63.2%	10% MeOH / 90% H_2O
Column #9 Sample 17	42738	46.4%	transition
Column #9 Sample 18	41560	45.1%	20% MeOH / 80% H_2O
Column #9 Sample 19	34502	37.5%	20% MeOH / 80% H_2O
Column #9 Sample 20	27625	30.0%	20% MeOH / 80% H_2O
Column #9 Sample 21	44168	47.9%	20% MeOH / 80% H_2O
Column #9 Sample 22	40392	43.8%	20% MeOH / 80% H_2O
Column #9 Sample 23	40108	43.5%	20% MeOH / 80% H_2O
Column #9 Sample 24	31723	34.4%	20% MeOH / 80% H_2O
Column #9 Sample 25	41747	45.3%	20% MeOH / 80% H_2O
Column #9 Sample 26	28162	30.6%	20% MeOH / 80% H_2O
Column #9 Sample 27	29709	32.3%	20% MeOH / 80% H_2O
Column #9 Sample 28	20967	22.8%	transition
Column #9 Sample 29	23259	25.2%	30% MeOH / 70% H_2O
Column #9 Sample 30	22495	24.4%	30% MeOH / 70% H_2O
Column #9 Sample 31	18007	19.5%	30% MeOH / 70% H_2O
Column #9 Sample 32	12870	14.0%	30% MeOH / 70% H_2O
Column #9 Sample 33	16621	18.0%	30% MeOH / 70% H_2O
Column #9 Sample 34	15167	16.5%	30% MeOH / 70% H_2O
Column #9 Sample 35	58548	63.6%	30% MeOH / 70% H_2O
Column #9 Sample 36	68835	74.7%	30% MeOH / 70% H_2O
Column #9 Sample 37	40103	43.5%	30% MeOH / 70% H_2O
Column #9 Sample 38	59931	65.1%	30% MeOH / 70% H_2O

Table A8, continued.

Column #9 Sample 39	43906	47.7%	transition
Column #9 Sample 40	42724	46.4%	40% MeOH / 60% H_2O
Column #9 Sample 41	55508	60.3%	40% MeOH / 60% H_2O
Column #9 Sample 42	38460	41.7%	40% MeOH / 60% H_2O
Column #9 Sample 43	43059	46.7%	40% MeOH / 60% H_2O
Column #9 Sample 44	61538	66.8%	40% MeOH / 60% H_2O
Column #9 Sample 45	39215	42.6%	40% MeOH / 60% H_2O
Column #9 Sample 46	32219	35.0%	40% MeOH / 60% H_2O
Column #9 Sample 47	43845	47.6%	40% MeOH / 60% H_2O
Column #9 Sample 48	30352	32.9%	40% MeOH / 60% H_2O
Column #9 Sample 49	36159	39.3%	40% MeOH / 60% H_2O
Column #9 Sample 50	11703	12.7%	transition
Column #9 Sample 51	28931	31.4%	50% MeOH / 50% H_2O
Column #9 Sample 52	33827	36.7%	50% MeOH / 50% H_2O
Column #9 Sample 53	18899	20.5%	50% MeOH / 50% H_2O
Column #9 Sample 54	7385	8.0%	50% MeOH / 50% H_2O
Column #9 Sample 55	62641	68.0%	50% MeOH / 50% H_2O
Column #9 Sample 56	60184	65.3%	50% MeOH / 50% H_2O
Column #9 Sample 57	42572	46.2%	50% MeOH / 50% H_2O
Column #9 Sample 58	24616	26.7%	50% MeOH / 50% H_2O
Column #9 Sample 59	36581	39.7%	50% MeOH / 50% H_2O
Column #9 Sample 60	56916	61.8%	50% MeOH / 50% H_2O
Column #9 Sample 61	53994	58.6%	transition
Column #9 Sample 62	64663	70.2%	60% MeOH / 40% H_2O
Column #9 Sample 63	55462	60.2%	60% MeOH / 40% H_2O
Column #9 Sample 64	69282	75.2%	60% MeOH / 40% H_2O
Column #9 Sample 65	26369	28.6%	60% MeOH / 40% H_2O
Column #9 Sample 66	29933	32.5%	60% MeOH / 40% H_2O
Column #9 Sample 67	14621	15.9%	60% MeOH / 40% H_2O
Column #9 Sample 68	23338	25.3%	60% MeOH / 40% H_2O
Column #9 Sample 69	27125	29.4%	60% MeOH / 40% H_2O
Column #9 Sample 70	25299	27.5%	60% MeOH / 40% H_2O
Column #9 Sample 71	13354	14.5%	60% MeOH / 40% H_2O

Table A8, continued.

Column #9 Sample 72	18310	19.9%	transition
Column #9 Sample 73	7107	7.7%	70% MeOH / 30% H_2O
Column #9 Sample 74	4873	5.3%	70% MeOH / 30% H_2O
Column #9 Sample 75	31223	33.9%	70% MeOH / 30% H_2O
Column #9 Sample 76	30535	33.1%	70% MeOH / 30% H_2O
Column #9 Sample 77	27443	29.8%	70% MeOH / 30% H_2O
Column #9 Sample 78	31461	34.2%	70% MeOH / 30% H_2O
Column #9 Sample 79	36154	39.2%	70% MeOH / 30% H_2O
Column #9 Sample 80	33025	35.8%	70% MeOH / 30% H_2O
Column #9 Sample 81	48963	53.2%	70% MeOH / 30% H_2O
Column #9 Sample 82	45255	49.1%	70% MeOH / 30% H_2O
Column #9 Sample 83	56945	61.8%	transition
Column #9 Sample 84	47327	51.4%	80% MeOH / 20% H_2O
Column #9 Sample 85	55210	59.9%	80% MeOH / 20% H_2O
Column #9 Sample 86	39221	42.6%	80% MeOH / 20% H_2O
Column #9 Sample 87	32432	35.2%	80% MeOH / 20% H_2O
Column #9 Sample 88	36000	39.1%	80% MeOH / 20% H_2O
Column #9 Sample 89	24458	26.6%	80% MeOH / 20% H_2O
Column #9 Sample 90	4417	4.8%	80% MeOH / 20% H_2O
Column #9 Sample 91	11388	12.4%	80% MeOH / 20% H_2O
Column #9 Sample 92	28617	31.1%	80% MeOH / 20% H_2O

Table A9. Results for B16-F10 cell proliferation assay with Column #10 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	32984	100.0%	N/A
Average 10 mg/mL Elderberry	8562	26.0%	N/A
Column #10 Sample 1	71	0.2%	0% MeOH / 100% H_2O
Column #10 Sample 2	129	0.4%	0% MeOH / 100% H_2O
Column #10 Sample 3	99	0.3%	0% MeOH / 100% H_2O
Column #10 Sample 4	93	0.3%	0% MeOH / 100% H_2O

Table A9, continued.

Column #10 Sample 5	76	0.2%	0% MeOH / 100% H_2O
Column #10 Sample 6	61	0.2%	transition
Column #10 Sample 7	58	0.2%	10% MeOH / 90% H_2O
Column #10 Sample 8	75	0.2%	10% MeOH / 90% H_2O
Column #10 Sample 9	100	0.3%	10% MeOH / 90% H_2O
Column #10 Sample 10	73	0.2%	10% MeOH / 90% H_2O
Column #10 Sample 11	78	0.2%	10% MeOH / 90% H_2O
Column #10 Sample 12	113	0.3%	10% MeOH / 90% H_2O
Column #10 Sample 13	154	0.5%	10% MeOH / 90% H_2O
Column #10 Sample 14	2099	6.4%	10% MeOH / 90% H_2O
Column #10 Sample 15	1900	5.8%	10% MeOH / 90% H_2O
Column #10 Sample 16	7116	21.6%	10% MeOH / 90% H_2O
Column #10 Sample 17	1694	5.1%	transition
Column #10 Sample 18	3307	10.0%	20% MeOH / 80% H_2O
Column #10 Sample 19	7524	22.8%	20% MeOH / 80% H_2O
Column #10 Sample 20	7204	21.8%	20% MeOH / 80% H_2O
Column #10 Sample 21	10730	32.5%	20% MeOH / 80% H_2O
Column #10 Sample 22	8209	24.9%	20% MeOH / 80% H_2O
Column #10 Sample 23	11506	34.9%	20% MeOH / 80% H_2O
Column #10 Sample 24	5070	15.4%	20% MeOH / 80% H_2O
Column #10 Sample 25	9630	29.2%	20% MeOH / 80% H_2O
Column #10 Sample 26	7353	22.3%	20% MeOH / 80% H_2O
Column #10 Sample 27	3282	10.0%	20% MeOH / 80% H_2O
Column #10 Sample 28	2678	8.1%	transition
Column #10 Sample 29	1946	5.9%	30% MeOH / 70% H_2O
Column #10 Sample 30	1733	5.3%	30% MeOH / 70% H_2O
Column #10 Sample 31	2986	9.1%	30% MeOH / 70% H_2O
Column #10 Sample 32	998	3.0%	30% MeOH / 70% H_2O
Column #10 Sample 33	1178	3.6%	30% MeOH / 70% H_2O
Column #10 Sample 34	2960	9.0%	30% MeOH / 70% H_2O
Column #10 Sample 35	20885	63.3%	30% MeOH / 70% H_2O
Column #10 Sample 36	10410	31.6%	30% MeOH / 70% H_2O
Column #10 Sample 37	2912	8.8%	30% MeOH / 70% H_2O

Table A9, continued.

Column #10 Sample 38	7247	22.0%	transition
Column #10 Sample 39	3721	11.3%	40% MeOH / 60% H_2O
Column #10 Sample 40	1999	6.1%	40% MeOH / 60% H_2O
Column #10 Sample 41	1785	5.4%	40% MeOH / 60% H_2O
Column #10 Sample 42	465	1.4%	40% MeOH / 60% H_2O
Column #10 Sample 43	9298	28.2%	40% MeOH / 60% H_2O
Column #10 Sample 44	5909	17.9%	40% MeOH / 60% H_2O
Column #10 Sample 45	1550	4.7%	40% MeOH / 60% H_2O
Column #10 Sample 46	1841	5.6%	40% MeOH / 60% H_2O
Column #10 Sample 47	250	0.8%	40% MeOH / 60% H_2O
Column #10 Sample 48	412	1.2%	40% MeOH / 60% H_2O
Column #10 Sample 49	1504	4.6%	transition
Column #10 Sample 50	345	1.0%	50% MeOH / 50% H_2O
Column #10 Sample 51	352	1.1%	50% MeOH / 50% H_2O
Column #10 Sample 52	237	0.7%	50% MeOH / 50% H_2O
Column #10 Sample 53	202	0.6%	50% MeOH / 50% H_2O
Column #10 Sample 54	2773	8.4%	50% MeOH / 50% H_2O
Column #10 Sample 55	9329	28.3%	50% MeOH / 50% H_2O
Column #10 Sample 56	2357	7.1%	50% MeOH / 50% H_2O
Column #10 Sample 57	857	2.6%	50% MeOH / 50% H_2O
Column #10 Sample 58	3090	9.4%	50% MeOH / 50% H_2O
Column #10 Sample 59	14278	43.3%	50% MeOH / 50% H_2O
Column #10 Sample 60	681	2.1%	transition
Column #10 Sample 61	1620	4.9%	60% MeOH / 40% H_2O
Column #10 Sample 62	6845	20.8%	60% MeOH / 40% H_2O
Column #10 Sample 63	1480	4.5%	60% MeOH / 40% H_2O
Column #10 Sample 64	13755	41.7%	60% MeOH / 40% H_2O
Column #10 Sample 65	6217	18.8%	60% MeOH / 40% H_2O
Column #10 Sample 66	3961	12.0%	60% MeOH / 40% H_2O
Column #10 Sample 67	1576	4.8%	60% MeOH / 40% H_2O
Column #10 Sample 68	4253	12.9%	60% MeOH / 40% H_2O
Column #10 Sample 69	4854	14.7%	60% MeOH / 40% H_2O
Column #10 Sample 70	7485	22.7%	60% MeOH / 40% H_2O

Table A9, continued.

Column #10 Sample 71	4637	14.1%	transition
Column #10 Sample 72	2461	7.5%	70% MeOH / 30% H_2O
Column #10 Sample 73	1066	3.2%	70% MeOH / 30% H_2O
Column #10 Sample 74	1004	3.0%	70% MeOH / 30% H_2O
Column #10 Sample 75	13774	41.8%	70% MeOH / 30% H_2O
Column #10 Sample 76	3541	10.7%	70% MeOH / 30% H_2O
Column #10 Sample 77	3738	11.3%	70% MeOH / 30% H_2O
Column #10 Sample 78	3637	11.0%	70% MeOH / 30% H_2O
Column #10 Sample 79	3318	10.1%	70% MeOH / 30% H_2O
Column #10 Sample 80	2557	7.8%	transition
Column #10 Sample 81	2650	8.0%	80% MeOH / 20% H_2O
Column #10 Sample 82	7813	23.7%	80% MeOH / 20% H_2O
Column #10 Sample 83	1585	4.8%	80% MeOH / 20% H_2O
Column #10 Sample 84	6387	19.4%	80% MeOH / 20% H_2O
Column #10 Sample 85	4038	12.2%	80% MeOH / 20% H_2O
Column #10 Sample 86	228	0.7%	80% MeOH / 20% H_2O
Column #10 Sample 87	768	2.3%	80% MeOH / 20% H_2O
Column #10 Sample 88	466	1.4%	80% MeOH / 20% H_2O
Column #10 Sample 89	149	0.5%	80% MeOH / 20% H_2O
Column #10 Sample 90	7678	23.3%	80% MeOH / 20% H_2O

Table A10. Results for B16-F10 cell proliferation assay with Column #11 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	16646	100.0%	N/A
Average 10 mg/mL Elderberry	4744	28.5%	N/A
Column #11 Sample 1	63	0.4%	0% MeOH / 100% H_2O
Column #11 Sample 2	78	0.5%	0% MeOH / 100% H_2O
Column #11 Sample 3	64	0.4%	0% MeOH / 100% H_2O
Column #11 Sample 4	45	0.3%	0% MeOH / 100% H_2O
Column #11 Sample 5	43	0.3%	0% MeOH / 100% H_2O

Table A10, continued.

Column #11 Sample 6	43	0.3%	transition
Column #11 Sample 7	53	0.3%	10% MeOH / 90% H_2O
Column #11 Sample 8	43	0.3%	10% MeOH / 90% H_2O
Column #11 Sample 9	53	0.3%	10% MeOH / 90% H_2O
Column #11 Sample 10	32	0.2%	10% MeOH / 90% H_2O
Column #11 Sample 11	42	0.3%	10% MeOH / 90% H_2O
Column #11 Sample 12	41	0.2%	10% MeOH / 90% H_2O
Column #11 Sample 13	272	1.6%	10% MeOH / 90% H_2O
Column #11 Sample 14	383	2.3%	10% MeOH / 90% H_2O
Column #11 Sample 15	8520	51.2%	10% MeOH / 90% H_2O
Column #11 Sample 16	1219	7.3%	10% MeOH / 90% H_2O
Column #11 Sample 17	626	3.8%	transition
Column #11 Sample 18	1931	11.6%	20% MeOH / 80% H_2O
Column #11 Sample 19	1075	6.5%	20% MeOH / 80% H_2O
Column #11 Sample 20	412	2.5%	20% MeOH / 80% H_2O
Column #11 Sample 21	2961	17.8%	20% MeOH / 80% H_2O
Column #11 Sample 22	2709	16.3%	20% MeOH / 80% H_2O
Column #11 Sample 23	1840	11.1%	20% MeOH / 80% H_2O
Column #11 Sample 24	808	4.9%	20% MeOH / 80% H_2O
Column #11 Sample 25	6646	39.9%	20% MeOH / 80% H_2O
Column #11 Sample 26	1513	9.1%	20% MeOH / 80% H_2O
Column #11 Sample 27	2000	12.0%	20% MeOH / 80% H_2O
Column #11 Sample 28	5409	32.5%	transition
Column #11 Sample 29	2539	15.3%	30% MeOH / 70% H_2O
Column #11 Sample 30	3825	23.0%	30% MeOH / 70% H_2O
Column #11 Sample 31	2080	12.5%	30% MeOH / 70% H_2O
Column #11 Sample 32	653	3.9%	30% MeOH / 70% H_2O
Column #11 Sample 33	524	3.1%	30% MeOH / 70% H_2O
Column #11 Sample 34	3350	20.1%	30% MeOH / 70% H_2O
Column #11 Sample 35	6971	41.9%	30% MeOH / 70% H_2O
Column #11 Sample 36	5311	31.9%	30% MeOH / 70% H_2O
Column #11 Sample 37	1920	11.5%	30% MeOH / 70% H_2O
Column #11 Sample 38	439	2.6%	transition

Table A10, continued.

Column #11 Sample 39	2154	12.9%	40% MeOH / 60% H_2O
Column #11 Sample 40	1240	7.4%	40% MeOH / 60% H_2O
Column #11 Sample 41	155	0.9%	40% MeOH / 60% H_2O
Column #11 Sample 42	1344	8.1%	40% MeOH / 60% H_2O
Column #11 Sample 43	280	1.7%	40% MeOH / 60% H_2O
Column #11 Sample 44	4706	28.3%	40% MeOH / 60% H_2O
Column #11 Sample 45	1445	8.7%	40% MeOH / 60% H_2O
Column #11 Sample 46	311	1.9%	40% MeOH / 60% H_2O
Column #11 Sample 47	442	2.7%	40% MeOH / 60% H_2O
Column #11 Sample 48	368	2.2%	40% MeOH / 60% H_2O
Column #11 Sample 49	325	2.0%	transition
Column #11 Sample 50	311	1.9%	50% MeOH / 50% H_2O
Column #11 Sample 51	341	2.0%	50% MeOH / 50% H_2O
Column #11 Sample 52	254	1.5%	50% MeOH / 50% H_2O
Column #11 Sample 53	297	1.8%	50% MeOH / 50% H_2O
Column #11 Sample 54	639	3.8%	50% MeOH / 50% H_2O
Column #11 Sample 55	5006	30.1%	50% MeOH / 50% H_2O
Column #11 Sample 56	501	3.0%	50% MeOH / 50% H_2O
Column #11 Sample 57	307	1.8%	50% MeOH / 50% H_2O
Column #11 Sample 58	421	2.5%	50% MeOH / 50% H_2O
Column #11 Sample 59	1156	6.9%	50% MeOH / 50% H_2O
Column #11 Sample 60	524	3.1%	transition
Column #11 Sample 61	379	2.3%	60% MeOH / 40% H_2O
Column #11 Sample 62	566	3.4%	60% MeOH / 40% H_2O
Column #11 Sample 63	395	2.4%	60% MeOH / 40% H_2O
Column #11 Sample 64	801	4.8%	60% MeOH / 40% H_2O
Column #11 Sample 65	487	2.9%	60% MeOH / 40% H_2O
Column #11 Sample 66	527	3.2%	60% MeOH / 40% H_2O
Column #11 Sample 67	1127	6.8%	60% MeOH / 40% H_2O
Column #11 Sample 68	708	4.3%	60% MeOH / 40% H_2O
Column #11 Sample 69	278	1.7%	60% MeOH / 40% H_2O
Column #11 Sample 70	2323	14.0%	60% MeOH / 40% H_2O
Column #11 Sample 71	660	4.0%	transition

Table A10, continued.

Column #11 Sample 72	200	1.2%	70% MeOH / 30% H_2O
Column #11 Sample 73	611	3.7%	70% MeOH / 30% H_2O
Column #11 Sample 74	532	3.2%	70% MeOH / 30% H_2O
Column #11 Sample 75	6073	36.5%	70% MeOH / 30% H_2O
Column #11 Sample 76	12112	72.8%	70% MeOH / 30% H_2O
Column #11 Sample 77	1422	8.5%	70% MeOH / 30% H_2O
Column #11 Sample 78	952	5.7%	70% MeOH / 30% H_2O
Column #11 Sample 79	520	3.1%	70% MeOH / 30% H_2O
Column #11 Sample 80	1748	10.5%	70% MeOH / 30% H_2O
Column #11 Sample 81	1897	11.4%	transition
Column #11 Sample 82	4619	27.7%	80% MeOH / 20% H_2O
Column #11 Sample 83	933	5.6%	80% MeOH / 20% H_2O
Column #11 Sample 84	3497	21.0%	80% MeOH / 20% H_2O
Column #11 Sample 85	3274	19.7%	80% MeOH / 20% H_2O
Column #11 Sample 86	915	5.5%	80% MeOH / 20% H_2O
Column #11 Sample 87	190	1.1%	80% MeOH / 20% H_2O
Column #11 Sample 88	574	3.4%	80% MeOH / 20% H_2O
Column #11 Sample 89	617	3.7%	80% MeOH / 20% H_2O
Column #11 Sample 90	2307	13.9%	80% MeOH / 20% H_2O
Column #11 Sample 91	652	3.9%	80% MeOH / 20% H_2O

Table A11. Results for B16-F10 cell proliferation assay with Column #12 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	119971	100.0%	N/A
Average 10 mg/mL Elderberry	62496	52.1%	N/A
Column #12 Sample 1	75	0.1%	0% MeOH / 100% H_2O
Column #12 Sample 2	103	0.1%	0% MeOH / 100% H_2O
Column #12 Sample 3	90	0.1%	0% MeOH / 100% H_2O
Column #12 Sample 4	82	0.1%	0% MeOH / 100% H_2O
Column #12 Sample 5	69	0.1%	transition

Table A11, continued.

Column #12 Sample 6	90	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 7	75	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 8	392	0.3%	10% MeOH / 90% H_2O
Column #12 Sample 9	72	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 10	63	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 11	78	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 12	69	0.1%	10% MeOH / 90% H_2O
Column #12 Sample 13	185	0.2%	10% MeOH / 90% H_2O
Column #12 Sample 14	1788	1.5%	10% MeOH / 90% H_2O
Column #12 Sample 15	43795	36.5%	10% MeOH / 90% H_2O
Column #12 Sample 16	38056	31.7%	transition
Column #12 Sample 17	22892	19.1%	20% MeOH / 80% H_2O
Column #12 Sample 18	13002	10.8%	20% MeOH / 80% H_2O
Column #12 Sample 19	21478	17.9%	20% MeOH / 80% H_2O
Column #12 Sample 20	16952	14.1%	20% MeOH / 80% H_2O
Column #12 Sample 21	15513	12.9%	20% MeOH / 80% H_2O
Column #12 Sample 22	11761	9.8%	20% MeOH / 80% H_2O
Column #12 Sample 23	14245	11.9%	20% MeOH / 80% H_2O
Column #12 Sample 24	19176	16.0%	20% MeOH / 80% H_2O
Column #12 Sample 25	21082	17.6%	20% MeOH / 80% H_2O
Column #12 Sample 26	10322	8.6%	20% MeOH / 80% H_2O
Column #12 Sample 27	12355	10.3%	transition
Column #12 Sample 28	9909	8.3%	30% MeOH / 70% H_2O
Column #12 Sample 29	5499	4.6%	30% MeOH / 70% H_2O
Column #12 Sample 30	6402	5.3%	30% MeOH / 70% H_2O
Column #12 Sample 31	4996	4.2%	30% MeOH / 70% H_2O
Column #12 Sample 32	4166	3.5%	30% MeOH / 70% H_2O
Column #12 Sample 33	370	0.3%	30% MeOH / 70% H_2O
Column #12 Sample 34	1419	1.2%	30% MeOH / 70% H_2O
Column #12 Sample 35	31822	26.5%	30% MeOH / 70% H_2O
Column #12 Sample 36	16675	13.9%	30% MeOH / 70% H_2O
Column #12 Sample 37	28196	23.5%	30% MeOH / 70% H_2O
Column #12 Sample 38	21062	17.6%	transition

Table A11, continued.

Column #12 Sample 39	16619	13.9%	40% MeOH / 60% H_2O
Column #12 Sample 40	15558	13.0%	40% MeOH / 60% H_2O
Column #12 Sample 41	14456	12.0%	40% MeOH / 60% H_2O
Column #12 Sample 42	18770	15.6%	40% MeOH / 60% H_2O
Column #12 Sample 43	24588	20.5%	40% MeOH / 60% H_2O
Column #12 Sample 44	47316	39.4%	40% MeOH / 60% H_2O
Column #12 Sample 45	46094	38.4%	40% MeOH / 60% H_2O
Column #12 Sample 46	24304	20.3%	40% MeOH / 60% H_2O
Column #12 Sample 47	15160	12.6%	40% MeOH / 60% H_2O
Column #12 Sample 48	13286	11.1%	40% MeOH / 60% H_2O
Column #12 Sample 49	10519	8.8%	transition
Column #12 Sample 50	11906	9.9%	50% MeOH / 50% H_2O
Column #12 Sample 51	3055	2.5%	50% MeOH / 50% H_2O
Column #12 Sample 52	9122	7.6%	50% MeOH / 50% H_2O
Column #12 Sample 53	9266	7.7%	50% MeOH / 50% H_2O
Column #12 Sample 54	29094	24.3%	50% MeOH / 50% H_2O
Column #12 Sample 55	37432	31.2%	50% MeOH / 50% H_2O
Column #12 Sample 56	35728	29.8%	50% MeOH / 50% H_2O
Column #12 Sample 57	20514	17.1%	50% MeOH / 50% H_2O
Column #12 Sample 58	4029	3.4%	50% MeOH / 50% H_2O
Column #12 Sample 59	24142	20.1%	transition
Column #12 Sample 60	30604	25.5%	60% MeOH / 40% H_2O
Column #12 Sample 61	26050	21.7%	60% MeOH / 40% H_2O
Column #12 Sample 62	45107	37.6%	60% MeOH / 40% H_2O
Column #12 Sample 63	25098	20.9%	60% MeOH / 40% H_2O
Column #12 Sample 64	40076	33.4%	60% MeOH / 40% H_2O
Column #12 Sample 65	10219	8.5%	60% MeOH / 40% H_2O
Column #12 Sample 66	8552	7.1%	60% MeOH / 40% H_2O
Column #12 Sample 67	3272	2.7%	60% MeOH / 40% H_2O
Column #12 Sample 68	17358	14.5%	60% MeOH / 40% H_2O
Column #12 Sample 69	7643	6.4%	transition
Column #12 Sample 70	7224	6.0%	70% MeOH / 30% H_2O
Column #12 Sample 71	2923	2.4%	70% MeOH / 30% H_2O

Table A11, continued.

Column #12 Sample 72	4603	3.8%	70% MeOH / 30% H_2O
Column #12 Sample 73	5234	4.4%	70% MeOH / 30% H_2O
Column #12 Sample 74	2825	2.4%	70% MeOH / 30% H_2O
Column #12 Sample 75	15589	13.0%	70% MeOH / 30% H_2O
Column #12 Sample 76	14034	11.7%	70% MeOH / 30% H_2O
Column #12 Sample 77	43648	36.4%	70% MeOH / 30% H_2O
Column #12 Sample 78	35152	29.3%	70% MeOH / 30% H_2O
Column #12 Sample 79	74538	62.1%	transition
Column #12 Sample 80	47150	39.3%	80% MeOH / 20% H_2O
Column #12 Sample 81	48355	40.3%	80% MeOH / 20% H_2O
Column #12 Sample 82	50574	42.2%	80% MeOH / 20% H_2O
Column #12 Sample 83	61052	50.9%	80% MeOH / 20% H_2O
Column #12 Sample 84	62154	51.8%	80% MeOH / 20% H_2O
Column #12 Sample 85	43980	36.7%	80% MeOH / 20% H_2O
Column #12 Sample 86	15251	12.7%	80% MeOH / 20% H_2O
Column #12 Sample 87	18561	15.5%	80% MeOH / 20% H_2O
Column #12 Sample 88	15346	12.8%	80% MeOH / 20% H_2O

Table A12. Results for B16-F10 cell proliferation assay with Column #13 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	119971	100.0%	N/A
Average 10 mg/mL Elderberry	62496	52.1%	N/A
Column #13 Sample 1	84	0.1%	0% MeOH / 100% H_2O
Column #13 Sample 2	74	0.1%	0% MeOH / 100% H_2O
Column #13 Sample 3	85	0.1%	0% MeOH / 100% H_2O
Column #13 Sample 4	59	0.0%	0% MeOH / 100% H_2O
Column #13 Sample 5	118	0.1%	0% MeOH / 100% H_2O
Column #13 Sample 6	123	0.1%	transition
Column #13 Sample 7	90	0.1%	10% MeOH / 90% H_2O
Column #13 Sample 8	171	0.1%	10% MeOH / 90% H_2O
Column #13 Sample 9	131	0.1%	10% MeOH / 90% H_2O

Table A12, continued.

Column #13 Sample 10	140	0.1%	10% MeOH / 90% H_2O
Column #13 Sample 11	151	0.1%	10% MeOH / 90% H_2O
Column #13 Sample 12	259	0.2%	10% MeOH / 90% H_2O
Column #13 Sample 13	6398	5.3%	10% MeOH / 90% H_2O
Column #13 Sample 14	1732	1.4%	10% MeOH / 90% H_2O
Column #13 Sample 15	18122	15.1%	10% MeOH / 90% H_2O
Column #13 Sample 16	20328	16.9%	10% MeOH / 90% H_2O
Column #13 Sample 17	4387	3.7%	transition
Column #13 Sample 18	11496	9.6%	20% MeOH / 80% H_2O
Column #13 Sample 19	5084	4.2%	20% MeOH / 80% H_2O
Column #13 Sample 20	41459	34.6%	20% MeOH / 80% H_2O
Column #13 Sample 21	22242	18.5%	20% MeOH / 80% H_2O
Column #13 Sample 22	19926	16.6%	20% MeOH / 80% H_2O
Column #13 Sample 23	10296	8.6%	20% MeOH / 80% H_2O
Column #13 Sample 24	8681	7.2%	20% MeOH / 80% H_2O
Column #13 Sample 25	8266	6.9%	20% MeOH / 80% H_2O
Column #13 Sample 26	10030	8.4%	20% MeOH / 80% H_2O
Column #13 Sample 27	12767	10.6%	20% MeOH / 80% H_2O
Column #13 Sample 28	10823	9.0%	transition
Column #13 Sample 29	6361	5.3%	30% MeOH / 70% H_2O
Column #13 Sample 30	16044	13.4%	30% MeOH / 70% H_2O
Column #13 Sample 31	6561	5.5%	30% MeOH / 70% H_2O
Column #13 Sample 32	5261	4.4%	30% MeOH / 70% H_2O
Column #13 Sample 33	2850	2.4%	30% MeOH / 70% H_2O
Column #13 Sample 34	2560	2.1%	30% MeOH / 70% H_2O
Column #13 Sample 35	2428	2.0%	30% MeOH / 70% H_2O
Column #13 Sample 36	2133	1.8%	30% MeOH / 70% H_2O
Column #13 Sample 37	1965	1.6%	30% MeOH / 70% H_2O
Column #13 Sample 38	3629	3.0%	transition
Column #13 Sample 39	9735	8.1%	40% MeOH / 60% H_2O
Column #13 Sample 40	6041	5.0%	40% MeOH / 60% H_2O
Column #13 Sample 41	14344	12.0%	40% MeOH / 60% H_2O
Column #13 Sample 42	8221	6.9%	40% MeOH / 60% H_2O

Table A12, continued.

Column #13 Sample 43	7190	6.0%	40% MeOH / 60% H_2O
Column #13 Sample 44	10670	8.9%	40% MeOH / 60% H_2O
Column #13 Sample 45	8143	6.8%	40% MeOH / 60% H_2O
Column #13 Sample 46	4096	3.4%	40% MeOH / 60% H_2O
Column #13 Sample 47	8364	7.0%	40% MeOH / 60% H_2O
Column #13 Sample 48	1328	1.1%	40% MeOH / 60% H_2O
Column #13 Sample 49	20215	16.8%	40% MeOH / 60% H_2O
Column #13 Sample 50	20515	17.1%	transition
Column #13 Sample 51	17458	14.6%	50% MeOH / 50% H_2O
Column #13 Sample 52	8942	7.5%	50% MeOH / 50% H_2O
Column #13 Sample 53	9557	8.0%	50% MeOH / 50% H_2O
Column #13 Sample 54	8122	6.8%	50% MeOH / 50% H_2O
Column #13 Sample 55	9742	8.1%	50% MeOH / 50% H_2O
Column #13 Sample 56	3608	3.0%	50% MeOH / 50% H_2O
Column #13 Sample 57	3434	2.9%	50% MeOH / 50% H_2O
Column #13 Sample 58	3338	2.8%	50% MeOH / 50% H_2O
Column #13 Sample 59	2460	2.1%	50% MeOH / 50% H_2O
Column #13 Sample 60	11048	9.2%	50% MeOH / 50% H_2O
Column #13 Sample 61	19198	16.0%	transition
Column #13 Sample 62	15499	12.9%	60% MeOH / 40% H_2O
Column #13 Sample 63	28343	23.6%	60% MeOH / 40% H_2O
Column #13 Sample 64	14668	12.2%	60% MeOH / 40% H_2O
Column #13 Sample 65	25493	21.2%	60% MeOH / 40% H_2O
Column #13 Sample 66	20568	17.1%	60% MeOH / 40% H_2O
Column #13 Sample 67	29516	24.6%	60% MeOH / 40% H_2O
Column #13 Sample 68	20676	17.2%	60% MeOH / 40% H_2O
Column #13 Sample 69	26396	22.0%	60% MeOH / 40% H_2O
Column #13 Sample 70	28419	23.7%	60% MeOH / 40% H_2O
Column #13 Sample 71	27487	22.9%	transition
Column #13 Sample 72	19126	15.9%	70% MeOH / 30% H_2O
Column #13 Sample 73	7511	6.3%	70% MeOH / 30% H_2O
Column #13 Sample 74	522	0.4%	70% MeOH / 30% H_2O
Column #13 Sample 75	608	0.5%	70% MeOH / 30% H_2O

Table A12, continued.

Column #13 Sample 76	731	0.6%	70% MeOH / 30% H_2O
Column #13 Sample 77	628	0.5%	70% MeOH / 30% H_2O
Column #13 Sample 78	4735	3.9%	70% MeOH / 30% H_2O
Column #13 Sample 79	5632	4.7%	70% MeOH / 30% H_2O
Column #13 Sample 80	697	0.6%	70% MeOH / 30% H_2O
Column #13 Sample 81	10194	8.5%	transition
Column #13 Sample 82	1179	1.0%	80% MeOH / 20% H_2O
Column #13 Sample 83	975	0.8%	80% MeOH / 20% H_2O
Column #13 Sample 84	277	0.2%	80% MeOH / 20% H_2O
Column #13 Sample 85	1087	0.9%	80% MeOH / 20% H_2O
Column #13 Sample 86	968	0.8%	80% MeOH / 20% H_2O
Column #13 Sample 87	2177	1.8%	80% MeOH / 20% H_2O
Column #13 Sample 88	179	0.1%	80% MeOH / 20% H_2O
Column #13 Sample 89	1629	1.4%	80% MeOH / 20% H_2O
Column #13 Sample 90	585	0.5%	80% MeOH / 20% H_2O
Column #13 Sample 91	26604	22.2%	80% MeOH / 20% H_2O

Table A13. Results for B16-F10 cell proliferation assay with Column #14 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	16646	100.0%	N/A
Average 10 mg/mL Elderberry	4744	28.5%	N/A
Column #14 Sample 1	86	0.5%	0% MeOH / 100% H_2O
Column #14 Sample 2	41	0.2%	0% MeOH / 100% H_2O
Column #14 Sample 3	42	0.3%	0% MeOH / 100% H_2O
Column #14 Sample 4	67	0.4%	0% MeOH / 100% H_2O
Column #14 Sample 5	38	0.2%	0% MeOH / 100% H_2O
Column #14 Sample 6	41	0.2%	transition
Column #14 Sample 7	53	0.3%	10% MeOH / 90% H_2O
Column #14 Sample 8	53	0.3%	10% MeOH / 90% H_2O
Column #14 Sample 9	39	0.2%	10% MeOH / 90% H_2O
Column #14 Sample 10	46	0.3%	10% MeOH / 90% H_2O

Table A13, continued.

Column #14 Sample 11	42	0.3%	10% MeOH / 90% H_2O
Column #14 Sample 12	63	0.4%	10% MeOH / 90% H_2O
Column #14 Sample 13	344	2.1%	10% MeOH / 90% H_2O
Column #14 Sample 14	526	3.2%	10% MeOH / 90% H_2O
Column #14 Sample 15	533	3.2%	10% MeOH / 90% H_2O
Column #14 Sample 16	667	4.0%	10% MeOH / 90% H_2O
Column #14 Sample 17	561	3.4%	transition
Column #14 Sample 18	418	2.5%	20% MeOH / 80% H_2O
Column #14 Sample 19	634	3.8%	20% MeOH / 80% H_2O
Column #14 Sample 20	4008	24.1%	20% MeOH / 80% H_2O
Column #14 Sample 21	15111	90.8%	20% MeOH / 80% H_2O
Column #14 Sample 22	7658	46.0%	20% MeOH / 80% H_2O
Column #14 Sample 23	655	3.9%	20% MeOH / 80% H_2O
Column #14 Sample 24	1592	9.6%	20% MeOH / 80% H_2O
Column #14 Sample 25	648	3.9%	20% MeOH / 80% H_2O
Column #14 Sample 26	747	4.5%	20% MeOH / 80% H_2O
Column #14 Sample 27	749	4.5%	transition
Column #14 Sample 28	2536	15.2%	30% MeOH / 70% H_2O
Column #14 Sample 29	609	3.7%	30% MeOH / 70% H_2O
Column #14 Sample 30	4359	26.2%	30% MeOH / 70% H_2O
Column #14 Sample 31	3119	18.7%	30% MeOH / 70% H_2O
Column #14 Sample 32	1377	8.3%	30% MeOH / 70% H_2O
Column #14 Sample 33	1051	6.3%	30% MeOH / 70% H_2O
Column #14 Sample 34	699	4.2%	30% MeOH / 70% H_2O
Column #14 Sample 35	733	4.4%	30% MeOH / 70% H_2O
Column #14 Sample 36	778	4.7%	30% MeOH / 70% H_2O
Column #14 Sample 37	1696	10.2%	30% MeOH / 70% H_2O
Column #14 Sample 38	658	4.0%	transition
Column #14 Sample 39	605	3.6%	40% MeOH / 60% H_2O
Column #14 Sample 40	508	3.1%	40% MeOH / 60% H_2O
Column #14 Sample 41	22218	133.5%	40% MeOH / 60% H_2O
Column #14 Sample 42	5180	31.1%	40% MeOH / 60% H_2O
Column #14 Sample 43	663	4.0%	40% MeOH / 60% H_2O

Table A13, continued.

Column #14 Sample 44	1674	10.1%	40% MeOH / 60% H_2O
Column #14 Sample 45	712	4.3%	40% MeOH / 60% H_2O
Column #14 Sample 46	1472	8.8%	40% MeOH / 60% H_2O
Column #14 Sample 47	1112	6.7%	40% MeOH / 60% H_2O
Column #14 Sample 48	3987	24.0%	40% MeOH / 60% H_2O
Column #14 Sample 49	822	4.9%	transition
Column #14 Sample 50	10374	62.3%	50% MeOH / 50% H_2O
Column #14 Sample 51	6657	40.0%	50% MeOH / 50% H_2O
Column #14 Sample 52	5512	33.1%	50% MeOH / 50% H_2O
Column #14 Sample 53	5900	35.4%	50% MeOH / 50% H_2O
Column #14 Sample 54	903	5.4%	50% MeOH / 50% H_2O
Column #14 Sample 55	6245	37.5%	50% MeOH / 50% H_2O
Column #14 Sample 56	2997	18.0%	50% MeOH / 50% H_2O
Column #14 Sample 57	601	3.6%	50% MeOH / 50% H_2O
Column #14 Sample 58	862	5.2%	50% MeOH / 50% H_2O
Column #14 Sample 59	1756	10.5%	transition
Column #14 Sample 60	556	3.3%	60% MeOH / 40% H_2O
Column #14 Sample 61	5044	30.3%	60% MeOH / 40% H_2O
Column #14 Sample 62	1600	9.6%	60% MeOH / 40% H_2O
Column #14 Sample 63	1465	8.8%	60% MeOH / 40% H_2O
Column #14 Sample 64	617	3.7%	60% MeOH / 40% H_2O
Column #14 Sample 65	980	5.9%	60% MeOH / 40% H_2O
Column #14 Sample 66	1886	11.3%	60% MeOH / 40% H_2O
Column #14 Sample 67	239	1.4%	60% MeOH / 40% H_2O
Column #14 Sample 68	466	2.8%	60% MeOH / 40% H_2O
Column #14 Sample 69	4017	24.1%	60% MeOH / 40% H_2O
Column #14 Sample 70	1839	11.0%	transition
Column #14 Sample 71	633	3.8%	70% MeOH / 30% H_2O
Column #14 Sample 72	496	3.0%	70% MeOH / 30% H_2O
Column #14 Sample 73	249	1.5%	70% MeOH / 30% H_2O
Column #14 Sample 74	759	4.6%	70% MeOH / 30% H_2O
Column #14 Sample 75	408	2.5%	70% MeOH / 30% H_2O
Column #14 Sample 76	340	2.0%	70% MeOH / 30% H_2O

Table A13, continued.

Column #14 Sample 77	130	0.8%	70% MeOH / 30% H_2O
Column #14 Sample 78	209	1.3%	70% MeOH / 30% H_2O
Column #14 Sample 79	243	1.5%	70% MeOH / 30% H_2O
Column #14 Sample 80	1016	6.1%	transition
Column #14 Sample 81	1563	9.4%	80% MeOH / 20% H_2O
Column #14 Sample 82	2745	16.5%	80% MeOH / 20% H_2O
Column #14 Sample 83	210	1.3%	80% MeOH / 20% H_2O
Column #14 Sample 84	799	4.8%	80% MeOH / 20% H_2O
Column #14 Sample 85	429	2.6%	80% MeOH / 20% H_2O
Column #14 Sample 86	1366	8.2%	80% MeOH / 20% H_2O
Column #14 Sample 87	417	2.5%	80% MeOH / 20% H_2O
Column #14 Sample 88	4573	27.5%	80% MeOH / 20% H_2O
Column #14 Sample 89	219	1.3%	80% MeOH / 20% H_2O

Table A14. Results for B16-F10 cell proliferation assay with Column #15 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	90426	100.0%	N/A
Average 10 mg/mL Elderberry	38638	42.7%	N/A
Column #15 Sample 1	294	0.3%	0% MeOH / 100% H_2O
Column #15 Sample 2	189	0.2%	0% MeOH / 100% H_2O
Column #15 Sample 3	66	0.1%	0% MeOH / 100% H_2O
Column #15 Sample 4	67	0.1%	0% MeOH / 100% H_2O
Column #15 Sample 5	59	0.1%	0% MeOH / 100% H_2O
Column #15 Sample 6	57	0.1%	0% MeOH / 100% H_2O
Column #15 Sample 7	57	0.1%	transition
Column #15 Sample 8	45	0.0%	10% MeOH / 90% H_2O
Column #15 Sample 9	186	0.2%	10% MeOH / 90% H_2O
Column #15 Sample 10	68	0.1%	10% MeOH / 90% H_2O
Column #15 Sample 11	56	0.1%	10% MeOH / 90% H_2O
Column #15 Sample 12	72	0.1%	10% MeOH / 90% H_2O
Column #15 Sample 13	549	0.6%	10% MeOH / 90% H_2O

Table A14, continued.

Column #15 Sample 14	1399	1.5%	10% MeOH / 90% H_2O
Column #15 Sample 15	1689	1.9%	10% MeOH / 90% H_2O
Column #15 Sample 16	820	0.9%	10% MeOH / 90% H_2O
Column #15 Sample 17	14695	16.3%	transition
Column #15 Sample 18	11516	12.7%	20% MeOH / 80% H_2O
Column #15 Sample 19	11835	13.1%	20% MeOH / 80% H_2O
Column #15 Sample 20	5660	6.3%	20% MeOH / 80% H_2O
Column #15 Sample 21	24322	26.9%	20% MeOH / 80% H_2O
Column #15 Sample 22	12245	13.5%	20% MeOH / 80% H_2O
Column #15 Sample 23	24769	27.4%	20% MeOH / 80% H_2O
Column #15 Sample 24	16067	17.8%	20% MeOH / 80% H_2O
Column #15 Sample 25	16285	18.0%	20% MeOH / 80% H_2O
Column #15 Sample 26	8849	9.8%	transition
Column #15 Sample 27	4507	5.0%	30% MeOH / 70% H_2O
Column #15 Sample 28	2892	3.2%	30% MeOH / 70% H_2O
Column #15 Sample 29	2597	2.9%	30% MeOH / 70% H_2O
Column #15 Sample 30	3533	3.9%	30% MeOH / 70% H_2O
Column #15 Sample 31	1954	2.2%	30% MeOH / 70% H_2O
Column #15 Sample 32	2707	3.0%	30% MeOH / 70% H_2O
Column #15 Sample 33	256	0.3%	30% MeOH / 70% H_2O
Column #15 Sample 34	5621	6.2%	30% MeOH / 70% H_2O
Column #15 Sample 35	15542	17.2%	30% MeOH / 70% H_2O
Column #15 Sample 36	20378	22.5%	30% MeOH / 70% H_2O
Column #15 Sample 37	10473	11.6%	transition
Column #15 Sample 38	10221	11.3%	40% MeOH / 60% H_2O
Column #15 Sample 39	9383	10.4%	40% MeOH / 60% H_2O
Column #15 Sample 40	15425	17.1%	40% MeOH / 60% H_2O
Column #15 Sample 41	7464	8.3%	40% MeOH / 60% H_2O
Column #15 Sample 42	21701	24.0%	40% MeOH / 60% H_2O
Column #15 Sample 43	16474	18.2%	40% MeOH / 60% H_2O
Column #15 Sample 44	22236	24.6%	40% MeOH / 60% H_2O
Column #15 Sample 45	9230	10.2%	40% MeOH / 60% H_2O
Column #15 Sample 46	3518	3.9%	40% MeOH / 60% H_2O

Table A14, continued.

Column #15 Sample 47	3846	4.3%	40% MeOH / 60% H_2O
Column #15 Sample 48	2820	3.1%	transition
Column #15 Sample 49	2920	3.2%	50% MeOH / 50% H_2O
Column #15 Sample 50	1993	2.2%	50% MeOH / 50% H_2O
Column #15 Sample 51	2038	2.3%	50% MeOH / 50% H_2O
Column #15 Sample 52	761	0.8%	50% MeOH / 50% H_2O
Column #15 Sample 53	1620	1.8%	50% MeOH / 50% H_2O
Column #15 Sample 54	4922	5.4%	50% MeOH / 50% H_2O
Column #15 Sample 55	26135	28.9%	50% MeOH / 50% H_2O
Column #15 Sample 56	11142	12.3%	50% MeOH / 50% H_2O
Column #15 Sample 57	9534	10.5%	50% MeOH / 50% H_2O
Column #15 Sample 58	11612	12.8%	50% MeOH / 50% H_2O
Column #15 Sample 59	12003	13.3%	transition
Column #15 Sample 60	19887	22.0%	60% MeOH / 40% H_2O
Column #15 Sample 61	23968	26.5%	60% MeOH / 40% H_2O
Column #15 Sample 62	15243	16.9%	60% MeOH / 40% H_2O
Column #15 Sample 63	7486	8.3%	60% MeOH / 40% H_2O
Column #15 Sample 64	27139	30.0%	60% MeOH / 40% H_2O
Column #15 Sample 65	4835	5.3%	60% MeOH / 40% H_2O
Column #15 Sample 66	5862	6.5%	60% MeOH / 40% H_2O
Column #15 Sample 67	4974	5.5%	60% MeOH / 40% H_2O
Column #15 Sample 68	5770	6.4%	60% MeOH / 40% H_2O
Column #15 Sample 69	3060	3.4%	transition
Column #15 Sample 70	4314	4.8%	70% MeOH / 30% H_2O
Column #15 Sample 71	674	0.7%	70% MeOH / 30% H_2O
Column #15 Sample 72	1741	1.9%	70% MeOH / 30% H_2O
Column #15 Sample 73	1142	1.3%	70% MeOH / 30% H_2O
Column #15 Sample 74	1416	1.6%	70% MeOH / 30% H_2O
Column #15 Sample 75	18176	20.1%	70% MeOH / 30% H_2O
Column #15 Sample 76	15620	17.3%	70% MeOH / 30% H_2O
Column #15 Sample 77	9445	10.4%	70% MeOH / 30% H_2O
Column #15 Sample 78	13754	15.2%	transition
Column #15 Sample 79	16706	18.5%	80% MeOH / 20% H_2O

Table A14, continued.

Column #15 Sample 80	13186	14.6%	80% MeOH / 20% H_2O
Column #15 Sample 81	12010	13.3%	80% MeOH / 20% H_2O
Column #15 Sample 82	19324	21.4%	80% MeOH / 20% H_2O
Column #15 Sample 83	6747	7.5%	80% MeOH / 20% H_2O
Column #15 Sample 84	8147	9.0%	80% MeOH / 20% H_2O
Column #15 Sample 85	15487	17.1%	80% MeOH / 20% H_2O
Column #15 Sample 86	16579	18.3%	80% MeOH / 20% H_2O
Column #15 Sample 87	19463	21.5%	80% MeOH / 20% H_2O

Table A15. Results for B16-F10 cell proliferation assay with Column #16 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	12243	100.0%	N/A
Average 10 mg/mL Elderberry	5003	40.9%	N/A
Column #16 Sample 1	230	1.9%	0% MeOH / 100% H_2O
Column #16 Sample 2	427	3.5%	0% MeOH / 100% H_2O
Column #16 Sample 3	262	2.1%	0% MeOH / 100% H_2O
Column #16 Sample 4	235	1.9%	0% MeOH / 100% H_2O
Column #16 Sample 5	257	2.1%	0% MeOH / 100% H_2O
Column #16 Sample 6	269	2.2%	transition
Column #16 Sample 7	510	4.2%	10% MeOH / 90% H_2O
Column #16 Sample 8	211	1.7%	10% MeOH / 90% H_2O
Column #16 Sample 9	295	2.4%	10% MeOH / 90% H_2O
Column #16 Sample 10	294	2.4%	10% MeOH / 90% H_2O
Column #16 Sample 11	316	2.6%	10% MeOH / 90% H_2O
Column #16 Sample 12	323	2.6%	10% MeOH / 90% H_2O
Column #16 Sample 13	603	4.9%	10% MeOH / 90% H_2O
Column #16 Sample 14	501	4.1%	10% MeOH / 90% H_2O
Column #16 Sample 15	1612	13.2%	10% MeOH / 90% H_2O
Column #16 Sample 16	819	6.7%	10% MeOH / 90% H_2O
Column #16 Sample 17	1066	8.7%	transition
Column #16 Sample 18	904	7.4%	20% MeOH / 80% H_2O

Table A15, continued.

Column #16 Sample 19	1360	11.1%	20% MeOH / 80% H_2O
Column #16 Sample 20	1972	16.1%	20% MeOH / 80% H_2O
Column #16 Sample 21	3474	28.4%	20% MeOH / 80% H_2O
Column #16 Sample 22	1248	10.2%	20% MeOH / 80% H_2O
Column #16 Sample 23	4226	34.5%	20% MeOH / 80% H_2O
Column #16 Sample 24	8393	68.6%	20% MeOH / 80% H_2O
Column #16 Sample 25	5453	44.5%	20% MeOH / 80% H_2O
Column #16 Sample 26	2440	19.9%	20% MeOH / 80% H_2O
Column #16 Sample 27	1854	15.1%	20% MeOH / 80% H_2O
Column #16 Sample 28	1098	9.0%	transition
Column #16 Sample 29	920	7.5%	30% MeOH / 70% H_2O
Column #16 Sample 30	1866	15.2%	30% MeOH / 70% H_2O
Column #16 Sample 31	1198	9.8%	30% MeOH / 70% H_2O
Column #16 Sample 32	1182	9.7%	30% MeOH / 70% H_2O
Column #16 Sample 33	1152	9.4%	30% MeOH / 70% H_2O
Column #16 Sample 34	3867	31.6%	30% MeOH / 70% H_2O
Column #16 Sample 35	7811	63.8%	30% MeOH / 70% H_2O
Column #16 Sample 36	3604	29.4%	30% MeOH / 70% H_2O
Column #16 Sample 37	2525	20.6%	30% MeOH / 70% H_2O
Column #16 Sample 38	2282	18.6%	transition
Column #16 Sample 39	1355	11.1%	40% MeOH / 60% H_2O
Column #16 Sample 40	3367	27.5%	40% MeOH / 60% H_2O
Column #16 Sample 41	1507	12.3%	40% MeOH / 60% H_2O
Column #16 Sample 42	5461	44.6%	40% MeOH / 60% H_2O
Column #16 Sample 43	2881	23.5%	40% MeOH / 60% H_2O
Column #16 Sample 44	10573	86.4%	40% MeOH / 60% H_2O
Column #16 Sample 45	5884	48.1%	40% MeOH / 60% H_2O
Column #16 Sample 46	1957	16.0%	40% MeOH / 60% H_2O
Column #16 Sample 47	1439	11.8%	40% MeOH / 60% H_2O
Column #16 Sample 48	803	6.6%	transition
Column #16 Sample 49	1137	9.3%	50% MeOH / 50% H_2O
Column #16 Sample 50	1149	9.4%	50% MeOH / 50% H_2O
Column #16 Sample 51	945	7.7%	50% MeOH / 50% H_2O

Table A15, continued.

Column #16 Sample 52	690	5.6%	50% MeOH / 50% H_2O
Column #16 Sample 53	1404	11.5%	50% MeOH / 50% H_2O
Column #16 Sample 54	1265	10.3%	50% MeOH / 50% H_2O
Column #16 Sample 55	5611	45.8%	50% MeOH / 50% H_2O
Column #16 Sample 56	1151	9.4%	50% MeOH / 50% H_2O
Column #16 Sample 57	597	4.9%	50% MeOH / 50% H_2O
Column #16 Sample 58	1918	15.7%	50% MeOH / 50% H_2O
Column #16 Sample 59	1144	9.3%	transition
Column #16 Sample 60	828	6.8%	60% MeOH / 40% H_2O
Column #16 Sample 61	602	4.9%	60% MeOH / 40% H_2O
Column #16 Sample 62	1210	9.9%	60% MeOH / 40% H_2O
Column #16 Sample 63	1059	8.6%	60% MeOH / 40% H_2O
Column #16 Sample 64	1270	10.4%	60% MeOH / 40% H_2O
Column #16 Sample 65	7338	59.9%	60% MeOH / 40% H_2O
Column #16 Sample 66	2376	19.4%	60% MeOH / 40% H_2O
Column #16 Sample 67	1822	14.9%	60% MeOH / 40% H_2O
Column #16 Sample 68	4747	38.8%	60% MeOH / 40% H_2O
Column #16 Sample 69	2181	17.8%	transition
Column #16 Sample 70	1496	12.2%	70% MeOH / 30% H_2O
Column #16 Sample 71	607	5.0%	70% MeOH / 30% H_2O
Column #16 Sample 72	1903	15.5%	70% MeOH / 30% H_2O
Column #16 Sample 73	668	5.5%	70% MeOH / 30% H_2O
Column #16 Sample 74	1075	8.8%	70% MeOH / 30% H_2O
Column #16 Sample 75	2797	22.8%	70% MeOH / 30% H_2O
Column #16 Sample 76	1136	9.3%	70% MeOH / 30% H_2O
Column #16 Sample 77	96	0.8%	70% MeOH / 30% H_2O
Column #16 Sample 78	285	2.3%	70% MeOH / 30% H_2O
Column #16 Sample 79	586	4.8%	transition
Column #16 Sample 80	229	1.9%	80% MeOH / 20% H_2O
Column #16 Sample 81	269	2.2%	80% MeOH / 20% H_2O
Column #16 Sample 82	616	5.0%	80% MeOH / 20% H_2O
Column #16 Sample 83	645	5.3%	80% MeOH / 20% H_2O
Column #16 Sample 84	126	1.0%	80% MeOH / 20% H_2O

Table A15, continued.

Column #16 Sample 85	241	2.0%	80% MeOH / 20% H_2O
Column #16 Sample 86	973	7.9%	80% MeOH / 20% H_2O
Column #16 Sample 87	221	1.8%	80% MeOH / 20% H_2O
Column #16 Sample 88	219	1.8%	80% MeOH / 20% H_2O
Column #16 Sample 89	7578	61.9%	80% MeOH / 20% H_2O

Table A16. Results for B16-F10 cell proliferation assay with Column #17 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	12636	100.0%	N/A
Average 10 mg/mL Elderberry	4762	37.7%	N/A
Column #17 Sample 1	156	1.2%	0% MeOH / 100% H_2O
Column #17 Sample 2	189	1.5%	0% MeOH / 100% H_2O
Column #17 Sample 3	111	0.9%	0% MeOH / 100% H_2O
Column #17 Sample 4	92	0.7%	0% MeOH / 100% H_2O
Column #17 Sample 5	136	1.1%	0% MeOH / 100% H_2O
Column #17 Sample 6	97	0.8%	transition
Column #17 Sample 7	147	1.2%	10% MeOH / 90% H_2O
Column #17 Sample 8	121	1.0%	10% MeOH / 90% H_2O
Column #17 Sample 9	90	0.7%	10% MeOH / 90% H_2O
Column #17 Sample 10	112	0.9%	10% MeOH / 90% H_2O
Column #17 Sample 11	122	1.0%	10% MeOH / 90% H_2O
Column #17 Sample 12	131	1.0%	10% MeOH / 90% H_2O
Column #17 Sample 13	121	1.0%	10% MeOH / 90% H_2O
Column #17 Sample 14	305	2.4%	10% MeOH / 90% H_2O
Column #17 Sample 15	542	4.3%	10% MeOH / 90% H_2O
Column #17 Sample 16	459	3.6%	10% MeOH / 90% H_2O
Column #17 Sample 17	1918	15.2%	transition
Column #17 Sample 18	503	4.0%	20% MeOH / 80% H_2O
Column #17 Sample 19	1507	11.9%	20% MeOH / 80% H_2O
Column #17 Sample 20	3877	30.7%	20% MeOH / 80% H_2O
Column #17 Sample 21	3732	29.5%	20% MeOH / 80% H_2O

Table A16, continued.

Column #17 Sample 22	2625	20.8%	20% MeOH / 80% H_2O
Column #17 Sample 23	4216	33.4%	20% MeOH / 80% H_2O
Column #17 Sample 24	7476	59.2%	20% MeOH / 80% H_2O
Column #17 Sample 25	3981	31.5%	20% MeOH / 80% H_2O
Column #17 Sample 26	6780	53.7%	20% MeOH / 80% H_2O
Column #17 Sample 27	4777	37.8%	20% MeOH / 80% H_2O
Column #17 Sample 28	5275	41.7%	transition
Column #17 Sample 29	6261	49.5%	30% MeOH / 70% H_2O
Column #17 Sample 30	5656	44.8%	30% MeOH / 70% H_2O
Column #17 Sample 31	5160	40.8%	30% MeOH / 70% H_2O
Column #17 Sample 32	4414	34.9%	30% MeOH / 70% H_2O
Column #17 Sample 33	3905	30.9%	30% MeOH / 70% H_2O
Column #17 Sample 34	1441	11.4%	30% MeOH / 70% H_2O
Column #17 Sample 35	10035	79.4%	30% MeOH / 70% H_2O
Column #17 Sample 36	7212	57.1%	30% MeOH / 70% H_2O
Column #17 Sample 37	8585	67.9%	30% MeOH / 70% H_2O
Column #17 Sample 38	9140	72.3%	30% MeOH / 70% H_2O
Column #17 Sample 39	9598	76.0%	transition
Column #17 Sample 40	8918	70.6%	40% MeOH / 60% H_2O
Column #17 Sample 41	7615	60.3%	40% MeOH / 60% H_2O
Column #17 Sample 42	8191	64.8%	40% MeOH / 60% H_2O
Column #17 Sample 43	8419	66.6%	40% MeOH / 60% H_2O
Column #17 Sample 44	8912	70.5%	40% MeOH / 60% H_2O
Column #17 Sample 45	2719	21.5%	40% MeOH / 60% H_2O
Column #17 Sample 46	12177	96.4%	40% MeOH / 60% H_2O
Column #17 Sample 47	9601	76.0%	40% MeOH / 60% H_2O
Column #17 Sample 48	6001	47.5%	40% MeOH / 60% H_2O
Column #17 Sample 49	9766	77.3%	40% MeOH / 60% H_2O
Column #17 Sample 50	8305	65.7%	transition
Column #17 Sample 51	5150	40.8%	50% MeOH / 50% H_2O
Column #17 Sample 52	7661	60.6%	50% MeOH / 50% H_2O
Column #17 Sample 53	1656	13.1%	50% MeOH / 50% H_2O
Column #17 Sample 54	1403	11.1%	50% MeOH / 50% H_2O

Table A16, continued.

Column #17 Sample 55	1063	8.4%	50% MeOH / 50% H_2O
Column #17 Sample 56	5058	40.0%	50% MeOH / 50% H_2O
Column #17 Sample 57	177	1.4%	50% MeOH / 50% H_2O
Column #17 Sample 58	179	1.4%	50% MeOH / 50% H_2O
Column #17 Sample 59	271	2.1%	50% MeOH / 50% H_2O
Column #17 Sample 60	121	1.0%	transition
Column #17 Sample 61	203	1.6%	60% MeOH / 40% H_2O
Column #17 Sample 62	74	0.6%	60% MeOH / 40% H_2O
Column #17 Sample 63	129	1.0%	60% MeOH / 40% H_2O
Column #17 Sample 64	3321	26.3%	60% MeOH / 40% H_2O
Column #17 Sample 65	96	0.8%	60% MeOH / 40% H_2O
Column #17 Sample 66	126	1.0%	60% MeOH / 40% H_2O
Column #17 Sample 67	2011	15.9%	60% MeOH / 40% H_2O
Column #17 Sample 68	3645	28.8%	60% MeOH / 40% H_2O
Column #17 Sample 69	121	1.0%	transition
Column #17 Sample 70	1333	10.5%	70% MeOH / 30% H_2O
Column #17 Sample 71	89	0.7%	70% MeOH / 30% H_2O
Column #17 Sample 72	277	2.2%	70% MeOH / 30% H_2O
Column #17 Sample 73	443	3.5%	70% MeOH / 30% H_2O
Column #17 Sample 74	108	0.9%	70% MeOH / 30% H_2O
Column #17 Sample 75	141	1.1%	70% MeOH / 30% H_2O
Column #17 Sample 76	109	0.9%	70% MeOH / 30% H_2O
Column #17 Sample 77	161	1.3%	70% MeOH / 30% H_2O
Column #17 Sample 78	1250	9.9%	70% MeOH / 30% H_2O
Column #17 Sample 79	6224	49.3%	70% MeOH / 30% H_2O
Column #17 Sample 80	2440	19.3%	transition
Column #17 Sample 81	6764	53.5%	80% MeOH / 20% H_2O
Column #17 Sample 82	10156	80.4%	80% MeOH / 20% H_2O
Column #17 Sample 83	5374	42.5%	80% MeOH / 20% H_2O
Column #17 Sample 84	7535	59.6%	80% MeOH / 20% H_2O
Column #17 Sample 85	5982	47.3%	80% MeOH / 20% H_2O
Column #17 Sample 86	3996	31.6%	80% MeOH / 20% H_2O
Column #17 Sample 87	93	0.7%	80% MeOH / 20% H_2O

Table A16, continued.

Column #17 Sample 88	107	0.8%	80% MeOH / 20% H_2O
Column #17 Sample 89	13533	107.1%	80% MeOH / 20% H_2O

Table A17. Results for B16-F10 cell proliferation assay with Column #18 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	7159	100.0%	N/A
Average 10 mg/mL Elderberry	2257	31.5%	N/A
Column #18 Sample 1	154	2.2%	0% MeOH / 100% H_2O
Column #18 Sample 2	243	3.4%	0% MeOH / 100% H_2O
Column #18 Sample 3	158	2.2%	0% MeOH / 100% H_2O
Column #18 Sample 4	82	1.1%	0% MeOH / 100% H_2O
Column #18 Sample 5	103	1.4%	0% MeOH / 100% H_2O
Column #18 Sample 6	136	1.9%	transition
Column #18 Sample 7	97	1.4%	10% MeOH / 90% H_2O
Column #18 Sample 8	95	1.3%	10% MeOH / 90% H_2O
Column #18 Sample 9	101	1.4%	10% MeOH / 90% H_2O
Column #18 Sample 10	79	1.1%	10% MeOH / 90% H_2O
Column #18 Sample 11	90	1.3%	10% MeOH / 90% H_2O
Column #18 Sample 12	330	4.6%	10% MeOH / 90% H_2O
Column #18 Sample 13	101	1.4%	10% MeOH / 90% H_2O
Column #18 Sample 14	162	2.3%	10% MeOH / 90% H_2O
Column #18 Sample 15	145	2.0%	10% MeOH / 90% H_2O
Column #18 Sample 16	1488	20.8%	10% MeOH / 90% H_2O
Column #18 Sample 17	579	8.1%	transition
Column #18 Sample 18	133	1.9%	20% MeOH / 80% H_2O
Column #18 Sample 19	130	1.8%	20% MeOH / 80% H_2O
Column #18 Sample 20	136	1.9%	20% MeOH / 80% H_2O
Column #18 Sample 21	135	1.9%	20% MeOH / 80% H_2O
Column #18 Sample 22	532	7.4%	20% MeOH / 80% H_2O
Column #18 Sample 23	896	12.5%	20% MeOH / 80% H_2O
Column #18 Sample 24	112	1.6%	20% MeOH / 80% H_2O
Column #18 Sample 25	633	8.8%	20% MeOH / 80% H_2O

Table A17, continued.

Column #18 Sample 26	2732	38.2%	20% MeOH / 80% H_2O
Column #18 Sample 27	3728	52.1%	transition
Column #18 Sample 28	1597	22.3%	30% MeOH / 70% H_2O
Column #18 Sample 29	2679	37.4%	30% MeOH / 70% H_2O
Column #18 Sample 30	3010	42.0%	30% MeOH / 70% H_2O
Column #18 Sample 31	82	1.1%	30% MeOH / 70% H_2O
Column #18 Sample 32	1403	19.6%	30% MeOH / 70% H_2O
Column #18 Sample 33	60	0.8%	30% MeOH / 70% H_2O
Column #18 Sample 34	2194	30.6%	30% MeOH / 70% H_2O
Column #18 Sample 35	180	2.5%	30% MeOH / 70% H_2O
Column #18 Sample 36	1210	16.9%	30% MeOH / 70% H_2O
Column #18 Sample 37	6253	87.3%	30% MeOH / 70% H_2O
Column #18 Sample 38	4524	63.2%	transition
Column #18 Sample 39	129	1.8%	40% MeOH / 60% H_2O
Column #18 Sample 40	2074	29.0%	40% MeOH / 60% H_2O
Column #18 Sample 41	4386	61.3%	40% MeOH / 60% H_2O
Column #18 Sample 42	3188	44.5%	40% MeOH / 60% H_2O
Column #18 Sample 43	1287	18.0%	40% MeOH / 60% H_2O
Column #18 Sample 44	1862	26.0%	40% MeOH / 60% H_2O
Column #18 Sample 45	2052	28.7%	40% MeOH / 60% H_2O
Column #18 Sample 46	1945	27.2%	40% MeOH / 60% H_2O
Column #18 Sample 47	3008	42.0%	40% MeOH / 60% H_2O
Column #18 Sample 48	2110	29.5%	40% MeOH / 60% H_2O
Column #18 Sample 49	3303	46.1%	transition
Column #18 Sample 50	2199	30.7%	50% MeOH / 50% H_2O
Column #18 Sample 51	2041	28.5%	50% MeOH / 50% H_2O
Column #18 Sample 52	1765	24.7%	50% MeOH / 50% H_2O
Column #18 Sample 53	2111	29.5%	50% MeOH / 50% H_2O
Column #18 Sample 54	1948	27.2%	50% MeOH / 50% H_2O
Column #18 Sample 55	4796	67.0%	50% MeOH / 50% H_2O
Column #18 Sample 56	6866	95.9%	50% MeOH / 50% H_2O
Column #18 Sample 57	1970	27.5%	50% MeOH / 50% H_2O
Column #18 Sample 58	8151	113.9%	50% MeOH / 50% H_2O

Table A17, continued.

Column #18 Sample 59	4958	69.3%	50% MeOH / 50% H_2O
Column #18 Sample 60	215	3.0%	transition
Column #18 Sample 61	3647	50.9%	60% MeOH / 40% H_2O
Column #18 Sample 62	1741	24.3%	60% MeOH / 40% H_2O
Column #18 Sample 63	8061	112.6%	60% MeOH / 40% H_2O
Column #18 Sample 64	4158	58.1%	60% MeOH / 40% H_2O
Column #18 Sample 65	2941	41.1%	60% MeOH / 40% H_2O
Column #18 Sample 66	1799	25.1%	60% MeOH / 40% H_2O
Column #18 Sample 67	2895	40.4%	60% MeOH / 40% H_2O
Column #18 Sample 68	1268	17.7%	60% MeOH / 40% H_2O
Column #18 Sample 69	1656	23.1%	60% MeOH / 40% H_2O
Column #18 Sample 70	1624	22.7%	60% MeOH / 40% H_2O
Column #18 Sample 71	1772	24.8%	transition
Column #18 Sample 72	323	4.5%	70% MeOH / 30% H_2O
Column #18 Sample 73	535	7.5%	70% MeOH / 30% H_2O
Column #18 Sample 74	983	13.7%	70% MeOH / 30% H_2O
Column #18 Sample 75	380	5.3%	70% MeOH / 30% H_2O
Column #18 Sample 76	114	1.6%	70% MeOH / 30% H_2O
Column #18 Sample 77	132	1.8%	70% MeOH / 30% H_2O
Column #18 Sample 78	116	1.6%	70% MeOH / 30% H_2O
Column #18 Sample 79	88	1.2%	70% MeOH / 30% H_2O
Column #18 Sample 80	176	2.5%	70% MeOH / 30% H_2O
Column #18 Sample 81	108	1.5%	transition
Column #18 Sample 82	123	1.7%	80% MeOH / 20% H_2O
Column #18 Sample 83	134	1.9%	80% MeOH / 20% H_2O
Column #18 Sample 84	88	1.2%	80% MeOH / 20% H_2O
Column #18 Sample 85	93	1.3%	80% MeOH / 20% H_2O
Column #18 Sample 86	148	2.1%	80% MeOH / 20% H_2O
Column #18 Sample 87	90	1.3%	80% MeOH / 20% H_2O
Column #18 Sample 88	70	1.0%	80% MeOH / 20% H_2O
Column #18 Sample 89	82	1.1%	80% MeOH / 20% H_2O
Column #18 Sample 90	76	1.1%	80% MeOH / 20% H_2O
Column #18 Sample 91	1348	18.8%	80% MeOH / 20% H_2O

Table A18. Results for B16-F10 cell proliferation assay with Column #19 individual samples.

Sample Identity	CPM	% Proliferation (Control = 100%)	Eluting Mobile Phase
Average Control	14189	100.0%	N/A
Average 10 mg/mL Elderberry	3525	24.8%	N/A
Column #19 Sample 1	110	0.8%	0% MeOH / 100% H_2O
Column #19 Sample 2	140	1.0%	0% MeOH / 100% H_2O
Column #19 Sample 3	133	0.9%	0% MeOH / 100% H_2O
Column #19 Sample 4	84	0.6%	0% MeOH / 100% H_2O
Column #19 Sample 5	69	0.5%	transition
Column #19 Sample 6	74	0.5%	10% MeOH / 90% H_2O
Column #19 Sample 7	81	0.6%	10% MeOH / 90% H_2O
Column #19 Sample 8	85	0.6%	10% MeOH / 90% H_2O
Column #19 Sample 9	95	0.7%	10% MeOH / 90% H_2O
Column #19 Sample 10	119	0.8%	10% MeOH / 90% H_2O
Column #19 Sample 11	85	0.6%	10% MeOH / 90% H_2O
Column #19 Sample 12	68	0.5%	10% MeOH / 90% H_2O
Column #19 Sample 13	96	0.7%	10% MeOH / 90% H_2O
Column #19 Sample 14	89	0.6%	10% MeOH / 90% H_2O
Column #19 Sample 15	96	0.7%	10% MeOH / 90% H_2O
Column #19 Sample 16	115	0.8%	transition
Column #19 Sample 17	100	0.7%	20% MeOH / 80% H_2O
Column #19 Sample 18	164	1.2%	20% MeOH / 80% H_2O
Column #19 Sample 19	651	4.6%	20% MeOH / 80% H_2O
Column #19 Sample 20	2371	16.7%	20% MeOH / 80% H_2O
Column #19 Sample 21	781	5.5%	20% MeOH / 80% H_2O
Column #19 Sample 22	2895	20.4%	20% MeOH / 80% H_2O
Column #19 Sample 23	99	0.7%	20% MeOH / 80% H_2O
Column #19 Sample 24	147	1.0%	20% MeOH / 80% H_2O
Column #19 Sample 25	120	0.8%	20% MeOH / 80% H_2O
Column #19 Sample 26	2374	16.7%	20% MeOH / 80% H_2O
Column #19 Sample 27	67	0.5%	transition
Column #19 Sample 28	77	0.5%	30% MeOH / 70% H_2O
Column #19 Sample 29	71	0.5%	30% MeOH / 70% H_2O
Column #19 Sample 30	1426	10.1%	30% MeOH / 70% H_2O
Column #19 Sample 31	61	0.4%	30% MeOH / 70% H_2O

Table A18, continued.

Column #19 Sample 32	88	0.6%	30% MeOH / 70% H_2O
Column #19 Sample 33	77	0.5%	30% MeOH / 70% H_2O
Column #19 Sample 34	2334	16.4%	30% MeOH / 70% H_2O
Column #19 Sample 35	97	0.7%	30% MeOH / 70% H_2O
Column #19 Sample 36	107	0.8%	30% MeOH / 70% H_2O
Column #19 Sample 37	1808	12.7%	30% MeOH / 70% H_2O
Column #19 Sample 38	696	4.9%	transition
Column #19 Sample 39	107	0.8%	40% MeOH / 60% H_2O
Column #19 Sample 40	154	1.1%	40% MeOH / 60% H_2O
Column #19 Sample 41	70	0.5%	40% MeOH / 60% H_2O
Column #19 Sample 42	104	0.7%	40% MeOH / 60% H_2O
Column #19 Sample 43	133	0.9%	40% MeOH / 60% H_2O
Column #19 Sample 44	5219	36.8%	40% MeOH / 60% H_2O
Column #19 Sample 45	234	1.6%	40% MeOH / 60% H_2O
Column #19 Sample 46	67	0.5%	40% MeOH / 60% H_2O
Column #19 Sample 47	302	2.1%	40% MeOH / 60% H_2O
Column #19 Sample 48	890	6.3%	40% MeOH / 60% H_2O
Column #19 Sample 49	139	1.0%	transition
Column #19 Sample 50	6012	42.4%	50% MeOH / 50% H_2O
Column #19 Sample 51	4316	30.4%	50% MeOH / 50% H_2O
Column #19 Sample 52	6799	47.9%	50% MeOH / 50% H_2O
Column #19 Sample 53	2706	19.1%	50% MeOH / 50% H_2O
Column #19 Sample 54	3463	24.4%	50% MeOH / 50% H_2O
Column #19 Sample 55	7213	50.8%	50% MeOH / 50% H_2O
Column #19 Sample 56	12487	88.0%	50% MeOH / 50% H_2O
Column #19 Sample 57	11205	79.0%	50% MeOH / 50% H_2O
Column #19 Sample 58	4939	34.8%	50% MeOH / 50% H_2O
Column #19 Sample 59	6616	46.6%	50% MeOH / 50% H_2O
Column #19 Sample 60	8650	61.0%	transition
Column #19 Sample 61	844	5.9%	60% MeOH / 40% H_2O
Column #19 Sample 62	995	7.0%	60% MeOH / 40% H_2O
Column #19 Sample 63	108	0.8%	60% MeOH / 40% H_2O
Column #19 Sample 64	257	1.8%	60% MeOH / 40% H_2O

Table A18, continued.

Column #19 Sample 65	59	0.4%	60% MeOH / 40% H_2O
Column #19 Sample 66	3018	21.3%	60% MeOH / 40% H_2O
Column #19 Sample 67	84	0.6%	60% MeOH / 40% H_2O
Column #19 Sample 68	76	0.5%	60% MeOH / 40% H_2O
Column #19 Sample 69	94	0.7%	60% MeOH / 40% H_2O
Column #19 Sample 70	167	1.2%	transition
Column #19 Sample 71	2344	16.5%	70% MeOH / 30% H_2O
Column #19 Sample 72	1006	7.1%	70% MeOH / 30% H_2O
Column #19 Sample 73	114	0.8%	70% MeOH / 30% H_2O
Column #19 Sample 74	1057	7.4%	70% MeOH / 30% H_2O
Column #19 Sample 75	665	4.7%	70% MeOH / 30% H_2O
Column #19 Sample 76	6545	46.1%	70% MeOH / 30% H_2O
Column #19 Sample 77	4760	33.5%	70% MeOH / 30% H_2O
Column #19 Sample 78	3325	23.4%	70% MeOH / 30% H_2O
Column #19 Sample 79	415	2.9%	70% MeOH / 30% H_2O
Column #19 Sample 80	1399	9.9%	70% MeOH / 30% H_2O
Column #19 Sample 81	1633	11.5%	transition
Column #19 Sample 82	119	0.8%	80% MeOH / 20% H_2O
Column #19 Sample 83	197	1.4%	80% MeOH / 20% H_2O
Column #19 Sample 84	341	2.4%	80% MeOH / 20% H_2O
Column #19 Sample 85	81	0.6%	80% MeOH / 20% H_2O
Column #19 Sample 86	98	0.7%	80% MeOH / 20% H_2O
Column #19 Sample 87	122	0.9%	80% MeOH / 20% H_2O
Column #19 Sample 88	78	0.5%	80% MeOH / 20% H_2O
Column #19 Sample 89	104	0.7%	80% MeOH / 20% H_2O
Column #19 Sample 90	118	0.8%	80% MeOH / 20% H_2O
Column #19 Sample 91	167	1.2%	80% MeOH / 20% H_2O

Table A19. Results for B16-F10 cell proliferation assay with Column #20 individual samples.

Sample Identity	CPM	% Proliferation (Control = 100%)	Eluting Mobile Phase
Average Control	35762	100.0%	N/A
Average 10 mg/mL Elderberry	14552	40.7%	N/A
Column #20 Sample 1	160	0.4%	0% MeOH / 100% H_2O
Column #20 Sample 2	131	0.4%	0% MeOH / 100% H_2O
Column #20 Sample 3	96	0.3%	0% MeOH / 100% H_2O
Column #20 Sample 4	51	0.1%	0% MeOH / 100% H_2O
Column #20 Sample 5	102	0.3%	0% MeOH / 100% H_2O
Column #20 Sample 6	53	0.1%	0% MeOH / 100% H_2O
Column #20 Sample 7	51	0.1%	transition
Column #20 Sample 8	23	0.1%	10% MeOH / 90% H_2O
Column #20 Sample 9	50	0.1%	10% MeOH / 90% H_2O
Column #20 Sample 10	94	0.3%	10% MeOH / 90% H_2O
Column #20 Sample 11	71	0.2%	10% MeOH / 90% H_2O
Column #20 Sample 12	87	0.2%	10% MeOH / 90% H_2O
Column #20 Sample 13	53	0.1%	10% MeOH / 90% H_2O
Column #20 Sample 14	78	0.2%	10% MeOH / 90% H_2O
Column #20 Sample 15	105	0.3%	10% MeOH / 90% H_2O
Column #20 Sample 16	1799	5.0%	10% MeOH / 90% H_2O
Column #20 Sample 17	154	0.4%	10% MeOH / 90% H_2O
Column #20 Sample 18	656	1.8%	transition
Column #20 Sample 19	11099	31.0%	20% MeOH / 80% H_2O
Column #20 Sample 20	1056	3.0%	20% MeOH / 80% H_2O
Column #20 Sample 21	4232	11.8%	20% MeOH / 80% H_2O
Column #20 Sample 22	165	0.5%	20% MeOH / 80% H_2O
Column #20 Sample 23	4796	13.4%	20% MeOH / 80% H_2O
Column #20 Sample 24	23703	66.3%	20% MeOH / 80% H_2O
Column #20 Sample 25	1200	3.4%	20% MeOH / 80% H_2O
Column #20 Sample 26	17718	49.5%	20% MeOH / 80% H_2O
Column #20 Sample 27	20270	56.7%	transition
Column #20 Sample 28	1072	3.0%	30% MeOH / 70% H_2O
Column #20 Sample 29	194	0.5%	30% MeOH / 70% H_2O
Column #20 Sample 30	95	0.3%	30% MeOH / 70% H_2O
Column #20 Sample 31	3092	8.6%	30% MeOH / 70% H_2O

Table A19, continued.

Column #20 Sample 32	98	0.3%	30% MeOH / 70% H_2O
Column #20 Sample 33	96	0.3%	30% MeOH / 70% H_2O
Column #20 Sample 34	2268	6.3%	30% MeOH / 70% H_2O
Column #20 Sample 35	290	0.8%	30% MeOH / 70% H_2O
Column #20 Sample 36	129	0.4%	30% MeOH / 70% H_2O
Column #20 Sample 37	165	0.5%	30% MeOH / 70% H_2O
Column #20 Sample 38	212	0.6%	transition
Column #20 Sample 39	130	0.4%	40% MeOH / 60% H_2O
Column #20 Sample 40	190	0.5%	40% MeOH / 60% H_2O
Column #20 Sample 41	109	0.3%	40% MeOH / 60% H_2O
Column #20 Sample 42	3422	9.6%	40% MeOH / 60% H_2O
Column #20 Sample 43	37291	104.3%	40% MeOH / 60% H_2O
Column #20 Sample 44	8214	23.0%	40% MeOH / 60% H_2O
Column #20 Sample 45	129	0.4%	40% MeOH / 60% H_2O
Column #20 Sample 46	162	0.5%	40% MeOH / 60% H_2O
Column #20 Sample 47	472	1.3%	40% MeOH / 60% H_2O
Column #20 Sample 48	12078	33.8%	40% MeOH / 60% H_2O
Column #20 Sample 49	2068	5.8%	transition
Column #20 Sample 50	95	0.3%	50% MeOH / 50% H_2O
Column #20 Sample 51	2042	5.7%	50% MeOH / 50% H_2O
Column #20 Sample 52	4100	11.5%	50% MeOH / 50% H_2O
Column #20 Sample 53	85	0.2%	50% MeOH / 50% H_2O
Column #20 Sample 54	15569	43.5%	50% MeOH / 50% H_2O
Column #20 Sample 55	140	0.4%	50% MeOH / 50% H_2O
Column #20 Sample 56	16609	46.4%	50% MeOH / 50% H_2O
Column #20 Sample 57	990	2.8%	50% MeOH / 50% H_2O
Column #20 Sample 58	4106	11.5%	50% MeOH / 50% H_2O
Column #20 Sample 59	1515	4.2%	transition
Column #20 Sample 60	140	0.4%	60% MeOH / 40% H_2O
Column #20 Sample 61	99	0.3%	60% MeOH / 40% H_2O
Column #20 Sample 62	2519	7.0%	60% MeOH / 40% H_2O
Column #20 Sample 63	115	0.3%	60% MeOH / 40% H_2O
Column #20 Sample 64	6449	18.0%	60% MeOH / 40% H_2O

Table A19, continued.

Column #20 Sample 65	84	0.2%	60% MeOH / 40% H_2O
Column #20 Sample 66	93	0.3%	60% MeOH / 40% H_2O
Column #20 Sample 67	2420	6.8%	60% MeOH / 40% H_2O
Column #20 Sample 68	16925	47.3%	60% MeOH / 40% H_2O
Column #20 Sample 69	78	0.2%	transition
Column #20 Sample 70	131	0.4%	70% MeOH / 30% H_2O
Column #20 Sample 71	5219	14.6%	70% MeOH / 30% H_2O
Column #20 Sample 72	5159	14.4%	70% MeOH / 30% H_2O
Column #20 Sample 73	1527	4.3%	70% MeOH / 30% H_2O
Column #20 Sample 74	766	2.1%	70% MeOH / 30% H_2O
Column #20 Sample 75	2215	6.2%	70% MeOH / 30% H_2O
Column #20 Sample 76	191	0.5%	70% MeOH / 30% H_2O
Column #20 Sample 77	651	1.8%	70% MeOH / 30% H_2O
Column #20 Sample 78	1326	3.7%	70% MeOH / 30% H_2O
Column #20 Sample 79	136	0.4%	transition
Column #20 Sample 80	161	0.5%	80% MeOH / 20% H_2O
Column #20 Sample 81	240	0.7%	80% MeOH / 20% H_2O
Column #20 Sample 82	164	0.5%	80% MeOH / 20% H_2O
Column #20 Sample 83	1283	3.6%	80% MeOH / 20% H_2O
Column #20 Sample 84	82	0.2%	80% MeOH / 20% H_2O
Column #20 Sample 85	238	0.7%	80% MeOH / 20% H_2O
Column #20 Sample 86	65	0.2%	80% MeOH / 20% H_2O
Column #20 Sample 87	44	0.1%	80% MeOH / 20% H_2O
Column #20 Sample 88	128	0.4%	80% MeOH / 20% H_2O
Column #20 Sample 89	293	0.8%	80% MeOH / 20% H_2O

Table A20. Results for B16-F10 cell proliferation assay with Column #21 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	38021	100.0%	N/A
Average 10 mg/mL Elderberry	14552	38.3%	N/A

Table A20, continued.

Column #21 Sample 1	96	0.3%	0% MeOH / 100% H_2O
Column #21 Sample 2	136	0.4%	0% MeOH / 100% H_2O
Column #21 Sample 3	99	0.3%	0% MeOH / 100% H_2O
Column #21 Sample 4	91	0.2%	0% MeOH / 100% H_2O
Column #21 Sample 5	85	0.2%	0% MeOH / 100% H_2O
Column #21 Sample 6	47	0.1%	transition
Column #21 Sample 7	50	0.1%	10% MeOH / 90% H_2O
Column #21 Sample 8	67	0.2%	10% MeOH / 90% H_2O
Column #21 Sample 9	60	0.2%	10% MeOH / 90% H_2O
Column #21 Sample 10	58	0.2%	10% MeOH / 90% H_2O
Column #21 Sample 11	58	0.2%	10% MeOH / 90% H_2O
Column #21 Sample 12	54	0.1%	10% MeOH / 90% H_2O
Column #21 Sample 13	3843	10.1%	10% MeOH / 90% H_2O
Column #21 Sample 14	196	0.5%	10% MeOH / 90% H_2O
Column #21 Sample 15	184	0.5%	10% MeOH / 90% H_2O
Column #21 Sample 16	12791	33.6%	10% MeOH / 90% H_2O
Column #21 Sample 17	380	1.0%	transition
Column #21 Sample 18	935	2.5%	20% MeOH / 80% H_2O
Column #21 Sample 19	6919	18.2%	20% MeOH / 80% H_2O
Column #21 Sample 20	4023	10.6%	20% MeOH / 80% H_2O
Column #21 Sample 21	6186	16.3%	20% MeOH / 80% H_2O
Column #21 Sample 22	19949	52.5%	20% MeOH / 80% H_2O
Column #21 Sample 23	17975	47.3%	20% MeOH / 80% H_2O
Column #21 Sample 24	24311	63.9%	20% MeOH / 80% H_2O
Column #21 Sample 25	7850	20.6%	20% MeOH / 80% H_2O
Column #21 Sample 26	10454	27.5%	20% MeOH / 80% H_2O
Column #21 Sample 27	12937	34.0%	20% MeOH / 80% H_2O
Column #21 Sample 28	7587	20.0%	transition
Column #21 Sample 29	13765	36.2%	30% MeOH / 70% H_2O
Column #21 Sample 30	14369	37.8%	30% MeOH / 70% H_2O
Column #21 Sample 31	9125	24.0%	30% MeOH / 70% H_2O
Column #21 Sample 32	7569	19.9%	30% MeOH / 70% H_2O
Column #21 Sample 33	14767	38.8%	30% MeOH / 70% H_2O

Table A20, continued.

Column #21 Sample 34	10543	27.7%	30% MeOH / 70% H_2O
Column #21 Sample 35	19302	50.8%	30% MeOH / 70% H_2O
Column #21 Sample 36	570	1.5%	30% MeOH / 70% H_2O
Column #21 Sample 37	14932	39.3%	30% MeOH / 70% H_2O
Column #21 Sample 38	21155	55.6%	30% MeOH / 70% H_2O
Column #21 Sample 39	14183	37.3%	transition
Column #21 Sample 40	10244	26.9%	40% MeOH / 60% H_2O
Column #21 Sample 41	23292	61.3%	40% MeOH / 60% H_2O
Column #21 Sample 42	16579	43.6%	40% MeOH / 60% H_2O
Column #21 Sample 43	18850	49.6%	40% MeOH / 60% H_2O
Column #21 Sample 44	20927	55.0%	40% MeOH / 60% H_2O
Column #21 Sample 45	21599	56.8%	40% MeOH / 60% H_2O
Column #21 Sample 46	17394	45.7%	40% MeOH / 60% H_2O
Column #21 Sample 47	22453	59.1%	40% MeOH / 60% H_2O
Column #21 Sample 48	13953	36.7%	40% MeOH / 60% H_2O
Column #21 Sample 49	21775	57.3%	40% MeOH / 60% H_2O
Column #21 Sample 50	12429	32.7%	transition
Column #21 Sample 51	20614	54.2%	50% MeOH / 50% H_2O
Column #21 Sample 52	24273	63.8%	50% MeOH / 50% H_2O
Column #21 Sample 53	17061	44.9%	50% MeOH / 50% H_2O
Column #21 Sample 54	17081	44.9%	50% MeOH / 50% H_2O
Column #21 Sample 55	16210	42.6%	50% MeOH / 50% H_2O
Column #21 Sample 56	5639	14.8%	50% MeOH / 50% H_2O
Column #21 Sample 57	22204	58.4%	50% MeOH / 50% H_2O
Column #21 Sample 58	14208	37.4%	50% MeOH / 50% H_2O
Column #21 Sample 59	19493	51.3%	50% MeOH / 50% H_2O
Column #21 Sample 60	24914	65.5%	50% MeOH / 50% H_2O
Column #21 Sample 61	626	1.6%	transition
Column #21 Sample 62	196	0.5%	60% MeOH / 40% H_2O
Column #21 Sample 63	2090	5.5%	60% MeOH / 40% H_2O
Column #21 Sample 64	80	0.2%	60% MeOH / 40% H_2O
Column #21 Sample 65	10509	27.6%	60% MeOH / 40% H_2O
Column #21 Sample 66	9665	25.4%	60% MeOH / 40% H_2O

Table A20, continued.

Column #21 Sample 67	5605	14.7%	60% MeOH / 40% H_2O
Column #21 Sample 68	3486	9.2%	60% MeOH / 40% H_2O
Column #21 Sample 69	1662	4.4%	60% MeOH / 40% H_2O
Column #21 Sample 70	5915	15.6%	60% MeOH / 40% H_2O
Column #21 Sample 71	13871	36.5%	60% MeOH / 40% H_2O
Column #21 Sample 72	79	0.2%	transition
Column #21 Sample 73	999	2.6%	70% MeOH / 30% H_2O
Column #21 Sample 74	5133	13.5%	70% MeOH / 30% H_2O
Column #21 Sample 75	196	0.5%	70% MeOH / 30% H_2O
Column #21 Sample 76	81	0.2%	70% MeOH / 30% H_2O
Column #21 Sample 77	3029	8.0%	70% MeOH / 30% H_2O
Column #21 Sample 78	130	0.3%	70% MeOH / 30% H_2O
Column #21 Sample 79	146	0.4%	70% MeOH / 30% H_2O
Column #21 Sample 80	85	0.2%	70% MeOH / 30% H_2O
Column #21 Sample 81	95	0.2%	70% MeOH / 30% H_2O
Column #21 Sample 82	172	0.5%	transition
Column #21 Sample 83	369	1.0%	80% MeOH / 20% H_2O
Column #21 Sample 84	3383	8.9%	80% MeOH / 20% H_2O
Column #21 Sample 85	58	0.2%	80% MeOH / 20% H_2O
Column #21 Sample 86	51	0.1%	80% MeOH / 20% H_2O
Column #21 Sample 87	2614	6.9%	80% MeOH / 20% H_2O
Column #21 Sample 88	83	0.2%	80% MeOH / 20% H_2O
Column #21 Sample 89	2305	6.1%	80% MeOH / 20% H_2O
Column #21 Sample 90	76	0.2%	80% MeOH / 20% H_2O
Column #21 Sample 91	57	0.1%	80% MeOH / 20% H_2O
Column #21 Sample 92	65	0.2%	80% MeOH / 20% H_2O

Table A21. Results for B16-F10 cell proliferation assay with Column #22 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	26280	100.0%	N/A

Table A21, continued.

Average 10 mg/mL Elderberry	11073	42.1%	N/A
Column #22 Sample 1	91	0.3%	0% MeOH / 100% H_2O
Column #22 Sample 2	162	0.6%	0% MeOH / 100% H_2O
Column #22 Sample 3	118	0.4%	0% MeOH / 100% H_2O
Column #22 Sample 4	123	0.5%	0% MeOH / 100% H_2O
Column #22 Sample 5	197	0.7%	0% MeOH / 100% H_2O
Column #22 Sample 6	244	0.9%	transition
Column #22 Sample 7	68	0.3%	10% MeOH / 90% H_2O
Column #22 Sample 8	223	0.8%	10% MeOH / 90% H_2O
Column #22 Sample 9	14339	54.6%	10% MeOH / 90% H_2O
Column #22 Sample 10	101	0.4%	10% MeOH / 90% H_2O
Column #22 Sample 11	96	0.4%	10% MeOH / 90% H_2O
Column #22 Sample 12	170	0.6%	10% MeOH / 90% H_2O
Column #22 Sample 13	4395	16.7%	10% MeOH / 90% H_2O
Column #22 Sample 14	1864	7.1%	10% MeOH / 90% H_2O
Column #22 Sample 15	3552	13.5%	10% MeOH / 90% H_2O
Column #22 Sample 16	7632	29.0%	10% MeOH / 90% H_2O
Column #22 Sample 17	7916	30.1%	transition
Column #22 Sample 18	118	0.4%	20% MeOH / 80% H_2O
Column #22 Sample 19	1246	4.7%	20% MeOH / 80% H_2O
Column #22 Sample 20	8756	33.3%	20% MeOH / 80% H_2O
Column #22 Sample 21	82	0.3%	20% MeOH / 80% H_2O
Column #22 Sample 22	298	1.1%	20% MeOH / 80% H_2O
Column #22 Sample 23	155	0.6%	20% MeOH / 80% H_2O
Column #22 Sample 24	12266	46.7%	20% MeOH / 80% H_2O
Column #22 Sample 25	762	2.9%	20% MeOH / 80% H_2O
Column #22 Sample 26	73	0.3%	20% MeOH / 80% H_2O
Column #22 Sample 27	120	0.5%	20% MeOH / 80% H_2O
Column #22 Sample 28	4048	15.4%	transition
Column #22 Sample 29	112	0.4%	30% MeOH / 70% H_2O
Column #22 Sample 30	6927	26.4%	30% MeOH / 70% H_2O
Column #22 Sample 31	142	0.5%	30% MeOH / 70% H_2O
Column #22 Sample 32	10364	39.4%	30% MeOH / 70% H_2O

Table A21, continued.

Column #22 Sample 33	8728	33.2%	30% MeOH / 70% H_2O
Column #22 Sample 34	12275	46.7%	30% MeOH / 70% H_2O
Column #22 Sample 35	19625	74.7%	30% MeOH / 70% H_2O
Column #22 Sample 36	5911	22.5%	30% MeOH / 70% H_2O
Column #22 Sample 37	14361	54.6%	30% MeOH / 70% H_2O
Column #22 Sample 38	90	0.3%	transition
Column #22 Sample 39	68	0.3%	40% MeOH / 60% H_2O
Column #22 Sample 40	845	3.2%	40% MeOH / 60% H_2O
Column #22 Sample 41	83	0.3%	40% MeOH / 60% H_2O
Column #22 Sample 42	4916	18.7%	40% MeOH / 60% H_2O
Column #22 Sample 43	224	0.9%	40% MeOH / 60% H_2O
Column #22 Sample 44	90	0.3%	40% MeOH / 60% H_2O
Column #22 Sample 45	75	0.3%	40% MeOH / 60% H_2O
Column #22 Sample 46	55	0.2%	40% MeOH / 60% H_2O
Column #22 Sample 47	10024	38.1%	40% MeOH / 60% H_2O
Column #22 Sample 48	11836	45.0%	transition
Column #22 Sample 49	75	0.3%	50% MeOH / 50% H_2O
Column #22 Sample 50	59	0.2%	50% MeOH / 50% H_2O
Column #22 Sample 51	87	0.3%	50% MeOH / 50% H_2O
Column #22 Sample 52	74	0.3%	50% MeOH / 50% H_2O
Column #22 Sample 53	753	2.9%	50% MeOH / 50% H_2O
Column #22 Sample 54	1635	6.2%	50% MeOH / 50% H_2O
Column #22 Sample 55	5630	21.4%	50% MeOH / 50% H_2O
Column #22 Sample 56	169	0.6%	50% MeOH / 50% H_2O
Column #22 Sample 57	129	0.5%	50% MeOH / 50% H_2O
Column #22 Sample 58	85	0.3%	transition
Column #22 Sample 59	87	0.3%	60% MeOH / 40% H_2O
Column #22 Sample 60	102	0.4%	60% MeOH / 40% H_2O
Column #22 Sample 61	76	0.3%	60% MeOH / 40% H_2O
Column #22 Sample 62	83	0.3%	60% MeOH / 40% H_2O
Column #22 Sample 63	104	0.4%	60% MeOH / 40% H_2O
Column #22 Sample 64	1560	5.9%	60% MeOH / 40% H_2O
Column #22 Sample 65	388	1.5%	60% MeOH / 40% H_2O

Table A21, continued.

Column #22 Sample 66	780	3.0%	60% MeOH / 40% H_2O
Column #22 Sample 67	509	1.9%	60% MeOH / 40% H_2O
Column #22 Sample 68	48	0.2%	60% MeOH / 40% H_2O
Column #22 Sample 69	82	0.3%	transition
Column #22 Sample 70	2197	8.4%	70% MeOH / 30% H_2O
Column #22 Sample 71	600	2.3%	70% MeOH / 30% H_2O
Column #22 Sample 72	66	0.3%	70% MeOH / 30% H_2O
Column #22 Sample 73	58	0.2%	70% MeOH / 30% H_2O
Column #22 Sample 74	404	1.5%	70% MeOH / 30% H_2O
Column #22 Sample 75	230	0.9%	70% MeOH / 30% H_2O
Column #22 Sample 76	110	0.4%	70% MeOH / 30% H_2O
Column #22 Sample 77	91	0.3%	70% MeOH / 30% H_2O
Column #22 Sample 78	97	0.4%	70% MeOH / 30% H_2O
Column #22 Sample 79	85	0.3%	transition
Column #22 Sample 80	151	0.6%	80% MeOH / 20% H_2O
Column #22 Sample 81	3221	12.3%	80% MeOH / 20% H_2O
Column #22 Sample 82	8275	31.5%	80% MeOH / 20% H_2O
Column #22 Sample 83	4991	19.0%	80% MeOH / 20% H_2O
Column #22 Sample 84	89	0.3%	80% MeOH / 20% H_2O
Column #22 Sample 85	390	1.5%	80% MeOH / 20% H_2O
Column #22 Sample 86	133	0.5%	80% MeOH / 20% H_2O
Column #22 Sample 87	78	0.3%	80% MeOH / 20% H_2O
Column #22 Sample 88	303	1.2%	80% MeOH / 20% H_2O

Table A22. Results for B16-F10 cell proliferation assay with Column #23 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	55949	100.0%	N/A
Average 10 mg/mL Elderberry	31371	56.1%	N/A
Column #23 Sample 45	8753	15.6%	40% MeOH / 60% H_2O
Column #23 Sample 46	9711	17.4%	40% MeOH / 60% H_2O

Table A22, continued.

Column #23 Sample 47	17184	30.7%	40% MeOH / 60% H_2O
Column #23 Sample 48	10781	19.3%	transition
Column #23 Sample 49	312	0.6%	50% MeOH / 50% H_2O
Column #23 Sample 50	188	0.3%	50% MeOH / 50% H_2O
Column #23 Sample 51	239	0.4%	50% MeOH / 50% H_2O
Column #23 Sample 52	9798	17.5%	50% MeOH / 50% H_2O
Column #23 Sample 53	117	0.2%	50% MeOH / 50% H_2O
Column #23 Sample 54	307	0.5%	50% MeOH / 50% H_2O
Column #23 Sample 55	94	0.2%	50% MeOH / 50% H_2O
Column #23 Sample 56	153	0.3%	50% MeOH / 50% H_2O
Column #23 Sample 57	8393	15.0%	50% MeOH / 50% H_2O
Column #23 Sample 58	4273	7.6%	transition
Column #23 Sample 59	4814	8.6%	60% MeOH / 40% H_2O
Column #23 Sample 60	9109	16.3%	60% MeOH / 40% H_2O
Column #23 Sample 61	12707	22.7%	60% MeOH / 40% H_2O
Column #23 Sample 62	107	0.2%	60% MeOH / 40% H_2O
Column #23 Sample 63	18676	33.4%	60% MeOH / 40% H_2O
Column #23 Sample 64	488	0.9%	60% MeOH / 40% H_2O
Column #23 Sample 65	12341	22.1%	60% MeOH / 40% H_2O
Column #23 Sample 66	84	0.2%	60% MeOH / 40% H_2O
Column #23 Sample 67	1162	2.1%	60% MeOH / 40% H_2O
Column #23 Sample 68	12622	22.6%	transition
Column #23 Sample 69	486	0.9%	70% MeOH / 30% H_2O
Column #23 Sample 70	660	1.2%	70% MeOH / 30% H_2O
Column #23 Sample 71	748	1.3%	70% MeOH / 30% H_2O
Column #23 Sample 72	79	0.1%	70% MeOH / 30% H_2O
Column #23 Sample 73	31	0.1%	70% MeOH / 30% H_2O
Column #23 Sample 74	42	0.1%	70% MeOH / 30% H_2O
Column #23 Sample 75	732	1.3%	70% MeOH / 30% H_2O
Column #23 Sample 76	1651	3.0%	70% MeOH / 30% H_2O
Column #23 Sample 77	7096	12.7%	70% MeOH / 30% H_2O
Column #23 Sample 78	2542	4.5%	transition
Column #23 Sample 79	6973	12.5%	80% MeOH / 20% H_2O

Table A22, continued.

Column #23 Sample 80	102	0.2%	80% MeOH / 20% H_2O
Column #23 Sample 81	703	1.3%	80% MeOH / 20% H_2O
Column #23 Sample 82	101	0.2%	80% MeOH / 20% H_2O
Column #23 Sample 83	64	0.1%	80% MeOH / 20% H_2O
Column #23 Sample 84	70	0.1%	80% MeOH / 20% H_2O
Column #23 Sample 85	101	0.2%	80% MeOH / 20% H_2O
Column #23 Sample 86	702	1.3%	80% MeOH / 20% H_2O
Column #23 Sample 87	252	0.5%	80% MeOH / 20% H_2O

Table A23. Results for B16-F10 cell proliferation assay with Column #24 individual samples.

Sample Identity	**CPM**	**% Proliferation (Control = 100%)**	**Eluting Mobile Phase**
Average Control	39379	100.0%	N/A
Average 10 mg/mL Elderberry	24280	61.7%	N/A
Column #24 Sample 45	5982	15.2%	40% MeOH / 60% H_2O
Column #24 Sample 46	11549	29.3%	40% MeOH / 60% H_2O
Column #24 Sample 47	16942	43.0%	40% MeOH / 60% H_2O
Column #24 Sample 48	9937	25.2%	40% MeOH / 60% H_2O
Column #24 Sample 49	2804	7.1%	transition
Column #24 Sample 50	119	0.3%	50% MeOH / 50% H_2O
Column #24 Sample 51	77	0.2%	50% MeOH / 50% H_2O
Column #24 Sample 52	100	0.3%	50% MeOH / 50% H_2O
Column #24 Sample 53	1048	2.7%	50% MeOH / 50% H_2O
Column #24 Sample 54	8632	21.9%	50% MeOH / 50% H_2O
Column #24 Sample 55	575	1.5%	50% MeOH / 50% H_2O
Column #24 Sample 56	120	0.3%	50% MeOH / 50% H_2O
Column #24 Sample 57	14597	37.1%	50% MeOH / 50% H_2O
Column #24 Sample 58	459	1.2%	transition
Column #24 Sample 59	1962	5.0%	60% MeOH / 40% H_2O
Column #24 Sample 60	3589	9.1%	60% MeOH / 40% H_2O
Column #24 Sample 61	3247	8.2%	60% MeOH / 40% H_2O
Column #24 Sample 62	4944	12.6%	60% MeOH / 40% H_2O

Table A23, continued.

Column #24 Sample 63	127	0.3%	60% MeOH / 40% H_2O
Column #24 Sample 64	12695	32.2%	60% MeOH / 40% H_2O
Column #24 Sample 65	10965	27.8%	60% MeOH / 40% H_2O
Column #24 Sample 66	3546	9.0%	60% MeOH / 40% H_2O
Column #24 Sample 67	2963	7.5%	60% MeOH / 40% H_2O
Column #24 Sample 68	3617	9.2%	60% MeOH / 40% H_2O
Column #24 Sample 69	3196	8.1%	transition
Column #24 Sample 70	4102	10.4%	70% MeOH / 30% H_2O
Column #24 Sample 71	105	0.3%	70% MeOH / 30% H_2O
Column #24 Sample 72	124	0.3%	70% MeOH / 30% H_2O
Column #24 Sample 73	56	0.1%	70% MeOH / 30% H_2O
Column #24 Sample 74	76	0.2%	70% MeOH / 30% H_2O
Column #24 Sample 75	55	0.1%	70% MeOH / 30% H_2O
Column #24 Sample 76	478	1.2%	70% MeOH / 30% H_2O
Column #24 Sample 77	335	0.9%	70% MeOH / 30% H_2O
Column #24 Sample 78	14658	37.2%	70% MeOH / 30% H_2O
Column #24 Sample 79	9612	24.4%	transition
Column #24 Sample 80	2468	6.3%	80% MeOH / 20% H_2O
Column #24 Sample 81	63	0.2%	80% MeOH / 20% H_2O
Column #24 Sample 82	311	0.8%	80% MeOH / 20% H_2O
Column #24 Sample 83	88	0.2%	80% MeOH / 20% H_2O
Column #24 Sample 84	41	0.1%	80% MeOH / 20% H_2O
Column #24 Sample 85	59	0.1%	80% MeOH / 20% H_2O
Column #24 Sample 86	75	0.2%	80% MeOH / 20% H_2O
Column #24 Sample 87	87	0.2%	80% MeOH / 20% H_2O
Column #24 Sample 88	86	0.2%	80% MeOH / 20% H_2O

Table A24. Pooling of Column #1 samples.

Pool #	Samples	Pool #	Samples
1	1, 2, 3, 4	21	64
2	5, 6, 7, 8	22	65, 66, 67, 68
3	9, 10, 11	23	69, 70, 71, 72
4	12, 13, 14, 15	24	73, 74
5	16	25	75, 76, 77
6	17, 18	26	78
7	19, 20, 21	27	79, 80, 81
8	22, 23, 24	28	82, 83
9	25, 26, 27, 28, 29	29	84, 85, 86
10	30, 31, 32, 33, 34	30	87, 88
11	35, 36, 37, 38, 39	31	89, 90, 91
12	40, 41, 42, 43, 44	32	92, 93, 94
13	45, 46	33	95
14	47, 48	34	96, 97, 98
15	49, 50, 51	35	99, 100, 101, 102
16	52, 53	36	103, 104
17	54, 55	37	105, 106
18	56, 57	38	107, 108, 109
19	58, 59, 60, 61	39	110, 111
20	62, 63		

Table A25. Column #2-24 samples pooled to generate P16, P24, and P29.

Column	Samples pooled to make P16	Samples pooled to make P24	Samples pooled to make P29
Column #2	49, 50, 51	73, 74, 75	85, 86, 87
Column #3	53, 54	72, 73, 74	84
Column #4	55, 56, 57	73, 74	85, 86
Column #5	53, 54	72, 73, 74	85, 86, 87, 88
Column #6	52, 53, 54	72, 73, 74	86, 87, 88, 89
Column #7	52, 53, 54	71, 72, 73, 74	85, 86, 87, 88
Column #8	52, 53, 54	73, 74	84, 85
Column #9	53, 54	73, 74	86, 87
Column #10	50, 51, 52, 53	73, 74	86, 87, 88, 89
Column #11	50, 51, 52, 53	72, 73, 74	86, 87, 88, 89
Column #12	51, 52, 53	71, 72, 73, 74	86, 87, 88
Column #13	55, 56, 57, 58	74, 75, 76, 77	83, 84, 85, 86
Column #14	54	72, 73, 74, 75	83, 84, 85
Column #15	50, 51, 52, 53	71, 72, 73, 74	83, 84
Column #16	50, 51, 52	73, 74	84, 85, 86
Column #17	53, 54, 55	71, 72, 73, 74, 75	87, 88
Column #18	51, 52, 53	72, 73, 74, 75	87, 88, 89, 90
Column #19	53, 54	71, 72, 73	85, 86, 87, 88
Column #20	49, 50, 51	73, 74, 75, 76	84, 85, 86, 87
Column #21	56	72, 73	85, 86
Column #22	49, 50, 51, 52, 53	72, 73, 74, 75	85, 86, 87, 88
Column #23	53,54,55,56	72,73,74	82,83,84,85
Column #24	50,51,52	71,72,73,74,75	84, 85, 86, 87

Appendix B: Figures

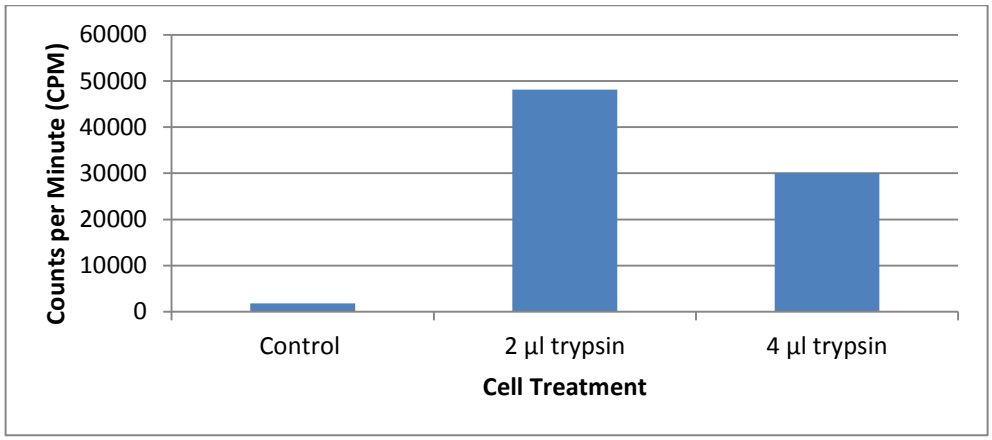

Figure B1. Optimal concentration of trypsin addition before harvesting for tumor cell proliferation assays.

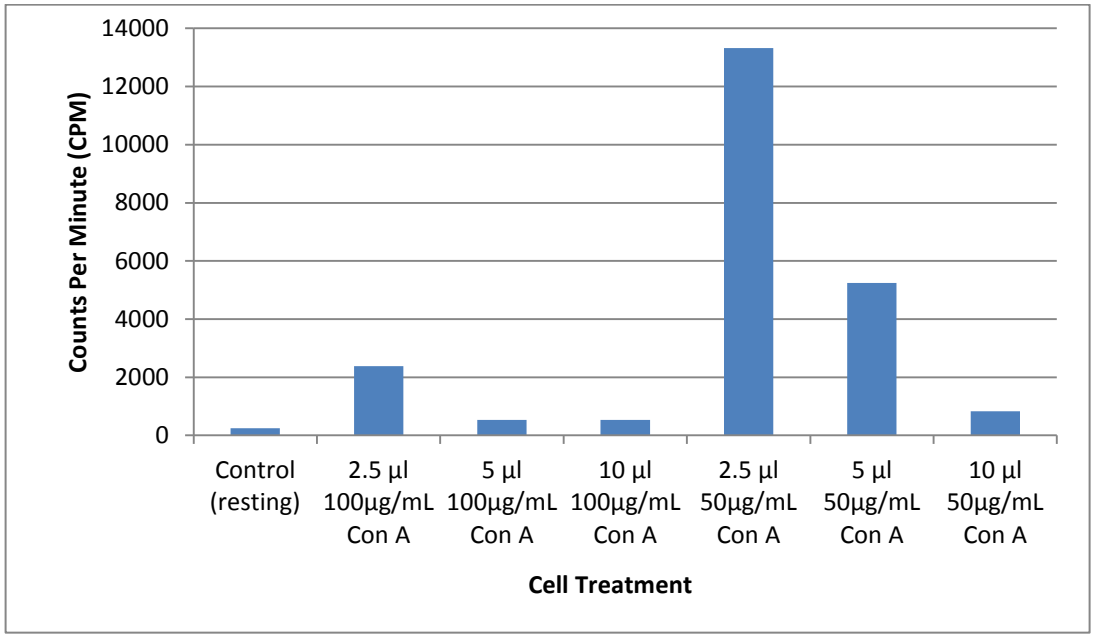

Figure B2. Optimal concentration of Con A on spleen cell proliferation.

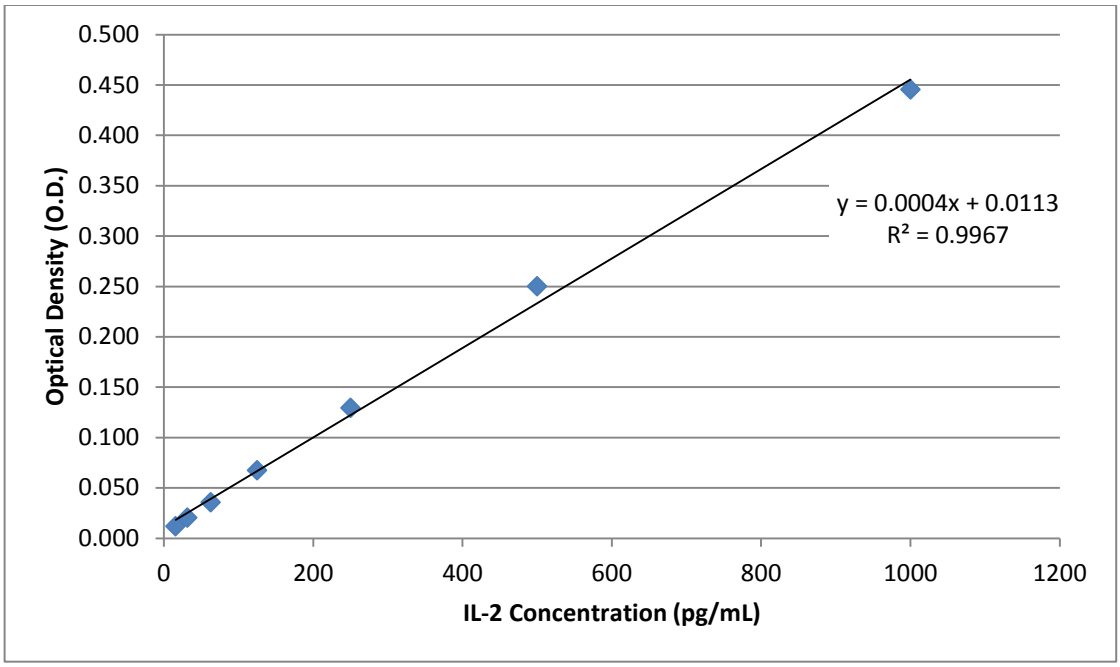

Figure B3. Standard IL-2 curve generated from the Quantikine murine IL-2 ELISA.

Appendix C: Solution Recipes

The working solution of tritiated thymidine (^{3}H-Thy) was prepared by adding 3.85 mL of filter-sterilized deionized water, or 3.85 mL of filter-sterilized RPMI-1640 media to 150 µL thymidine stock (Moravek Biochemicals, Brea, CA).

Mobile phases for column chromatography were prepared by mixing varying concentrations of deionized water and methanol and a consistent amount of 0.01*N* HCl. Mobile phases were added to the column in order of decreasing polarity in 165 mL increments.

Mobile Phase	**Volume of H_2O (mL)**	**Volume of MeOH (mL)**	**Volume of 0.01*N* HCl (mL)**
0% MeOH / 100% H_2O	150.0	0.0	15.0
10% MeOH / 90% H_2O	135.0	15.0	15.0
20% MeOH / 80% H_2O	12.0	30.0	15.0
30% MeOH / 70% H_2O	105.0	45.0	15.0
40% MeOH / 60% H_2O	90.0	60.0	15.0
50% MeOH / 50% H_2O	75.0	75.0	15.0
60% MeOH / 40% H_2O	60.0	90.0	15.0
70% MeOH / 30% H_2O	45.0	105.0	15.0
80% MeOH / 20% H_2O	30.0	120.0	15.0
90% MeOH / 10% H_2O	15.0	135.0	15.0
100% MeOH / 0% H_2O	0.0	150.0	15.0