Purification of Resveratrol, Arachidin-1, and Arachidin-3 from Hairy Root Cultures of Peanut (*Arachis hypogaea)* and Determination of Their Antioxidant Activity and Cytotoxicity

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Antioxidant stilbenoids, such as resveratrol, arachidin-1, and arachidin-3, have demonstrated beneficial effects on human health. Although resveratrol is commercially available, arachidin-1 and arachidin-3 are not, resulting in an opportunity to explore purification methods and to confirm biological activity. Recently, Arachis hypogaea *hairy root cultures (produced via* Agrobacterium rhizogenes*-mediated transformation) were reported to secrete stilbenoids into liquid growth media upon elicitation in quantities sufficient for commercial production. The purpose of this study was to purify substantial quantities of resveratrol, arachidin-1, and arachidin-3 from* A. hypogaea *hairy root cultures using centrifugal partition chromatography (CPC), determine the antioxidant activity of these compounds using the thiobarbituric acid reactive substances (TBARS) assay, and determine the cytotoxicity of the compounds using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In a single run of CPC, resveratrol, arachidin-1, and arachidin-3 were separated to a purity of 97.1%, 97.0%, and 91.8%, respectively. Lipid oxidation was inhibited by a 27 and 7 μM dose for reference standards of resveratrol and arachidin-1, respectively, while oxidation was not inhibited up to a 27 μM dose for reference standard of arachidin-3. Oxidation was inhibited at a 14, 7, and 14 μM doses for CPC-purified resveratrol, arachidin-1, and arachidin-3, respectively. Arachidin-1 and arachidin-3 demonstrated cytotoxicity at 27 and 55 μM in RAW 264.7 and HeLa cell lines, respectively; while resveratrol exhibited no cytotoxicity to either cell line. These results demonstrate the integration of a production and purification system for the manufacturing of* A. hypogaea*-derived stilbenoids.* © 2010 American Institute of Chemical Engineers *Biotechnol. Prog.,* 26: 1344–1351, 2010
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Introduction

Resveratrol, in particular *trans*-resveratrol, has gained popularity because of its wide range of antioxidant benefits such as atherosclerosis prevention and neurological protection in vitro and in vivo.[1-3] In addition, resveratrol has been shown to prolong the lifespan in mice on a high calorie diet in vivo.[4] Also, certain human populations that moderately consume red wine, a well-known source of resveratrol, have lower incidences of heart disease; this observation has been termed "the French Paradox".[5] Although resveratrol appears to be the cause of these positive effects, it has been reported to have a low bioavailability in the body.[6,7] That is, within

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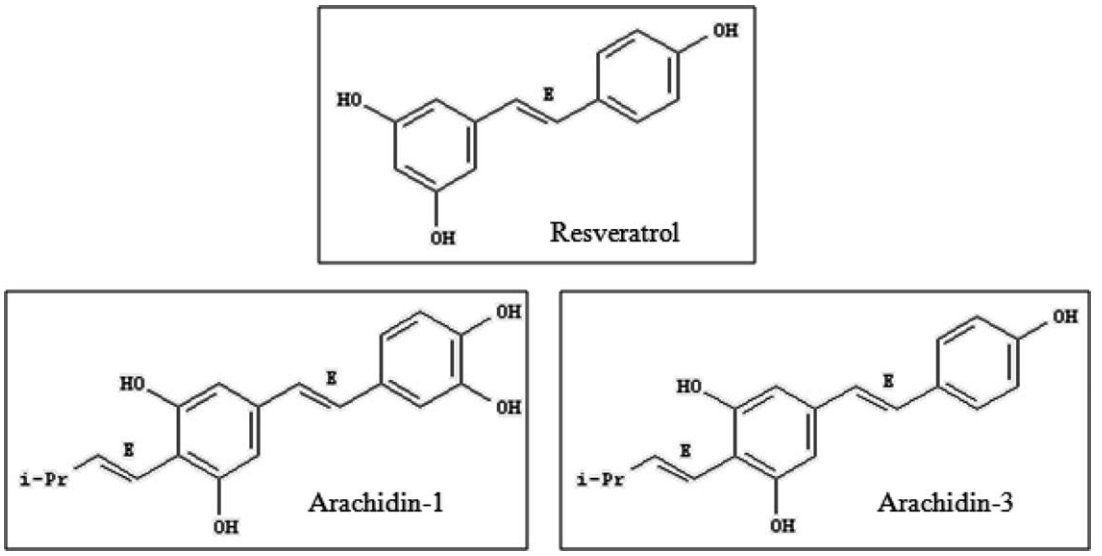

Figure 1. Structures of *trans*-resveratrol, *trans*-arachidin-1, and *trans*-arachidin-3 (prepared by A. Engelberth).

30 min after ingesting resveratrol, more than 90% of the resveratrol is conjugated into other forms, namely glucuronides.[6] Less than 5 ng/mL of unchanged resveratrol was detected in human plasma after test candidates received an oral dose of 25 mg of resveratrol.[7] This suggests that, while resveratrol may produce positive results in vitro, the benefits attributed to resveratrol in vivo studies actually may be due to the metabolites that are produced, as resveratrol is metabolized by the body soon after consumption. In addition to resveratrol, its prenylated analogs, arachidin-1 and arachidin-3, possess antioxidant and anti-inflammatory activities similar to that of resveratrol in vitro.[8] Figure 1 demonstrates the structural similarities of these compounds. In vitro antioxidative potency (AOP) tests indicated that arachidin-1 has a higher antioxidant activity than resveratrol and butylated hydroxytoluene (BHT), but the differences are not significant with 100 μM doses.[8] In later studies, arachidin-3 showed significantly lower antioxidant activity than arachidin-1, resveratrol, and BHT.[8] In this same study, inhibition of LPS-induced nitric oxide (NO) and prostaglandin E2 (PGE_2) production in macrophages was outperformed by arachidin-1 when compared to arachidin-3 and resveratrol. Arachidin-3 failed to outperform resveratrol. In another study, arachidin-1 was reported to have equal or superior immunological activities to resveratrol in in vitro LPS-induced production of NO, PGE_2 assays.[9] These results indicate that side-by-side testing of *trans*-resveratrol, arachidin-1 and arachidin-3 would shed additional light as to their biological activity.

Of these compounds, *trans*-resveratrol, which is notably found in wine made from merlot and cabernet sauvignon grapes, is commercially available. *Trans*-resveratrol is found in the highest concentrations in Itadori root, yielding 523 μg of *trans*-resveratrol per gram of fresh root[10] as compared to 0.5 μg of *trans*-resveratrol per gram fresh weight of grape.[10] Peanuts are also a source of *trans*-resveratrol. Boiled peanuts have been shown to contain an average of 5.1 μg of *trans*-resveratrol per gram of peanut, while peanut butter contains 0.3 μg of *trans*-resveratrol per gram of peanut butter.[10] Although (unlike *trans*-resveratrol) arachidin-1 and arachidin-3 are not commercially available, and must be purified in-house. Arachidin-1 and arachidin-3 have recently been reported to be extracted from the germinated seeds and/or stems of *Arachis hypogaea,* when the plant material was sliced and the natural flora, including various fungi, were allowed to challenge or infect the plant tissues.[11,12] Recently, *A. hypogaea* hairy root cultures, transformed by *Agrobacterium rhizogenes,* have shown to be a scalable source of resveratrol[13] arachidin-1 and arachidin-3.[14] Stilbenoid production and secretion into the media was obtained through the use of hairy root cultures elicited with 10.2 mM of sodium acetate.[14]

In this work, resveratrol, arachidin-1, and arachidin-3 were obtained from *A. hypogaea* hairy root culture media extracts, and were purified using centrifugal partition chromatography (CPC). This technique permitted the acquisition of sufficient quantities of resveratrol, arachidin-1, and arachidin-3 to perform biological activity assays, such as low-density lipoproteins (LDL) oxidation analysis using the thiobarbituric acid reactive substances (TBARS) procedure and cytotoxicity analysis, using the 3-[4,5 dimethylthiaol-2-yl]2,5-dipheyltetrazolium bromide (MTT)-based assay. This is the first report on CPC purification of the three listed stilbenoids from *A. hypogaea* hairy root culture extract, as well as MTT-based cytotoxicity assays of arachidin-1 with HeLa cell lines and arachidin-3 with RAW 264.7 and HeLa cell lines.

Materials and Methods

Chemicals and reference standards

Methanol and ethyl acetate were purchased from either EMD Chemicals (Gibbstown, NJ) or Fisher Scientific (Pittsburgh, PA). Water was purified to a resistivity of 18.20 MΩ with a Direct-Q 5 Millipore system (Bedford, MA). Acetonitrile was obtained from EMD Chemicals, and formic acid was manufactured by EM Science (Gibbstown, NJ). Heptane was purchased from Aeasar (Ward Hill, MA), and ethanol was obtained from Pharmaco-Aaper (Shelbyville, KY). Dimethyl sulfoxide (DMSO), copper (II) sulfate pentahydrate, BHT, 1,1,3,3-tetraethoxypropane (TEP), thiobarbituric acid (TBA), sodium acetate, trizma hydrochloride, Murashige and Skoog medium, antibiotic/antimycotic solution (10,000 units penicillin, 10 mg streptomycin and 25 μg amphotencin B per mL), and MTT based in vitro assay kit were purchased from Sigma Chemical Company (St. Louis, MO). Human LDL was purchased from Biomedical Technologies (Stoughton, MA), and sodium hydroxide (NaOH) was obtained from

VWR Scientific Products (West Chester, PA). Sodium chloride (NaCl) and trichloroacetic acid (TCA) were purchased from J.T. Baker (Phillipsburg, NJ). DIAION HP-20 polystyrene resin was manufactured by Supelco (Bellefonte, PA). *Trans*-resveratrol was purchased from Biophysica Research (La Jolla, CA). Reference standards of *trans*-arachidin-1 and *trans*-arachidin-3 were purified from fungus-treated peanut seeds at the laboratory of Dr. Victor S. Sobolev of the National Peanut Research Laboratory (Dawson, GA) as described recently,[14] and were gifts from Dr. Fabricio Medina Bolivar, Arkansas State University, Jonesboro, AR. Helene Langer cervical cancer (HeLa) cell line was kindly provided by Dr. Jody Lingbeck, Center for Food Safety, Department of Food Science, University of Arkansas, Fayetteville. Mouse leukaemic monocyte macrophage cell line (RAW 264.7) was obtained from American Type Cell Culture (ATCC, Manassas, VA). Fetal bovine serum (FBS), and Dulbecco's modified Eagle's high glucose medium (DMEM), -L-glutamine, -sodium pyruvate were purchased from HyClone Laboratories (Logan, UT). Cellgro™ L-glutamine, 200 mM, was obtained from Mediatech (VA, AK, HI). Microtest™ 96-well, flat-bottom culture plates were obtained from Becton-Dickinson & Company (Franklin Lakes, NJ).

Preparation of hairy root culture extracts

Hairy roots of peanut cv. Hull line 3 were cultured in 250 mL flasks containing 50 mL of MSV medium as previously described by the Medina-Bolivar laboratory.[14] At day 12, the culture medium was exchanged for fresh medium containing 10.2 mM sodium acetate as the elicitor and incubated for an additional 48 h (elicitation period) under continuous darkness at 28°C on a rotary shaker (90 rpm). During the elicitation period, a pack containing 250 mg of polystyrene resin DIAION HP-20 was added to each culture to absorb the stilbenoids secreted into the medium. Preliminary trials showed that this resin was effective in recovering most of the stilbenoids from the culture medium. Following elicitation, the resin-containing packs were combined and stilbenoids were extracted from the resin with ethyl acetate. The ethyl acetate was evaporated to dryness under vacuum at 40°C in a Rotavapor R-200 (Büchi, Flawil, Switzerland). The dried extracts were resuspended in methanol, centrifuged at 3220*g* in an Eppendorf 5810R centrifuge (Westbury, NY), for 30 min at 4°C. The supernatant was filtered through a 0.22 μm nylon filter, transferred into an amber vial and dried to completeness under a nitrogen stream using a Reacti-Vap™ III apparatus (Pierce, Rockford, IL). The weight of the extract was recorded before HPLC analyses. A total of 420 mg of dry extract was produced from the processing of forty 250 mL flasks, each containing 50 mL of culture medium. The entire extraction process was done under low light to avoid isomerization of the stilbenoids.

Quantitative analysis

The identity and concentrations of the compounds in the *A. hypogaea* hairy root culture extract were determined using the HPLC method adapted from Condori et al.[15] HPLC analysis was performed using a Waters system (Milford, MA) equipped with an Alliance 2,690 separations module and a 996 Photodiode Array controlled by Empower chromatography software. A volume of 10 μL of an extract sample in methanol was injected into a 250 mm column (Symmetry® C_{18}, 250 × 4.6 mm, 5.0 μm, Waters). Detection occurred at 320 nm. Solvent A consisted of 0.05% formic acid in water, and solvent B consisted of 99:1 v/v acetonitrile:solvent A. The run was initiated with 90:10 solvent A:solvent B. Solvent A decreased linearly for 8 min to 82:18, which was maintained for 2 min. Solvent A further decreased linearly to 75:25 for 5 min, and then solvent B increased linearly to 65:35 over 3 min. This state was held for 20 min. Solvent A decreased linearly for 59 min to 40:60, and then returned to the initial condition of 90:10 in 3 min. This condition was held for 5 min. The flow rate was 0.5 mL/min, and the solution temperature was set to 30°C. The authenticity of the peaks was compared to reference standards.

Purification of stilbenoids

The stilbenoids *trans*-resveratrol, *trans*-arachidin-1, and *trans*-arachidin-3, produced by the *A. hypogaea* hairy root cultures, were separated via bench scale Fast Centrifugal Partition Chromatograph (Kromaton, Angers, France). The solvent system of heptane/ethyl acetate/ethanol/water (4:5:3:3, v/v/v/v), was chosen based upon trial runs and previously published work on the separation of polyphenols with CPC.[16,17]

The solvents were mixed well, allowed to settle for 30 min in a separatory funnel, and were separated just before use. The CPC was run in the descending mode, meaning that the lighter organic portion of the solvent system was the stationary phase. The 200 mL column was flushed and filled with 500 mL of the organic phase at 200 rpm and at 16 mL/min using a Waters 510 adjustable solvent delivery pump. The rotation rate was then increased to 1,000 rpm and the aqueous phase was then introduced at 3.1 mL/min. Equilibrium was obtained when 74 mL of stationary phase had been displaced.

Four milliliters of each phase were added to the 420 mg dry *A. hypogaea* hairy root culture extract sample to be purified. The solution was mixed well and allowed to settle for at least 3 min. The sample was then loaded into the 10 mL sample loop and injected into the column. The eluent exiting the rotor was monitored with a UV-VIS absorbance detector set to 320 nm (VUV24 Reflect Scientific, Orem, UT), and fractions were collected every 2 min using a Waters Fraction Collector III.

Oxidation of low-density lipoproteins

The antioxidant activity of resveratrol, arachidin-1, and arachidin-3 was determined using the TBARS assay, developed by Wallin et al.[18] Human LDL was dialyzed in 1.8 L of a 50 mM (13.1 g trizma hydrochloride and 15.66 g NaCl in 1.8 L of water) EDTA-free Tris buffer solution (pH 7.4 via NaOH) in Slide-A-Lyzer Mini Dialysis Units from Pierce (10,000 MWCO) for 24 h at 4°C. To stimulate the oxidation reaction, 55 μM copper (II) sulfate pentahydrate solution was added to each test well in 96-well MICROTEST Flat Bottom Tissue Culture Plates from Greiner Bio-one (Frickenhausen, Germany).

Next, 10 μL of the test compound dilutions in DMSO were added to the wells such that the final concentration of each compound (resveratrol, arachidin-1, or arachidin-3) was 0, 1, 2, 3, 7, 14, and 27 μM for the reference standards; or 0, 1, 3, 7, 14, 27, and 55 μM for the CPC-purified compounds. The oxidation reaction was stopped in specified wells at the onset of the experiment with the addition of 10 μL of 1 mM BHT. The plate was then placed in a water bath (Sheldon Manufacturing, Cornelius, OR) at 37°C. After

a 24-h incubation period, 10 μL of 1 mM BHT solution were added to the remaining wells to stop the reaction.

A volume of 50 μL of 50% weight per volume TCA was added to each well along with 75 μL of 1.3% weight per volume of TBA. The plate was then placed again in the water bath for 40 min at 60°C. Standard concentrations TEP in Tris in the presence of BHT were prepared to estimate the amount of TBARS formed. The plate was then read at optical densities (OD) of 600 and 530 nm in a Synergy HT plate reader from BioTek Instruments (Winooski, VT), and the difference in absorbance between the two wavelengths was calculated by Kineticalc for Windows, version #3.3 by Biotek Instruments.

MTT-based cytotoxicity assay

HeLa and RAW 264.7 cell lines were cultured in DMEM, supplemented with 10% FBS, 1% L-glutamine, and 1.4% Sigma's antibiotic/antimycotic solution at 37°C with 5% CO_2. Cells were plated at approximately 2×10^3 per well (100 μL per well) in the 96-well tissue culture plate, and incubated overnight to allow for attachment of cells to plate. Test compounds (resveratrol, arachidin-1, or arachidin-3) were diluted in DMEM containing 2% DMSO (0.2 μm filtered) to concentrations of 1, 3, 7, 14, 27, and 55 μM. Due to the solubility issue of the compounds, DMSO was a necessary solvent for the dilution. It was determined that 2% DMSO solution was an optimum solvent to use for this study. Test wells received 100 μL per well of these dilutions in descending order. Controls included wells with cells in DMEM with 2% DMSO (controls for DMSO), wells with cells in DMEM (negative controls), wells with only DMEM (blanks), and wells with cells in DMEM with 10% Triton X-100 (positive controls). Test plates were incubated for 18 h at 37°C with 5% CO_2. Test solutions were aspirated and MTT solution (1 mL MTT concentrate in 9 mL DMEM without phenol red) was added to each well at 100 μL per well (preliminary tests determined that using DMEM without phenol red and without FBS apparently caused the particular cells to reduce their metabolism, but not die. Therefore, adding FBS to media, without phenol red, aided the metabolism of the cells). Plates were incubated for 4 h and the resulting formazan crystals were dissolved by adding the MTT solubilization solution. Solution in wells was pipetted up and down to further help dissolve the crystals. The plate was then read at ODs of 600 and 530 nm in a Synergy HT plate reader and analyzed as stated for TBARS. Representative runs of 4–8 replications were reported.

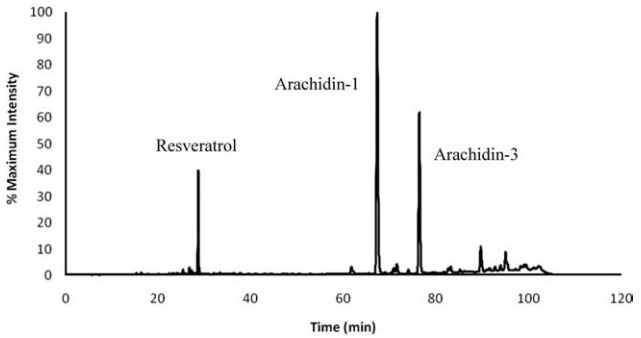

Figure 2. HPLC trace of *A. hypogaea* hairy root culture extract with *trans*-resveratrol, *trans*-arachidin-1, and *trans*-arachidin-3 peaks indicated at retention times of 28.6, 67.3, and 76.4 min, respectively.

Detection occurred at 320 nm.

Statistical analysis

The treatment levels required of resveratrol, arachidin-1, and archidin-3 in the TBARS assay to produce a significant decrease in oxidation were tested using analysis of variance with the Fit Least Squares Model and LSMeans Differences Student's *t*-test procedure of JMP (SAS Institute, Cary, NC).

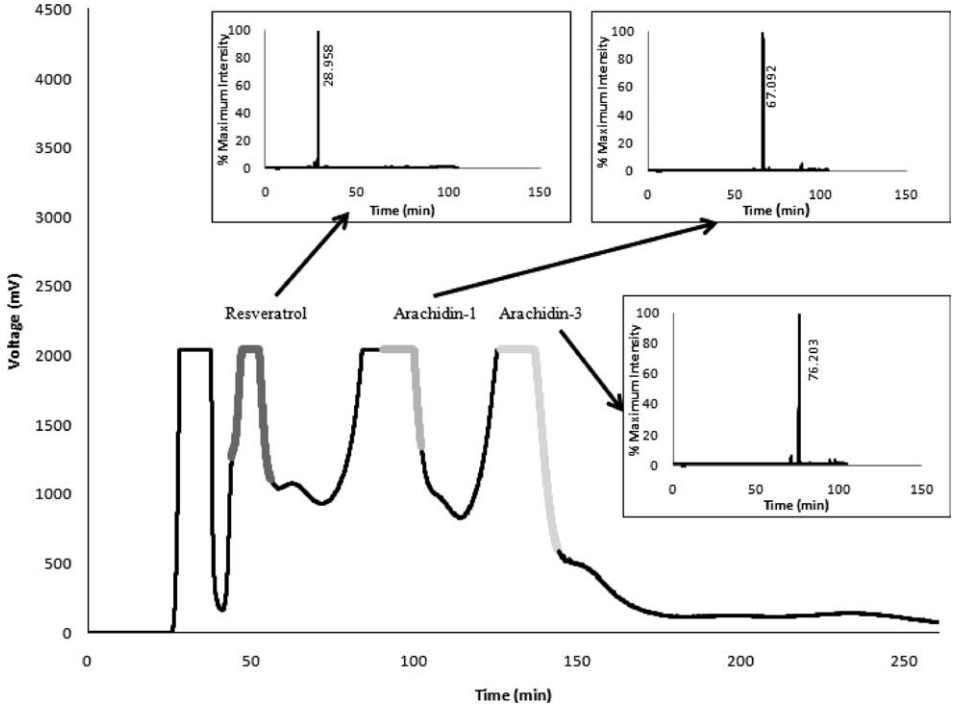

Figure 3. CPC Chromatogram with *trans*-resveratrol, *trans*-arachidin-1, and *trans*-arachidin-3 peaks indicated.

Peak contents were verified via HPLC and the *trans*-resveratrol, *trans*-arachidin-1, and *trans*-arachidin-3 fraction HPLC traces are shown inset alongside the CPC trace.

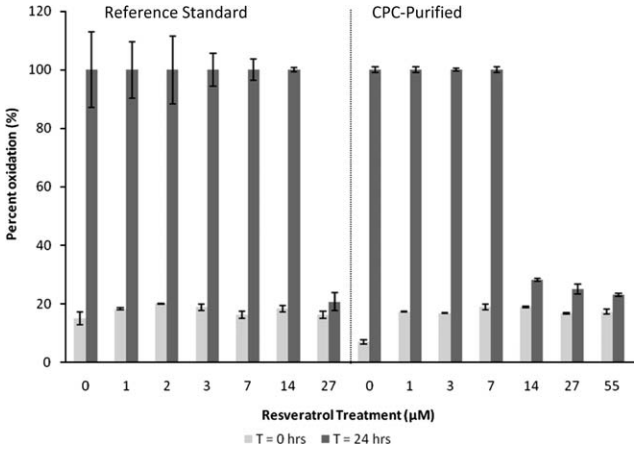

Figure 4. TBARS formation with various concentrations of *trans*-resveratrol reference compound and *trans*-resveratrol obtained from combined fractions of a CPC separation.

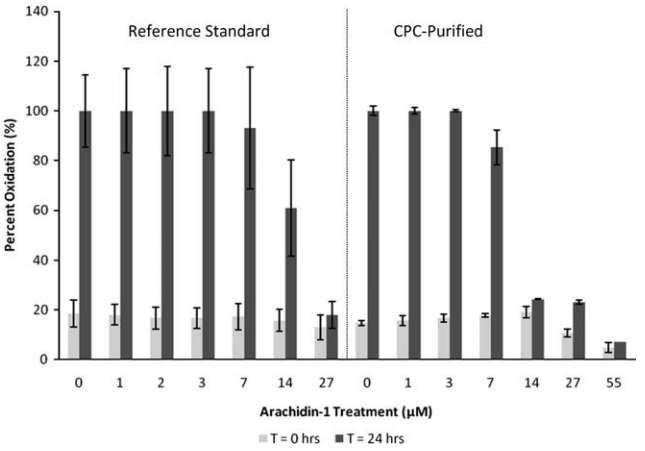

Figure 5. TBARS formation with various concentrations of reference standard of *trans*-arachidin-1 and *trans*-arachidin-1 obtained from combined fractions of a CPC separation.

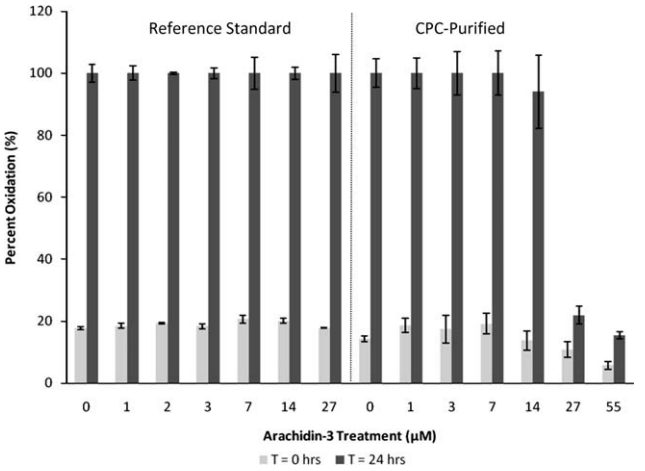

Figure 6. TBARS formation with various concentrations of reference standard *trans*-arachidin-3 and *trans*-arachidin-3 obtained from combined fractions of a CPC separation.

The reference standards were tested in duplicate while the CPC-purified compounds were tested in triplicate. Significance was determined at $P < 0.05$.

Treatment levels of resveratrol, arachidin-1, and archidin-3 in the MTT-based assay to determine the cytotoxicity of these compounds on RAW 264.7 and HeLa cell lines were analyzed as stated for the TBARS LDL oxidation assay, excluding the reference standards.

Results and Discussion

Quantification and purification of stilbenoids

The production of stilbenoids in the peanut hairy root cultures was carried out as described by the Medina-Bolivar laboratory,[14] using sodium acetate as the elicitor with the following modifications. The elicitation period was changed from 24 to 48 h and a resin was used to trap the secreted stilbenoids from the culture medium. These changes allowed for an increase in the yields of arachidin-1 and arachidin-3 vs. resveratrol. In addition, the resin-based extraction permitted to use less amount of organic solvent during the extraction. The stilbenoids in the extract were analyzed and quantified by HPLC, essentially as described by Condori et al.[15] Extracts from *A. hypogaea* hairy root culture with a 420 mg total dry weight were shown to contain 5.03 mg of resveratrol, 30.02 mg of arachidin-1, and 19.84 mg of arachidin-3. A chromatogram of the *A. hypogaea* hairy root culture extract is presented in Figure 2. Resveratrol, arachidin-1, and arachidin-3 eluted at 28.6, 67.3, and 76.4 min, respectively. A 9-min difference in the retention times of arachidin-1 and arachidin-3 ensured that these compounds did not co-elute. Of the possible available separation techniques, CPC was used to separate resveratrol, arachidin-1, and arachidin-3 from the *A. hypogaea* hairy root culture medium extract. Based upon previously published work regarding the separation of polyphenols with CPC, the solvent system of heptane/ethyl acetate/ethanol/water (4:5:3:3, v/v/v/v) was chosen.[16,17] The partition coefficient, *K*, also was used to validate the solvent system. *K* was determined with reference standards using the shake flask method.[19] The partition coefficient was calculated by dividing the area of the HPLC peak of the compound in the organic phase by that in the aqueous phase. The partition coefficient for resveratrol, arachidin-1, and arachidin-3 were calculated to be 0.42, 2.04, and 3.43, respectively. The calculated coefficients were adequate for CPC separation, as they were reasonably close to the acceptable range ($0.5 < K < 2.5$).[20] Figure 3 shows the chromatogram obtained during the CPC separation with resveratrol, arachidin-1, and arachidin-3 indicated; HPLC traces are shown inset alongside the CPC trace. During the CPC purification, resveratrol, arachidin-1, and arachidin-3 eluted at 44–55, 90–101, and 126–143 min, respectively. Based upon HPLC analysis of the CPC fractions, tubes with similar stilbenoid profiles were combined. The extract to be purified initially contained 5.03, 30.02, and 19.84 mg of resveratrol, arachidin-1, and arachidin-3, respectively, and 4.61, 19.99, and 16.51 mg were recovered, respectively.

The developed CPC purification protocol was repeated for a similar *A. hypogaea* hairy root culture extract, and comparable results were obtained, indicating that this protocol is reproducible. Fractions from both separations were combined, and HPLC was again used to determine the purity of the compounds from the combined extracts. Using CPC, resveratrol, arachidin-1, and arachidin-3 were recovered at 91.7%, 66.6%, and 83.2%, respectively, based on total percentage of recovered compounds. Arachidin-1 and arachidin-3 were previously isolated by HPLC, but no yields were

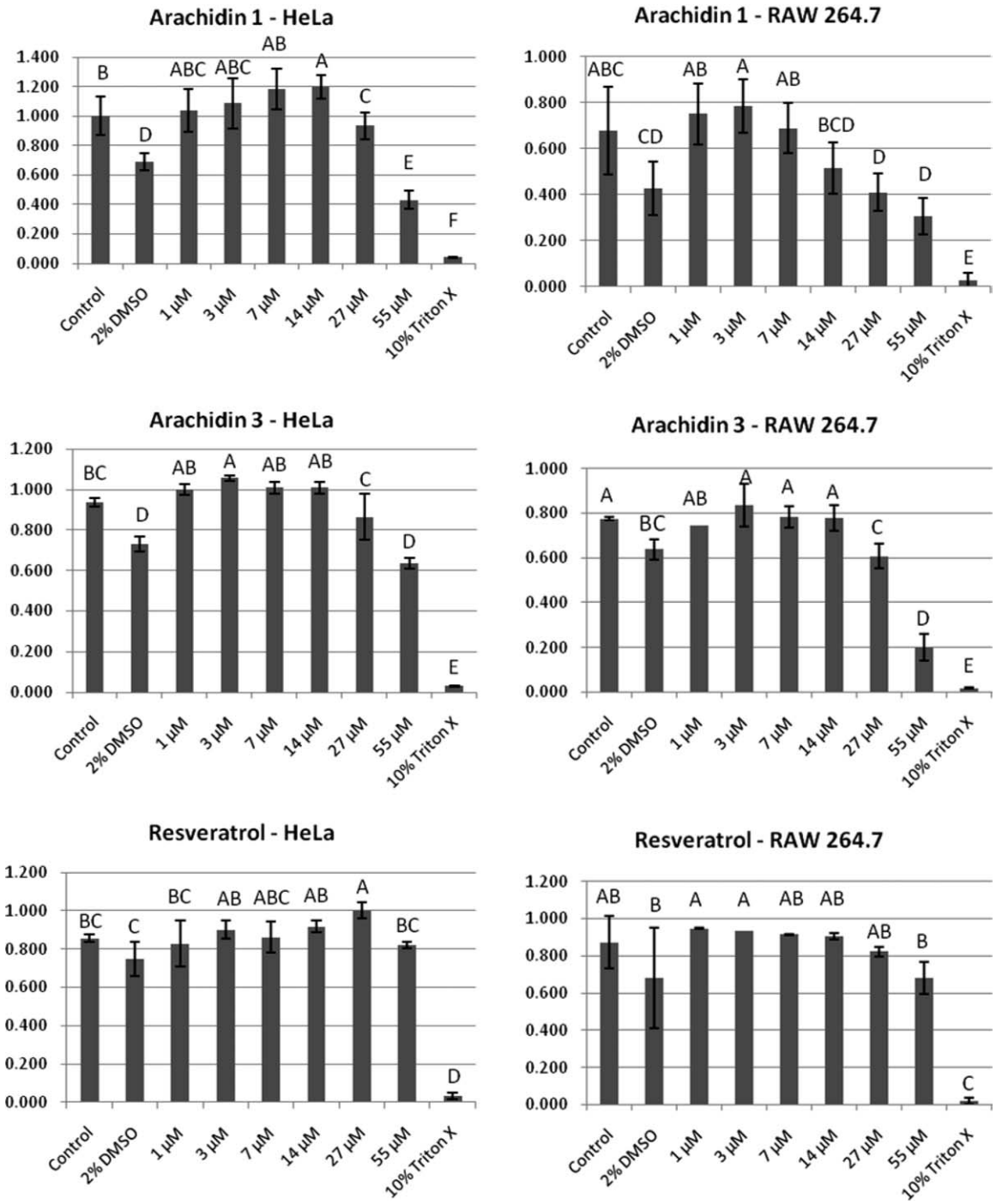

Figure 7. Effects of CPC-purified *trans*-arachidin-1 and *trans*-arachidin-3 and *trans*-resveratrol on HeLa and RAW 264.7 cell lines.
Y axis was OD readings (Delta 600/530). Samples with OD readings not connected by same letters are significantly different, $P < 0.05$.

reported.[9] Preparative HPLC was used to recover arachidin-1 and arachidin-3, where no yields were reported; however, novel peanut-derived phytoalexins, such as arachidin-2, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, chiricanine A, arahypin-1, arahypin-2, arahypin-3, arahypin-4, arahypin-5, arahypin-6, and arahypin-7, were reported.[21] The CPC-purified compounds were subsequently used in the TBARS and MTT assays, alongside reference standards, to determine their biological activity.

Antioxidant activity

The antioxidant activity of resveratrol, arachidin-1, and arachidin-3 was determined using Cu^{2+}-induced LDL oxidation using the in vitro TBARS assay.[18] Figure 4 shows the results of the TBARS assay performed on the reference standard of resveratrol at concentrations of 0, 1, 2, 3, 7, 14, and 27 μM, as well as on the CPC-purified resveratrol at concentrations of 0, 1, 3, 7, 14, 27, and 55 μM. Oxidation was inhibited at 27 and 14 μM dose by the reference standard and CPC-purified compound, respectively. The resveratrol concentrations that resulted in biological activity in this work were lower than those reported elsewhere; Zou et al.[22] showed that resveratrol reduced the Cu^{2+}-induced oxidation of LDL TBARS by 70.5% at a 50 μM dose. Although an in vivo study, Zhu et al.[23] showed that resveratrol lowered plasma and hepatic TBARS levels when rats were fed at least 30 mg resveratrol/kg body weight.

Interestingly, the results presented in this report show that CPC-purified resveratrol showed biological activity at a lower concentration than the purchased reference compound. The reference standard of resveratrol, used in this work, had been stored in methanol at –20°C, but protected from light for a period of at least 4 months, while the CPC-purified compounds only remained in storage (in methanol, at –20°C, protected from light) for 4 weeks. While studies have indicated that *trans*-resveratrol remains stable when protected from light for a matter of months, and although precautions were made, degradation could have affected the results.[22,23]

Figure 5 shows the results of the TBARS assay performed on the reference standard of arachidin-1 as well as CPC-purified arachidin-1 in the same manner as Figure 4. Oxidation was inhibited at a dose of 7 μM for both the reference standard as well as the CPC-purified compound. Therefore, stability may not have affected the overall antioxidant activity of compound at the time of the study; however, the larger standard error present in this study may be attributed to the instability of the compound.

The results of the TBARS assay performed on the reference standard of arachidin-3 as compared to that of CPC purified arachidin-3 can be seen in Figure 6. The CPC-purified compound showed greater antioxidant activity with oxidation inhibition occurring at a 14 μM dose. The reference standard did not inhibit lipid oxidation up to a dose of 27 μM. The stability of the compound may have had an effect on the results of the assay. Arachidin-3 was stored in the same conditions for the same length of time as resveratrol. There are no studies exploring the stability of arachidin-3, but it is interesting to note that reference standards of resveratrol and arachidin-3, which were stored the longest, also showed the least antioxidant activity.

Arachidin-1 and arachidin-3 were subjected to the AOP test at 100 μM doses, and arachidin-1 reported to outperform, but not significantly, resveratrol and BHT.[8] Arachidin-3 showed the least antioxidant potency when compared to arachidin-1 and resveratrol. Although the results reported herein stem from a different assay, to the three compounds in this study, arachidin-1 remains the most bioactive, confirming the results reported by Chang et al.[8]

Cytotoxicity

The cytotoxicity of the three compounds was analyzed using MTT-based assay. Results in Figure 7 demonstrate the effects of the various concentrations arachidin-1, arachidin-3, and resveratrol on HeLa and RAW 264.7 cell cultures. It was noted from this study that 2% DMSO had a detrimental effect in all samples. In contrast, with the addition of the test compounds, there was an increase in viability, compared to controls, of both cell lines at all but the highest concentrations for arachdin-1 and arachidin-3. At 27 and 55 μM concentrations of arachidin-1 and arachidin-3, MTT-based assays of RAW 264.7 cell cultures resulted in OD readings that were significantly lower than controls; at 27 and 55 μM of arachidin-1 and 55 μM of arachidin-3, OD readings from HeLa cell cultures were significantly lower than controls. Djoko et al.[9] reported similar results using the MTT-based assay with RAW 264.7 cells, stating that arachidin-1 displayed cytotoxicity at 31.7 μM. Arachidin-3 was not included in the Djoko study.[9] Chang et al.[8] reported, using direct microscopic cell counts that at 15 μM, arachidin-1, arachidin-3, and resveratrol did not present any obvious cytotoxicity to RAW 264.7 cell cultures. In our study, resveratrol did not display cytotoxicity towards either RAW 264.7 or HeLa cell cultures at any of the concentrations listed (1, 3, 7, 15, 27, and 55 μM). With all samples, the positive control treatment (10% Triton X-100) presented OD readings that were significantly lower than any other treatments.

In conclusion, this research has delineated the integration of a production and purification stilbenoid system; the produced compounds displayed biological activity in both the TBARS and MTT assays. Given that novel peanut-derived phytoalexins were reported, this production and purification system may be modified such that it is also used for the manufacture and testing of these original compounds. Ongoing work on scaling up root biomass and production of stilbenoids from 250 mL flask cultures to airlift balloon-type bioreactors (Medina-Bolivar et al., unpublished) demonstrates the use of the *A. hypogaea* hairy roots as a potentially scalable and sustainable source for the production of resveratrol, arachidin-1, and arachidin-3. CPC could potentially be a technique by which these compounds could be separated and purified.

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