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Anti-Cancer and Bioavailabilty of Arachidin-1 and Arachidin-3 in Colon Cancer Cells

Macy R. Shirley
University of Arkansas, Fayetteville

Cindi Brownmiller
University of Arkansas, Fayetteville

Wing Shun Lam
University of Arkansas, Fayetteville

Sun-Ok Lee
University of Arkansas, Fayetteville

Anti-cancer and bioavailability of arachidin-1 and arachidin-3 in colon cancer cells

Macy R. Shirley[*], *Cindi Brownmiller*[†], *Wing Shun Lam*[§], *and Sun-Ok Lee*[‡]

Abstract

Cancer is a common cause of death in the United States and locally in the state of Arkansas. Modifiable factors such as tobacco use, physical activity, and diet lead to reduced incidence of colon cancer diagnosis. Plant-based foods may contain phytochemicals that confer health promoting properties. Specifically, peanuts contain phytochemicals known as resveratrol, arachidin-1, and arachidin-3 that have been linked to anticancer activities. However, few research studies have been done on arachidin-1 and arachidin-3 that could develop understanding of their health promoting properties or nutraceutical applications. The objectives of this study were to (1) determine the most effective concentration of arachidin-1 and arachidin-3 for inhibiting cell proliferation and (2) assess the bioavailability of these compounds. Concentrations of 0, 5, 10, and 20 μM arachidin-1 and arachidin-3 were applied and cell viability was measured at 0, 24, and 48 hours. Significant reduction of cell proliferation occurred with treatments of 10 and 20 μM arachidin-1 and 10 and 20 μM arachidin-3 in comparison with the control. Due to the limitations of high performance liquid chromatography (HPLC) detection, no transport values were determined when arachidin-1 and arachidin-3 were applied in 50 and 100 μM concentrations. The findings suggest that arachidin-1 and arachidin-3 inhibit cell proliferation in human colon cancer cells. Further research is needed to understand the bioavailability of arachidin-1 and arachidin-3.

[*] Macy Shirley is a 2017 honors program graduate with a major in Human Nutrition and Hospitality Innovation .
[†] Cindi Brownmiller is a Laboratory Specialist in the Department of Food Science.
[§] Wing Shun Lam is a Graduate student in the Department of Food Science.
[‡] Sun-Ok Lee, the faculty mentor, is an Associate Professor in the Department of Food Science.

Meet the Student-Author

Macy Shirley

I am from Texarkana, Texas and I graduated with honors from Texas High School. I graduated in May 2017 with a Bachelor of Science in Human Environmental Sciences, majoring in Human Nutrition and Hospitality Innovation. During my undergraduate career, I was able to participate in the Pre-Dental Society and Student Dietetics Association, be an active member of Phi Mu Fraternity, participate actively as a member of Cross Church-Fayetteville, volunteer as a Diamond Doll for the University of Arkansas Baseball program, and participate in Cooking for Health, a study abroad program in France during summer 2016.

I would like to thank Dr. Sun-Ok Lee for the copious hours she invested in order to patiently guide me in this process. I am also very appreciative that Mrs. Mechelle Bailey and Dr. Sabrina Trudo served on my Honors Thesis Committee. Many other people have also supported me throughout this process, and I would like to thank them. I am grateful for Cindi Brownmiller, Wing Shun Lam, Danielle Ashley, and Inah Gu. I could not have completed this project successfully without their patience, guidance, and support.

Introduction

Cancer is one of the leading causes of mortality in Arkansas and throughout the United States. Specifically, an incidence rate of 50.1 per 100,000 for males and 37 per 100,000 for females exists for colon cancer in Arkansas (Siegel et al., 2017). The mortality rate for colon cancer in Arkansas is approximately 18.2 per 100,000 people, which ranks third highest compared with other states in the United States (National Cancer Institute, 2015).

Genetic and lifestyle factors are potentially influential in the development of colon cancer (Burt et al., 2010). Prevention reduces incidence and mortality rates associated with colon cancer (Siegel et al., 2016). Amin et al. (2009) reported an inverse relationship between a diet rich in fruits and vegetables and diagnosis with colon cancer. This relationship can be attributed to the phytochemicals found in fruits and vegetables (Amin et al., 2009). The peanut plant (*Arachis hypogaea* L.) is a source of phytochemicals known as stilbenoids, which confer cancer preventative properties following extraction from the root or kernel of the peanut plant. Arachidin-1, arachidin-3, and resveratrol are stilbenoids derived from the peanut plant (Huang et al., 2010). While a significant amount of research has been conducted involving resveratrol, there is limited information available about the health-promoting properties of arachidin-1 and arachidin-3 (Chang et al., 2006).

Understanding the bioavailability of phytochemicals is essential for determining their biological and health effects in humans. Therefore, the objectives of the present study were to: 1) determine the effective concentrations of arachidin-1 and arachidin-3 for inhibiting colon cancer cell growth, and 2) assess the intestinal transport of arachidin-1 and arachidin-3 to determine which compound is more bioavailable.

Materials and Methods

Arachidin-1 and arachidin-3 were generously donated by Dr. Medina-Bolivar in their pure form (Arkansas State University, Jonesboro, Ark.). All reagents and media components were purchased from ThermoFisher (Waltham, Mass.).

High Performance Liquid Chromatography Analysis

To measure arachidin-1 and arachidin-3, a System Gold high performance liquid chromatograph (HPLC; Beckman-Coulter, Fullerton, Calif.) 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. Arachidin-1 and arachidin-3 were separated using the method proposed by Abbott et al. (2010) to ensure use of pure compounds. Separation of the phytochemicals occurred with the Phenomenex

(Torrance, Calif.) Aqua 5 µM C18 (250 × 4.6 mm) column containing a binary gradient of 2% formic acid [mobile phase A] and 99:1 (v/v) acetonitrile/mobile phase A [mobile phase B]. The gradient began with 10% B and increased uniformly to 18% for 8 minutes. The gradient was then held at 18% B for 2 minutes before increasing linearly to 25% B over the course of 5 minutes, and increasing to 35% B for 3 minutes. Then the gradient remained constant and uniform in the mobile phase for 20 minutes at 35%. Mobile phase B increased uniformly to 60% over the course of 59 minutes before returning to the initial binary gradient of 10%. The peaks of arachidin-1 and arachidin-3 were monitored at 340 nm (Bettis, 2016).

Cell Culture

Caco-2 cells, a human epithelial colorectal adenocarcinoma cell line, purchased from American Type Culture collection (ATCC, Rockville, Md.) were incubated at 37 °C in an atmosphere containing 5% CO_2. Cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS), 1% nonessential amino acids solution, and 1% antibiotic-antimycotic (Bettis, 2016; Thurow, 2012). Cell cultures were maintained under sterile conditions and incubated at 37 °C, 70% humidity, and 5% CO_2 in the incubator (VWR® symphony™, VWR® International LLC, Radnor, Pa.).

Cell Viability Assay

Caco-2 cells, passage number 36-42, were used for the viability assay. For a standard curve, cells attached to the chambers of a 96-well microplate (Corning Inc., Tewksbury, Mass.) for 24 hours after being seeded in a range from 0 to 3.2×10^3 cells with 100 µL working media (WMEM). For the experiment, cells (2×10^3) were seeded in wells of a 96 well plate for 24 hours. Three separate plates were prepared for each of the five experiments performed during this study. At the end of the 24 hour period, media was aspirated and cells were treated with 100 µL of 0, 5, 10, or 20 µM concentration of arachidin-1 or arachidin-3. These concentrations were chosen based on results from previous studies evaluating cell viability. Dried arachidin-1 or arachidin-3 was suspended in DMEM in the quantity deemed calculated when the density was converted to moles. The compounds were suspended in media to make a 100 µM stock solution and dilutions with media occurred accordingly. Each treatment was applied in quadruplicate. The control was treated with 100 µL of 0.1% DMSO. Measurements were obtained at 0 (T0), 24 (T24), and 48 (T48) hours following treatment to determine cell viability. When the absorbance was read at T0, T24, and T48, 20 µL of cell titer reagent (CellTiter96® Aqueous One Solution Proliferation Assay, Promega Co., Madison, Wis.) was added to each well. Using a plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc. Winooski, Vt.) the absorbance of the cells was measured at 490 nm. This absorbance was converted to the number of viable cells using an equation from the standard curve. Based on the number of viable cells present at T0, a proliferation percentage was calculated for T24 and T48 (Bettis, 2016).

Intestinal Transport Assay

Caco-2 cells were seeded at a density of $1 \times 10^5/cm^2$ cells on polycarbonate membrane inserts (12 mm diameter, pore size 0.4 µm) fitted in bicameral chambers (Corning Inc., Tewksbury, Mass.). Cell monolayer integrity was confirmed by determining the transepithelial electrical resistance (TEER) values using the Millicell ERS-2 Voltohmmeter (EMD Millipore, Billerica, Mass.). After seeding, media was aspirated from both the apical and basal chambers. One mL of phosphate-buffered saline (PBS) was added to the basal chamber and 0.5 mL of 50 and 100 µM arachidin-1 or arachidin-3 was added to the apical chamber.

For time 0 h, transport media containing arachidin-1 (or arachidin-3) was loaded and 0.5 mL of PBS were collected immediately in a vial. Each vial was preserved with 25 µL trifluoroacetic acid (TFA) and 25 µL 70% ethanol. At this point, 0.5 mL of PBS was replaced in the basal chamber. The plate was incubated at 37 °C for 30 minutes. For time 0.5 h and time 1 h, steps performed for time 0 were repeated. The plate was incubated for 1 hour following time 1. At the end of 2 hours, media from the apical was collected in a vial and rinsed with 0.5 mL of PBS three times. Phosphate-buffered saline was removed from the basal chamber, collected in a vial, and rinsed with 1 mL of PBS. Each vial was preserved with 25 µL TFA and 25 µL 70% ethanol. All aliquots were frozen at -20 °C until analysis via HPLC. Apparent permeability coefficients (P_{app}) were calculated using the following equation:

$$P_{app} = \frac{dQ/dt}{C_0 * A}$$

Where dQ/dt is the rate of permeation across the cell, A is the area of the cell monolayer, and C_0 is the initial concentration of the apical chamber.

Statistical Analysis

All statistical analyses were carried out by JMP software v. 13 (SAS Institute, Inc., Cary, N.C.) using a one-way analysis of variance test (ANOVA). The ANOVA results were compared with the student's t test. Data were represented as means ± standard error of mean (SEM). Statistical significance was accepted at $P < 0.05$.

Results and Discussion

Identification of Arachidin-1 and Arachidin-3

Arachidin-1 and arachidin-3 used for this experiment were identified as pure compounds at 304 nm (Fig. 1).

Cell Viability after Arachidin-1 and Arachidin-3 Treatment

Arachidin-1 and arachidin-3 treatments (10 µM and 20 µM) reduced cell viability compared to control (Figs. 2 and 3). At the 48 hour time point, 10 and 20 µM concentrations of arachidin-1 resulted in a significant reduction of cell viability (%) compared to the control ($P < 0.05$) (Fig. 2). There was no significant difference of cell viability between treatments and control at time point 24 hours. The cell viability was significantly reduced in treatments of 10 and 20 µM arachidin-3 compared to 5 µM arachidin-3 and the control at time point 48 hours ($P < 0.05$; Fig. 3). At time point 48 hours, 10 and 20 µM concentrations of arachidin-1 showed the lowest cell viability in comparison with cell viability measured after treatments of 10 or 20 µM arachidin-3 or control ($P < 0.05$; Fig. 4).

Intestinal Transport Assay of Arachidin-1 and Arachidin-3 Treatment

Arachidin-1 was not detected in samples from both apical and basal chambers treated with 50 µM or 100 µM at time point 2 hours (Fig. 5). At time point 2 hours, a trace amount

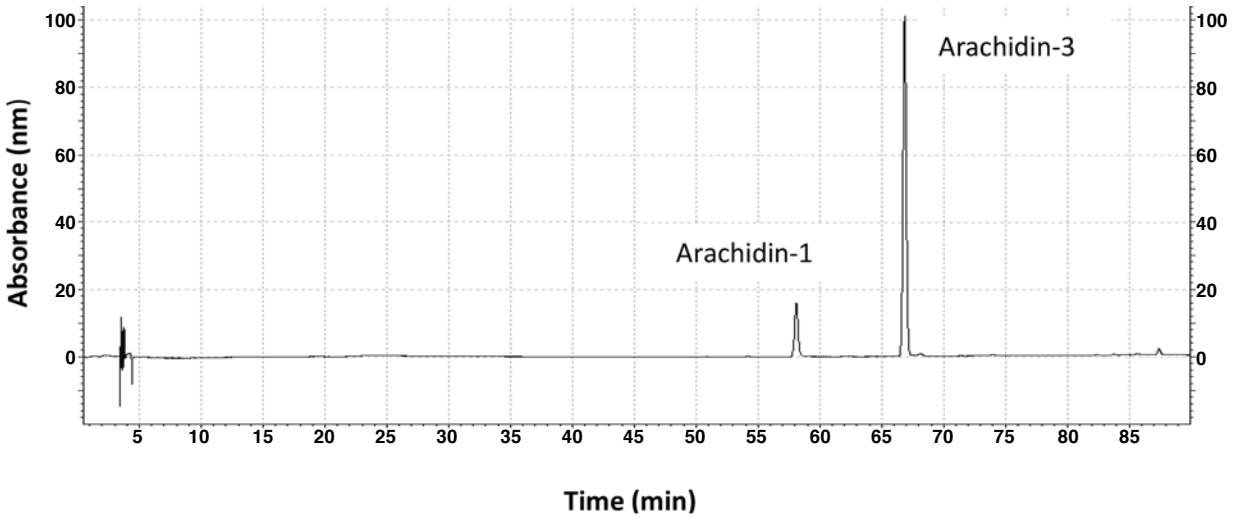

Fig. 1. High Performance Liquid Chromatogram of arachidin-1 and arachidin-3 at 340 nm.

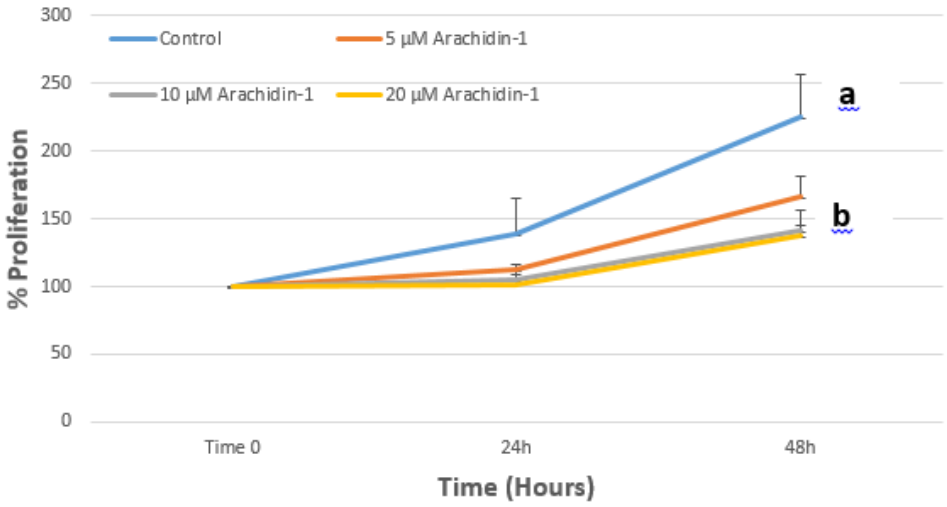

Fig. 2. Effect of arachidin-1 on the survival of Caco-2 cells. Values with different letters at the same incubation time are significantly different ($P < 0.05$).

of arachidin-3 was detected in apical sample treated with 100 µM (Fig. 6). Apparent permeability coefficient (P_{app}) values of arachidin-1 and archidin-3 were not determined.

The peanut root contains phytochemicals that confer anticancer and anti-inflammatory effects to the body for a nutraceutical approach. Arachidin-1 and arachidin-3 have been involved in limited research regarding their health effects for cancer prevention. Therefore, the anti-colon cancer activity and bioavailability of arachidin-1 and archidin-3 have been investigated in this study.

In order to understand the anticancer properties linked with arachidin-1 and arachidin-3, it is important to evaluate the effect of these compounds on colon cancer cell proliferation. In the present study, both arachidin-1 and arachidin-3 treatments (10 and 20 µM) resulted in reduced cell proliferation rates compared with the control ($P < 0.05$). The most significant reduction of cell proliferation occurred at time point 48 hours when arachidin-1 was applied at concentrations of 10 and 20 µM in comparison with the control or 5, 10, and 20 µM arachidin-3 treatments ($P < 0.05$).

A few studies have investigated the anticancer properties of arachidin-1 and arachidin-3 in different cell lines with different concentrations of stilbenoids (Ball et al., 2015; Huang et al., 2010; Ko et al., 2013). Cell viability results

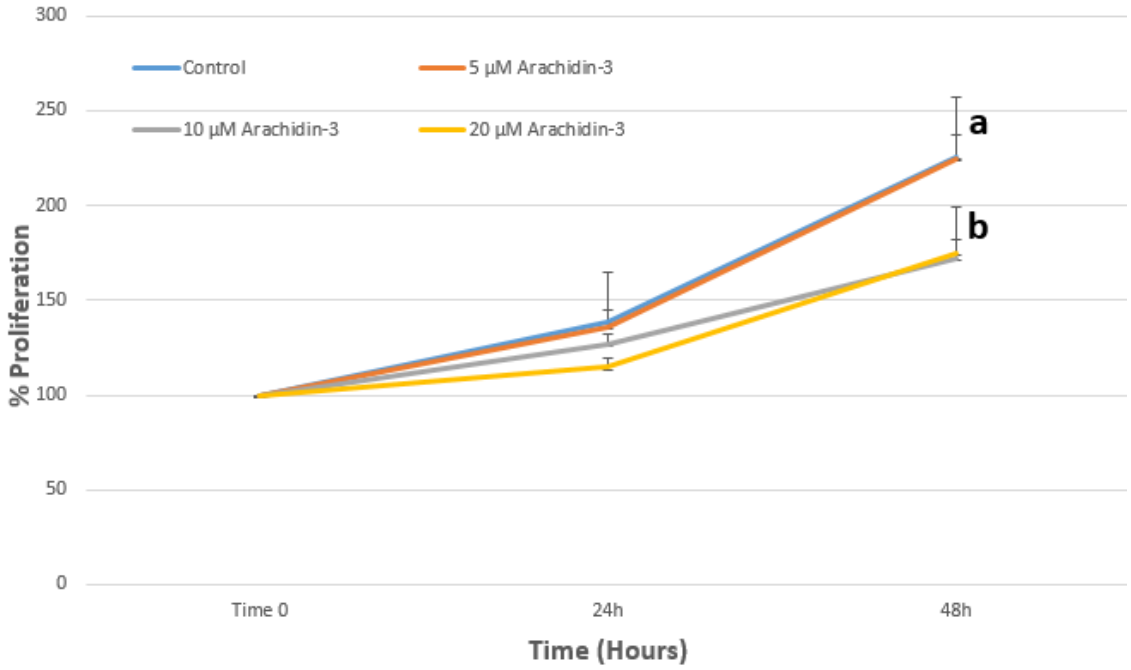

Fig. 3. Effect of arachidin-3 on the survival of Caco-2 cells. Values with different letters at the same incubation time are significantly different ($P < 0.05$).

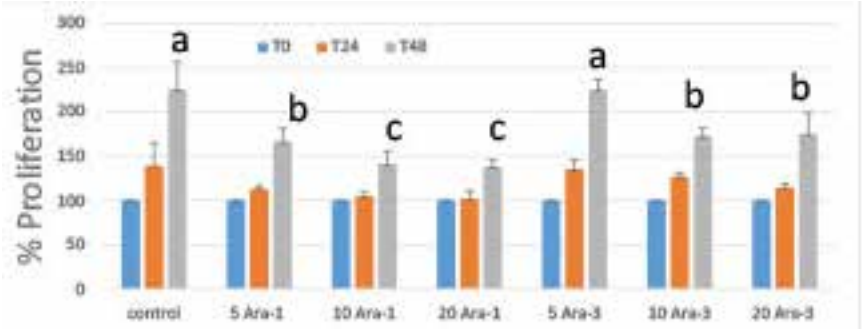

Fig. 4. Summarized effects of arachidin-1 and arachidin-3 on the survival of Caco-2 cells. Values with different letters at the same incubation time are significantly different ($P < 0.05$).

from a study on the effect of 0 to 20 μM concentrations of arachidin-1, arachidin-3, and resveratrol on human leukemia cell (HL-60) proliferation reported that arachidin-1 resulted in reduced cell viability (Huang et al., 2010). Another study investigated the effects of metformin and arachidin-1 upon dose-dependent application to A549 and H1975 human lung cancer cell lines. Metformin is an anti-diabetic drug associated with anticancer properties. Treatments of 0.5, 1, 5, and 10 μM arachidin-1 with 25 μM metformin over the course of a 4-day incubation period resulted in reduced cell viability. (Ko et al., 2013).

The 10 and 20 μM concentrations of arachidin-1, resveratrol, piceatannol, and arachidin-3 were applied to rotavirus infected HT29.F8 cells from the human adenocarcinoma line of the intestine. These concentrations of stilbenoids did not decrease cell viability substantially. However, application of arachidin-1 or arachidin-3 resulted in reduced viral replication and can be attributed to an-

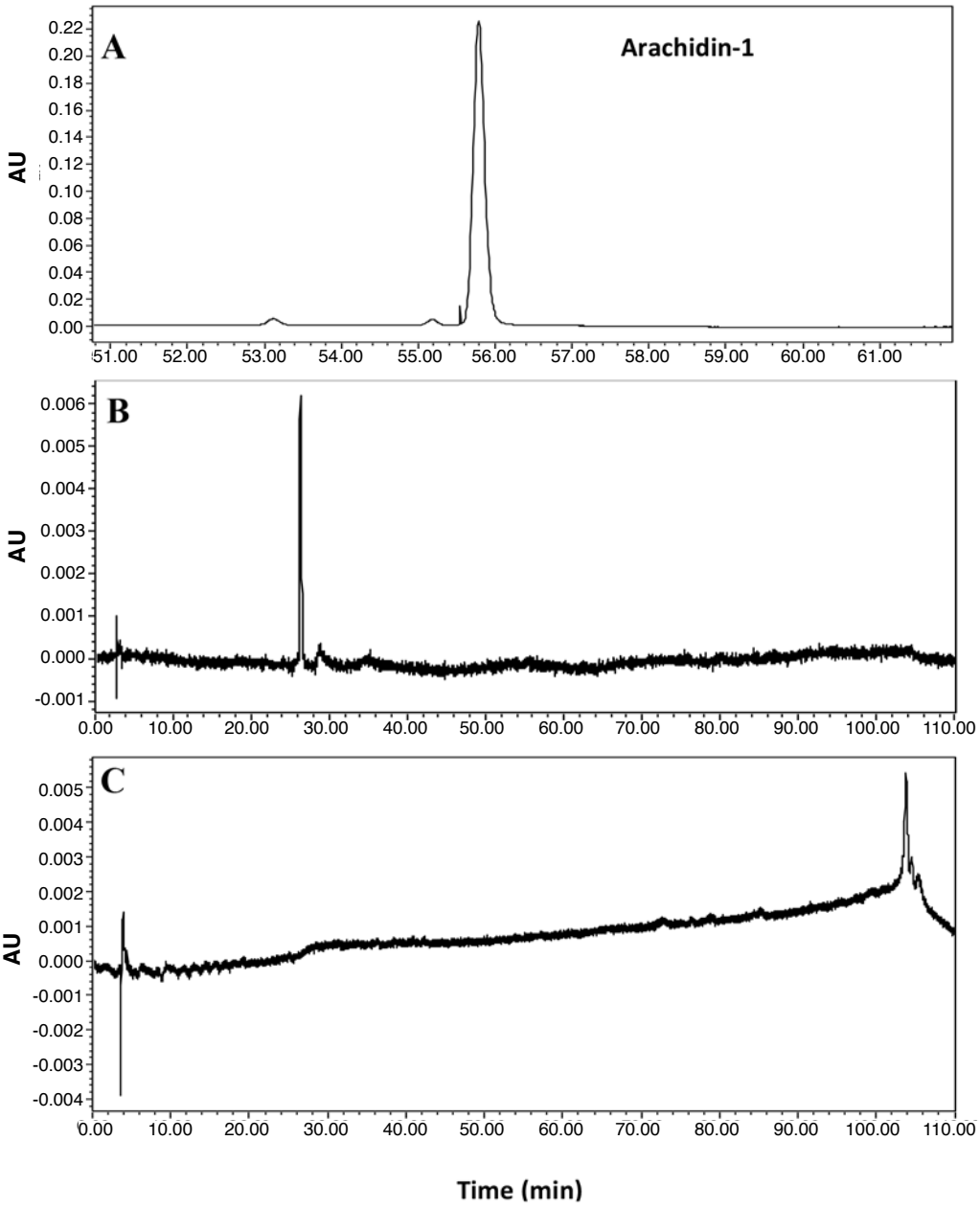

Fig. 5. High performance liquid chromatogram of (A) arachidin-1 standard, (B) apical sample treated with 100 μM of arachidin-1 and (C) basal sample treated with 100 μM of arachidin-1 at time point 2 hours.

tioxidant activity of these compounds (Ball et al., 2015). Additional research must be conducted to understand the effects of stilbenoids from the peanut plant on cell viability.

Previous studies regarding the transport abilities of arachidin-1 and arachidin-3 have not been conducted. Due to the limitations of detection in the HPLC system, the apparent transport of arachidin-1 and arachidin-3 were not calculated. Additional research must be conducted with higher concentrations of arachidin-1 and arachidin-3 to evaluate bioavailability.

Conclusions

The results of this study indicate that concentrations greater than 10 μM arachidin-1 and arachidin-3 reduced cell viability in colon cancer cells in comparison with the control. Arachidin-1 was more effective for inhibiting cell proliferation than arachidin-3. No apparent intestinal transport occurred, so it is likely that concentrations of arachidin-1 and arachidin-3 greater than 100 μM are needed to determine bioavailability. Additional research is

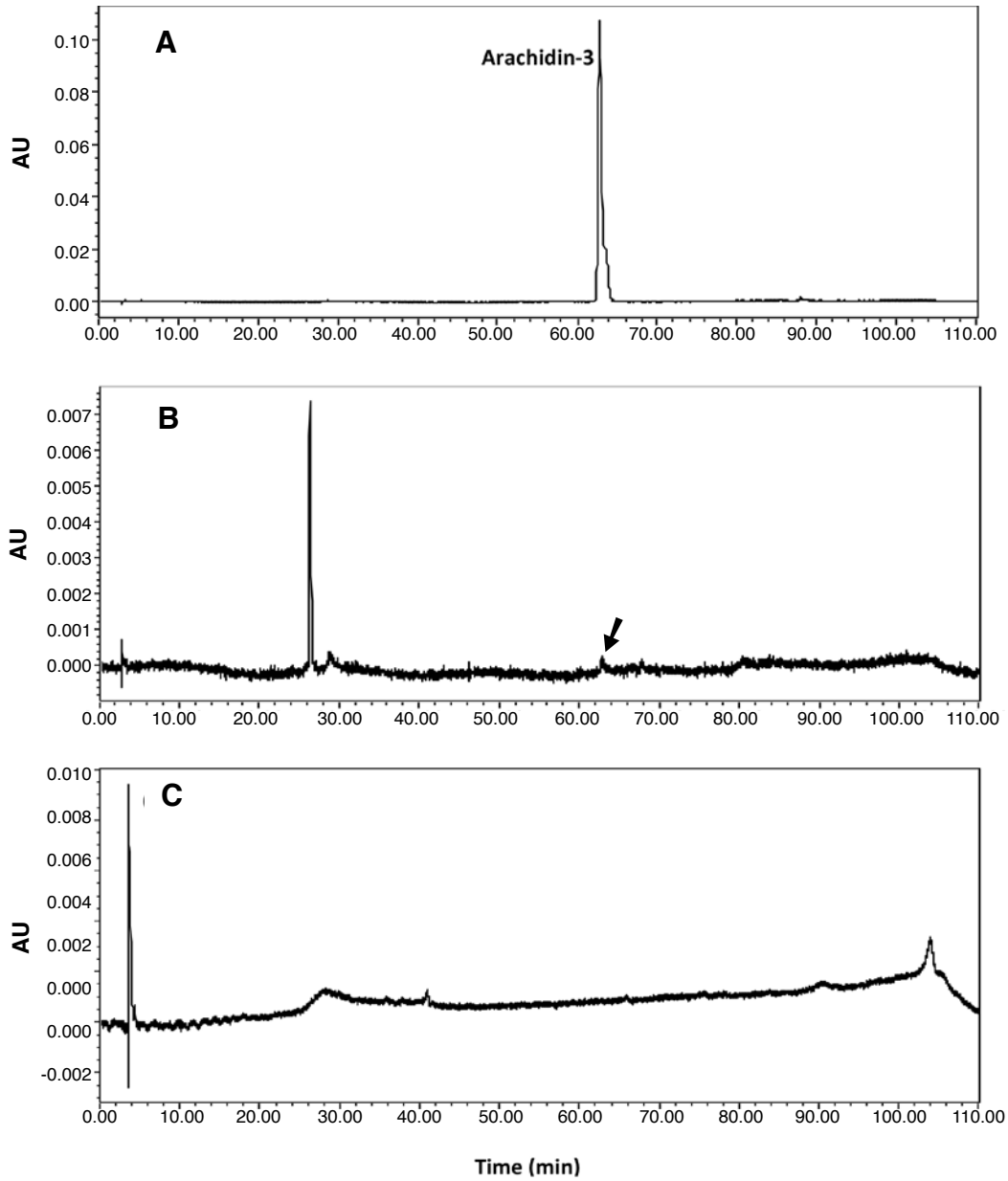

Fig. 6. High performance liquid chromatogram of (A) arachidin-3 standard, (B) apical sample treated with 100 μM of arachidin-3 and (C) basal sample treated with 100 μM of arachidin-3 at time point 2 hours.

needed to establish the chemopreventative or nutraceutical applications of arachidin-1 and arachidin-3.

Acknowledgements

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