

5-2016

# Effects of Arachidin-1 and Arachidin-3 on Human Colon Cancer Cells

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# Effects of Arachidin-1 and Arachidin-3 on Human Colon Cancer Cells

An Honors Thesis submitted in partial fulfillment  
of the requirements for Honors Studies in Biological Sciences

by

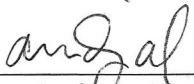
Jessica Lynne Bettis

May 2016  
University of Arkansas



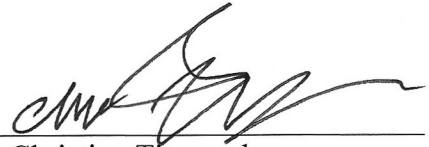
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Honors Council Representative

## **Acknowledgements**

The thought of writing a thesis two years ago was a daunting task that I never thought I would accomplish. I am thankful for the help that I received from my mentor, Dr. Sun-Ok Lee. I am thankful that she has been patient with me through this learning process and spent many hours reviewing my lab work. I know that the skills that I have obtained in this research experience are invaluable for my career ahead of me in medicine. I am also very grateful for Dr. Christian Tipsmark, Dr. Andrew Alverson, and Dr. Eric Funkhouser for being gracious enough to serve on my Honors Thesis Committee. There are many that deserve recognition for guiding me and supporting me throughout these past couple of years. I would like to thank Cindi Brownmiller, Tung Pham, Ellen Pottgen, and Xuan Gu. Without their wisdom and support this thesis would not have been possible. Lastly, I would like to thank my family especially my parents, Jimmy and Joy Bettis for giving me unconditional love and support throughout this journey. They believed in me when I did not believe in myself. I am deeply grateful and thankful that all of these individuals and more have invested time and support in me. I am forever grateful.

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## **Abstract**

Cancer remains a leading cause of death in the state of Arkansas and across the United States. Recent research found that peanuts consumption has been correlated with a decreased risk of cancer. Although limited research has been conducted, phenolic antioxidant compounds found in peanuts, arachidin-1 and arachidin-3, are correlated to a decrease in human leukemia and lung cancer cell growth. The objective of this study was to evaluate the anti-colon cancer effects of these compounds. Experiments were conducted to examine the effects of purified arachidin-1 and arachidin-3 on Caco-2 cell proliferation. Treatment of cells with arachidin-1 (0, 1, 5, 10 $\mu$ M) and arachidin-3 (0, 5, 10, 20 $\mu$ M) were measured at time points 0, 24, and 48 hours. There was cell reduction for 10 and 20 $\mu$ M treatments of arachidin-3 at time point 24 hours, but no other significant reduction was found. The findings suggest that arachidin-1 and arachidin-3 did not inhibit the growth of human colon cancer cells. Further research needs to be conducted to examine the effects of peanuts stilbenoids, arachidin-1 and archidin-3, as potential anticarcinogenic agents.

## Chapter I. Introduction

Globally, each year about 14 million individuals learn they have cancer while 8 million people die from the disease (CDC, 2014). Cancer is the second leading cause of mortality in the United States. Colorectal cancer is the third leading cause of cancer death and approximately 5%, or every 1 in 20 Americans, will be diagnosed with cancer of the rectum or colon in their lifetime (ACS, 2014). In the state of Arkansas, there is a rate of 41.7 per 100,000 men and women per year compared to the national rate (42.4 per 100,000). But Arkansas has a higher death rate (17.9 per 100,000) than the national rate (15.5 per 100,000) (Holt et al., 2011).

Epidemiologic studies have identified an inverse association between diet rich in fruits and vegetables and cancer (Chen et al., 2015). A variety of fruits and vegetables contain bioactive components, antioxidants, which have been linked to chemoprevention of colon cancer (Amin et al., 2009).

Peanuts (*Arachis hypogaea L.*) contain high concentrations of polyphenolic antioxidants. Of the bioactive compounds in peanuts much attention has been on the phenolic antioxidant, resveratrol (trans-3,5,49- trihydroxystilbene), a family of stilbenoids. Previous research on resveratrol and polydatin (resveratrol-3-O-b-mono- D-glucoside) has indicated that stilbenoid compound polydatin alone or with resveratrol caused the Caco-2 cell (a human epithelial colorectal adenocarcinoma cell) death by apoptosis (De Maria et al., 2013). Arachidin-1 [trans-4-(3-methyl-1-butenyl)-3,5,3',4'- tetrahydroxystilbene)], and arachidin-3 [(Arachidin-3, trans-4-(3-methyl-1-butenyl)- 3,5,4'-trihydroxystilbene)] also belong to a family of stilbenoids derived from the root of

a peanut plant. However, there is limited information available on health-promoting activities of arachidin-1 and arachidin-3.

The goal of this study is to determine the effects of arachidin-1 and arachidin-3 on human colon cancer cells. The objectives of this study were to: 1) determine if arachidin-1 and arachidin-3 can inhibit colon cancer cell growth and 2) determine what concentrations of arachidin-1 and arachidin-3 are effective at inhibiting cell proliferation.

## **Chapter II: Literature Review**

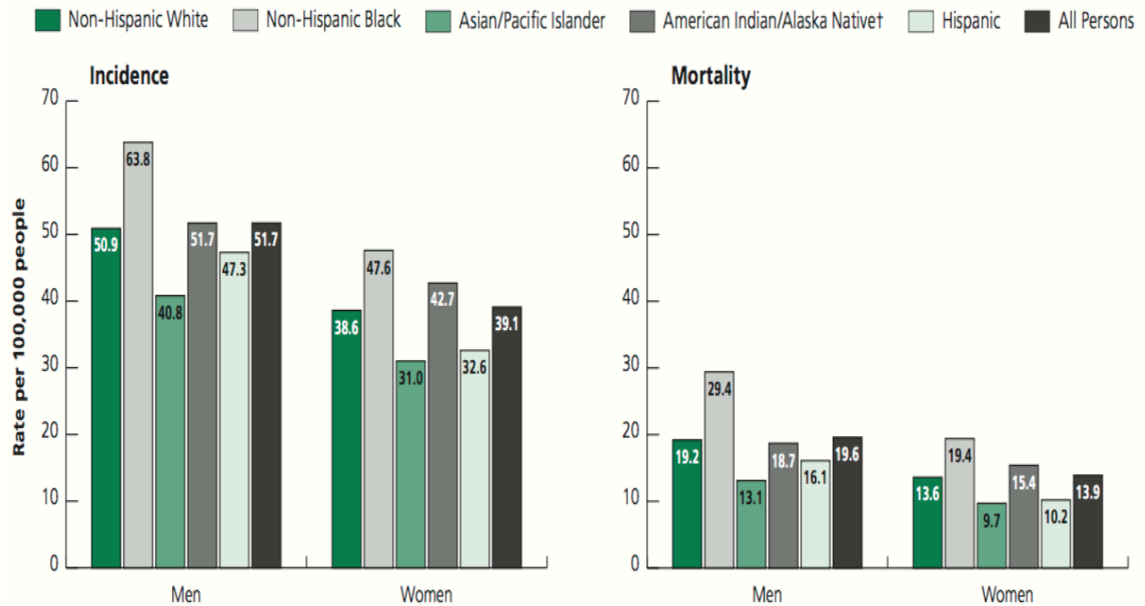
### **Colon Cancer**

Colorectal cancer is the third most common cancer diagnosed in both men and women in the United States (NIH, 2016). CRC is cancer that starts in the colon or rectum. The colon and the rectum are parts of the large intestine, which is the lower part of the body's digestive system (CDC, 2014). The function of the colon is to absorb water and salt from the food matter and to serve as a storage place for waste matter. It is composed of four different sections: ascending colon, transverse colon, descending colon, and sigmoid colon (NIH, 2016).

Colorectal cancer incident and mortality rates are dependent on sex, race, age, and ethnicity (Figure 1). From 2002 to 2011 in the United States the incident rate of colorectal decreased significantly by 3.6% per year among men and by 3.2% per year among women. While in the same years, the death rate decreased significantly by 3.1% per year among men and by 2.9% per year among women (CDC, 2014). Although there is a decrease, in the United States for 2015 the number of CRC cases was projected to be 132,700 new cases of colon and rectum cancer and 49,700 deaths (NIH, 2016).

Risk factors of colorectal cancer include age, race, sex, heredity, preexisting conditions and lifestyle factors such as: physical activity, diet, overweight, alcohol consumption, and tobacco use (CDC, 2014). There is much evidence linking the 'Western' diet (high fat and low fiber content) to increased risk of CRC. Diets are under review as there is a correlation to a higher risk of CRC if a diet consists of a large amount of red meat or processed meats, but a lower risk of CRC if the diet is high in fruits and vegetables (NIH, 2016).





**Figure 1:** Colorectal Cancer Incident and Mortality Rates\* by Race/Ethnicity and Sex, 2006-2010 (ACS, 2014). \*Rate are per 100,000

Since diet is a risk factor linked to colorectal cancer, researchers have investigated whether consuming dietary antioxidants can help lower the risk of developing cancer in humans. An antioxidant is a chemical substance that slows down or prevents the damage that oxygen does to organisms or food. Currently research is being conducted looking at specific antioxidants and the effects on cancer, heart disease, stroke, and other diseases associated with aging.

## General Information about Peanuts

### Nutrients in Peanuts

Peanuts (*Arachis hypogaea* L.), a type of legume, are known for their source of protein and energy. For every 100 grams of peanuts there is approximately 585 kilocalories, 21.51 grams of carbohydrates, 8.0g of fiber, and 49.66g of fats, and 23.68g of proteins. With the exception of meat, legumes, such as peas, beans, and peanuts contain a large amount of protein, more than any other nuts (Settaluri et al., 2012). Peanuts are composed of nutrients, minerals, and vitamins that are essential for optimum health.

**Table 1.** Composition by weight\* of biomolecules in peanuts (Adapted from Settaluri et al., 2012)

<b>Amino acids</b>		<b>Vitamins</b>	
Type	Weight (g)*	Type	Weight (g)*
Tryptophan	0.230	Thiamin	$0.438 \times 10^{-3}$
Threonine	0.811	Riboflavin	$0.098 \times 10^{-3}$
Isoleucine	0.833	Niacin	$13.525 \times 10^{-3}$
Leucine	1.535	Pantothenic acid	$1.395 \times 10^{-3}$
Lysine	0.850	B6	$0.256 \times 10^{-3}$
Methionine	0.291	Folate	$1450 \times 10^{-6}$
Cysteine	0.304	E**	$6.93 \times 10^{-3}$
Phenylalanine	1.227	Choline	$55.3 \times 10^{-3}$
Tyrosine	0.963		
Valine	0.993	<b>Minerals</b>	
Arginine	2.832	Calcium	$54 \times 10^{-3}$
Histidine	0.599	Iron	$2.26 \times 10^{-3}$
Alanine	0.941	Magnesium	$176 \times 10^{-3}$
Aspartic acid	2.888	Phosphorous	$358 \times 10^{-3}$
Glutamic acid	4.949	Potassium	$658 \times 10^{-3}$
Glycine	1.427	Sodium	$6 \times 10^{-3}$
Proline	1.045	Zinc	$3.31 \times 10^{-3}$
Serine	1.167	Manganese	$2.083 \times 10^{-3}$
<b>Lipids</b>		<b>Others</b>	
Total saturated fatty acid	6.893	Carbohydrates (total)	21.51
Total monounsaturated	24.640	Dietary fiber	8.0
Total polyunsaturated	15.694	Sugar (total)	4.18

\*Quantities expressed are per 100g of dry-roasted peanuts, all types, and without salt. \*\*This is a fat soluble vitamin and the rest are water soluble vitamins

Peanuts dietary protein contains amino acids essential for growth and development. Additionally, they are great sources of folate, manganese, niacin – which contributes to brain health and blood flow to brain, and vitamin E – a powerful lipid solvent antioxidant.

### **Health Benefits of Peanuts**

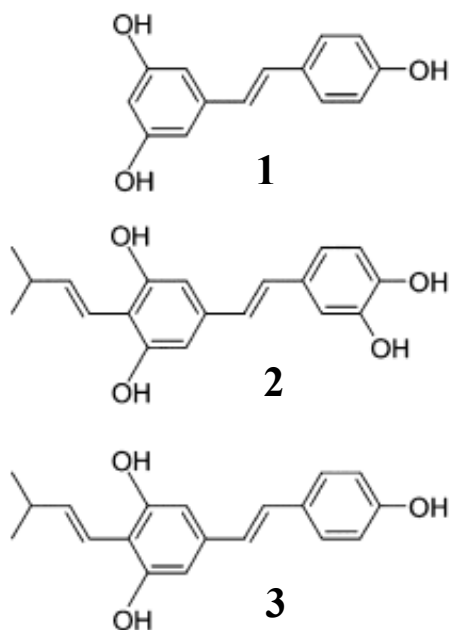
Peanuts are one of the widely cultivated oil-seed and established prime commercial crops in the world. The health benefits of peanuts are becoming a rising topic for researchers as well as nutritionists. In recent years, research has shown that peanuts could be linked to reduced weight gain and especially correlated with different functions in the digestive system (Bes-Rastrollo et al., 2007). Women age 30-55 years had no history of gallstone disease was studied in a 20-year cohort study. Women who consumed at least one ounce of peanuts and/or other nuts each week had a significantly lower risk of cholecystectomy than women who did not eat nuts (Tsai et al., 2004). In a different cohort study of 8,866 individuals, it was found that those who ate nuts—walnuts, almonds, hazelnuts, and peanuts— at least twice a week were much less likely to gain weight of 5 kilograms or more than those who almost never ate nuts (Bes-Rastrollo et al., 2007). In another 10-year cohort study beginning in 1990, the consumption of peanuts and a reduced risk of colorectal cancer were correlated. 12,026 men and 11,917 women aged 30 to 65 years, who were residents in 7 townships in Taiwan, were annually followed by evaluating weekly food measures. Their findings suggest that women who consumed peanuts had a much lower risk of colorectal cancer. It is indicated that some specific components in peanuts inhibit the carcinogenic mechanisms of colorectal cancer, but other studies must be conducted in order to rule out other factors that might have been

present (Yeh et al., 2006). A following step could be to determine what specific component(s) do peanuts possess that could be linked to one or more of these advancements in human health mentioned above. Recent discovery of bioactive components in naturally occurring food has allowed research to continue to conduct more specific studies by researching one or more of the components in food directly. The ability to test only certain bioactive components are crucial for both the research of food industries as well as research regarding human biological processes and diseases. As mentioned above, there are many studies correlating health benefits and peanuts. With increased research with certain bioactive components experimenters can see more specific correlations and possibly extend information to other areas especially for human health.

### **Bioactive Components in Peanuts**

Bioactive compounds are extra nutritional constituents that typically occur in small quantities in foods (Kris-Etherton et al., 2002). Currently bioactive components (which are varied by the number of hydroxyl groups and isopentenyl moiety) in peanuts are on the forefront of research as they are able to prevent or slow the growth of cancers, contain antioxidants, and even extend lifespans (Chang et al., 2006). Polyphenols, antioxidants, are abundant in fruits and vegetables, and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging (Manach et al., 2004). The health effects of polyphenols depend on the amount consumed and on their bioavailability (Manach et al., 2004). Not only do polyphenols have antioxidant properties, but also they have other have a variety of biological activities that are yet to be understood (Manach et al., 2004). Polyphenols are composed of a

subcategory named natural stilbenoids, which are a family of polyphenols known for their diverse biological activities. Recently, it was found that the stilbenoid antioxidants and resveratrol (trans-3,5,4'- trihydroxystilbene), found in peanuts seeds and red grapes has been determined to improve blood flow in the brain by as much as 30%, thus greatly reducing the risk of stroke (Peanuts, 2015). Previous research on polydatin, (resveratrol-3-O-b-mono- D-glucoside, Product Origin: Root of *Polygonum cuspidatum*), a natural precursor of resveratrol, reported that polydatin alone or with resveratrol caused the Caco-2 cell (a human epithelial colorectal adenocarcinoma cell) death by induction of apoptosis (De Maria et al., 2013). Although resveratrol (trans-3,5,4'- trihydroxystilbene) has received considerable attention as a putative anticancer agent, a study found that other peanuts prenylated stilbenoids, arachidin-1 and archidin-3, may be preferable alternatives due to increased bioavailability via slowed metabolism (Brents et al., 2012). Due to the similar chemical structures of resveratrol, arachidin-1 (trans-4-(3-methyl-1-butenyl)-3,5,3',4'-tetrahydroxystilbene), and arachidin-3 (Arachidin-3, trans-4-(3-methyl-1-butenyl)-3,5,4'-trihydroxystilbene) are now specifically being studied. Arachidin-1 and arachidin-3 belong to a family of stilbenoids derived from the root of a peanuts plant. Current research is limited due to the recent discovery of these bioactive components.



**Figure 2.** Chemical Structures of 1. Resveratrol (trans-3,5,4'- trihydroxystilbene) 2. Arachidin-1 trans-4-(3-methyl-1-butenyl)-3,5,3',4'-tetrahydroxystilbene; 3. Arachidin-3, trans-4-(3-methyl-1-butenyl)-3,5,4'-trihydroxystilbene (Adapted from Soboley 2013).

### ***Biological Activities of Arachidin-1 and Arachidin-3***

Prenylated stilbene compounds in peanuts such as arachidin-1 (Ara-1) and arachidin-3 (Ara-3) are beginning to be studied *in vitro* experiments in order to understand these compounds and the functions that they possess. It was found that both of these compounds possess a defense mechanism against physical injuries and fungal contamination when challenged by a fungal strain (Liu et al., 2013). Prenylated Ara-1 and Ara-3 improved glucuronidation profiles (Bretns et al., 2012). In addition, both Ara-1 and Ara-3 showed a significant decrease in Rotavirus Gastroenteritis viral replication when applied (Ball et al., 2015). This study found that the effects of the compounds, Ara-1 and Ara-3, were dose- and time- dependent to decrease viral progeny when they were applied to both the Rotavirus within human intestinal cells. It is indicated that the prenylated side

chain of Ara-1 and Ara-3 promotes invasion through cell membranes more than resveratrol due to the increased lipophilicity to the attached molecules (Ball et al., 2015). Compounds, Ara-1 and Ara-3, are being used in a variety of experiments with viruses, fungal strains, and possible therapeutic values for chronic disease, but they are commonly used in exposure to cancer cells. (Liu et al., 2013). In recent studies Ara-1 had the highest efficacy in inducing programmed cell death in Human Leukemia cells HL-60 with an approximately 4-fold lower EC<sub>50</sub> than resveratrol. Specifically Ara-1 caused mitochondrial membrane damage, released apoptosis-inducing factor, and activation of caspases (Huang et al., 2010). Ko and colleagues in 2013, reported that arachidin-1 decreased thymidine phosphorylase (TP) and Excision repair cross-complementation 1 (ERCC1), both with high amounts in cancer cells are associated with poor prognosis. In addition, arachidin-1 combined with Metformin, a type two diabetic drug, significantly decreased TP and ERCC1 in human lung cancer (NSCLC) cell lines (Ko et al., 2013).

**Table 2.** Summary of anticarcinogenic activities of arachidin-1 arachidin-3, and resveratrol (*in vitro* studies).

Phenolic Compound	Cell Line	Concentration	Effect	Time
Resveratrol, Polydatin (De Maria et al., 2013)	Caco-2 cells, differentiated and undifferentiated	Different concentrations: 0 to 500µM of each or combination (50:50, 75:25 molar ratio)	IC50: 190µM Pol and 373µM Res in differentiated polydatin alone has a stronger cytotoxicity, antiproliferative effect poly:res 3:1	24 hours

<b>Phenolic Compound</b>	<b>Cell Line</b>	<b>Concentration</b>	<b>Effect</b>	<b>Time</b>
Arachidin-1, Arachidin-3, Resveratrol (Cheng-Po et al., 2010)	Human leukemia HL-60	0 to 20 $\mu$ M treating with Ara-1 and Ara-3	EC <sub>50</sub> values of 4.2 $\mu$ M Ara-1, 18.9 $\mu$ M Ara-3, and 17.6 $\mu$ M resveratrol	0-72 hours
Metformin (diabetic drug), Arachidin-1 (Ko et al., 2013)	A549 and H1975 Lung cancer cells	0.5, 1, 5 and 10 $\mu$ M Ara-1 and 25 $\mu$ M metformin  5, 10, 25 and 50 $\mu$ M metformin and 0.5 $\mu$ M Ara-1  Ara-1 (0.5 $\mu$ M) were added for 24 hr	Greater loss of cell viability with both metformin and Ara-1	Treated with metformin, Ara-1 or both for 24hr
Arachidin-1 Arachidin-3 Resveratrol Piceatannol (Ball et al., 2015)	Rotavirus Gastroenteritis HT29 (Human intestinal line infected with Rotavirus)	10, 20 $\mu$ M Ara-1, 10, 20 $\mu$ M Ara-3	Ara-1 10 $\mu$ M tenfold decrease, 20 $\mu$ M twenty-fivefold decrease Ara-3 10 $\mu$ M ninefold decrease, 20 $\mu$ M ninety-eightfold decrease in virus infectivity titer	12 and 24 hours postinfection

\*IC<sub>50</sub>: the dose that causes half-maximal inhibition by antagonist

\*\* EC<sub>50</sub>: the concentration resulting in a 50% decrease in cell viability (Cheng-Po et al., 2010)



## **Chapter III: Materials and Methods**

### **Materials**

Arachidin-1 (Ara-1) and arachidin-3 (Ara-3) compounds were generously donated by Dr. Medina-Bolivar (Arkansas State University, Jonesboro, AR). All media components and reagents were obtained from Gibco® through ThermoFisher (Waltham, MA).

### **Methods**

#### **HPLC Analysis**

A System Gold high performance liquid chromatograph (Beckman-Coulter, Fullerton, CA, USA) with autosampler (model 508), dual pump (model 126), photodiode array detector (model 168) with Beckman-Coulter System 32 Karat software (version 8, 2006) was used to analyze the arachidin-1 and arachidin-3. The compounds were separated using an adapted method of Abbott et al (2010). Separation was performed using a Phenomenex (Torrance, CA) Aqua 5 $\mu$ m C18 (250  $\times$  4.6 mm) column using a binary gradient of 2% formic acid for mobile phase A and 99:1 (v/v) acetonitrile/mobile phase A. The initial conditions of the gradient were 10% B, then increased linearly from 10-18% for 8 min, and was held at 18% B for 2 minutes. Next, B increased linearly to 18-25% over 5 min, 25-35% B for 3 min, and held isocratically for 20 min, then %B increased 35-60% linearly for 60 min before returning to the initial 10%B. Peaks were monitored at 340 nm refer to Figure 3.

## **Cell Culture**

A human epithelial colorectal adenocarcinoma cell line (Caco-2 cells) was purchased from the American Type Culture Collection (ATCC, Rockville, MD). The cells were at passage number 18 (passage number refers to the number of times the cell line has been re-plated) and cultured in Working Media (W-MEM). W-MEM is composed of 10% fetal bovine serum (FBS), 2% antibiotic-antimycotic solution, 1% Non-essential amino acids, and Dulbecco's modified eagle's medium (DMEM). Cell cultures were maintained under strict sterile conditions incubated at 37°C, 70% humidity, and 5% CO<sub>2</sub> in the incubator (VWR® symphony™, VWR® International LLC, Radnor, PA).

## **Cell Proliferation Assay**

Cells, passage number 22-25, were used for the proliferation assays. The effects of Ara-1 and Ara-3 on the cellular proliferation and viability of Caco-2 cells were measured using CellTiter 96® Aqueous One Solution Proliferation Assay (Promega Cor. Madison, WI). CellTiter 96® contains tetrazolium compound [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfohenyl)-2H-tetrazolium, inner salt; **MTS**] and an electron coupling reagent (phenazine ethosulfate; PES). This assay is a colorimetric method to determine the number of viable cells in proliferation or cytotoxicity assays. The tetrazolium compound becomes reduced by living cells into a colored formazan product, which is soluble in culture media. The amount of reduced tetrazolium is measured by a plate reader resulting in a directly proportional number of living cells in the culture.

To conduct this MTS assay, Caco-2 cells ( $2 \times 10^3$ ) in 200µl of W-MEM were seeded in the wells of a 96 well plate. Cells were incubated at 37°C, 70% humidity, and

5% CO<sub>2</sub> for 24 hours to allow attachment of the cells to the bottom of the wells. After 24 hours, the media was aspirated and replaced with different concentrations of a compound (either Ara-1 or Ara-3) and control. Treatment media containing Ara-1 at concentrations of 1, 5, and 10 μM and Ara-3 at concentrations of 5, 10, and 10 μM. A negative control of 0.1% DMSO in 10% FBS W-MEM was used to measure the viability of Caco-2 cells in the absence of compounds. Treatments and negative controls were done in triplicate. Viability measurements were made at time 0hr, 24hr, and 48hr after the addition of the compounds or control. Time 0hr refers to the time of applying treatment.

CellTiter reagent was directly added to the cells, 40 μl per well. The reagent was added to all wells (including wells with cells and to wells only with compounds where no cells are present, a sample control). Microplates were incubated for 2 hours after the addition of the reagent, before measuring the absorbance at 490nm with the plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc. Winooski, VT). Once the absorbance readings were made, data was corrected for the background absorbance of the wells with negative control media or with wells with compounds without cells. Corrected absorbance numbers were converted into cell numbers using an equation from the Caco-2 cell standard curve.

A standard curve was composed of serial dilution of cells prepared in triplicate in a 96 well plate ranging from 32000 cells to 0 cells with W-MEM. The standard curve was created by seeding cells in the actual experiment adding CellTiter reagent directly to the wells, and then measuring the absorbance of these cells. The results of standard curve of the 0hr plate were plotted to compare the change in absorbance as the total cell number increased. Each experiment conducted used the equation given from data plotted to

calculate the cell numbers to plot cell growth curves. Cell viability was checked at time points of 24hr and 48hr with cell counts ranging from 32000 to 2000.

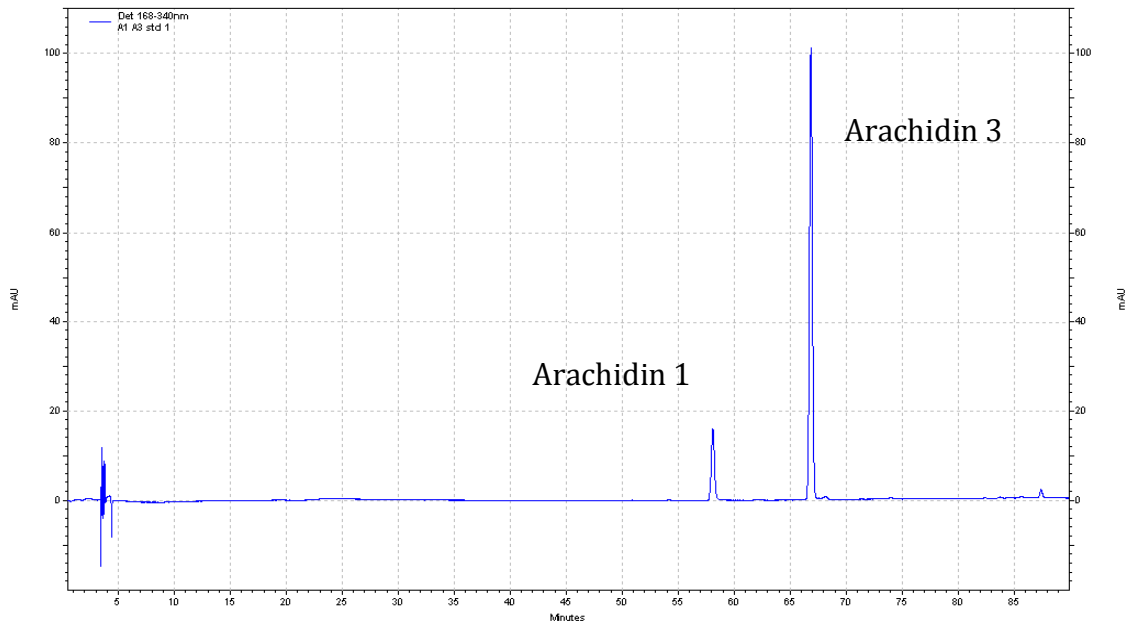
### **Statistical Analysis**

Values were expressed means  $\pm$  standard error of the mean (SEM). Data was analyzed using Statistical Analysis System (Version 9.4; SAS Institute Inc., Cary, NC). A one-way analysis of variance was used to show significant differences from the control cells at each time point. An alpha level of 0.05 was used to determine statistically significant differences. Fisher's least significant difference (LSD) test was used to analyze the data.

## Chapter IV: Results

### HPLC Results

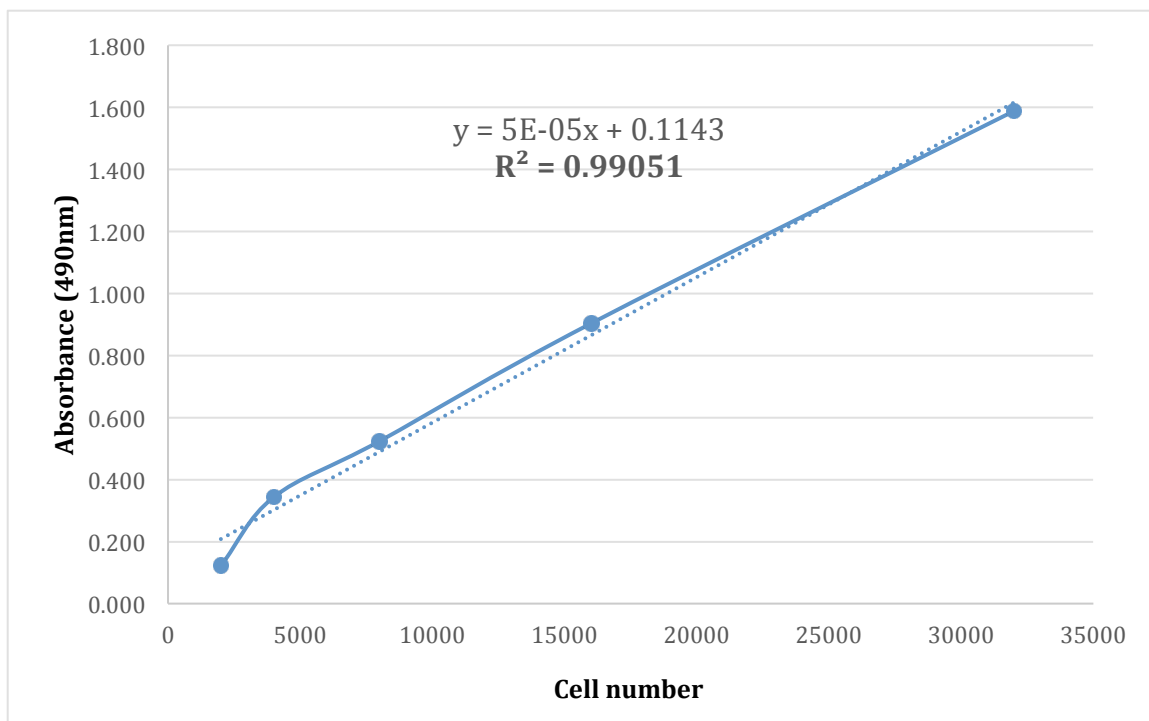
The High Performance Liquid Chromatogram (HPLC) analysis confirmed the identity of compounds used for this experiment: arachidin-1 and arachidin-3. The results are summarized in Figure 3.



**Figure 3.** High Performance Liquid Chromatogram of arachidin-1 and arachidin-3 at 340nm.

### Cell Standard Curve

In order to differentiate outside factors from treatment cells, a standard curve was used as a control on every plate. This was used as a reference for cell growth and conditions on each cell plate throughout the experiment. Figure 4 shows the standard curve of Caco-2 cells.

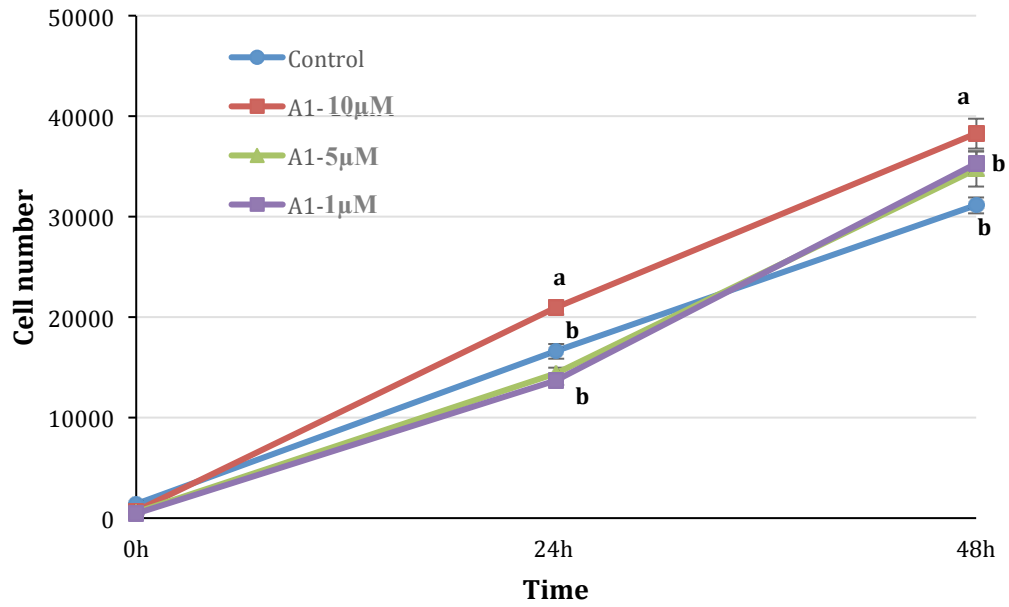


**Figure 4.** Caco-2 cell standard curve

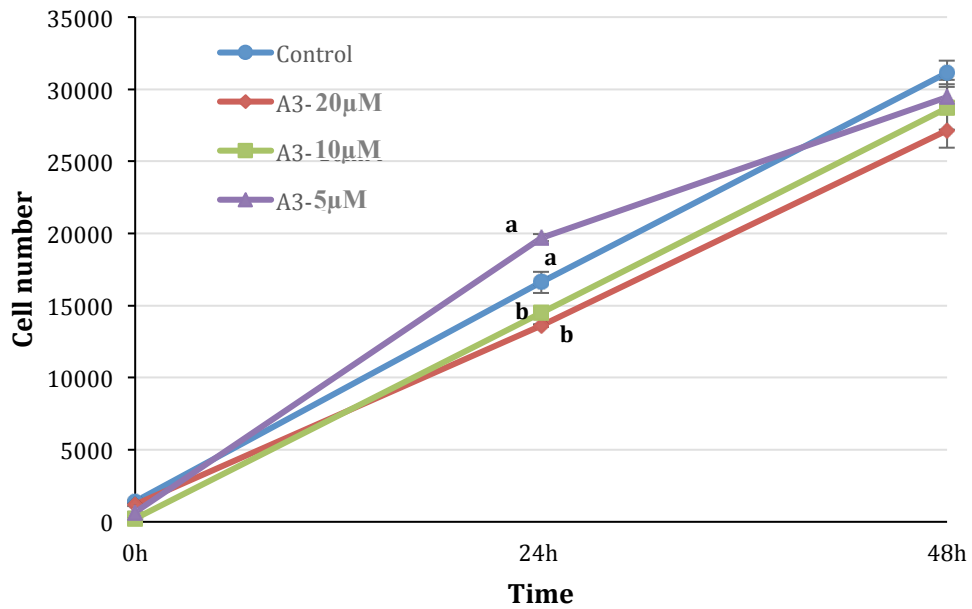
### Cell Proliferation

Treatment of Caco-2 Cells with arachidin-1 did not decrease cell proliferation compared to control (Figure 5). There is a difference between 10mM cell growth compared to the control (0 $\mu$ M), 1 $\mu$ M, and 5 $\mu$ M. At both time points of 24 and 48 hours for arachidin-1, 10 $\mu$ M treatment seemed to promote cell growth compared to the control, treatment of 1 $\mu$ M and 5 $\mu$ M. The effectiveness of reduced cell proliferation for arachin-1 was not correlated with concentration at time points 24 and 48 hours.

Figure 6 shows the effects of arachidin-3 on the survival of Caco-2 cells. Compared with the control and 5 $\mu$ M of arachidin-3, treatments of 10 $\mu$ M and 20 $\mu$ M of arachidin-3 resulted in a significant reduction of cell number at time point 24 hours ( $P < 0.05$ ). At time point 48 hours there was not a significant difference between the any of the treatments of arachidin-3.



**Figure 5.** Effect of arachidin-1 on the survival of Caco-2 cells. Values are mean  $\pm$  standard error of the mean (SEM). Values with different letters at the same incubation time are significantly different ( $P < 0.05$ ).



**Figure 6.** Effect of arachidin-3 on the survival of Caco-2 cells. Values are mean  $\pm$  standard error of the mean (SEM). Values with different letters at the same incubation time are significantly different ( $P < 0.05$ ).

## Chapter V: Discussions

Peanuts (*Arachis hypogaea* L.) contain many nutrients, antioxidants, vitamins, and minerals essential to optimal health (Settaluri et al., 2012). Peanuts have been linked to reduced weight gain to reduced risk of heart disease (Bes-Rastrollo et al., 2007; Settaluri et al., 2012).

The present study investigated the effects of stilbenoids (arachidin-1 and arachidin-3) found in peanuts root on colon cancer cells (Caco-2 cells) proliferation. No significant reduction of cell growth with arachidin-1. There was a significant increase of proliferation at time point 24 and 48 hours for 10 $\mu$ M treatment of arachidin-1. However, there was a significant difference in inhibition of cell growth for treatments 10 $\mu$ M and 20 $\mu$ M of arachidin-3, but none of the treatments showed a significant difference at time point 48 hours. Further research should include higher concentrations of arachidin-1 and arachidin-3 on Caco-2 cell growth.

There have been a few *in-vitro* studies regarding the anti-proliferative behavior of arachidin-1 and arachidin-3, but there is a variation in results and concentrations of these compounds between studies. These studies have reviewed different cells lines and combined alternative drugs or naturally occurring compounds combined with arachidin-1 and arachidin-3 (De Maria et al., 2013, Cheng-Po et al., 2010, Ko et al., 2013, Ball et al., 2015). There was only one study found to look at Caco-2 cells combining antioxidant agents, resveratrol and Polydatin (De Maria et al., 2013). Due to the limited research of compounds arachidin-1 and arachidin-3 effects on Caco-2 cells, there could be a different concentration needed to inhibit proliferation. A study on human leukemia cell line



suggested that arachidin-1 should be more potent than arachidin-3, but this study was not able to concur with these results (Cheng-Po et al., 2010). Based on previous studies concentrations of treatments varies depending on cell line experimented (De Maria et al., 2013, Cheng-Po et al., 2010). Due to the limited research with Caco-2 cell line with peanuts stilbenoids more research need to be conducted to fully understand the effects of these compounds.

## **Chapter IV: Conclusions**

The results of the present study suggest that arachidin-1 and arachidin-3 did not inhibit the growth of human colon cancer cells. In order to understand the biological activities of these compounds more studies need to be conducted.

## References:

- Abbott, J. A., Medina-Bolivar, F., Martin, E. M., Engelberth, A. S., Villagarcia, H., Clausen, E. C., & Carrier, D. J. (2010). Purification of resveratrol, arachidin-1, and arachidin-3 from hairy root cultures of peanut (*Arachis hypogaea*) and determination of their antioxidant activity and cytotoxicity. *Biotechnology Progress*, *26*(5), 1344-1351.
- Amin, A. R. M. R., Kucuk, O., Khuri, F. R., & Shin, D. M. (2009). Perspectives for Cancer Prevention With Natural Compounds. *Journal of Clinical Oncology*, *27*(16), 2712–2725.
- Antioxidant Definition in the Cambridge English Dictionary. (n.d.). Retrieved March 04, 2016, from <http://dictionary.cambridge.org/us/dictionary/english/antioxidant>
- Ball, J. M., Medina-Bolivar, F., Defrates, K., Hambleton, E., Hurlburt, M. E., Fang, L., Yang, L., Nopo-Olazabal, L., Atwill, R., Ghai, P., Parr, R. D. (2015). Investigation of Stilbenoids as Potential Therapeutic Agents for Rotavirus Gastroenteritis. *Advances In Virology*, *2015*, 1-10. doi:10.1155/2015/293524
- Bes-Rastrollo, M., Sabate, J., Gomez-Gracia, E., Alonso, A., Martinez, J. A., & Martinez-Gonzalez, M. A. (2007). Nut consumption and weight gain in a Mediterranean cohort: the SUN Study. *Obesity*, *15*(1), 107-116. doi:10.1038/oby.2007.507
- Brents, L. K., Medina-Bolivar, F., Seely, K. A., Nair, V., Bratton, S. M., Nopo-Olazabal, L., & ... Radomska-Pandya, A. (2012). Natural prenylated resveratrol analogs arachidin-1 and -3 demonstrate improved glucuronidation profiles and have affinity for cannabinoid receptors. *Xenobiotica; The Fate Of Foreign Compounds In Biological Systems*, *42*(2), 139-156. doi:10.3109/00498254.2011.609570
- Cancer Facts & Figures 2014-2016. American Cancer Society (ACS). (2014). Journal of Consumer Health On the Internet. Retrieved March 4, 2016, from <http://www.cancer.org/acs/groups/content/documents/document/acspc-042280.pdf>
- Centers for Disease Control and Prevention (2016, February 16). Colorectal (Colon) Cancer. Retrieved February 24, 2016, from <http://www.cdc.gov/cancer/colorectal/>
- Chang, J., Lai, Y., Djoko, B., Wu, P., Liu, C., Liu, Y., & Chiou, R. Y. (2006). Biosynthesis enhancement and antioxidant and anti-inflammatory activities of peanut (*Arachis hypogaea* L.) arachidin-1, arachidin-3, and isopentadienylresveratrol. *Journal of Agricultural And Food Chemistry*, *54*(26), 10281-10287.
- Chen, Z., Wang, P. P., Woodrow, J., Zhu, Y., Roebbothan, B., Mclaughlin, J. R., and Parfrey, P. S. 2015. Dietary patterns and colorectal cancer: results from a Canadian population-based study. *Nutrition journal*, *14*(1), 1.

Cheng-Po, Huang., Lo-Chun, A., Chiou, R. Y., Ping-Chen, C., Su-Yu, C., Wei-Chien, T., & Shwu-Bin, L. (2010). Arachidin-1, a peanut stilbenoid, induces programmed cell death in human leukemia HL-60 cells. *Journal of Agricultural and Food Chemistry*, 58(23), 12123-12129.

Chung-Jyi, Tsai., Leitzmann, M. F., Hu, F. B., Willett, W. C., & Giovannucci, E. L. (2004). Frequent nut consumption and decreased risk of cholecystectomy in women. *American Journal of Clinical Nutrition*, 80(1), 76-81.

De Maria, S., Scognamiglio, I., Lombardi, A., Amodio, N., Caraglia, M., Carteni, M., & Stiuso, P. (2013). Polydatin, a natural precursor of resveratrol, induces cell cycle arrest and differentiation of human colorectal Caco2 cell. *Journal of Translational Medicine*, 11(1), 1-24. doi:10.1186/1479-5876-11-264

Holt, A., Mitchell, T., and Delongchamp, B. (2011). Arkansas Cancer Facts & Figures. *Arkansas Central Cancer Registry Arkansas Department of Health*: 27.

Ko, J., Huang, Y., Chen, H., Tseng, S., Chiu, H., Wo, T., & Lin, Y. (2013). Metformin Induces Cytotoxicity by Down-Regulating Thymidine Phosphorylase and Excision Repair Cross-Complementation 1 Expression in Non-Small Cell Lung Cancer Cells. *Basic & Clinical Pharmacology & Toxicology*, 113(1), 56-65. doi:10.1111/bcpt.12052

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., & Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine*, 11371-88. doi:10.1016/S0002-9343(01)00995-0

Liu Z., Wu J., & Huang D. (2013). New stilbenoids isolated from fungus-challenged black skin peanut seeds and their adipogenesis inhibitory activity in 3T3-L1 cells. *Journal of Agricultural And Food Chemistry*, 61(17), 4155-4161.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79(5), 727-747.

National Institute of Health. (2014, November 5). A Snapshot of Colorectal Cancer. (Retrieved November 10, 2015, from <http://www.cancer.gov/research/progress/snapshots/colorectal>

Niesen, D. B., Hessler, C., & Seeram, N. P. (2013). Beyond resveratrol: A review of natural stilbenoids identified from 2009-2013. *Journal of Berry Research*, 3(4), 181-196. doi:10.3233/JBR-130062

Peanuts. (n.d.). Retrieved November 10, 2015, from <http://www.whfoods.com/genpage.php?tname=foodspice&dbid=101>

Riviere, C., Pawlus, A. D., & Merillon, J. M. (2012). Natural stilbenoids: distribution

in the plant kingdom and chemotaxonomic interest in Vitaceae. *Natural Product Reports*, 29(11), 1317-1333.

Settaluri, V. S., Kandala, C. K., Puppala, N., & Sundaram, J. (2012). Peanuts and their nutritional aspects - a review. *Food and Nutrition Sciences*, 3(12), 1644-1650. doi:10.4236/fns.2012.312215

Sobolev, V. S. (2013). Production of phytoalexins in peanut (*Arachis hypogaea*) seed elicited by selected microorganisms. *Journal of Agricultural and Food Chemistry*, 61(8), 1850-1858. doi:10.1021/jf3054752

U. (n.d.). Peanuts nutrition facts and health benefits. Retrieved February 25, 2016, from <http://www.nutrition-and-you.com/peanuts.html>

What are complementary and alternative methods? (2016, January 20). Retrieved February 25, 2016, from <http://www.cancer.org/treatment/treatmentsandsideeffects/complementaryandalternativemedicine/complementaryandalternativemethodsandcancer/complementary-and-alternative-methods-and-cancer-what-are-cam>

Yeh, C., You, S., Chen, C., & Sung, F. (2006). Peanut consumption and reduced risk of colorectal cancer in women: a prospective study in Taiwan. *World Journal of Gastroenterology*, 12(2), 222-227.