



Research Journal of  
**Medicinal  
Plant**

ISSN 1819-3455



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## **Antiproliferative and Apoptotic Effects of *Phytolacca americana* Extracts and their Fractions on Breast and Colon Cancer Cells**

<sup>1</sup>L. Maness, <sup>1</sup>I. Goktepe, <sup>1</sup>B. Hardy, <sup>1</sup>J. Yu and <sup>2</sup>M. Ahmedna

<sup>1</sup>Department of Family and Consumer Sciences, Food and Nutritional Sciences Program, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA

<sup>2</sup>North Carolina Agricultural and Technical State University, Center of Excellence in Post-Harvest Technologies, North Carolina Research Campus, Kannapolis, NC 28081, USA

*Corresponding Author: Ipek Goktepe, Department of Family and Consumer Sciences, Food and Nutritional Sciences Program, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA  
Tel: 1-336-334-7963 Fax: 1-336-334-7239*

### **ABSTRACT**

*Phytolacca americana*, native to North and South America and East Asia, has been used by Native Americans as a laxative, and to treat inflammation, rashes and breast problems. Recent investigations include determining anti-viral and anti-cancer properties of purified pokeweed antiviral protein conjugated to monoclonal antibodies. However, there is limited information on the activities of *P. americana* extracts directly against cancer cells. This study was carried out to evaluate the antiproliferative activities of crude ethanol, methanol and water extracts from *P. americana* against MCF-7 human breast and HCT-116 colon cancer cells using the 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The ethanol extract of *P. americana* (PRE) had significantly higher antiproliferative activity against HCT-116 cells than the methanol extract (PRM) and the water extract (PRW). None of the extracts showed a significant antiproliferative activity against MCF-7 cells. The ethanol extract was further fractionated and the water fraction showed the greatest antiproliferative activity against HCT-11 Cells compared to the ethyl acetate and butanol fractions. Changes in caspase 2, 3, 6, 8 and 9 activities in HCT-116 cells after exposure to the ethanol extract and its most active fraction were also examined. Caspases 6 and 9 showed increased activities in HCT-116 cells exposed to the ethanol extract. Caspases 3, 8 and 9 were activated in HCT-116 cells exposed to the water fraction. Relatively strong antiproliferative and apoptotic activities were exhibited against HCT-116 cells by the ethanol extract and its water fraction. These findings suggest that further investigation into their potential use in pharmaceutical applications as chemopreventive supplements or in complementary treatment of colon cancer is warranted.

**Key words:** *Phytolacca americana*, caspases, complementary medicine, alternative medicine, HCT-116, MCF-7

### **INTRODUCTION**

As of 2006, cancer was still the second leading cause of death in the United States, comprising 23% of all deaths, only 3% below deaths from heart diseases (ACS, 2009). The second and third most common causes of cancer deaths in women are breast and colon cancers, respectively, while in men, colon cancer is the second most common cause of cancer deaths.

Since many of the current cancer treatments have undesirable side effects, alternative methods are being sought (Mao *et al.*, 2007; Molassiotis *et al.*, 2005).

Herbal remedies have been used as treatments for centuries by native people of various countries worldwide and scientific studies are recently emerging, in many cases supporting their antiproliferative effects. For example, *Uncaria tomentosa* extracts, traditionally used in South America for the treatment of inflammatory conditions, arthritis and cancer have been shown to inhibit proliferation of MCF-7 breast cancer cells up to 90% (Riva *et al.*, 2001). Similarly, *Astragali radix*, an ancient Chinese remedy, has been found to inhibit the growth of gastric cell lines up to 68% (Lin *et al.*, 2003) while *Aegle marmelos*, a medicinal plant from Bangladesh, has been shown to inhibit proliferation of a variety of cancer cell lines (Lampronti *et al.*, 2003).

Some herbs have been shown to inhibit proliferation of cancer cells through apoptosis as shown through the activation of cysteine proteases known as caspases. For example, Chios mastic gum was able to activate caspases 3, 8 and 9 in HCT-116 cells (Balan *et al.*, 2005). In addition, *Viscum album* exposure resulted in measurable activities of the same caspases in T- and B- cell leukemia cell lines (Bantel *et al.*, 1999). Furthermore, *Hypericum perforatum* L., commonly known as St. John's wort, contains an active component, hyperforin, which has been shown to increase caspase 8 and 3 activities in K562 human histiocytic lymphoma cells (Hostanska *et al.*, 2003).

*Phytolacca americana* (pokeweed) is an herbaceous plant that has been used by Native Americans as a laxative, to treat inflammation and rashes, to induce vomiting and to treat breast problems, especially in breast-feeding mothers (Jones, 2001). More recently, anti-viral and anti-carcinogenic properties have been investigated by Schlick *et al.* (2000) and Rajamohan *et al.* (1999).

Through studying the anti-viral properties of various parts of *P. americana*, several inhibitors of protein translation have been isolated. Pokeweed antiviral proteins PAPI, PAPII, PAPIII and PAP-S can be extracted from spring, early summer, late summer leaves and seeds, respectively and each has individually been shown to inhibit replication in plant and animal viruses, such as brome mosaic and hepatitis B (Misawa *et al.*, 1975; He *et al.*, 2008; Picard *et al.*, 2005; Barbieri *et al.*, 1982). PAP has also been conjugated with various antibodies and this has been demonstrated to be effective against the proliferation of osteosarcoma cells in hamsters (Ek *et al.*, 1998) and against HIV replication (Zarling *et al.*, 1990).

In addition to PAP, there are other compounds that have been isolated from the various parts of *P. americana*. For example, the seeds are known to contain triterpenes, glycosides and neolignans. Furthermore, several 1, 4-benzo-dioxane-type compounds have been isolated from the stems, such as americanoic acid methyl ester, isoamericanoic acid A methyl ester and 9'-O-methylamericanol A (Takahashi *et al.*, 2001). Extracts of *P. americana* that contain these compounds have not been previously tested on cancer cells.

To examine this potential, the objectives of this study were to: 1) investigate the inhibitory activities of extracts of ethanol (PRE), methanol (PRM) and water (PRW) from *Phytolacca americana* root against the proliferation of HCT-116 (colon) and MCF-7 (breast) cancer cells *in vitro*; 2) fractionate the most active *P. americana* extract and determine the antiproliferative effects of those fractions; and 3) determine the changes in caspase activities of the most active *P. americana* root extract and its most active fraction against HCT-116 cells.

## **MATERIALS AND METHODS**

**Sample collection and preparation:** Roots of *P. americana* were harvested from the North Carolina Agricultural and Technical State University farm in Greensboro, North Carolina in the spring of 2009. The roots were washed, cut and freeze-dried at  $-80^{\circ}\text{C}$  using a Labconco freeze-zone freeze dryer (Kansas City, Missouri) for 72 h. Following freeze drying, the samples were powdered using a grinder (Reustch 1640, Germany). The powdered materials were then divided into three equal portions and soaked either in 100% deionized water, 80% ethanol (1:5 w/v) or 80% methanol overnight with continuous stirring. Solid material was removed from the mixtures by centrifugation at 7000xg for 20 min at  $4^{\circ}\text{C}$ . The liquid supernatant was then collected in a flask and the final residue was discarded. The water, ethanol or methanol in the supernatant was evaporated under reduced pressure using a Rotovapor (Buchi, Germany) at  $42^{\circ}\text{C}$ . The concentrated extracts were collected as crude ethanol (PRE), methanol (PRM), or water (PRW) extracts of *P. americana* roots and kept at  $-80^{\circ}\text{C}$  until further use.

**Cell culture:** Two cancer cell lines (1) human colorectal adenocarcinoma (HCT-116), cultured in 89% McCoy's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin; and (2) human breast adenocarcinoma (MCF-7), cultured in 89% Roswell Park Memorial Institute (RPMI-1640) medium with 0.1% HEPES buffer solution, 10% fetal bovine serum and 1% penicillin/streptomycin, were purchased from American Type Culture Collection (ATCC, Manassas, VA). The cancer cells were maintained in a 5%  $\text{CO}_2$  incubator at  $37^{\circ}\text{C}$  with fresh media added every 2 to 3 days for the duration of the study to allow healthy growth and proliferation for testing.

**Antiproliferative activity assay:** The antiproliferative activity of the crude extracts (PRW, PRE, and PRM) was evaluated at concentrations of 0, 400, 800, 1600 and 3200  $\mu\text{g mL}^{-1}$  using the MTT assay (Mosmann, 1983). Extracts were dissolved in 0.5% Dimethyl Sulfoxide (DMSO). The dissolved extracts were sterilized using a 0.22  $\mu\text{m}$  syringe filter. The cancer cells' viability was confirmed using Trypan Blue Exclusion Test (ATCC, Manassas, VA). Forty microliters of viable HCT-116 or MCF-7 cells, at concentrations of  $6.25 \times 10^5$  cells  $\text{mL}^{-1}$ , were seeded in a 96-well microplate and incubated for 2 h to allow for cell attachment, after which 20  $\mu\text{L}$  of the various concentrations of dissolved crude extracts was added. Phosphatidylinositol 3-Kinase Inhibitor (PI3KI) (Cell Signaling Technology, Danvers, MA) was used as a positive control at 200  $\mu\text{g mL}^{-1}$ . The microplates were then incubated for 24, 48 and 72 h at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere. After each incubation time period, aliquots of MTT (Sigma-Aldrich, St. Louis, MO) were added to each well at 4:15 ratio and incubated for an additional 3 h. Next, 0.01 N HCl in isopropanol was added and mixed by pipetting to dissolve the tetrazolium crystals. Absorbance was read at 490 nm using an Elx808 Ultra Microplate Reader (BioTek, Vienna, VA). All concentrations and time intervals were repeated three times with six replicates per run.

**Fractionation of PRE and antiproliferative activity assay:** Based on the antiproliferative assays described above, PRE was selected for fractionation. As a result, a total of 5.68 g of PRE was fractionated using 110 mL each of hexane and water, followed by ethyl acetate and then butanol. Each separate liquid fraction was dried by rotavapor evaporation to obtain the following yields: 0 mg hexane, 22.04 mg ethyl acetate, 354.44 mg butanol and 4734.08 mg water fraction (PREW).

Each fraction, excluding hexane, was tested for its antiproliferative activity following the same method as described under the section 2.3., except at concentrations of 25, 50, 100 and 200  $\mu\text{g mL}^{-1}$  and for 48 and 72 h.

**Caspase analysis:** After determining the most active extract and/or fraction derived from *P. americana* against both cell lines, analyses of caspases 2, 3, 6, 8 and 9 were carried out to study the apoptotic effect of the crude ethanol extract of *P. americana* (PRE) and its water fraction (PREW) on HCT-116 cells. The ApoTarget™ Colorimetric Protease Assay Sampler Kit from Invitrogen (Carlsbad, CA) was used following the manufacturer's instructions. This kit detects caspase levels by measuring free p-nitroaniline at an absorbance of 405 nm. Free p-nitroaniline increases as caspases cleave the p-nitroaniline-labeled peptides in cells. PRE was tested at 1600 and 3200  $\mu\text{g mL}^{-1}$  and its corresponding water fraction, PREW, was tested at 400, 800 and 1600  $\mu\text{g mL}^{-1}$  for 48 h. A negative control was also used that consisted of HCT-116 cells exposed only to 0.5% DMSO. Phosphatidylinositol 3 kinase inhibitor (PI3KI) (Cell Signaling Technology, Danvers, MA) was used as a positive control at 200  $\mu\text{g mL}^{-1}$ . All concentrations and time intervals were repeated at least six times.

**Statistical analysis:** Data were analyzed by Analysis of Variance (ANOVA) using Statistical Analysis Software (SAS, 2000) with the mean for each treatment at each concentration tested separately by comparing percent reduction. The least significant difference of means was run as a post ANOVA test to determine significance of treatments at  $p \leq 0.05$ .

## RESULTS

**Activities of *P. americana* ethanol (PRE), methanol (PRM) and water (PRW) extracts against HCT-116 and MCF-7 cancer cells:** Overall, the ethanol extract of *P. americana* (PRE) inhibited the proliferation of HCT-116 cells more than the methanol (PRM) and water extracts (PRW) of *P. americana* (Fig. 1-3), with increasing inhibitory activity as the concentrations of extracts increased ( $p \leq 0.05$ ). After 48 and 72 h, PRE at a concentration of 3200  $\mu\text{g mL}^{-1}$  inhibited HCT-116 cells by 17 and 20%, respectively. After 48 and 72 h, PRM inhibited the cells by 7% and 8%, respectively, at the same concentration. The water extract (PRW) had a 10% inhibitory rate against HCT-116 cells after 48 h at 3200  $\mu\text{g mL}^{-1}$  but no antiproliferative activity was observed after 72 h.

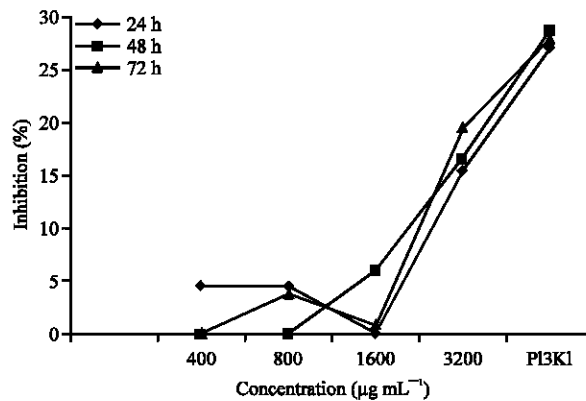


Fig. 1: Inhibitory activity of ethanol extract (PRE) at 400, 1600, 800 and 3200  $\mu\text{g mL}^{-1}$  against HCT-116 cancer cells ( $p \leq 0.05$ ). PI3KI = Phosphatidylinositol 3 kinase inhibitor (used as a positive control) at 200  $\mu\text{g mL}^{-1}$

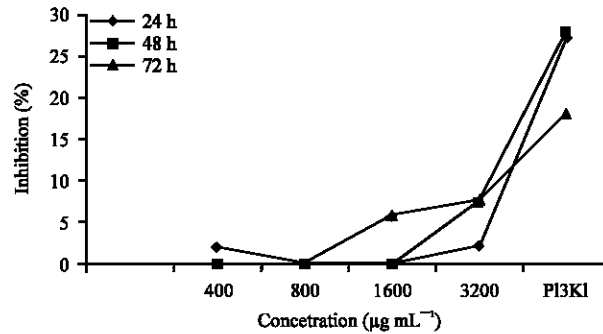


Fig. 2: Inhibitory activity of methanol extract at 400, 1600, 800 and 3200 µg mL against HCT-116 cancer cells at 24, 48 and 72 h

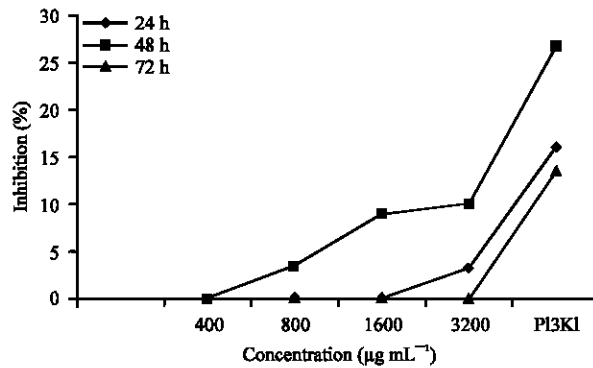


Fig. 3: Inhibitory activity of water extract at 400, 1600, 800 and 3200 µg mL<sup>-1</sup> against HCT-116 cancer cells at 24, 48 and 72 h

None of the extracts of *P. americana* used in this study inhibited the proliferation of MCF-7 cancer cells significantly ( $p \geq 0.05$ ) at any of the concentrations tested either after 48 or 72 h. The average inhibition for PRE, PRM and PRW at 3200 µg mL<sup>-1</sup> was 3, 0 and 0%, respectively, after 48 h and 5, 1 and 0%, respectively, after 72 h. Since the inhibitions were not significant ( $p \geq 0.05$ ), the data are not shown.

**Activities of ethyl acetate, butanol and water fractions derived from PRE against HCT-116 cancer cells:** After 48 h at concentrations of 200 µg mL<sup>-1</sup>, the ethyl acetate fraction inhibited the proliferation of HCT-116 cells by up to 8% (Fig. 4), the butanol fraction inhibited the growth by up to 7% (Fig. 5) and the water fraction (PREW) showed the greatest inhibitory activity of up to 18% (Fig. 6).

**Change in caspase activities after exposure of HCT-116 cells to PRE and PREW:** In HCT-116 cells exposed to PRE, one of the caspases tested increased in activity consistently with increasing concentrations (Table 1). The activity of Caspase 6 was significantly the highest ( $p \leq 0.05$ ) among all caspases tested in this study. Caspase 9 increased significantly ( $p \leq 0.05$ ) from an absorbance of 0.02 after exposure to the negative control to an absorbance of 0.06 at 1600 µg mL<sup>-1</sup> of PRE. However, no activity of this caspase was detected in HCT-116 cells exposed to PRE at a

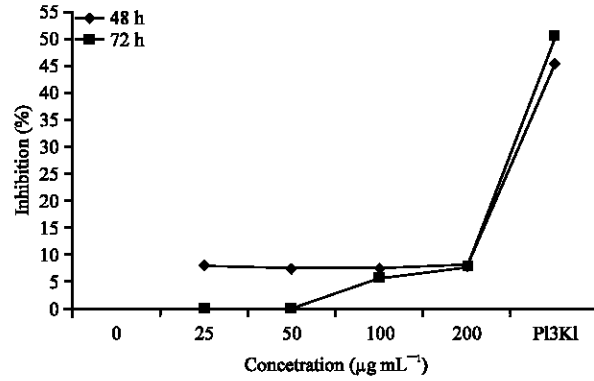


Fig. 4: Inhibitory activity of ethyl acetate fraction at 25, 50, 100 and 200 µg mL<sup>-1</sup> against HCT-116 cancer cells at 48 and 72 h

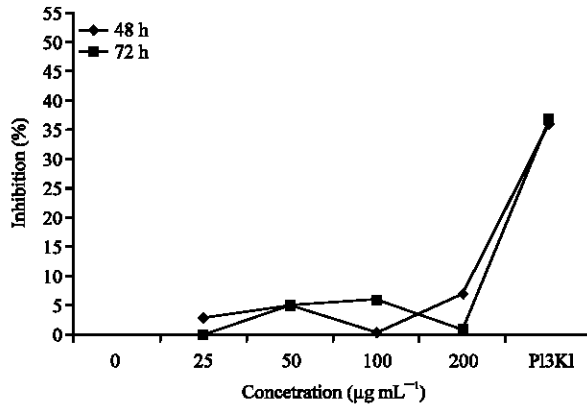


Fig. 5: Inhibitory activity of butanol fraction at 25, 50, 100 and 200 µg mL<sup>-1</sup> against HCT-116 cancer cells at 48 and 72 h

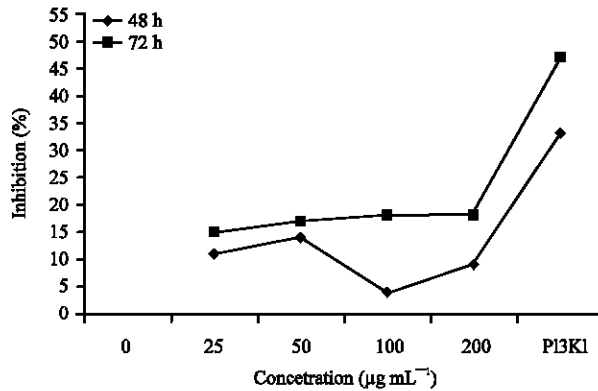


Fig. 6: Inhibitory activity of water fraction (PREW) at 25, 50, 100 and 200 µg mL<sup>-1</sup> against HCT-116 cancer cells at 48 and 72 h

Table 1: Measured absorption (405 nm) and percent change in caspase activity after HCT-116 cells were exposed to PRE for 48 h

Caspases	Concentrations ( $\mu\text{g mL}^{-1}$ )			
	0	1600	3200	PI3KI
2	0.04±0.0004	0.02±0.0002	0.04±0.0008	0.10±0.0040
3	0.05±0.0020	0.05±0.0015	0.04±0.0012	0.09±0.0063
6	0.00±0.0000	0.02±0.0004**	0.06±0.0240**	0.05±0.0025
8	0.03±0.0003	0.03±0.0012	0.03±0.0003	0.10±0.0065
9	0.02±0.0008	0.06±0.0030**	0.02±0.0008	0.04±0.0020

\*\* $p \leq 0.05$ ; PI3KI tested at  $200 \mu\text{g mL}^{-1}$

Table 2: Measured absorption (405 nm) and percent change in caspase activity after HCT-116 cells were exposed to PREW for 48 h

Caspases	Concentrations ( $\mu\text{g mL}^{-1}$ )				
	0	400	800	1600	PI3KI
2	0.02±0.0006	0.02±0.0008	0.02±0.0006	0.02±0.0006	0.07±0.0035
3	0.03±0.0015	0.05±0.0015**	0.02±0.0008	0.04±0.0008**	0.10±0.0010
6	0.06±0.0030	0.01±0.0003	0.03±0.0012	0.03±0.0015	0.04±0.0000
8	0.01±0.0002	0.00±0.0000	0.03±0.0015**	0.02±0.0006**	0.03±0.0009
9	0.02±0.0010	0.00±0.0000	0.03±0.0012**	0.03±0.0012**	0.07±0.0042

\*\* $p \leq 0.05$ ; PI3KI tested at  $200 \mu\text{g mL}^{-1}$

concentration of  $3200 \mu\text{g mL}^{-1}$ . The remaining caspases had activities that were not significant ( $p \geq 0.05$ ). The activity of caspase 2 decreased with a concentration of  $1600 \mu\text{g mL}^{-1}$  but had no change in activity at a concentration of  $3200 \mu\text{g mL}^{-1}$ . The activity of caspase 3 remained constant at the smaller concentration tested but had a slight decrease from an absorbance of 0.05 to 0.04 when the cells were exposed to  $3200 \mu\text{g mL}^{-1}$ . The absorbance readings of caspase 8 remained stable when compared to the negative control in HCT-116 cells exposed to both concentrations of PRE.

None of the caspases' activities increased with increasing concentration following exposure of HCT-116 cells to increasing concentrations of the water fraction (PREW) derived from PRE (Table 2). Caspase 9 had a decrease in activity at  $400 \mu\text{g mL}^{-1}$  compared to the negative control, but had increases in absorbance from 0.02 to 0.03 at concentrations of 800 and  $1600 \mu\text{g mL}^{-1}$ . Similarly, caspase 8 had a decrease in activity after exposure to the lowest concentration but then increased in activity at the highest concentrations. The activity of caspase 3 fluctuated in activity in HCT-116 cells exposed to increasing concentrations of PRE. Caspase 6 did not exhibit strong activity at all concentrations compared to HCT-116 cells exposed to the control. Caspase 2 had no significant change in activity after exposing HCT-116 cells to any of the concentrations of PREW.

## DISCUSSION

In this study, the ethanol extract (PRE) derived from the roots of *P. americana* inhibited the HCT-116 cells in a concentration-dependent manner. This inhibition by PRE was greater than that of the methanol and water extracts and thus, PRE was chosen for further fractionation and testing. None of the extracts had a significant effect on the proliferation of MCF-7 cells. This indicates that the anticancer properties of *P. americana* may be restricted to certain types of cancer cells, as is generally the case with different herbal extracts (Lampronti *et al.*, 2003; De Martino *et al.*, 2006).



Of the fractions derived from PRE, the water fraction (PREW) demonstrated a greater effect than the butanol or ethyl acetate fractions. The results of the antiproliferative activities of the PRE fractions against HCT-116 cells initially suggest that the ethanol extract may have compounds that act synergistically and that fractionating may reduce its antiproliferative activity. However, lower concentrations were used in performing antiproliferative studies for each fraction than those used for the crude extract, PRE. The water soluble fraction made up 83.35% of the starting material of the crude ethanol extract used in the fractionation process. If we correlate the concentrations of PRE and PREW at 3200  $\mu\text{g mL}^{-1}$ , the adjusted concentration of PREW would be 2667  $\mu\text{g mL}^{-1}$ . This correlative concentration would likely have resulted in greater inhibitions of HCT-116 cells exposed to PREW.

Caspase 9 is an initiator caspase necessary to activate caspase 6 and other effector caspases (Slee *et al.*, 1999; Zou *et al.*, 1997). At a concentration of 1600  $\mu\text{g mL}^{-1}$ , both of these caspases had increases in activity in HCT-116 cells after exposure to PRE. This suggests that the mitochondrial apoptotic pathway was disrupted by this extract, with the increased activity of both initiator and activator caspases. It is unclear why caspase 9 had increases in activity at 1600  $\mu\text{g mL}^{-1}$  but did not have increased activity at 3200  $\mu\text{g mL}^{-1}$ . It is possible that compounds associated with this fraction act antagonistically depending on the concentration used in this study. Similar results were confirmed in a study in which chlorophyllin was shown to work as a chemotherapeutic agent using a mechanism involving caspase 6 as well as caspase 8 (an initiator caspase), BID and BAK, ultimately causing the cleavage of nuclear lamins (Diaz *et al.*, 2003).

The water fraction of ethanol (PREW) increased the activities of caspases 3, 8 and 9 in exposed HCT-116. Caspases 8 and 9 are initiator caspases and caspase 3 is an effector caspase. Caspase 3 affects a number of substrates and, thus, impacts cell death in several ways, including the activation of gelsolin and DFF, leading to both nuclear and DNA fragmentation (Kothakota *et al.*, 1997; Liu *et al.*, 1997). Further testing that involves any or all of the above mentioned parameters could verify the cellular impact of both PRE and PREW on HCT-116 cells and help confirm that apoptosis is in fact occurring. The results of this study are in agreement with the studies previously mentioned using Chios mastic gum and *V. album* whereby each of these caspases was also activated (Balan *et al.*, 2005; Bantel *et al.*, 1999).

Present findings indicated that the ethanol extract of roots of *P. americana* (PRE) inhibited the proliferation of HCT-116 cells through apoptosis, involving caspases 6 and 9. We also demonstrated that PREW, the water fraction of PRE, likewise inhibited the proliferation of HCT-116 cells through apoptosis, involving caspase 3, 8 and 9, depending on the concentrations used.

To understand how these beneficial changes would truly aid in the treatment of colon cancer, further *in vitro* testing is necessary. In addition, animal testing would ultimately be necessary to determine whether or not the exposures of PRE or PREW reduce tumor size as well as to understand what physiological side effects may occur on animals due to PRE and PREW exposures.

## ACKNOWLEDGMENTS

This study was supported by a grant from the United States Department of Agriculture, Cooperative State Research, Education and Extension Service (No. NCX-2285081701).

## REFERENCES

ACS, 2009. Cancer statistics 2009 presentation. <http://www.cancer.org/Research/CancerFactsFigures/cancer-statistics-2009-presentation>.

- Balan, K.V., C. Demetzos, J. Prince, K. Dimas and M. Cladaras *et al.*, 2005. Induction of apoptosis in human colon cancer HCT-116 cells treated with an extract of the plant product, chios mastic gum. *In vivo*, 19: 93-102.
- Bantel, H., I.H. Engels, W. Voelter, K. Schulze-Osthoff and S. Wesselborg, 1999. Mistletoe lectin activates caspase-8/FLICE independently of death receptor signaling and enhances anticancer drug-induced apoptosis. *Cancer Res.*, 59: 2083-2090.
- Barbieri, L., G.M. Aron, J.D. Irvin and F. Stirpe, 1982. Purification and partial characterization of another form of the antiviral protein from the seeds of *Phytolacca americana* L. (pokeweed). *Biochem. J.*, 203: 55-59.
- De Martino, L., J.L.S. Martinot, S. Franceschelli, A. Leone, C. Pizza and V. De Feo, 2006. Proapoptotic effect of *Uncaria tomentosa* extracts. *J. Ethnopharmacol.*, 107: 91-94.
- Diaz, G., Q. Li and R. Dashwood, 2003. Caspase-8 and apoptosis-inducing factor mediate a cytochrome c-independent pathway of apoptosis in human colon cancer cells induced by the dietary phytochemical chlorophyllin. *Cancer Res.*, 63: 1254-1261.
- Ek, O., B. Waurzyniak, D.E. Myers and F.M. Uckun, 1998. Antitumor activity of TP3(anti-p80)-pokeweed antiviral protein immunotoxin in hamster cheek pouch and severe combined immunodeficient mouse xenograft models of human osteosarcoma. *Clin. Cancer Res.*, 4: 1641-1647.
- He, Y.W., C.X. Guo, Y.F. Pan, C. Peng and Z.H. Weng, 2008. Inhibition of hepatitis B virus replication by pokeweed antiviral protein *in vitro*. *World J. Gastroenterol.*, 14: 1592-1597.
- Hostanska, K., J. Reichling, S. Bommer, M. Weber and R. Saller, 2003. Hyperforin a constituent of St John's wort (*Hypericum perforatum* L.) extract induces apoptosis by triggering activation of caspases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. *Eur. J. Pharm. Biopharm.*, 56: 121-132.
- Jones, E., 2001. *Cancer: Its Causes, Symptoms and Treatment*. Healing Mountain Publishing, Washington, DC., SBN: 0974117854.
- Kothakota, S., T. Azuma, C. Reinhard, A. Klippel and J. Tang *et al.*, 1997. Caspase-3-generated fragment of gelsolin: Effector of morphological change in apoptosis. *Science*, 278: 294-298.
- Lampronti, I., D. Martello, N. Bianchi, M. Borgatti and E. Lambertini *et al.*, 2003. *In vitro* antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. *Phytomedicine*, 10: 300-308.
- Lin, J., H.F. Dong, J.J. Oppenheim and O.M. Howard, 2003. Effects of *Astragali radix* on the growth of different cancer cell lines. *World J. Gastroenterol.*, 9: 670-673.
- Liu, X., H. Zou, C. Slaughter, X. Wang, 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell*, 89: 175-184.
- Mao, J.J., J.T. Farrar, S.X. Xie, M.A. Bowman and K. Armstrong, 2007. Use of complementary and alternative medicine and prayer among a national sample of cancer survivors compared to other populations without cancer. *Complement Ther. Med.*, 15: 21-29.
- Misawa, M., M. Hayashi, H. Tanaka, K. Ko and T. Misato, 1975. Production of plant virus inhibitor by *Phytolacca americana* suspension culture. *Biotechnol. Bioeng.*, 17: 1335-1347.
- Molassiotis, A., P. Fernandez-Ortega, D. Pud, G. Ozden and J. Scott *et al.*, 2005. Use of complementary and alternative medicine in cancer patients: A European survey. *Ann. Oncol.*, 164: 655-663.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Method*, 65: 55-63.

- Picard, D., C.C. Kao and K.A. Hudak, 2005. Pokeweed antiviral protein inhibits brome mosaic virus replication in plant cells. *J. Biol. Chem.*, 280: 20069-20075.
- Rajamohan, F., T.K. Venkatachalam, J.D. Irvin and F.M. Uckun, 1999. Pokeweed antiviral protein isoforms PAPI, PAPII and PAPIII depurinate RNA of human immunodeficiency virus (HIV)-1. *Biochem. Biophys. Res. Commun.*, 260: 453-458.
- Riva, L., D. Coradini, G. Di Fronzo, V. De Feo, N. De Tommasi, F. De Simone and C. Pizza, 2001. The antiproliferative effects of *Uncaria tomentosa* extracts and fractions on the growth of breast cancer cell line. *Anticancer Res.*, 21: 2457-2461.
- SAS, 2000. Statistical Analysis Software. Version 8, SAS Inst., Cary, NC., USA.
- Schlick, J.L., P. Dulieu, B. Desvoyes, P. Adami, J. Radom and M. Jouvenot, 2000. Cytotoxic activity of a recombinant GnRH-PAP fusion toxin on human tumor cell lines. *FEBS Lett.*, 472: 241-246.
- Slee, E.A., M.T. Harte, R.M. Kluck, B.B. Wolf and C.A. Casiano *et al.*, 1999. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8 and -10 in a caspase-9-dependent manner. *J. Cell Biol.*, 144: 281-292.
- Takahashi, H., Y. Namikawa, M. Tanaka and Y. Fukuyama, 2001. Triterpene glycosides from the cultures of *Phytolacca americana*. *Chem. Pharm. Bull.*, 49: 246-248.
- Zarling, J.M., P.A. Moran, O. Haffar, J. Sias and D.D. Richman *et al.*, 1990. Inhibition of HIV replication by pokeweed antiviral protein targeted to CD4+ cells by monoclonal antibodies. *Nature*, 347: 92-95.
- Zou, H., W.J. Henzel, X. Liu, A. Lutschg and X. Wang, 1997. Apaf-1, a human protein homologous to *C. Elegans* CED-4 participates in cytochrome dependent activation of caspase 3. *Cell*, 90: 405-413.