INFLUENCE OF GENETIC VARIABILITY ON SPECIALTY POTATO FUNCTIONAL COMPONENTS AND THEIR EFFECT ON PROSTATE CANCER CELL LINES

A Dissertation

by

LAVANYA REDDIVARI

Submitted to the Office of Graduate Studies of
Texas A&M Universtiy
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Horticulture

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Approved by:

Chair of Committee,	J. Creighton Miller Jr.
Committee Members,	Daniel Lineberger
	Hisashi Koiwa
	Stephen H. Safe
Head of Department,	Tim Davis

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ABSTRACT

Influence of Genetic Variability on Specialty Potato Functional Components and Their Effect on Prostate Cancer Cell Lines. (May 2007)

Lavanya Reddivari, B.S., Acharya N.G.Ranga Agricultural University, India;

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Chair of Advisory Committee: Dr. J. Creighton Miller Jr.

The influence of genotype (selection), location, and year on antioxidant activity (AOA), total phenolics (TP), total carotenoids (TC), phenolic and carotenoid composition was studied using specialty (colored) potatoes (*Solanum tuberosum* L.) from the Texas Potato Variety Development Program, grown at two Texas locations (McCook and Dalhart), and in two years (2003 and 2004). Chlorogenic acid, gallic acid, catechin, caffeic acid, and malvidin-3-(p-coumaryl rutinoside)–5-galactoside were the major phenolics, and lutein and violaxanthin were the major carotenoids identified.

The AOA, TP, and TC and phenolic composition differed significantly with genotype, location and year. However, genotypic effects were larger than location and year effects. Selection CO112F2-2 was high in all the measured parameters and also stable across locations and years, suggesting that this selection could be used as a parent in breeding varieties with improved health benefits. The AOA, TP and chlorogenic acid content were highly significantly correlated with one another.

The effects of whole specialty potato extracts, fractions and individual compounds on LNCaP (androgen-dependent) and PC-3 (androgen-independent) prostate cancer cells were also investigated. Ethanol extract of the selection CO112F2-2 (5 µg chlorogenic acid eq/ml), the anthocyanin fraction (AF; 5 µg chlorogenic acid eq/ml), gallic acid and chaconine showed potent anti-proliferative properties and increased the cyclin-dependent kinase inhibitor p27 levels in LNCaP and PC-3 cells. Induction of apoptosis was cell context dependent and associated with JNK (c-Jun NH2-terminal Kinase) and Erk (extracellular signal regulated kinase) activation. Cell death pathways, induced by potato extract and the AF, were associated with Erk and JNK activation, and these kinases activated caspase-independent apoptosis through nuclear translocation of endonuclease G (endo G) and apoptosis-inducing factor (AIF) in both cell lines. Induction of caspase-dependent apoptosis was also kinase-dependent but was observed only in LNCaP cells. Kinase inhibitors reversed this nuclear translocation of endo G and AIF. This is the first report showing that the cytotoxic activities of potato extract/AF in cancer cells were due to activation of caspase-independent apoptosis.

DEDICATION

This dissertation is dedicated to my loving parents

R. VENKATARAMANA REDDY and R. PRAVEENA, my husband JAIRAM VANAMALA, and my daughter ANJALI JOI VANAMALA whose constant love and support made this endeavor possible.

ACKNOWLEDGEMENTS

This is perhaps the easiest and hardest part that I have to write. It will be simple to name all the people that helped to get this dissertation completed, but it will be tough to thank them enough.

I bow before the God Almighty for his unconditional love and guidance in my life.

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Heartfelt thanks go to Dr. Stephen H. Safe**,** as the cell culture work would not have been possible without his support and encouragement. His incessant encouragement, meticulous suggestions, and academic advice have been abundantly helpful, and assisted me in numerous ways, including summarizing the contents of documents.

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With immense love, I thank my awesome husband, Jairam, for his support, encouagement, confidence and care. His loving support was always there for me whenever I needed it. I also thank my little angel, Anjali, who says “Mommy I am your big helper”.

With gratitude and affection, I recall the immense love, help and encouragement given to be by my beloved parents, Venkataramana Reddy and Praveena and in-laws Ramaiah and Subbamma. I also thank my sister Binatha and her husband Yashwanth and their little one, Hitesh, for their support.

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CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum*) is indigenous to the central Andean region of South America (*1*) and was introduced into Europe by the Spanish in the 16th century (*2*). Domestication and selection by humans made this crop a high-yielding carbohydrate-rich crop. Now, potato is the fourth most important food crop worldwide and one of the leading vegetable crops in the US, with a per capita consumption of approximately 135 pounds (*3*). Potato is a good source of vitamins, minerals, and high quality proteins (*4*). It is considered to be an excellent source of vitamin C, and also contains significant levels of vitamin B, E (*5*) and small amounts of vitamin A (*6*). Based on National Health and Nutrition Examination Survey (NHANES II), potatoes (excluding French fries) are the third largest source of vitamin B6 for adults 19 to 74. Potato contains some 20 minerals, including phosphorous, calcium and iron, which are of dietary importance (*1*). Potatoes are also rich in potassium.

Potatoes are an excellent source of lysine, and on the basis of amino acid composition, the calculated protein quality is about 70% that of whole-egg protein (*7*). Though potatoes contain only 2% protein on a fresh-weight basis, the protein content is 10% on a dry-weight basis. Potatoes also contain significant levels of functional compounds.

This dissertation follows the style of *Journal of Agricultural and Food Chemistry*.

Potato Functional Components

Polyphenols

Polyphenols are secondary plant metabolites (*8*) and constitute one of the most widely distributed groups of substances in plants, with more than 8,000 known phenolic structures (*9, 10*). Polyphenols are synthesized in plants through two main pathways; the shikimate pathway and the acetate pathway (*9*). Polyphenols range from simple molecules (phenolic acids) to highly polymerized compounds (tannins). Primarily, polyphenols occur as conjugates with one or more sugars, attached either to the hydroxyl group or to an aromatic carbon atom. The attached sugar can be a mono, di or even an oligosaccharide, with glucose is the most common type. Polyphenols can be classified into 14 different classes depending on their basic chemical structure. The phenolic acid and flavonoid classes are further subdivided into two and thirteen sub – classes, respectively (*9*) **(Figure 1.1)**.

Potatoes accumulate a wide variety of secondary metabolites including polyphenols (*8*). The Polyphenol content of potato tubers ranges from 530 – 1770 μg/gfw (*11*). Major polyphenols in potatoes are phenolic acids and flavonoids. Red- and purple-fleshed potatoes (specialty) are rich sources of anthocyanins (glycosylated forms of anthocyanidins, a subclass of flavonoids) (*12, 13*).

Phenolic acids

Major tuber phenolic acids include caffeic acid (CA) (280 mg/kg), chlorogenic acid (CHA) (22-71 mg/kg), ferulic acid (FA) (28 mg/kg), and cryptochlorogenic acid (11 mg/kg). Other phenolic acids in potato include neochlorogenic acid (7mg/kg),

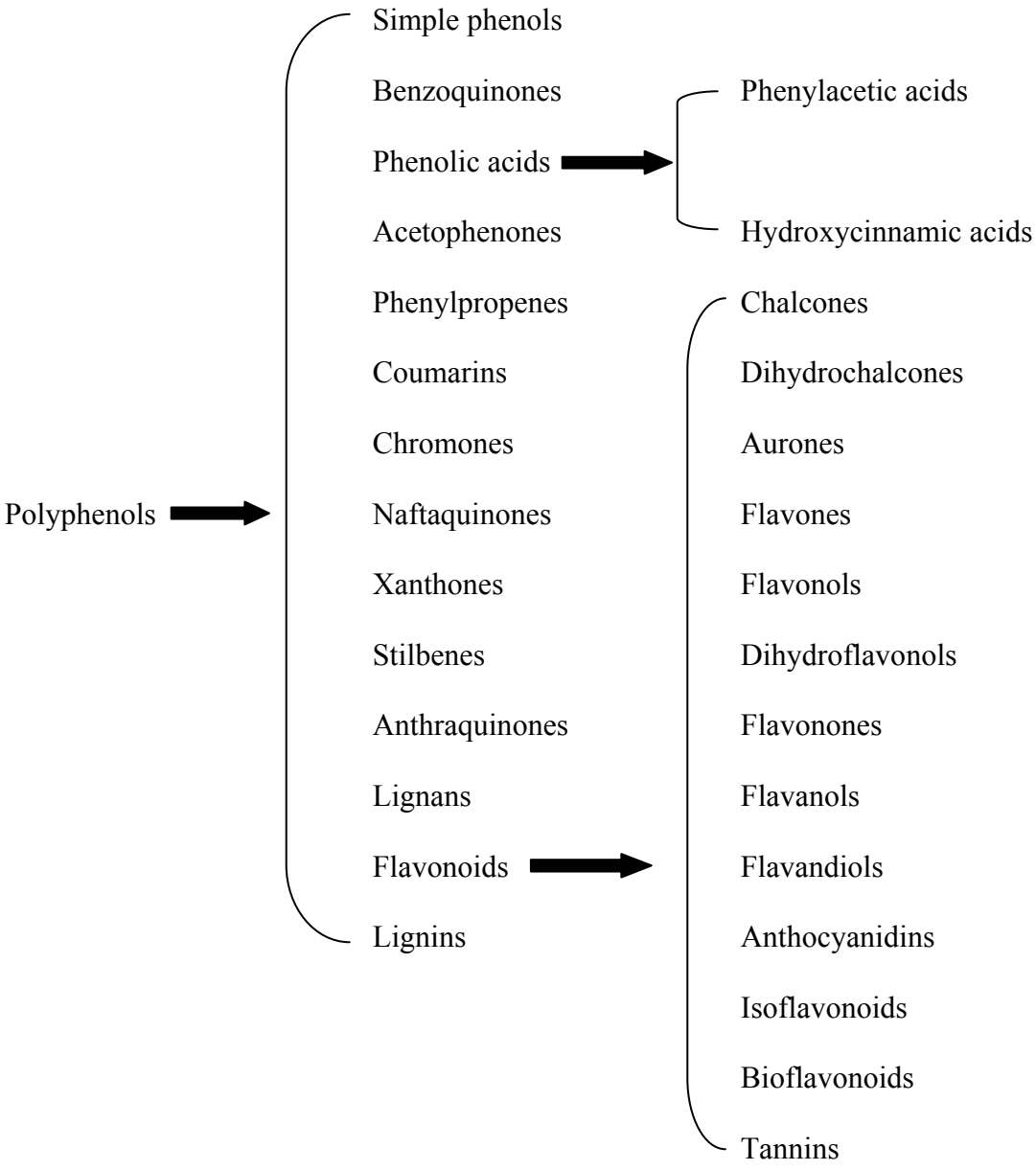

Figure 1.1. Classification of the polyphenols, phenolic acids and flavonoids (9).

p-coumaric acid (4mg/kg), and ferulic acid amides (*5*). Freidman (*8*) reported that chlorogenic acid constitutes up to 90% of the total phenolic content of potato tubers. According to Yamamoto et al. (*14*), caffeic acid levels in potato are as high as 0.2 to 0.3 mg/kg. The phenolic acid profile was reported to be chlorogenic acid (50.3%), caffeic acid (41.7%), gallic acid (7.8%), and protocatechuic acid (0.21%) (*15*). According to Kanatt et al. (*16*), the major peaks identified in potato peel were chlorogenic acid, caffeic acid, and gallic acid. Freeze dried methanol or ethanol extracts of potato peel revealed that gallic acid was the most abundant phenolic compound, followed by caffeic acid, chlorogenic acid, protocatechuic acid and vanillin (*17*) **(Figure 1.2)**.

Potato skin contained approximately double the amount of phenolic acids as flesh (*5*). According to Lewis et al. (*18*) cultivated potato tuber flesh and skin contained 157 – 600 µg/gfw and 2000-5000 µg/gfw phenolic acids, respectively. Tuber skins had high concentrations of chlorogenic acid (1000-4000 mg/kg), moderate amounts of protocatechuic acid (100-400mg/kg), caffeic acid (40-500mg/kg), vanillic acid (20-200mg/kg), sinapic acid (20-25mg/kg), and lower concentrations of gallic, syringic, p-coumaric, ferulic, salycilic, and cinnamic acids (each at 30mg/kg) (*18*). In addition to differences in concentration of phenolic acids in skin and flesh, differences exist within the tuber as well. Reeve et al. (*19*) were first to report that phenolic acids are not evenly distributed in the tuber tissue. A higher concentration of chlorogenic acid was observed in the stem end of the tuber than in the bud end (*11*). Cortex tissue had a higher content of phenolic acids compared to premedullary and pith tissue (*20*).

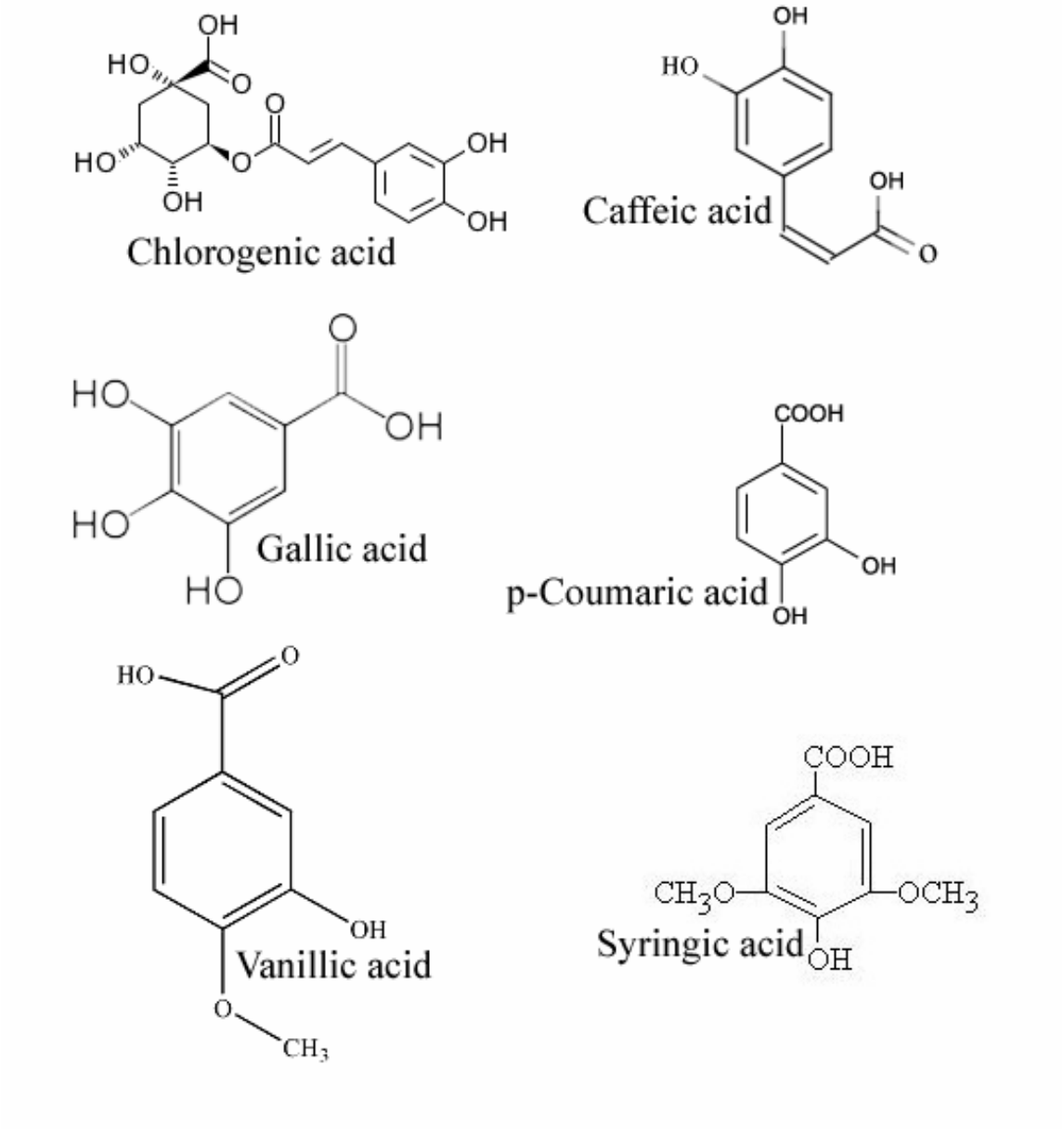

Figure 1.2. Chemical structure of phenolic acids in potatoes.

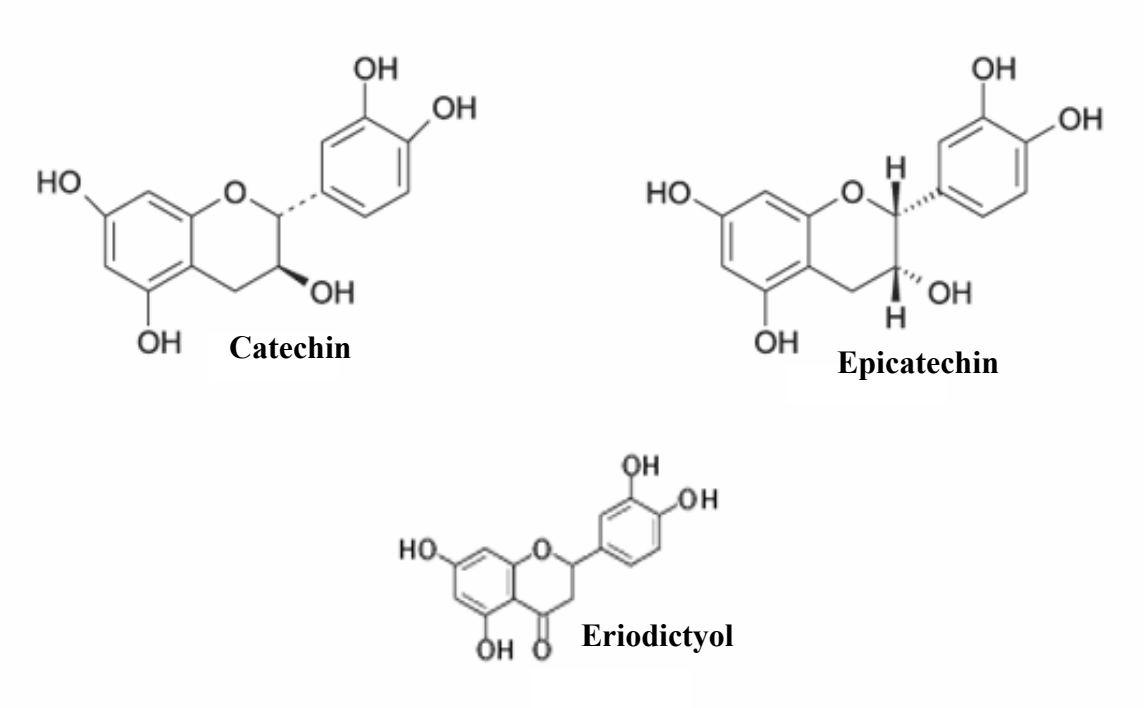

Figure 1.3. Chemical structure of flavonoids in potatoes.

Flavonoids

Potato flavonoids, other than anthocyanins, in order of abundance, were catechin, epicatechin (flavanols), eridictyol (flavonone) and kaempferol (flavonol) (*21*). Potato skin had a higher flavonoid content compared to flesh, and their concentrations ranged from 200 – 300 µg/gfw and 0 – 25 µg/gfw in skin and flesh, respectively (*18*) **(Figure 1.3)**.

Anthocyanins

Earlier reports on potato anthocyanins focused on inheritance of these pigments in progeny, but the recent research focuses on anthocyanin content and composition. The anthocyanin pigments in potatoes were identified by HPLC and mass spectroscopy analyses. According to Rodriquez Saona (*22*) red–fleshed potato anthocyanin content ranged from 3 – 40 mg/100 gfw. Lewis et al. (*18, 23*), reported higher anthocyanin content (368 mg/100 gfw) in certain purple fleshed cultivars. Anthocyanin content of different purple- and red-fleshed potato genotypes ranged from 11 – 174 mg cyanidin 3-glucoside eq/100gfw (*23*), however, Brown et al. (*24*) reported much lower anthocyanin range in red–fleshed (7 – 35 mg/100gfw) and purple-fleshed (6 – 17 mg/100 gfw) potato cultivars. Potato skin contained more anthocyanins than the flesh. Skin contained 900 mg and 500 mg/100 gfw of anthocyanins in purple-fleshed and red–fleshed cultivars, respectively.

The major anthocyanins identified were coumaryl–rutino–glucosides of pelargonidin, peonidin, petunidin and malvidin (*18*) **(Figure 1.4)**. Fossen and Anderson (*25*) identified a novel anthocyanin in Congo, a purple cultivar as 3-O-[6-(4-feruryl-o-alpha-rhamnopyranosyl)-beta-glucopyranoside]-5-o-beta-glucopyranosides of petunidin and malvidin, and stated that acylated pigments contributed to more than 98% of the total anthocyanin content in tubers. Fossen et al. (*26*) confirmed the presence of rhamno-glucopyranosides of petunidin and peonidin acylated with caffeic acid and/or p-coumaric acid in proportions 10, 6, 37, and 25%, respectively, in purple sprouts of a Norwegian potato cultivar.

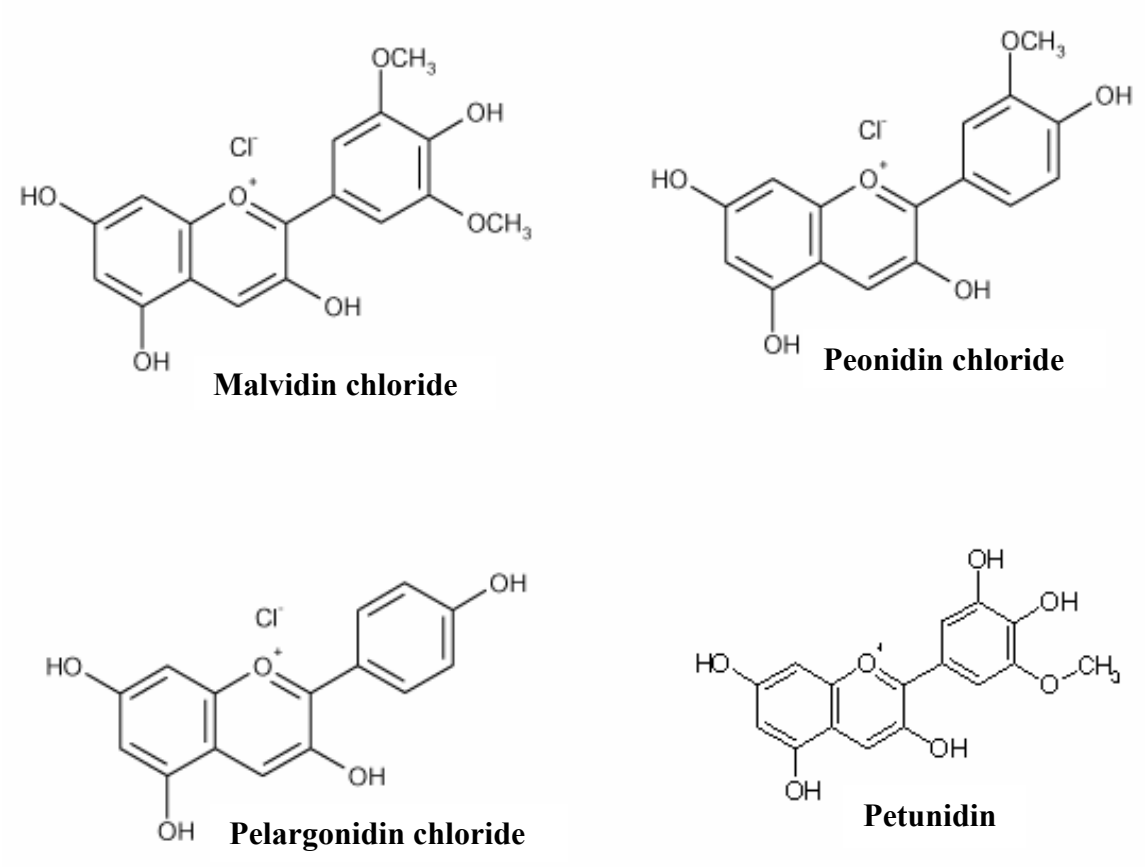

Figure 1.4. Chemical structure of anthocyanins in potatoes.

Carotenoids

Carotenoids are a group of natural pigments (≈ 600) found in plants and some other photosynthetic organisms such as algae. Carotenoid synthesis starts from an isoprenoid unit and is divided into xanthophylls and hydrocarbons. Xanthophylls are oxygenated carotenoids such as lutein, zeaxanthin, β–cryptoxanthin, antheraxanthin, violaxanthin, neoxanthin, canthaxanthin, etc., **(Figure 1.5)** and hydrocarbons are non-oxygenated carotenoids such as carotenes, lycopene, etc.

Pendlington et al.(*27*) reported that potato contained eight major pigments, β–carotene β–carotene– 5,6–monoepoxide, cryptoxanthin-5,6–diepoxide, lutein, cis-violaxanthin, cis-antheraxanthin–5,6–monoepoxide, cis-neoxanthin and an unknown pigment. Kasim (*28*) quantified the total and individual carotenoid content in nine German varieties. Total carotenoid content ranged from 199 – 560 µg/100gfw, and the major carotenoids were β–carotene– 5,6,5,6–diepoxide (33 - 108 µg/100gfw), lutein (30 – 119 µg/100fw), violaxanthin (8 – 29 µg/100gfw), and lutein 5,6–epoxide (81 – 257 µg/100gfw). A comparative study of nine Indian potato cultivars showed a good correlation between total carotenoid content and tuber flesh color (*29*). Iwanzik et al. (*30*) also showed a positive correlation between total carotenoid content and intensity of (yellow) flesh color using thirteen German potato varieties. Gross (*31*) reported that intense yellow-fleshed cultivars had a higher carotenoid content (300 µg/100gfw) than white-fleshed cultivars (30 – 70 µg/gfw).

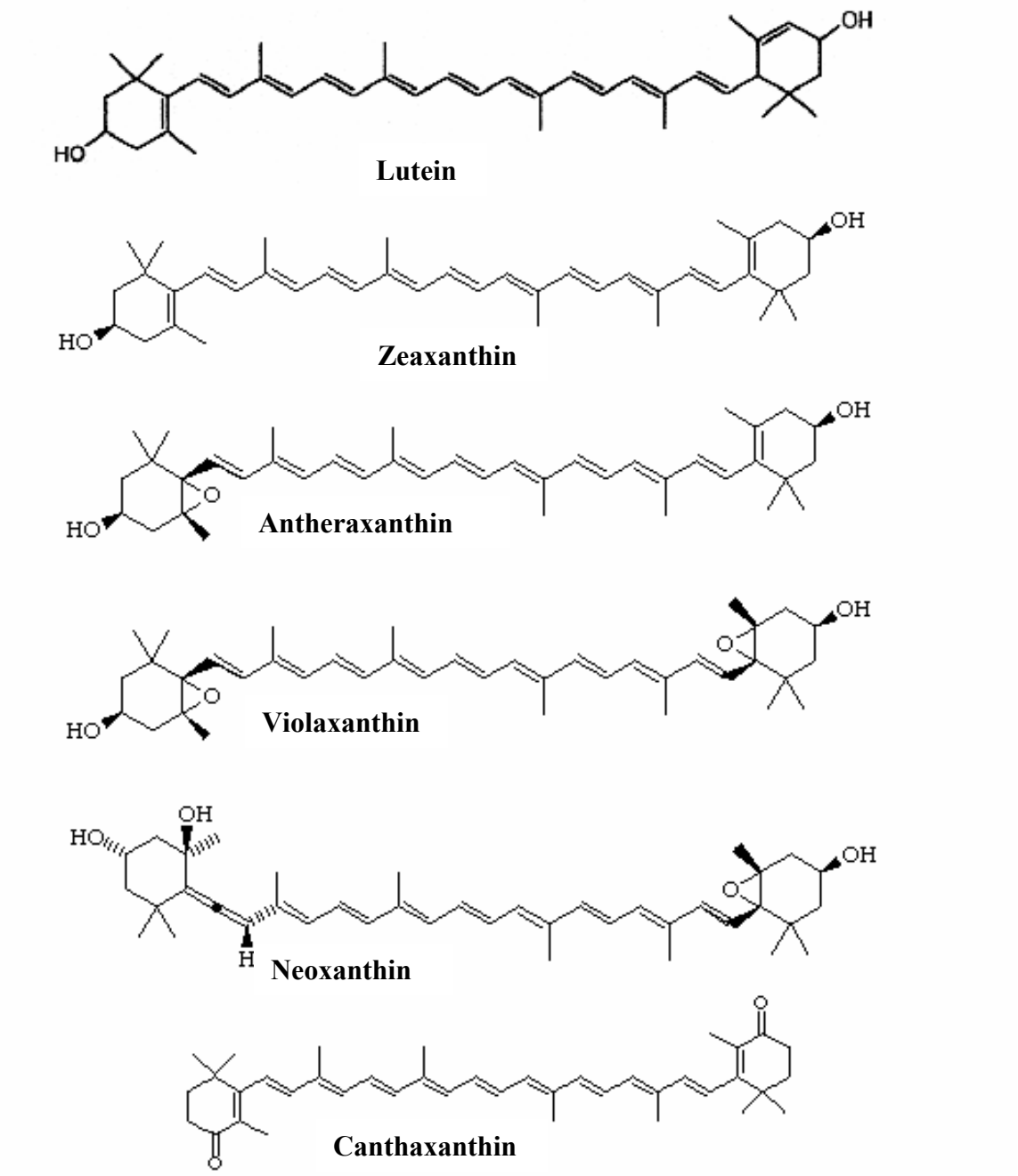

Figure 1.5. Chemical structure of carotenoids in potatoes.

In yellow–fleshed potatoes, epoxides contribute to 80% of the carotenoids, with violaxanthin (40 – 70%) the major carotenoid, followed by lutein epoxide. Several reports confirmed the presence of lutein, zeaxanthin, lutein epoxide, violaxanthin and neoxanthin in yellow-fleshed potatoes (*31, 32*).

Breithaupt and Bamedi (*33*) investigated carotenoid patterns in four yellow– and four white–fleshed potato cultivars using HPLC and LC-MS. Violaxanthin, antheraxanthin, lutein and zeaxanthin dominated the carotenoid pattern, while neoxanthin, β–cryptoxanthin and β–carotene were present in minor quantities. Nesterenko and Sink (*34*) observed the same seven carotenoids in 19 genotypes, including 15 breeding lines and four cultivars (Atlantic, Spunta, and Yukon Gold, OR-4). Quantification of carotenoids in orange-fleshed cultivars showed significantly higher carotenoid content than in yellow–fleshed cultivars (*35*). According Brown et al. (*36*), orange flesh was associated with higher levels of zeaxanthin, and orange-fleshed cultivars contained four times more lutein + zeaxanthin content compared to yellow–fleshed varieties.

Glycoalkaloids

Glycoalkaloids are nitrogen–containing compounds naturally present in many species of the family Solanaceae (*37*) that protect the plant from insects, bacteria and fungi (*38*). The two major potato glycoalkaloids are α–solanine and α–chaconine, which together account for 95% of the total glycoalkaloids (*8, 39, 40*). The other glycoalkaloids present in potato are β- and γ–solanines and chaconines, α–and β–solamarines, demissidine, leptines, commersonine, demissine, and tomatine (*41*). Though the

permitted safe level of glycoalkaloids is 200 mg/kg fresh tubers (*42*), varieties with 20 – 130 mg/kgfw glycoalkaloids are preferred (*43*) and most of the edible cultivars contain about 75 mg/kgfw glycoalkaloids (*41*). According to Freidman (*44*) the total glycoalkaloid content in eight popularly grown varieties in the US ranged from 7 – 187 mg/kgfw. The total glycoalkaloid content and the ratio of α–solanine and α–chaconine **(Figure 1.6)** differed depending on the part of the plant, though they are present in almost all parts of the potato plant (*42*). Higher concentrations of glycoalkaloids are associated with high metabolically active parts (*45, 46*). In tubers, skin (12 – 429 mg/kgfw) contained higher glycoalkaloid content than flesh (1 – 148 mg/kgfw) (*44*).

Effect of Genotype and Environment on Potato Functional Compounds

Both genotype and environment have been reported to influence potato phenolic and carotenoid levels. Varieties differed in their total phenolic content, with some showing two-fold higher concentrations than others (*11*). Hamouz et al. (*12*) reported that total phenolic content was higher in the Karin variety than in Agria. Genotype influences both total phenolics and anthocyanin content in potato (*5, 18, 21, 23*). Colored potato cultivars differed in their concentration of phenolic acids in the flesh (*18*). Colored potato cultivars contained two to four times more phenolic acids and twice the concentration of flavonoids compared to white cultivars. Total phenolic acid concentration of wild *Solanum* species ranged from 100-600 μg/gfw in the flesh, depending on the species (*21*).

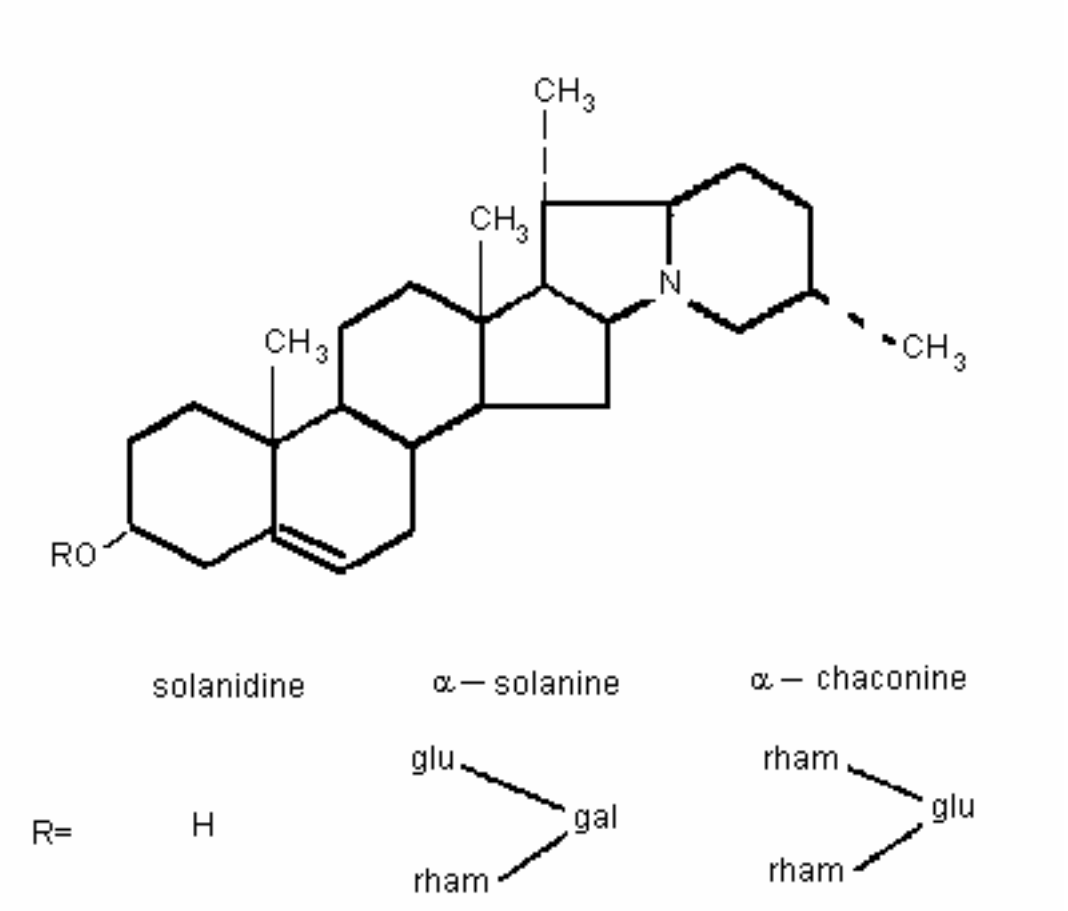

Figure 1.6. Basic chemical structure of solanidine and substituted groups for solanine and chaconine.

Dao and Freidman (*47*) reported two-fold differences in chlorogenic acid content among seven potato varieties. Red - and purple - fleshed cultivars differed in both content and composition of anthocyanins. Major anthocyanins identified were coumaryl –rutino–glucosides of pelargonidin (200-2000µg/gfw) and peonidin (20-400µg/gfw) in red tubers, and of petunidin (1000 - 2000µg/gfw) and malvidin (20 - 200µg/gfw) in light to medium purple tubers. Dark purple to black tubers contained similar pigments as that of medium purple types but had higher malvidin content (2000 - 5000µg/gfw). Red-fleshed clones predominantly contained acylated glycosides of pelargonidin, while purple-fleshed clones contained acylated glycosides of petunidin and peonidin, with smaller amounts of delphinidin and malvidin.

Greater total phenolic content was observed in higher, cooler and more humid regions with less fertile sandy loams compared to lower, warmer and drier regions with fertile loamy soils. In these, the varietal influence was much more pronounced than locality. Total polyphenolic content was not significantly influenced by year (*48*). Longer days and cooler temperatures caused a 2.5- and 1.4-fold increase in anthocyanins and total phenolics, respectively (*49*). Hamouz et al. (*12*) investigated the influence of environment on seven potato cultivars by growing them in 12 localities in the Czeck Republic for three years. Potatoes grown in lower, warmer and drier regions contained 5.8% less phenolics than potatoes grown in higher, cooler and humid regions. Chlorogenic acid content differed consistently between organically and conventionally produced potatoes (*50*).

Significant differences were observed among cultivars (*36, 51*) and environments for carotenoid content and composition. Iwanzik et al. (*30*) observed that the total carotenoid content ranged from 27µg/100 gfw (Kero) to 343 µg/100 gfw (Monza), confirming the existence of varietal differences on carotenoid content. Kufri Chamatkar had the highest carotenoid content out of ten cultivars analyzed by Marwaha (*52*). Lu et al. (*32*) reported three to 22-fold differences in carotenoid content among 11 diploid clones. Singh also reported ten-fold differences among 11 Indian potato cultivars (*53*). Nesterenko and Sink (*34*) showed the influence of cultivar not only on carotenoid content, but also on composition. Lutein and violaxanthin were detected in all 19 genotypes studied, but neoxanthin, antheraxanthin, beta-cryptoxanthin, zeaxanthin and beta-carotene were found only in 26%, 63%, 5%, 10%, and 16% of the genotypes, respectively. Some potato genotypes showed an increase in carotenoid content when transferred from higher temperatures to lower temperatures without altering the photoperiod in growth chambers (*54*). Salt stress reduced the carotenoid content in the Zhihuabai and Jingshi-2 cultivars (*55*). Significant differences among environments, clones and clone x environment interactions for yellow flesh intensity were also reported by Haynes et al. (*56*).

Antioxidant Activity of Potato Functional Components

Antioxidants are compounds that can quench free radicals and delay the oxidation of an oxidizable substrate or protect a biological systems against the potentially harmful effects of these free radicals (*57, 58*). Antioxidant activity is the radical absorbance capacity of an antioxidant. A wide array of assays has been reported

to measure antioxidant activity. Based on the chemical reactions involved, they are grouped into two major categories: hydrogen atom transfer (HAT) reaction-based assays and single electron transfer (ET) reaction-based assays. HAT-based assays include oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays. Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), Cu (II) complex antioxidant potential, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) assays come under ET-based assays. In addition, superoxide, hydrogen peroxide, the hydroxyl radical, singlet oxygen and peroxynitrite scavenging capacity assays are used to measure the ability of antioxidants to scavenge respective radicals (*59*).

Chromogen compounds are commonly used to mimic reactive oxygen and nitrogen species in measuring antioxidant activity because of their ease, speed and sensitivity. Most widely used chromogens are the $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals. DPPH and ABTS are stable radicals and absorbs at 515 nm (*60*) and 734 nm, respectively. Antioxidant activity of both hydrophilic and lipophilic compounds can be measured with $ABTS^{+\cdot}$ because of its solubility in both aqueous and organic solvents, but $DPPH^{\cdot}$ is soluble only in alcoholic media (*61*).

Potato tubers exhibited wide variation in their antioxidant activity. Hale et al. (*62*) reported a range from 104 to 565 µg trolox equivalents/gfw for 67 advanced selections and 24 named varieties using the DPPH assay. Campos et al. (*51*) used the ABTS assay to measure antioxidant activity and reported a range from 483 to 9800 µg

trolox equivalents/gfw. Functional compounds responsible for this antioxidant activity in potatoes are phenolic acids, flavonoids, anthocyanins, ascorbic acid, carotenoids, tocopherols, and α – lipoic acid (*13*). ORAC values for anthocyanins ranged from 7.6 to 14.2 µmole trolox equivalents/gfw (*63*). Caffeic acid and chlorogenic acid showed three times lesser oxidation potential than the highest oxidation potential of phenolic acids such as 4-hydroxybenzoic acid. Kahkonen and Heionan (*64*) reported that, among anthocyanidins, malvidin is the most potent antioxidant. Cyanidin showed three times more antioxidant potential than pelargonidin (*65*). Several reports suggested strong positive correlation between total phenolics and/or anthocyanins and antioxidant capacity in potatoes (*23, 63, 66*). Red-fleshed and purple-fleshed varieties showed 2.5 to three times higher antioxidant activity compare to white-fleshed varieties (*67*) because of the presence of acylated anthocyanins along with phenolic acids. In addition to antioxidant activity, polyphenols also exhibit anti-platelet, anti-inflamatory, anti-microbial, anti-viral, anti-neoplastic and anti-carcinogenic activities. In this review, the discussion will center on the anti-carcinogenic role of phenolics in prostate caricinogenesis.

Prostate Carcinogenesis

The prostate is a sex-accessory gland at the base of the bladder around the urethra, and consists of epithelial and stromal cells (*68*). The prostate is divided into three regions based on their position in relation to the urethra, namely transition zone, central zone and peripheral zone, with 5-10 %, 20 %, and ≈ 70 % of glandular tissue, respectively (*69*). Androgen receptor (AR) signaling, cell proliferation and cell death

play a critical role in maintaining the health of the prostate. Androgen plays a critical role in regulating the growth and differentiation of epithelial cells in the normal prostate.

Prostate cancer is the third leading cause of cancer deaths in men in the US, and it is estimated that there will be 234,460 new cases and 27,350 additional deaths in 2006 due to prostate cancer. Prostate cancer incidence is influenced both by genetic and non-genetic factors. The risk attributed to genetic factors is sometimes as high as 43%. However, only 9% of these cases are directly linked to family history. Genetic factors are important at younger ages (< 55 years). Non-genetic factors include age, race, ethnicity, environment, geographical location, birthplace, diet and lifestyle (*70*). Aging increases the risk of prostate cancer development. Studies (*71*) have shown that more than 70 % of men over 85 years of age have prostate adenocarcinoma occurrence. African American men are more prone to prostate cancer incidence than men of Asian ancestry (*72*). Migration studies adjusted for age have shown that Australian born individuals had higher risk for prostate cancer death than migrants from the British Isles and Southern Europe (*73*). Another study by Shimizu (*74*) showed no difference in prostate cancer incidence among Japanese migrants and Spanish migrants in the US and US born individuals. However, the prostate cancer risk was higher in Japanese and Spanish immigrants in the US than those in the two homelands for each racial and ethnic group. Incidence of prostate cancer is high in western countries because of high animal fat intake. A 60-70 % decrease in prostate, colorectal and breast cancers and 40 -50 % reduction in lung cancer can be achieved by following the diet guidelines of the American Institute for Cancer Research (*75, 76*). Asian men who migrated to the US and

adapted a western diet showed similar risk of developing prostate cancer as US-born men. Diet and related factors are modifiable risk factors (*75, 77, 78*).

Prostate carcinogenesis is a multi-step process involving initiation, promotion and progression. Initiation is a rapid and irreversible process involving carcinogen exposure, uptake, distribution and transport to organs and interaction with DNA and genotoxic damage. Promotion is a relatively lengthy and reversible process, and progression is the final stage (*79*). Prostate cancer initially develops as a high-grade intraepithelial neoplasia (HGPIN) in the peripheral and transition zones and promotes to a small latent carcinoma, which may subsequently progress to a large, higher grade, metastasizing carcinoma (*75, 80, 81*). Promotion and progression stages are controlled by signal transduction molecules which are triggered by hormones such as androgens (*75, 82, 83*). The terms androgen-dependent and androgen-independent are widely used to classify prostate cancer (*69, 84-87*). In androgen-dependent prostate cancers, the cells depend on androgens for their growth and survival and can be treated by either blocking the androgen pathway or using anti-androgens. Androgen ablation inhibits AR initially and reduces prostate specific antigen (PSA). The reoccurrence of prostate cancer in later stages is normally resistant to hormonal alterations, and this type is known as androgen-independent, androgen-refractory or androgen depletion-independent (*88*). This may be due to the activation of AR in presence of weak concentrations of androgens due to mutations in AR or increase in co-activator expression. Though, androgen-independent tumors are resistant to androgen ablation, AR still plays a critical role in growth and survival of these tumors. Several reports (*69, 85-87*) showed that inhibition of AR

decreased PSA expression in androgen-independent, cell-based models. Essential processes in progression of prostate cancer are inappropriate androgen receptor signaling, uncontrolled proliferation of prostatic epithelial cells, and avoidance of apoptosis (*70, 89*).

Anrogen-receptor

The androgen receptor (AR) is a phospho protein belonging to the nuclear receptor transcription factor super family (*90*). Like regular transcription factors, AR contains transactivation domain, DNA binding domain, and ligand binding activation domain (*91*). AR plays a key role in prostate morphology, metabolism, secretion, tissue differentiation, cell proliferation and survival through androgens. Testosterone accounts for 90 % of the androgens in circulation and is produced by the leidig cells in the prostate. To activate AR, testosterone can directly bind to AR or convert to dihydro-testosterone (DHT) by 5α-reductase before binding to AR. The receptor confirmation induced by DHT is more resistant to degradation than by testosterone. After binding to androgens, AR can either act as transcription factor (genomic effects) or as a cytoplasmic signaling molecule (non-genomic effects) **(Figure 1.7)** (*69*). AR as transcription factor translocates into the nucleus and binds to androgen responsive elements (AREs) as a dimer and activates the target genes involved in cell proliferation, differentiation and apoptosis. As a cytoplasmic signaling molecule, AR results in activation of Src and MAPK pathways.

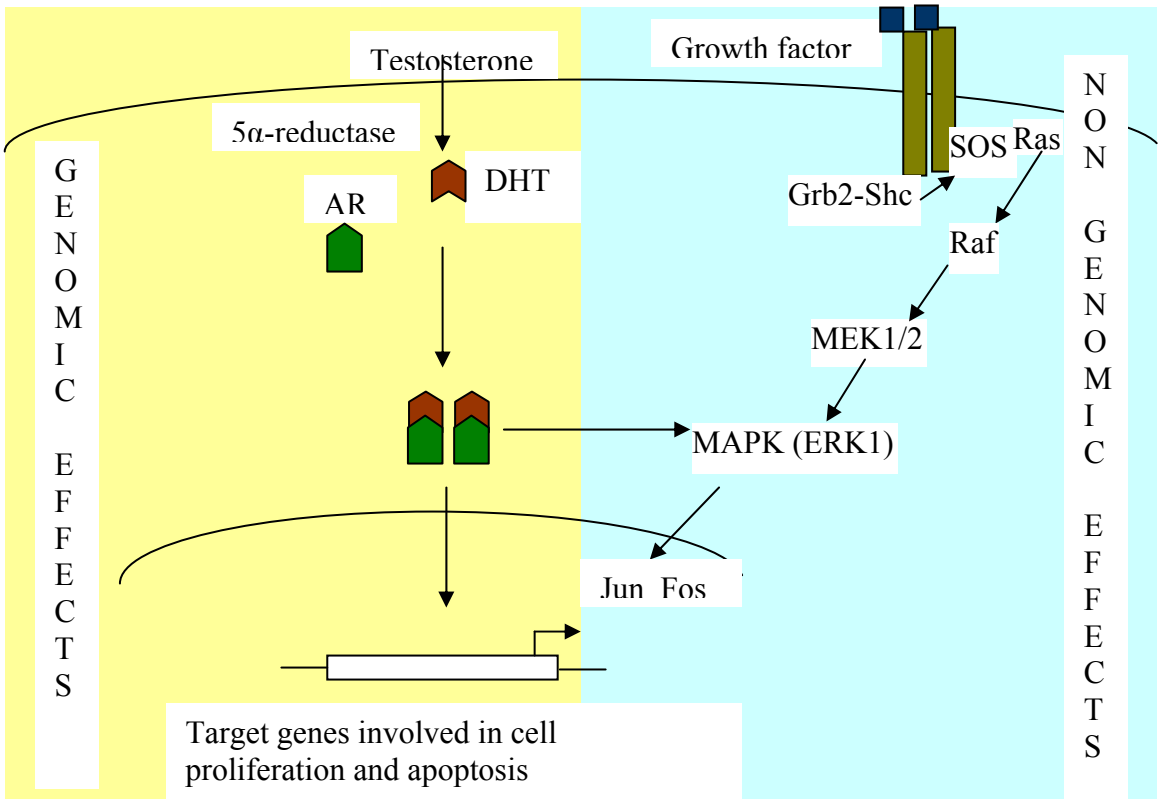

Figure 1.7. Genomic and non genomic effects of androgen receptor (92).

Cell Proliferation and Cell Cycle Regulation

In normal prostate epithelial cells, proliferation is balanced by programmed cell death to maintain a healthy prostate. In prostate cancer progression, the cells gain a proliferative advantage resulting in excessive growth (*93, 94*). Cell proliferation can be measured in vivo by tumor volume and in vitro by using colorimetric assays, where the tetrazolium salt is converted to formazan by living cells (*95-99*). The number of cells in vitro can be counted using haemocytometer or coulter counter.

The cell cycle is regulated at two check points (G1–S and G2-M phases) in normal cells. Most of the human cancers are characterized by genetic alterations in control of the G1-S phase progression in cell cycle. This check point is controlled by a sequence of cell cycle regulatory cyclins such as cyclin-dependent kinases (CDK) and CDK inhibitors. Recent studies have identified many types of CDKs (CDK1, CDK2, CDK4, and CDK6) and cyclins including cyclin A, cyclin B, cyclin D (CD1, CD2, CD3) and cyclin E. However, over expression of cyclin D1 is the most common aberration in many cancers. Mutations which deregulate CDKs are present in many of the prostate cancers (*100*). Research also suggests that, in prostate cancer, p27 over expression can sometimes be coupled with decreased CD1 and cyclin E levels, which is a cyclin dependent kinase inhibitor. This reduction in p27 levels might be through transcriptional regulation involving ubiquitin-mediated degradation (*101-106*). The p27 degradation mechanism is turned on in different types of cancers, including prostate cancer (*101, 107*). Kibel et al. (*108*) identified homozygous deletion at 12p12-12p13.1 (position of gene coding for p27) in 47 % of patients dying from prostate adenocarcinoma. In vitro

studies have shown that the over expression of p27 results in reduced cell proliferation (*109*).

Mitogen activated Protein Kinase (MAPK) signaling pathways also play a critical role in both cell proliferation and apoptosis. Extracellular signal-regulated kinase (Erk), the c-Jun N-terminal Kinase (JNK), and the p38 MAPK are the three sub groups of MAPKs in mammalian cells (*110*). MAPK signaling pathway is a three step cascade, whereas in the first step a MAP3K activates a MAP2K and in turn MAP2K activates MAPK. Erk pathway is activated by growth factors and JNK by a variety of environmental stressors. These kinases can induce both survival and apoptotic responses in cells depending on cell type and environment (*111, 112*).

Mutations involving conversion of Ras into an active oncogene are common in many cancers. Active oncogenic Ras activate ERK pathways constantly, contributing to the increased proliferation (*113*). It also promotes cell survival by up-regulating anti-apoptotic proteins. Anti-apoptotic effects of Erk are due to down regulation of pro-apoptotic proteins by decreasing their activity or reducing the expression of protein through transcriptional repression (*111*). In neuroral cells Erk inactivates BAD, a pro-apoptotic protein by phosphorylating at Ser112, and increases the stability and activity of Mcl-1, an anti-apoptotic protein by phosphorylating at Thr163. Erk can also exhibit pro-apoptotic activity. In U937 (human leukemia cells) Erk activated by EGF causes tis21 phosphorylation, inducing tis21 and Pin-1 interaction, mitochondrial depolarization, and apoptosis (*114*). Suppression of Erk activity decreses cispatin-, isothiocyanates- and hydroxycinnamaldehyde-induced Bax expression, cytochrome *c* release, caspase-3

activation and apoptosis (*111, 115*). In ovarian cancer cells phosphorylation and accumulation of p53 induced by cisplatin was blocked by erk suppression (*111, 116*).

JNK, also known as stress-activated protein kinase controls cell growth, differentiation, or apoptosis. JNK activation causes apoptosis in response to UV-iradiation, toxins, anticancer drugs, heat shock, ceramide, or inflammatory cytokines such as TNFα (*112*). JNK phosphorylates and increases activity of c-jun, a component of AP-1 transcription complex, which controls many cytokine genes (*113*). In UV – irradiated mouse fibroblasts JNK induced apoptosis through repression of p53-mediated p21 induction (*117*). The proapoptotic effects of JNK in mouse embryo fibroblasts included inactivation of anti-apoptotic protein Bcl2 by phosphorylation, and proteolytic activation of pro-apoptotic Bid, which releases cytochrome *c* from mitochondria and causes activation of caspases-9 and -3 (*118*). In Hepatoma cells (HepG2) stress-induced JNK activated Bax, a proapoptotic protein by phosphorylating at Thr167 to induce apoptosis (**Figure 1.8)** (*119*).

Apoptosis

Apoptosis or programmed cell death is a conserved and controlled mechanism through which cells are eliminated in both healthy and diseased tissues in an organized manner. Apoptosis results in morphological and biochemical changes in cells. Morphological changes include shrinkage of cells, development of blebs, and condensation chromatin. Biochemical changes include a phosphatidyl serine flip flop from the inner to the outer membrane, and DNA fragmentation (*120*). Major apoptotic regulators are p53 and Bcl-2 family members (*109*). P53 regulates genes involved in cell

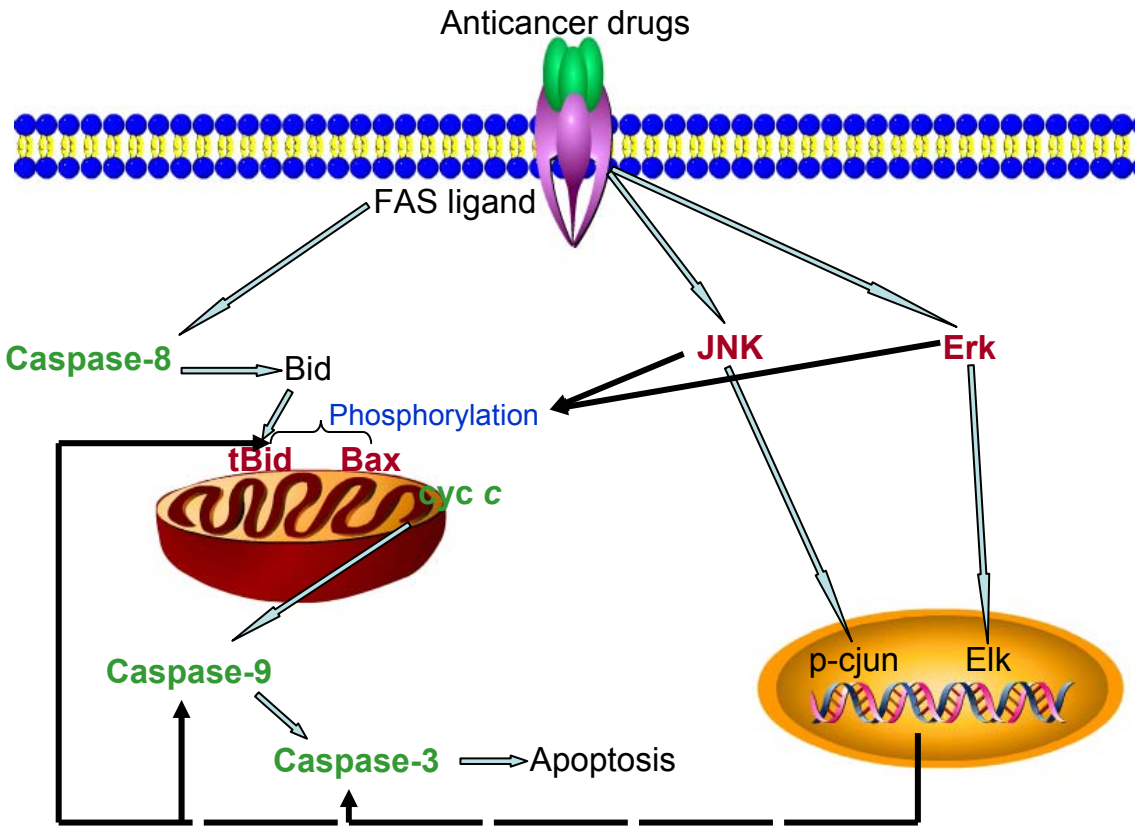

Figure 1.8. Pro-apoptotic mechanism of JNK and ERK, activated by anticancerous drugs.

proliferation, apoptosis and angiogenesis. Bcl-2 family members can act either as anti-apoptotic (Bcl-2, Bcl-xL) or proapoptotic (Bax, Bak, Bad and Bcl-xS) agents. Genetic alterations within the cell are initiated by the activation of anti-apoptotic genes, inactivation of tumor suppressor genes, or both. The p53 tumor suppressor genes are mutated in advanced stages of prostate carcinomas (*121*).

Apoptosis pathways can be initiated through different entry sites. Initiation can be at the plasma membrane by death receptor ligation (transmembrane or Fas-ligand dependent pathway) or at the mitochondria (mitochondrial or Fas-ligand independent pathway) (*122-126*). The transmembrane pathway of apoptosis involves the tumor necrosis factor (TNF) ligand and receptor superfamily members (TNFα, Fas ligand and TNF-related apoptosis-inducing ligand; TRAIL). Ligand receptor interactions trigger a number of intracellular events leading to apoptotic cell death. Finally these pathways activate caspases (*126-128*). Mitochondrial pathways involve release of cytochrome C followed by increased membrane permeability controlled by Bcl-2 family members. These pathways can be caspase-dependent or caspase-independent. In caspase-dependent pathways cytochrome c release triggers the formation of apoptosome which in turn activates caspase-9 and caspase-3. Activated caspases trigger the down stream reaction involved in apoptosis. Caspase independent pathway involves activation of AIF (apoptosis inducing factor) or endo G (endonuclease G) through translocation from mitochondria to nucleus (*126, 129*).

Phenolics in Chemoprevention

Epidemiological data suggest that consumption of fruits and vegetables can offer protection against several kinds of cancers, including prostate cancer, because of the presence of chemopreventive compounds. Chemoprevention is not only less toxic compared to other treatments, but also increases the effectiveness of the other treatments (*100*). Chemopreventive agents are classified conventionally into blocking and suppressing agents. Blocking agents block the carcinogen from entering the target points, undergoing activation, and interacting with DNA, RNA and proteins. Suppressing agents suppress the initiated cell transformation to the promotion and progression stages (*79*).

Phenolic Acids

Both in vitro and in vivo studies showed the anticarcinogenic activity of phenolic acids (*130-132*). In F344 rats, phenolic acids (chlorogenic acid 250 ppm, caffeic acid 500 ppm and ferulic acid 500ppm) significantly inhibited the development of preneoplastic tongue lesions, and the number of tongue neoplasms (*133*). Ferulic acid also reduced the azoxymethane-induced aberrant crypt foci of the colon in F344 male rats (*134*). Chlorogenic acid (250-500 ppm) reduced the number of tumors in both colon and stomach at the initiation and post initiation stages in F344 rats (*135, 136*). Ferulic and chlorogenic acid (100 mg/Kg) decreased lung tumors in A/J mice by 30 to 40 % (*137*). In vitro studies reported that caffeic acid increased the sensitivity of tumor cells to chemotherapeutic agents, for example, it reduced the IC50 value of doxorubicin by approximately ten times in MCF-7/Dox cells (*138*). Caffeic and ferulic acids have shown

to be both carcinogenic and anticarcinogenic, depending on the type of cancer and the concentration of the compound used. Long term intake of caffeic acid at 0.4 % for 2 years caused a 14 % increase in stomach papilomas. Ferulic acid was anticarcinogenic to the tongue, skin and colon and carcinogenic to the lung (*137*).

Inhibition of carcinogen uptake, formation and activation, prevention carcinogen binding to DNA, removal of free radicals, activation of detoxifying enzymes, inhibition of cell proliferation and metastasis, and induction of apoptosis are the possible mechanisms of penolic acids in chemoprevention. Ferulic acid increased glutathione-s-transferase and quinine reductase activities, and in turn resulted in the induction of detoxifying enzymes (*134, 137*). Because of their antioxidant properties, phenolic acids act as potential free radical scavengers (*139*). Gallic acid from grape seed extract caused growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. Apoptotic cell death involved activation of caspase-3 and caspase-9, and cleavage of PARP (*140*). Caffeic acid activated proapoptotic proteins such as caspases, Bax and Fas in MCF-7 cells in induction of apoptosis (*141*). C6 glioma cells showed cytochrome C release from mitochondria into the cytosol and increased levels of p53, Bax and Bak upon treatment with caffeic acid phenethyl ester (CAPE) (*137*).

Anthocyanins

Anthocyanins showed inhibitory effects on colon carcinogenesis in F-344 rats (*142*). Proanthocyanidin grape seed extract resulted in a dose dependent decrease in the development of aberrant crypts in female Sprague-Dawley rats. Grape seed extracts and berry induced cytotoxicity in lung and breast cancer cells (*143*). Anthocyanins and their

aglycones such as cyanidin, delphinidin, malvidin, pelargonidin and peonidin exhibited antiproliferative and proapototic properties in gastric adenocarcinoma (*144*), HT-29 and Caco-2 (colon cancer) (*145*) and BAEC bovine aortic endothelial cells (*146*), and also protective effects against esophageal cancer in rodents (*147*). Cyanidin-3-glucoside reduced the size of A549 tumor xenograft growth and significantly inhibited metastasis in nude mice (*148*).

The chemopreventive mechanisms of anthocyanins include scavenging free radicals, reducing cell proliferation, up-regulating/inducing apoptosis and modulating mitogen activated protein kinase activities (*146*). Peonidin 3-glucoside and cyanidin 3-glucoside from black rice showed marked inhibition on the invasion and motility of SKHep-1 cells through reduced expression of matrix metalloproteinase (MMP)-9 and urokinase-type plasminogen activator (u-PA). These two compounds also exerted an inhibitory effect on the DNA binding activity and the nuclear translocation of AP-1 (*149*). In F344, black raspberries down regulated the growth of premalignant esophageal cells, through down-regulation of cyclooxygenase-2 (*147*). Cyanidin-3-glucoside (C3G), was able to scavenge ultraviolet B-induced *OH and O_2^{-}* radicals in cultured JB6 cells. C3G pretreatment inhibited UVB- and TPA-induced NF-kappaB and AP-1 activation and cyclooxygenase-2 and tumor necrosis factor-alpha expression through regulation of MAPK activity in JB6 cells. These inhibitory effects appear to be mediated through the inhibition of MAPK activity.

Delphinidin caused apoptosis in HL-60 leukemia cells in a time and dose dependent manner through caspase activation. Delphinidin also caused JNK

phosphorylation and c-jun expression, and this phosphorylation was reversed by N-acetyl cysteine (*150*). Similar results were observed with malvidin in the same cell line by Chang et al. (*151*).

Objective

The objective of this study was to screen specialty potato cultivars for antioxidant activity, phenolic and carotenoid content and composition, and to evaluate their antiproliferative and proapoptotic activities, in order to develop varieties with higher content of these beneficial compounds.

Specific Aims

- Evaluate antioxidant activity (AOA), total phenolic content (TP), and carotenoid (TC) content of specialty potato selections in order to identify potential varieties and elite parents for use in the Texas Potato Variety Development Program.
- Identify and quantify important phenolics and carotenoids present in specialty potatoes.
- Investigate the influence of year and location on antioxidant activity, total phenolic content, carotenoid content and phenolic composition.
- Evaluate the effect of specialty potato extracts on cell proliferation and apoptosis of LNCap and PC-3 cells.
- Investigate the mechanism of action of potato extract and fractions in apoptosis

CHAPTER II

DETERMINATION OF PHENOLIC CONTENT, COMPOSITION AND THEIR CONTRIBUTION TO ANTIOXIDANT ACTIVITY IN SPECIALTY POTATO SELECTIONS

Introduction

In recent years, much emphasis has been placed on functional properties of various fruits and vegetables and their contribution to human health. A variety of phytochemicals, e.g., phenolics, carotenoids and flavonoids, have been shown to exhibit functional properties such as antimicrobial, antimutagenic, and free radical scavenging (*8*) etc. Free radicals induce oxidative stress, which may result in DNA, protein, and lipid damage, leading to the incidence of chronic illnesses including cancer, cardiovascular diseases, and inflammation (*152*). Phenolic compounds can suppress free radical-induced oxidative stress and reduce the onset of these chronic illnesses and synergistic effects of beneficial compounds in whole foods provide better protection against diseases than single-nutrient supplements (*89*).

Potato (*Solanum tuberosum* L), a whole food, also contains significant levels of vitamins (*153*) and important antioxidants (*35, 154*), including phenolic acids, carotenoids, and flavonoids (*155, 156*). Potato is the fourth most important food crop worldwide and is the leading vegetable crop in the US, with a per capita consumption of about 135 pounds (*157*). Earlier studies on potato phenolics in our lab indicated the presence of chlorogenic acid, caffeic acid, vanillic acid, p-coumaric acid, gallic acid and rutin (*62, 154*). Freeze dried methanol or ethanol extracts of potato peel revealed that

gallic acid was the most abundant phenolic compound, followed by caffeic acid, chlorogenic acid, protocatechuic acid and vanillin (*17*). According to Kanatt et al. (*158*), the major peaks identified in potato peel extract were chlorogenic acid, caffeic acid, and gallic acid. *Solanum* species exhibit variation in antioxidant levels, and species with intense skin and flesh colors tend to have higher antioxidant activity than those with white flesh (*62*), making it necessary to screen the available selections for their antioxidant activity (AOA) and total phenolic content (TP) and to investigate the effect of selection on AOA and TP.

Therefore, the objectives of this study were to evaluate the effect of genotype on AOA, TP and phenolic composition (PC) of specialty selections from the Texas Potato Variety Development Program, and to investigate the contribution of individual phenols to antioxidant activity in order to identify elite parental lines high in these compounds to use in breeding programs emphasizing human health benefits. The present study was conducted as a part of our ongoing efforts to investigate the health benefiting compounds in potato.

Materials and Methods

Materials

Specialty potato selections (n = 25) with a wide range of skin and flesh colors were grown near McCook, TX, located near the Mexican border. Tubers from each selection were harvested and bulked. Samples were transported at ambient temperature (25^{o}C) from McCook to Texas A&M University, College Station for further analysis. Tubers were washed, and uniformly sized (weight ~110 to 120g) tubers were used in the

study. DPPH, ABTS and Folin-Ciocalteu reagents were purchased from Fisher Scientific (Pittsburgh, PA). Solvents for HPLC were purchased from VWR International (Bristol, CT). Phenolic acid standards were from Sigma (St. Louis, MO) and malvidin was from Indofine Chemical Company (Hillsborough, NJ).

Sample Preparation

Uniformly sized tubers (n = 6 per selection) were diced into 0.5 cm cubes, and three representative samples, each from two tubers, were collected for each selection and stored at –80^{o}C until analysis. Tuber samples (5 g) were homogenized with ethanol (15 mL) using an Ultra Turrax Tissumizer T25 (Cincinnati, OH) (17,500 rpm), and for convenience were stored for 12 - 15 h at –20^{o}C since no differences in readings were found before and after storage. The samples were then centrifuged at 31,000 g for 20 min, and the supernatant was collected for analysis of AOA, TP, and PC.

Antioxidant Activity

Antioxidant activity was measured using two assays, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) assay. Intense sample color interferes with the DPPH assay readings, therefore to confirm the AOA results, the samples were also analyzed using the ABTS assay.

DPPH Assay

Antioxidant activity of tuber extracts was estimated using the DPPH (*159*). DPPH is a stable free radical, which on reaction with an anti-oxidant reduces from a violet color to the yellow colored diphenylpicrylhydrazine. The reduction in absorbance

is an indication of the antioxidant activity of a sample. DPPH stock solution was prepared fresh by dissolving 24 mg of DPPH in 100 mL ethanol. The stock solution was diluted until the spectral reading at 515 nm was 1.1. Clear supernatant solution (150 µL), collected as described above was allowed to react with 2850 µL of diluted DPPH solution for 24 h at ambient temperature (25^{o}C). The resulting absorbance was measured at 515 nm using a spectrophotometer. The blank or negative control contained all the reaction reagents except the sample. Trolox (6-Hydroxy-2, 3, 7, 8-tetramethyl chroman-2-carboxylic acid) was used as positive control. The results were expressed as micrograms of trolox equivalents per gram fresh weight (µg TE/gfw). Percent inhibition of DPPH activity was calculated using the following equation

$$\text{Percent DPPH scavenging activity} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

ABTS Scavenging Activity

Antioxidant activity was also evaluated using the ABTS radical cation decolorization assay (*160, 161*). The extent of reduction in absorption maxima with antioxidants was measured at 734 nm. The $ABTS^{\cdot+}$ radical solution was prepared by mixing equal volumes of ABTS (8 mM) and $K_2S_2O_8$ (3 mM) and incubating in the dark for 16 h at room temperature. The working solution was prepared by adding 5 mL of $ABTS^{\cdot+}$ radical solution to 145 mL of phosphate buffer (0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, 150 mM NaCl with pH 7.4). The working solution (2900 µL) was added to the sample solution (150 µL), and absorbance was measured after 30 min and expressed as µg TE/gfw.

Total Phenolic Content

The total phenolic content was measured spectrometrically using the Folin-Ciocalteu colorimetric method (*162*). Clear supernatant (150 µL) prepared as described for the sample preparation was diluted with 2400 µL of nanopure water. The sample was allowed to react with 150 µL of 0.25N Folin-Ciocalteu reagent for 3 min, and then 300 µLof 1N Na_2CO_3 was added to this reaction mixture. The absorbance was recorded at 725 nm after 2 h of reaction at room temperature in the dark. Results were expressed as milligrams of chlorogenic acid equivalents (CGA) per 100 gfw.

Phenolic Composition (PC)

The ethanol extract (7 mL) prepared in the sample preparation was evaporated to dryness under vacuum at 45^{o}C and reconstituted with 1.0 mL of ethanol. The filtered (0.45 µm nylon) samples (10 µL) were injected into a high performance liquid chromatography (HPLC) system equipped with a binary pump (Waters 515), using an auto sampler (Waters 717 plus). Separation of polyphenols was done using an Atlantis C-18 reverse-phase column (4.6 x 150 mm, and 5 µm) with a guard-column at 40^{o}C. The mobile phase consisted of a binary solvent system with a flow rate of 1 mL/min. Initial conditions were 85% nanopure water (pH adjusted to 2.3): 15% acetonitrile (ACN) and were maintained for 5 min. Between 5 and 35 minutes, a linear gradient was used to increase ACN from 15% to 100%. The total polyphenols were detected at 220, 320 and 515 nm. Peaks were identified both by spiking and matching the spectra and retention times with the standards.

Identification of malvidin-3-(p-coumaryl rutinoside)–5-galactoside was accomplished by using a Time of Flight LC-MS with an ESI + ve ion mode (PE Sciex Qstar Pulsar) and a matrix-assisted laser desorption/ionization LC-MS (MALDI-MS Voyager-DE STR) with an Atlantis C-18 reverse column. Solvents used were water with 0.1% formic acid and acetonitrile with 0.1% formic acid.

Statistical Analysis

Data are presented as the means of three replications. They were subjected to one-way analysis of variance for means comparison, and a least square difference was used to calculate significant differences between means. Pearson's correlation analysis was performed for antioxidant activity versus total phenolic content using SAS (*163*). Differences at $p < 0.05$ were considered statistically significant.

Results and Discussion

Antioxidant Activity

The DPPH assay has been widely used in assessing the free radical scavenging capacity of natural compounds (*158*). Specialty potato selections showed free radical scavenging activity against DPPH. A wide range of variation in percent DPPH scavenging activity was observed among the 25 potato selections analyzed in this study. The purple flesh selection CO112F2-2P/P had the highest ($p = 0.001$) DPPH scavenging capacity (95.7%) followed by CO111F2-2P/P. The purple skin/yellow flesh selection COTX00104-2P/Y had the lowest scavenging capacity (17.03%). Antioxidant activity ranged from 157 (COTX00104-2P/Y) to 832 (CO112F2-2P/P) μg trolox eq/gfw

Table 2.1. Skin and flesh color, antioxidant activity (AOA) of specialty potato selections measured by the DPPH and ABTS assays

ID No.	Selection	Skin color	Flesh color	AOA	
				DPPH*	ABTS*
				(µg trolox eq/gfw)	
1	ATTX98013-1	red	red	471 ± 8	1256 ± 47
2	ATTX96753-3	yellowish red	yellow	192 ± 7	1050 ± 39
3	ATTX96753-4	yellowish red	yellow	273 ± 21	1114 ± 16
4	ATTX98012-5	red	yellow	268 ± 28	872 ± 40
5	ATTX98444-4	yellow	yellow	184 ± 31	810 ± 6
6	ATTX98462-3	reddish yellow	reddish yellow	366 ± 24	1023 ± 27
7	ATTX98492-1	yellow	yellow	215 ± 34	944 ± 44
8	ATTX98500-2	purple	yellow	398 ± 29	1251 ± 17
9	ATTX98500-4	purple	yellow	260 ± 32	979 ± 73
10	ATTX99295-2	yellowish red	yellow	275 ± 3	941 ± 38
11	ATTX98491-3	yellowish red	yellow	341 ± 34	1055 ± 13
12	ATTX99325-1	red	yellow	241 ± 18	972 ± 21
13	ATX99324-1	purple	yellow	247 ± 12	1021 ± 18
14	CO111F2-1	purple	purple	683 ± 17	1462 ± 31
15	CO112F2-2	purple	purple	832 ± 10	1622 ± 38
16	CO141F2-1	purple	purple	446 ± 25	1190 ± 58
17	CO142F2-1	red	red	317 ± 25	946 ± 27
18	COTX00104-2	purple	yellow	157 ± 2	827 ± 17
19	COTX00104-5	red	yellow	300 ± 31	888 ± 42
20	COTX99086-4	yellow with red eyes	yellow	292 ± 37	930 ± 28
21	COTX99338-1	yellow with red eyes	yellow	378 ± 15	1003 ± 11
22	NDTX4528-3	purple	purplish white	554 ± 26	1377 ± 31
23	NDTX4528-4	purple	purplish white	314 ± 12	1024 ± 22
24	NDTX4528-4B	purple	purplish white	418 ± 28	983 ± 19
25	PATX99P32-2	reddish yellow	yellowish red	363 ± 28	933 ± 50

***** Each value is a mean of three replications ± SE

by the DPPH assay and 810 (ATTX98444-4Y/Y) to 1622 (CO112F2-2P/P) µg trolox eq/gfw using the ABTS assay (**Table 2.1**).

Significant positive correlations (r = 0.90, p = 0.05) were observed between AOA measurements using the DPPH and ABTS methods. Leong and Shui (*164*) also reported significant positive correlations of AOA measured by these two methods. Antioxidant activity values measured by ABTS were greater than those measured by DPPH. This may be due to differences in the absorption maxima of the two radicals. The DPPH maximum absorption wavelength (515 nm) is in the visible region, and the interference because of sample color is much more pronounced in this region as compared to the ABTS maximum absorption wavelength (725 nm), which is not in the visible region (*61*). However, consistency in relative ranking is probably more important than consistency in absolute numerical scores. To determine the variation in antioxidant activity due to color, selections were grouped into yellow/yellow (Y/Y), reddishyellow/yellow (RY/Y), red/red (R/R), purple/yellow (P/Y) and purple/purple (P/P) categories, depending on the intensity of skin and flesh color. Selections ATTX98444-4, ATTX98492-1, COTX99086-4 and COTX99338-1 were grouped under Y/Y. The R/R group consisted of CO142F2-1, ATTX98013-1 and ATTX98462-3. Group P/Y included ATX99324-1, COTX00104-2, ATTX98500-2 and ATTX98500-4. Selections CO141F2-1, CO111F2-1, CO112F2-2, NDTX4528-3, NDTX4528-4 and NDTX4528-4B were included in the P/P group. Remaining selections were under RY/Y. The average AOA values for each group are presented in **Figure 2.1**. With both assays,

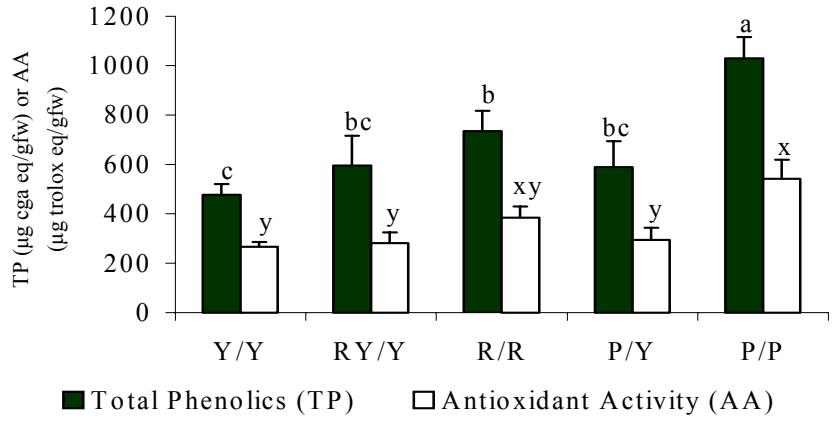

Figure 2.1. Total phenolic content (TP) and antioxidant activity (AOA) of specialty potatoes. Skin/flesh designations are represented by Y/Y, RY/Y, R/R, P/Y, P/P, where Y: yellow, RY: red with yellow, R: red, P: purple. Each value is an average of the AOA or TP for all selections with common skin and flesh color. The vertical bars represent the standard deviation. Different letters on the bars for TP and AOA represent significant differences among selections ($P < 0.01$).

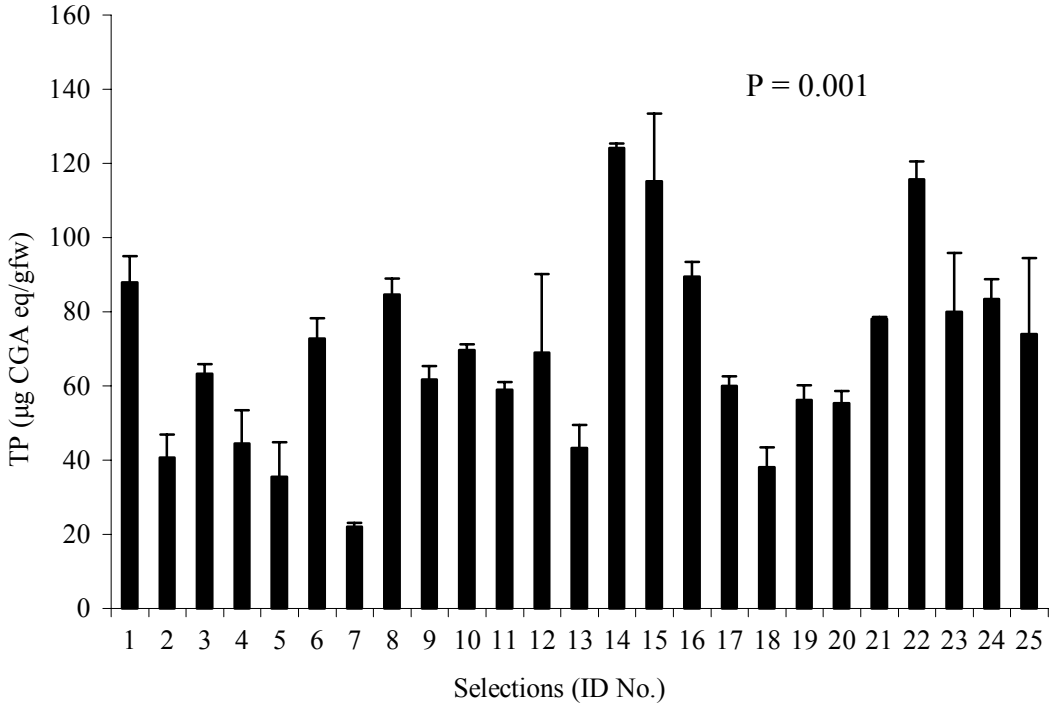

Figure 2.2. Total phenolics of specialty selections. Values shown are the means of three replicates in µg CGA eq/gfw (micrograms of chlorogenic equivalents per gram fresh weight). Vertical bars represent standard deviation. Refer to Table 2.1 for numbers corresponding to each selection.

purple skin/purple flesh varieties showed significantly higher AOA, followed by red skin/red flesh varieties. Selections with similar flesh color did not show significant variation in antioxidant activity, irrespective of differences in skin color. This may be because potato skin accounts for only 7 – 10% of the total weight of tubers, depending on the selection.

Total Phenolics

Phenolics are one of the major groups of compounds which act as free radical scavengers. The amount of total phenolics varied widely among the specialty selections and ranged from 221 µg chlorogenic acid (CGA) eq/gfw (ATTX98492-1Y/Y) to 1252 (CO112F2-2P/P) µg CGA eq/gfw. (**Figure 2.2**). Selections with similar flesh colors were not significantly different in their TP.

Selections with purple skin and purple flesh had the highest phenolic content followed by red skin/red flesh, purple skin/yellow flesh, red skin/yellow flesh and yellow skin/yellow flesh (**Figure 2.1**). A significant positive correlation was observed between TP and AOA against the DPPH radical ($r = 0.93$, $p < 0.001$) (**Figure 2.3**) and TP and AOA against the ABTS radical ($r = 0.90$, $p < 0.001$) for these selections. Scalzo et al. (*165*) also observed similar results in peaches.

Phenolic Composition

The qualitative and quantitative analysis of ethanol extracts of specialty potatoes were done using high performance liquid chromatography coupled with PDA detection. (**Table 2**.**2**). Peaks were identified by comparing both their retention times and UV

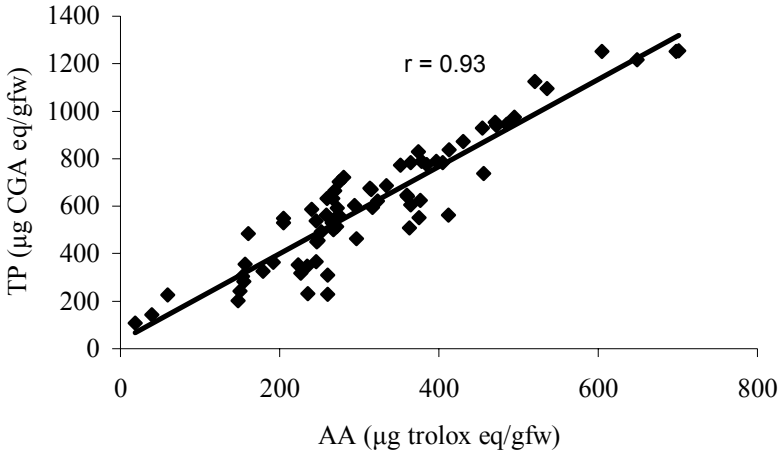

Figure 2.3. Pearson's correlation coefficient for the values of antioxidant activity (AOA) and total phenolic content (TP)**.** AOA was expressed as trolox equivalents and TP was expressed as chlorogenic acid equivalents.

Table 2.2. Composition of phenolics in different specialty potato selections.(CGA: Chlorogenic acid, CA: Caffeic acid, GA: Gallic acid, CAT: Catechin)

ID No.	Selection	(µg/gfw)*			
		CGA	CA	GA	CAT
1	ATTX98013-1	261 ± 24	35 ± 0.5	87 ± 0.4	87 ± 1.0
2	ATTX96753-3	270 ± 13	34 ± 0.3	24 ± 10	85 ± 0.2
3	ATTX96753-4	161 ± 7	35 ± 0.4	37 ± 6	85 ± 0.3
4	ATTX98012-5	198 ± 17	35 ± 0.3	42 ± 0.1	86 ± 0.4
5	ATTX98444-4	328 ± 4	34 ± 0.1	58 ± 9	85 ± 0.1
6	ATTX98462-3	366 ± 34	33 ± 0.1	37 ± 3	85 ± 0.9
7	ATTX98492-1	215 ± 36	35 ± 0.7	36 ± 13	85 ± 0.5
8	ATTX98500-2	257 ± 23	36 ± 0.2	74 ± 6	87 ± 0.1
9	ATTX98500-4	218 ± 57	37 ± 1.3	55 ± 13	88 ± 1.8
10	ATTX99295-2	211 ± 43	35 ± 0.8	29 ± 19	88 ± 0.1
11	ATTX98491-3	215 ± 40	33 ± 0.8	38 ± 2	84 ± 0.7
12	ATTX99325-1	297 ± 42	35 ± 0.3	15 ± 1	85 ± 0.3
13	ATX99324-1	138 ± 8	37 ± 1.6	28 ± 16	86 ± 1.2
14	CO111F2-1	369 ± 43	40 ± 0.7	81 ± 9	91 ± 1.2
15	CO112F2-2	548 ± 10	42 ± 0.5	84 ± 3	91 ± 0.5
16	CO141F2-1	174 ± 29	40 ± 1.5	75 ± 4	87 ± 0.4
17	CO142F2-1	203 ± 35	36 ± 0.4	76 ± 3	87 ± 1.0
18	COTX00104-2	181 ± 3	34 ± 0.6	40 ± 13	84 ± 0.5
19	COTX00104-5	230 ± 21	35 ± 0.3	38 ± 8	84 ± 0.3
20	COTX99086-4	240 ± 20	34 ± 0.1	49 ± 3	85 ± 0.7
21	COTX99338-1	192 ± 6	38 ± 0.3	21 ± 5	86 ± 0.3
22	NDTX4528-3	262 ± 36	36 ± 0.9	73 ± 5	87 ± 1.4
23	NDTX4528-4	399 ± 10	41 ± 0.6	59 ± 3	87 ± 1.6
24	NDTX4528-4B	426 ± 26	37 ± 0.5	79 ± 6	91 ± 0.5
25	PATX99P32-2	519 ± 40	35 ± 0.1	87 ± 8	88 ± 0.3

***** Each value is a mean of three replications ± SE

spectra with that of pure standards and quantified using calibration curves. Chlorogenic acid, gallic acid, catechin, caffeic acid, and malvidin-3-(p-coumaryl rutinoside)–5-galactoside were the major phenolics identified. Malvidin-3-(p-coumaryl rutinoside)–5-galactoside (m/z 947.4) was identified by comparing mass/charge ratios. According to Giusti et al. (*166*) mass/charge ratios of malvidin, malvidin + rutinose, malvidin + rutinose + 1hexose, and malvidin + rutinose + hexose + p-coumaric acid – H2O were 493.2, 639.2, 801.4, and 947.6, respectively. BHT was used as an internal standard. Only four of the five peaks identified were quantified because the malvidin-3-(p-coumaryl rutinoside)–5-galactoside peak was quantifiable only in purple flesh and purple skin selections and absent in those with yellow flesh and yellow skin (**Figures 2.4** & **2.5**).

Chlorogenic acid content accounted for 50 – 70% of TP depending on the selection. Earlier research suggests that chlorogenic acid is the major polyphenol in potato tubers and contributes 90% of the total phenolics (*8*). In the present study, chlorogenic acid content ranged from 138.3 µg/gfw (ATX99324-1) to 548.9 µg/gfw (CO112F2-2). Selection CO112F2-2 had significantly higher ($p = 0.001$) chlorogenic acid content than all other selections except PATX99P32-2 (519.5 µg/gfw). Caffeic acid concentration ranged from 33 µg/gfw to 42 µg/gfw. The difference among the selections for caffeic acid content was less than for chlorogenic acid. Caffeic acid content accounted for 5 - 10% of the total phenolic content. NDTX4528-4B had higher gallic acid content (96.6 µg/gfw), but it was not significantly different from CO112F2-2 or CO111F2-1. Gallic acid content ranged from 15.3 to 96.6 µg/gfw and contributed approximately 3 to 18% to total phenolic content. Rodriguez de Sotillo et al. (*15*)

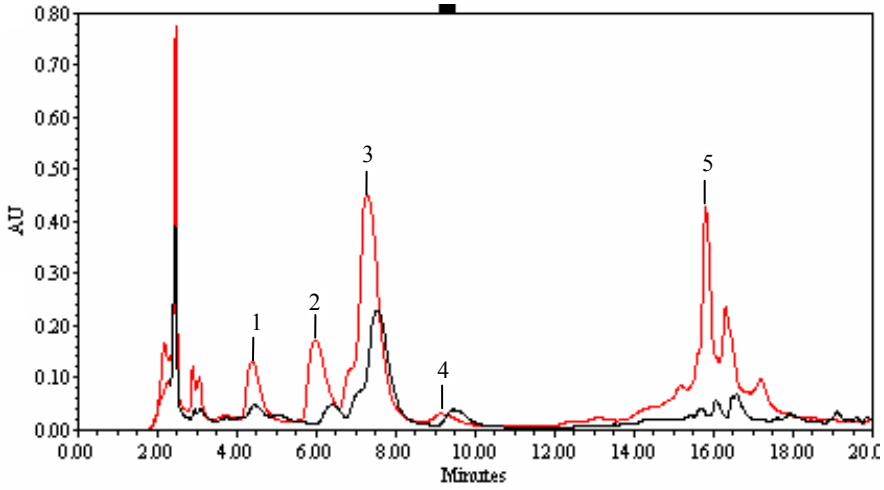

Figure 2.4. HPLC chromatogram of phenolics from specialty potato selections. CO112F2-2 (P/P) (red line) and ATTX 98492-1 (Y/Y) (black line). Peaks 1,2,3,4 and 5 correspond to L-tryptophan, catechin, chlorogenic acid, caffeic acid and malvidin-3- (p-coumaryl rutinoside)–5-galactoside, respectively.

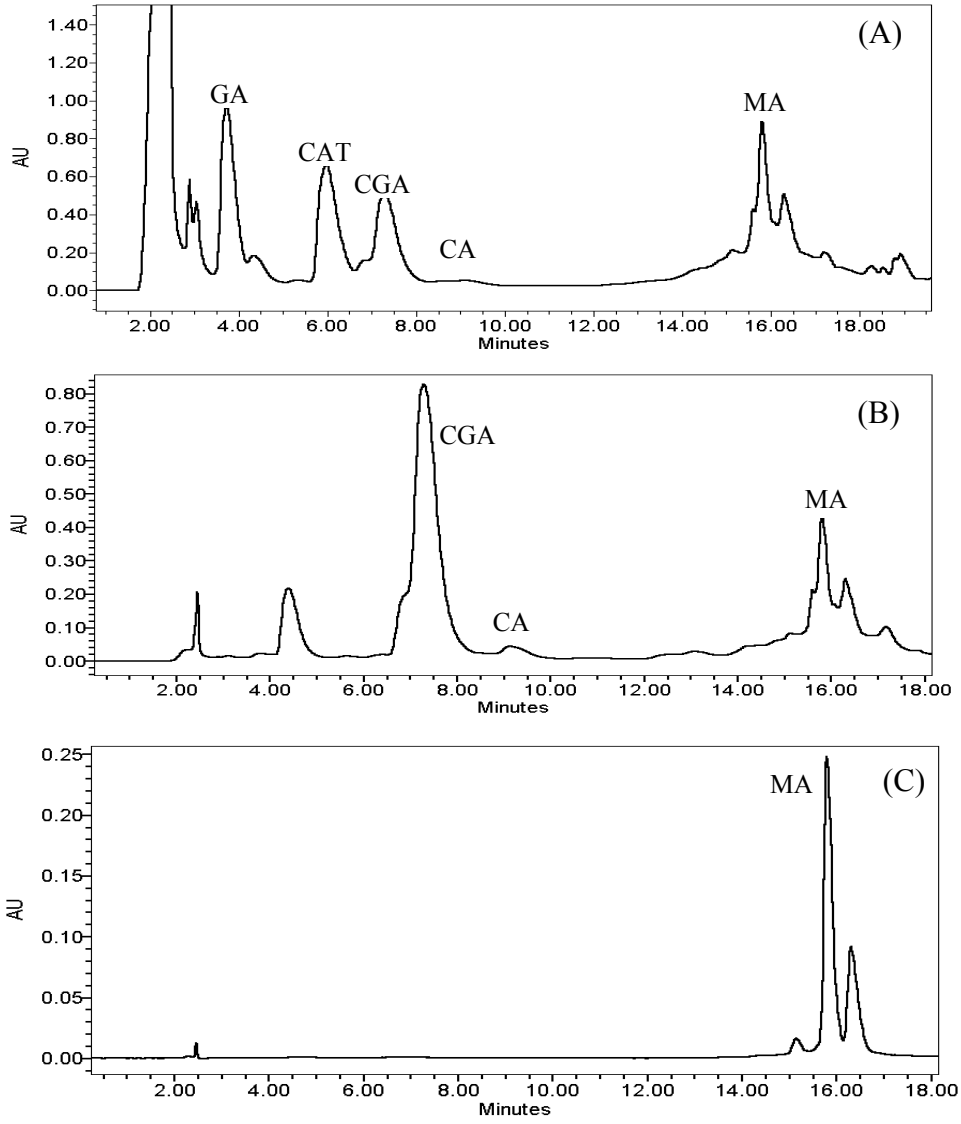

Figure 2.5. HPLC chromatogram of CO112F2-2P/P. (A) total phenolics at 220nm; (B) phenolic acids at 320nm; (D) anthocyanins at 520nm (GA: Gallic acid, CAT: Catechin, CGA: Chlorogenic acid, CA: Caffeic acid, MA: malvidin-3- (p-coumaryl rutinoside)–5-galactoside).

detected 41% of the gallic acid in the peel of potato. In this study, whole tubers were used and the samples contained approximately 10% peel. This might have contributed to the observed gallic acid content. Selections differed significantly in their gallic acid content. Catechin was the other phenolic compound identified in the specialty potatoes (*156, 167*) and ranged from 83.8 µg/gfw to 91.4 µg/gfw. Purple selections CO112F2-2 and CO111F2-1 had higher catechin content, but were not significantly different from NDTX4528-4. Catechin content accounted for approximately 13 to 30% of the TP.

Contribution of Individual Phenolics to AOA

Pure standards were used to estimate percentage contribution of phenolic compounds in potato extract to AOA, with the assumption that isolated pure phenolic compounds exhibit similar activity as standards. Individual phenolics differed in their AOA when measured by the DPPH assay. Gallic acid had the highest AOA, while chlorogenic acid was the lowest in AOA among the four standards tested (**Figure 2.6**). The order of the standards tested from high to low AOA was gallic acid, caffeic acid, catechin, and chlorogenic acid. Depending on the content in selections, chlorogenic acid contributed approximately 28 to 45% of AOA; however, it accounted for 50 to 70% of TP because of its low antioxidant capacity. In contrast, gallic acid accounted for only 3 – 18% of TP, yet contributed to 20 – 30% of AOA. Caffeic acid and catechin accounted for 8 -15% and 18 – 30% of AOA, respectively. AOA of different combinations of the two standards were also calculated to assess the synergistic effect of these standards. The combinations involving gallic acid had higher AOA. The four compounds (chlorogenic

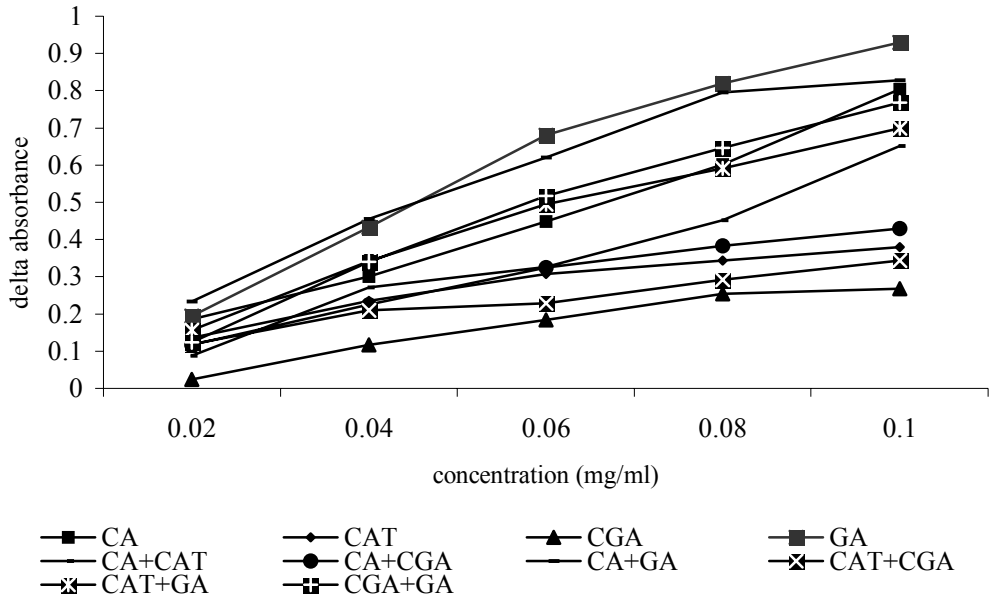

Figure 2.6. Antioxidant activity of different standards alone and in combination as measured by the DPPH assay.

acid, caffeic acid, gallic acid and catechin) together accounted for 58 to 82% of AOA, indicating that malvidin and other unidentified compounds also contributed to AOA.

In summary, our results suggest that selections with purple flesh color are highest in AOA and TP followed by red flesh color. Thus, genotype has a significant effect on AOA and TP of specialty potatoes. Chlorogenic acid is the major phenolic acid in potatoes, and contributed approximately 28 to 45% of total AOA followed by gallic acid, catechin and caffeic acid. Purple skin/purple flesh selections can be used to cross with popular varieties (4X) because of their tetraploid nature, while most of the wild species high in these traits are diploids and require chromosomal doubling.

CHAPTER III

GENOTYPE AND LOCATION INFLUENCE ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT, CAROTENOID CONTENT, AND PHENOLIC COMPOSITION IN SPECIALTY POTATOES

Introduction

Numerous epidemiological studies suggest that frequent consumption of fruits and vegetables is associated with a reduced risk of various diseases (*168-170*). Bioactive compounds in fruits and vegetables which cause reduction in oxidative stress are responsible for these protective effects (*171*). Consumer awareness of the disease preventive role of antioxidants and their concerns about the safety of synthetic antioxidants promotes the preference for natural antioxidants from fruits and vegetables (*172*). In addition, synergistic effects of bioactive compounds in whole foods provide better protection against diseases than synthetic single-nutrient supplements (*89, 170*).

Potato (*Solanum tuberosum* L.) is the leading vegetable crop in the US, with a per capita consumption of about 137 pounds (*3*). Specialty (colored) potato tubers are high in antioxidants, total phenolics, with content ranging from 530 μg/g to 1770 μg/g (*154*), and carotenoids ranging from 97 to 536 μg/100gfw (*62*). Major tuber polyphenols include chlorogenic acid (CGA), caffeic acid (CA), scopolin, ferulic acid (FA) and cryptochlorogenic acid, with the skin containing approximately double the amount as tuber flesh (*5*). Potato peel contained 50% chlorogenic acid and 41% gallic acid (*15*). Lutein, zeaxanthin, neoxanthin, violaxanthin, and lutein 5,6 epoxide were the major carotenoids identified in highly pigmented genotypes (*32*).

Varieties differed in their total phenolic content, with some showing two-fold higher concentrations than others (*154*). Along with genotype, environment also influenced total phenolic and carotenoid levels. Greater total phenolic content has been observed in higher, cooler and more humid regions with less fertile sandy loams, as compared to lower, warmer and drier regions with fertile loamy soils, with varietal influence much more pronounced than locality (*48*). Significant differences among environments and clones for yellow-flesh intensity have been reported. (*56*). Earlier studies (*12, 48, 56*) reported the effects of environment on total phenolics and carotenoids, but no information has been found on the effect of location and year on individual potato phenolics (phenolic composition) and the percent contribution of genotype, location, year and their interactions on AOA, TP, and phenolic composition.

Therefore, the objectives of this study were 1) to evaluate the effects of genotype, location and year on AOA, TP, TC and phenolic composition of 25 specialty selections from the Texas Potato Variety Development Program grown near McCook and Dalhart, Texas, 2) to identify the genotypic effects on carotenoid composition, and 3) to investigate and calculate the relative contributions of genotype, location, year and their interactions on these parameters in specialty potatoes.

Materials and Methods

Materials

Potato selections (n=25) with different skin flesh colors (R = red, P = purple, Y = yellow, and W = white) were grown at two Texas locations, McCook (near the Mexican border) and Dalhart (in the N.W. corner of the Panhandle), in two different

years, 2003 (year 1) and 2004 (year 2). Environmental conditions of these locations, planting and harvest dates are presented in **Table 3.1**. Tubers of each selection were harvested from four plants and transported at ambient temperature (25^{o}C) to Texas A&M University, College Station for further analysis.

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical and Folin-Ciocalteu reagents were obtained from Fisher Scientific (Pittsburg, PA). Phenolic acid standards were purchased from Sigma (St. Louis, MO). Solvents for extraction and HPLC were obtained from VWR International (Bristol, CT).

Sample Preparation

From the bulked material, uniformly sized tubers (n = 6) were selected, washed and diced into 0.5 cm cubes. Three 5 g samples and three 10 g samples, each from two tubers, were stored in falcon tubes at –80^{o}C until analysis. For AOA, TP, and PC analysis, 5 g tuber samples were homogenized with 15 ml ethanol using a tissumizer (Ultra Turrax), and stored at –20^{o}C overnight. The supernatant was collected by centrifugation. For carotenoid content and composition analyses, 10 g tuber samples were homogenized with 15 ml ethanol plus butylated hydroxy toluene (BHT) (1 g/L). Five ml of ethanol with BHT and 10 ml of hexane were added to the homogenized tissue and kept at –20^{o}C. Ethanol and hexane layers were collected separately after centrifugation. A second extraction was made by adding 5 ml ethanol and 10 ml hexane to the previous extracts.

Table 3.1. Climatic conditions, planting and harvesting dates for potatoes grown at two Texas locations and for two years

location	year	soil type	temp (^{o}F)[a]		rainfall (in)[a]	planting date	harvesting date
			min	max			
McCook	1 (2003)	McAllen fine sandy loam	50.8	74.6	0.06	9-Dec-02	29-Apr-03
	2 (2004)		51.8	74.3	0.07	11-Dec-02	19-Apr-03
Dalhart	1 (2003)	Tivoli fine sand	57.9	87.1	0.07	5-May-03	22-Sep-03
	2 (2004)		57.8	85.8	0.13	10-May-04	18-Oct-04

[a] Average per location per year from planting to harvest. Crops were produced under center pivot irrigation as required.

Antioxidant Activity

Total antioxidant activity (AOA) of the specialty potatoes was determined via a modified 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (*173*). The sample solution (150 µl) was allowed to react with the DPPH radical in an ethanol solution, with absorbance adjusted to 1.1. The absorbance was measured at 515 nm against the blank (pure ethanol) using a spectrophotometer. The difference in absorbance is proportional to the AOA of the sample and was expressed as micrograms trolox equivalents per gram fresh weight (µg TE/gfw).

Total Phenolic Content

The Folin-Ciocalteu colorimetric method (*162*) was used to measure total phenolic content of the samples. Nanopure (2400 µl) water was added to 150 µl of the

clear supernatant sample, followed by the addition of 150 µl of 0.25N Folin-Ciocalteu reagent and 300 µl of 1N Na_2CO_3. The absorbance reading was recorded every 30 min and compared to a prepared blank at 725 nm until no significant change in absorbance was observed. The results were expressed as milligrams chlorogenic acid equivalents per 100 g of tissue (mg CGAE/100 gfw).

Phenolic Composition

Samples were concentrated by drying 7 ml to completion in a heated speed vacuum and resuspending in 1 ml ethanol for HPLC analysis. The HPLC system equipped with a binary pump (Waters 515), an autoinjector (Waters 717 plus), and a photodiode array (PDA) detector (Waters 996) was used with a 4.6 x 150 mm, 5 µm, Atlantis C-18 reverse-phase column at 40^{o}C. A 20 µl sample was injected using the mobile phase acetonitrile (solvent A) and nano pure water (pH adjusted to 2.3; solvent B). Flow rate was 1 ml/min with a gradient 0/85, 6-35/85-0, 36-45/85 (min/%A) (*62*).

Total Carotenoid Content

Total carotenoid content was determined by the absorbance of the ethanol and hexane extracts at 445 nm and 450 nm, respectively (*174*). Carotenoid content in the ethanol and hexane fractions were calculated using lutein and β carotene standard curves, respectively. Total content was expressed as µg lutein equivalents per 100 g of tuber (µg LE/100 gfw).

Carotenoid Composition

Both ethanol and hexane extracts were concentrated by drying under a nitrogen stream to completion and resuspending in 1 ml of 50% ethanol:water. The concentrated

sample was filtered through a 0.45 μm syringe filter before injection. A 20-μl sample was injected onto the HPLC. Solvent A consisted of methanol: water: triethylamine (90: 10: 0.1 v/v/v) and solvent B consisted of methanol: MTBE: triethylamine (6: 90: 0.1 v/v/v) (*33*). A YMC carotenoid column (4.6 x 250 mm, 5 μm, C-30 reverse-phase) was used at 35^{o}C with 1 ml/min flow rate. The top five selections from McCook 2003 were analyzed for carotenoid composition.

Statistical Analysis

The effects of genotype, year, and location on AOA, TP, and TC were determined by analysis of variance (ANOVA) using the SAS general linear model (GLM) procedure (*163*). Pair-wise multiple comparisons were determined by Fishers LSD test at the 5% significance level. For phenolic compounds, years were determined to be insignificant, so data from both years were pooled. An LSD mean separation between the locations was performed using Fischers LSD. Pearson correlation coefficient was determined using the Proc Corr procedure of SAS. Principal component analysis was carried out using Biplot 1.1 software by centering the data for genotype and environment.

Results and Discussion

Effect of Genotype, Location and Year on Antioxidant Activity, Total Phenolics and Total Carotenoids

Previous reports have shown that genotype and environment play a role in antioxidant, phenolic and carotenoid levels in potatoes. In order to identify the elite genotypes high in the above parameters suitable for different locations, it was necessary

Table 3.2. Antioxidant activity (AOA), total phenolics (TP) and total carotenoids of 25 specialty potato genotypes grown in two Texas locations and two years (2003 and 2004)

ID	genotype	AOA (µg TE/gfw) [b]				TP (mg CGAE/100gfw) [b]				TC (µg LE/100gfw) [b]			
		Dalhart		McCook		Dalhart		McCook		Dalhart		McCook	
		Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
1	ATTX98013-1	**654b**	**579b**	**754a**	**656b**	**131a**	**91c**	**111b**	**96c**	286ab	345a	267b	320ab
2	ATTX961014-1	127c	113c	210b	449a	59c	58c	103a	88b	367a	305a	344a	224a
3	ATTX96750-1	159b	128b	352a	84b	63b	55bc	85a	41c	261c	484a	273c	352b
4	ATTX98012-5	167b	140b	543a	232b	84a	69ab	82ab	66b	257ab	266a	160b	157c
5	ATTX98444-16	223b	333a	336a	114c	87b	56c	95a	60c	354b	512a	270b	161b
6	ATTX98444-4	75b	47b	444a	162b	88a	60b	88a	65b	345a	591a	378a	448a
7	ATTX98462-3	186b	202b	380a	254b	62b	56bc	85a	48c	**530a**	**276bc**	**422ab**	**516a**
8	ATTX98491-3	293c	361b	309c	446a	87a	69b	77ab	67b	**502c**	**662b**	**387c**	**888a**
9	ATTX98493-1	356b	202c	494a	314b	92a	77b	98a	64c	**525b**	**887a**	**385c**	**930a**
10	ATTX98500-2	326c	549ab	489b	620a	71b	78ab	90a	67b	437a	436a	396a	344a
11	ATTX98500-4	151c	246b	345a	330a	76a	69a	83a	72a	191b	655a	320b	533a
12	ATTX99325-1	196ab	146b	270a	153b	60a	67a	63a	57a	273a	339a	210a	328a
13	CO111F2-1	543b	592b	707a	592b	102a	108a	117a	103a	377a	363a	332a	372a
14	CO112F2-2	**783a**	**586b**	**763a**	**730a**	**112b**	**114b**	**155a**	**111b**	**611a**	**569a**	**587a**	**554a**
15	CO141F2-1	272b	202b	365a	411a	74b	77b	119a	77b	173b	291a	166b	271a
16	CO142F2-1	496b	661a	448b	717a	72a	94a	102a	89a	226b	381a	207b	373a
17	COTX00104-2	342b	484a	335b	204c	86a	53b	78a	52b	125b	536a	101b	421a
18	COTX00104-4	251bc	167c	425ab	468a	78a	61b	72ab	65ab	482a	187b	469a	186b
19	COTX99086-4	741a	586b	456c	590b	105a	81b	75b	72b	310a	216b	181b	216b
20	COTX99338-1	356b	511ab	362b	625a	79b	80b	96a	82b	274a	326a	202b	334a
21	NDTX4528-3	311b	347b	668a	299b	73b	74b	95a	59c	134bc	284a	96c	170b
22	NDTX4528-4	138b	109b	340a	370a	56b	66ab	78a	66ab	190b	289a	184b	272ab
23	NDTX4528-4B	173c	220bc	303ab	384a	60b	53b	80a	64ab	294a	223ab	137b	218ab
24	PATX99P32-2	351c	648ab	521b	697a	95a	92a	98a	91a	**560a**	**533a**	**533a**	**376a**
25	PATX99P41-1	425b	574ab	490b	725a	85b	85b	104a	88b	485a	474a	450a	454a
	lsd[a]	70	44	190	44	16	7	12	8	105	116	88	103

[a] lsd is used to compare genotypes within columns.

[b] Different letters across four columns for each parameter represent significant difference among four location and year combinations

to investigate the effect of climate on AOA, TP and TC. Significant differences ($p < 0.001$) were observed among the genotypes at each location and year for AOA, TP and TC (*32, 35, 62, 63*) (**Table 3.2**). The main effects of genotype (G), location (L) and year (Y), and their interaction effects G x L, G x Y, L x Y, and G x L x Y were significant for TP. For AOA, all above effects were significant except year and L x Y. For TC, all interaction effects were significant except for G x L and L x Y, indicating the importance of these effects in selecting varieties for different parameters.

AOA ranged from 47 μg TE/gfw for ATTX98444-4 in year 2 at Dalhart to 783 μg TE/gfw for CO112F2-2 in year 1 at Dalhart (**Table 3.2**). Averaged across locations and years, the genotype CO112F2-2 had the highest AOA. This genotype showed intense purple skin and flesh color, indicating the presence of anthocyanins that can also contribute to AOA along with phenolic acids (*63*). ATTX 98444-4 had the lowest AOA in both years at Dalhart, whereas ATTX 961014-1 and ATTX96750-1 had the lowest AOA at McCook in year 1 and year 2, respectively. CO112F2-2, COTX99086-4, CO142F2-1 and CO111F2-1 were significantly different from other genotypes for AOA in Dalhart. In McCook CO112F2-2, ATTX98013-1, CO142F2-1, CO111F2-1, PATX99P32-2, and PATX99P41-1 were significantly different from other genotypes (**Table 3.2**). This is consistent with findings of Reyes et al. (*23*) who reported variation in AOA among different genotypes of potato. Averaged across genotypes and years, McCook showed 29% more AOA than Dalhart. The higher TP concentrations found in McCook compared with Dalhart might be responsible for the higher AOA in genotypes

grown in McCook. The significantly high positive correlation between AOA and TP (*23, 154*) further indicates the contribution of these compounds to AOA.

Total phenolics ranged from 41 mg CGAE/100 gfw in ATTX96750-1 in year 2 at McCook to 155 mg CGAE/100 gfw in CO112F2-2 in year 1 at McCook. CO112F2-2, CO111F2-1 and ATTX98013-1 were significantly different from the other genotypes at Dalhart in both years. In McCook, CO112F2-2 was significantly higher for TP in the first year, and CO111F2-1 did not significantly differ from CO112F2-2 in the second year. Hale (*62*) and Friedman (*8*) also reported significant differences in TP among different genotypes. Averaged across genotypes and years, McCook had higher TP than Dalhart. This may have resulted from the lower temperatures observed in McCook during the growing year (**Table 3.1**). Hamouz et al. (*48*) also observed significantly higher TP in tubers from traditional potato regions, which are cooler. Tubers from Dalhart year 1 significantly differed from those in year 2 in their TP when averaged across genotypes and locations, confirming the results that polyphenol content of potato tubers is influenced by the specific features of the given year (*175*). Tubers from year 1 had 20% greater TP compared with year 2.

Total carotenoid content ranged from 96 μg LE/100 gfw in NDTX4528-3 in year 1 at McCook to 930 μg LE/100 gfw in ATTX98493-1 in year 2 at McCook. Nesterenko and Sink (*34*) reported TC ranging form 48 to 879 in white, yellow and orange fleshed potatoes. In year 1, CO112F2-2 (purple flesh/purple skin) had the highest TC in both locations, whereas ATTX98493-1 (dark yellowish red flesh/red skin) had the highest TC in year 2. COTX00104-2, COTX00104-4, NDTX4528-3 and ATTX98012-5 had the

lowest TC in Dalhart year 1, and year 2, McCook year 1 and year 2, respectively. Brown et al. (*67*) reported that dark yellow flesh varieties had three to four times higher TC than light yellow cultivars. Unlike AOA and TP, Dalhart showed higher TC (13%) compared with McCook, when averaged across genotypes and years. Year 2 had higher TC than year 1 when averaged across genotypes and locations. Significant differences among environments, clones and clone x environment interactions for yellow flesh intensity were reported by Haynes et al. (*56*).

Theses results show that genotypes differ in their AOA, TP and TC in different locations and years. Some genotypes performed better in McCook and others in Dalhart. Certain genotypes had better performance in both locations. Genotype CO112F2-2 had the highest AOA and TP in both the locations and years and the highest TC in both the locations in year1.

Effect of Genotype, Location and Year on Phenolic Composition

Selection of genotypes for a single phenolic compound necessitates the need of knowing the effect and contribution of climate to that particular compound. Chlorogenic acid (CGA), caffeic acid (CA), gallic acid (GA), and catechin (CAT) were significantly different among genotypes. Year and the genotype x year interaction were not significant for the above four phenolic compounds. Therefore, the phenolic compounds were averaged across years (**Table 3.3**).

Chlorogenic acid was the major phenolic compound present, with concentrations ranging from 132 to 683 μg/gfw. ATTX98013-1 had the highest CGA content and was significantly different from other genotypes tested except CO112F2-2 in Dalhart. In

Table 3.3. Phenolic composition of specialty potato genotypes grown in two Texas locations[a]

genotype	skin/flesh color	(µg/gfw)							
		CGA		CA		GA		CAT	
		D	M	D	M	D	M	D	M
ATTX98013-1	**R/R**	**642**	**654**	**34**	**36**	**61**	**63**	**83**	**88***
ATTX961014-1	R/Y	170	359*	35	35	63*	39	85	86
ATTX96750-1	W/Y	149	275*	33	35	57	60	84	84
ATTX98012-5	R/Y	262*	176	33	34	57*	43	83	85
ATTX98444-16	R/Y	224	263	33	36*	59	66*	83	86
ATTX98444-4	Y/Y	209	183	39	34	45*	33	85	86
ATTX98462-3	RY/RY	164	162	36	35	55	46	84	85
ATTX98491-3	YR/Y	253	184	35	34	63	61	83	85
ATTX98493-1	R/YR	289	242	35	34	52*	34	85	85
ATTX98500-2	P/Y	214	346	34	35	50	58*	84	86
ATTX98500-4	P/Y	211	485*	33	35	46	54	82	86*
ATTX99325-1	R/Y	175	140	35	34	48	59	84	86
CO111F2-1	P/P	519	641	35	35	42	45	86	87
CO112F2-2	**P/P**	**591**	**683**	**40**	**36**	**56**	**66**	**89***	**85**
CO141F2-1	P/P	216	353	35	34	42	60*	81	85*
CO142F2-1	R/R	321	411	34	34	43	53	82	86
COTX00104-2	P/Y	223	174	34	35	49	51	84	84
COTX00104-4	R/Y	190	192	34	34	54	50	83	85
COTX99086-4	YR/Y	450	221	37	34	57	43	86	86
COTX99338-1	YR/Y	271	344	33	35	48	62	83	85
NDTX4528-3	P/PW	241	231	34	34	48	49	84	85
NDTX4528-4	P/PW	132	281*	33	34	45	47	85	84
NDTX4528-4B	P/PW	142	247*	31	33	46	60*	84	85
PATX99P32-2	RY/YR	255	390*	35	34	68*	64	86	85
PATX99P41-1	RY/YR	227	427*	34	35	61	60	83	85
lsd		95	101	4	1	9	7	2	2

[a] Each value is a mean of six observations (3 replications and 2 years). Two years within the same location were pooled together because the year effect was not significant. * represents significant difference (p = 0.001) between two locations for the same genotype for a single parameter. lsd was used to compare genotypes within columns. CGA – chlorogenic acid, CA – caffeic acid, GA – gallic acid, CAT - catechin, D – Dalhart, M – McCook. In skin/flesh color column, R = Red, Y = Yellow, W = White, P = Purple

McCook, CO112F2-2, ATTX98013-1 and CO111F2-1 were significantly different from other genotypes in their CGA content, but they did not differ significantly from each other. Location and interaction effects (G x L, G x L x Y) were significant for CGA content. McCook had significantly higher CGA content for seven genotypes. One genotype (ATTX98012-5) had higher CGA content in Dalhart than in McCook. This corroborates the findings of Emmons and Peterson (*176*), where location significantly affected the concentration of five of the phenolics in oats. All the other genotypes did not differ significantly between the two locations. Averaged across genotypes and years, McCook showed 20% more CGA content than Dalhart. This higher level of CGA accounts for the corresponding higher level of TP in McCook.

Caffeic acid concentration ranged from 31 to 40 μg/gfw. CO112F2-2 had the highest CA content in both ocations, but was not significantly different from most of the genotypes tested. Location effect and all the interaction effects were not significant for CA content.

Gallic acid concentration ranged from 33 to 68 μg/gfw. PATX99P32-2 had the highest GA content in Dalhart, whereas C0112F2-2 was highest in McCook. However, C0112F2-2 was not significantly different from many other genotypes tested. Though the overall location effect was not significant, some genotypes significantly differed in their GA content between locations. The interaction effects G x L, L x Y, and G x L x Y were significant for GA content.

Catechin content ranged from 81 to 89 μg/gfw. CO112F2-2 and CO111F2-1 had the highest CAT content in Dalhart and McCook, respectively. The location effect was

Table 3.4. Correlation coefficients between antioxidant activity (AOA), total phenolics (TP), total carotenoids (TC), and four phenolic compounds

	TP	TC	chlorogenic acid	caffeic acid	gallic acid	catechin
AOA	0.653*	0.050	0.668*	0.108	0.156	0.246*
TP		0.042	0.781*	0.220*	0.216*	0.128
TC			0.058	0.036	0.145	0.102
chlorogenic acid				0.192*	0.186*	0.255*
caffeic acid					0.058	0.113
gallic acid						0.020

* Significant ($p < 0.01$)

significant. Over all, McCook had higher CAT content than Dalhart. Genotypes ATTX98013-1, ATTX98500-4, CO141F2-1, CO111F2-1 differed significantly in their CAT content between locations, while the first three had the highest content in McCook. The interaction effects G x L, L x Y, and G x L x Y were also significant for CAT content. Over all, chlorogenic acid showed significant differences among genotypes and between locations with 550 μg/gfw differences between the highest and the lowest genotypes.

Correlation Coefficients among Different Parameters

Significant positive correlations were observed among different traits. Significant correlations above 0.5 were considered strong and below 0.5 were considered weak. Strong correlations were observed among AOA, TP and CGA (**Table 3.4**). The CGA accounted for 90% of the TP (*8*). Abundance of this phenolic acid might be responsible for its strong correlation with AOA and TP. Vinokur et al. (*177*) and Scalzo et al. (*165*) reported strong correlations between AOA and TP. Weak correlations were observed between AOA and CAT, TP and CA, TP and GA, CGA and CA, CGA and GA, and CGA and CAT. In wheat, strong correlations were observed between TP and AOA, TP and ferulic acid, and AOA and ferulic acid, and weak correlations were observed between TP and CA (*178*). Total carotenoid content was not correlated with AOA measured by the DPPH radical, probably due to the DPPH radical having no similarity to the highly reactive peroxyl radicals and singlet oxygens in the lipid phase where carotenoids exert their antioxidant functions (*179*). This may be the reason for the lack of correlation between TC and AOA. The TC did not show any correlation with TP

Table 3.5. Relative influence of variance components (genotype (G), location (L), Year (Y) and their interactions) on antioxidant activity (AOA), total phenols (TP), total carotenoids (TC) and phenolic composition. 25 specialty potato genotypes grown in two Texas locations and two years

	genotype (24)[a]	location (1)	Year (1)	rep (2)	GxL (24)	GxS (24)	LxS (1)	GxLxS (24)
AOA	65.1*	6.0*	0.0	0.1	6.4*	10.3*	0.3	5.2*
TP	56.2*	1.7*	12.6*	0.0	7.5*	5.9*	2.6*	5.6*
TC	54.9*	1.3*	4.1*	0.4	1.0	21.4*	0.0	2.5*
CGA	64.8*	2.5*	0.3	0.0	10.7*	5.3	0.3	5.4*
CA	12.7*	0.0	2.4	5.2	10.6	5.5	2.1	5.9
GA	36.8*	0.1	0.3	0.5	23.1*	5.5	7.3*	5.3*
CAT	12.2*	7.4*	1.1	0.9	12.8*	5.9	5.4*	11.0*

Each value was obtained by individual mean square/total mean square x 100. CGA = chlorogenic acid, CA = caffeic acid, GA = gallic acid, CAT = catechin

[a] the number in the parenthesis represents degrees of freedom

* Significant (p = 0.01)

or individual phenolic compounds. The AOA did not exhibit any correlation with CA or GA. The CAT showed no correlation with TP, CA, and GA.

Relative Influence of Variance Components on Different Parameters

Variance components (genotype, location, year and interactions) significantly influenced the measured parameters. The percent contribution of variance components to the total variation was calculated as the proportion of individual sum of squares to the total sum of squares for each parameter in order to identify the most influential component. The results are presented in **Table 3.5**. Variation due to genotype was significant for all measured parameters and was largest when compared with location, year and their interactions. This study is the first to quantify the relative influence of genotype, location, year and their interactions on potato phenolic compounds. Main effects and interaction effects together accounted for 85 to 93% of total variation for AOA, TP and TC. Genotype variation alone explained more than 50% of the total variation for AOA, TP, and TC. In blackberry, 40 to 92% of total variation in anthocyanins also accounted for cultivar main effects (*180*). Location influence was significant for AOA, TP, and TC and ranged from 1.3 to 6.0%. Year variation was significant for only TP and TC (12.6% and 4.1%, respectively). Genotype variation contribution was 64.8, 12.7, 36.8, and 12.2% for CGA, CA, GA, and CAT, respectively. For CA, the only variance component that was significant was that of genotype and all other variance components were not significant. All the variations together accounted for only 44% of the total variation. Location variation was significant for CGA and

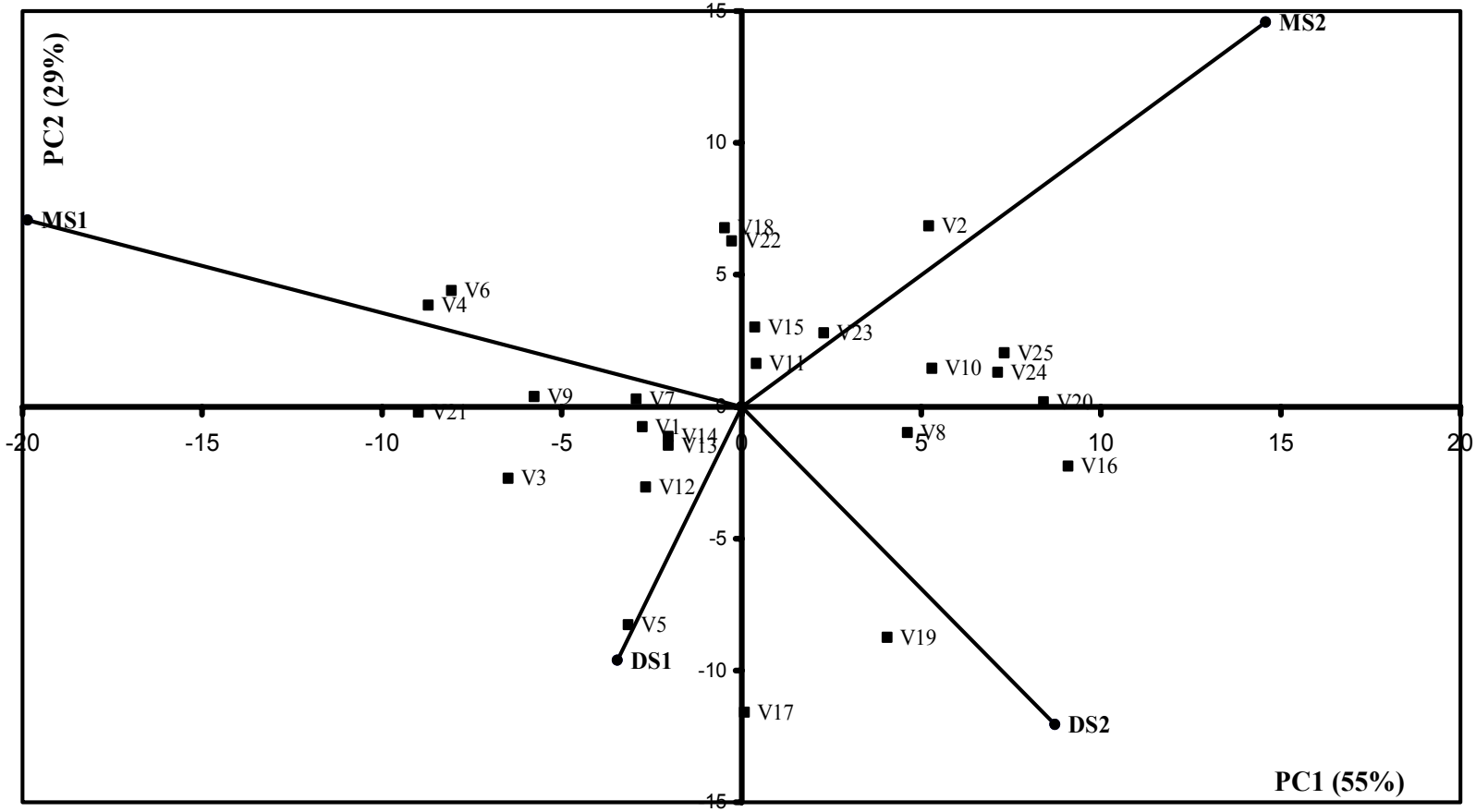

Figure 3.1. Principal component analysis biplot for antioxidant activity of 25 genotypes in four environments. Environments are in upper case with first letter representing location (M = McCook, D = Dalhart) Y1 and Y2 represent Year 1 and Year 2, respectively. Genotypes are in lower case. Principal component 1 values are on the x axis and principal component 2 values are on the y axis. V1 to V25 correspond to the genotypes 1 to 25 in Table 3.2.

CAT. All two-way interactions were significant for TP. In blackberries, year, cultivar x year and cultivar xlocation interactions accounted for 22%, 34% and 20% of total variation for AOA, respectively. Though the three-way interaction variation was significant for all measured parameters except CA, it accounted for only a small proportion of total variation. Mpofu et al. (*178*) reported similar results in wheat.

Principal component analysis was done to determine the deviation of the genotypes and environments from their averages for AOA, and to identify the stable genotypes across locations and years. Principal component 1 (PC 1) (55%) and principal component 2 (PC2) (29%) together explained 84% of variation (**Figure 3.1**). Genotypes ATTX98013-1, ATTX98462-3, ATTX98500-4, CO111F2-1 and CO112F2-2 were relatively stable for AOA across environments, because of less interaction effect. Genotype CO112F2-2 was not only stable, but also had significantly higher AOA, TP and TC. Genotypes farthest from the origin (**Figure 3.1**) had a large interaction effect and deviated much from the average performance (*181*).

Carotenoid Composition

Five genotypes with high total carotenoids were analyzed for carotenoid composition, and they differed significantly in their carotenoid content and composition. Lutein and violaxanthin were the two major carotenoids present in all five genotypes analyzed. Antheraxanthin and canthaxanthin were present only in some genotypes. Violaxanthin content ranged from 58 to 183 µg/100 gfw. ATTX98493-1 had significantly higher lutein content compared to the other four genotypes. Lutein was

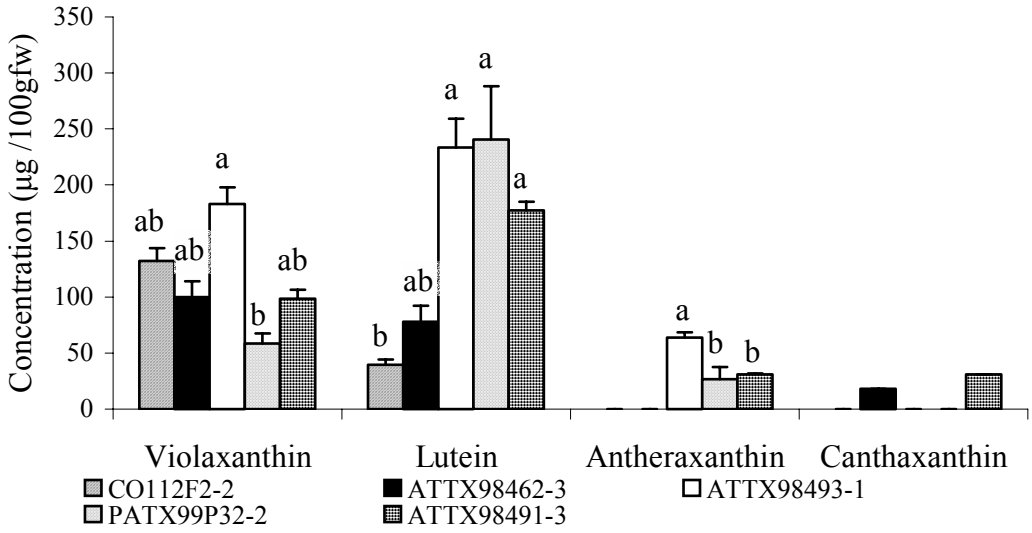

Figure 3.2. Individual carotenoid content in specialty potato genotypes. Each value is a mean of three replications. Different letters on the bars represent significant differences (p=0.001) among genotypes for each carotenoid.

highest in PATX99P32-2 (240 µg/100 gfw), but not significantly different from ATTX98493-1 (234 µg/100 gfw) (**Figure 3.2**). ATTX98493-1, PATX99P32-2 and ATTX98491-3 contained antheraxanthin and ATTX98493-1 had significantly higher antheraxanthin content (63.7 µg/100 gfw) than all other genotypes (**Figure 3.3**). Canthaxanthin was detected in two of the five genotypes (ATTX98462-3 and ATTX98493-1). Earlier reports suggested the presence of lutein, violaxanthin, lutein 5,6 epoxide, zeaxanthin and neoxanthin in potatoes (*32, 63*).

In conclusion, genotype and location significantly affected AOA, TP, TC and phenolic composition in potatoes. Overall, CO112F2-2 had significantly higher AOA and TP. McCook had significantly higher AOA (29%), TP (7%) and CGA (20%), and Dalhart had significantly higher TC (13%). Year had a significant influence on TP and TC. Significant positive correlations were observed between AOA-TP, AOA-CGA and TP-CGA. Relative influence of genotype was highly significant for AOA, TP, TC, and phenolic composition. Genotypic variance accounted for more than 55% of total variation for AOA, TP, TC and CGA. Location, year and interaction effects were significant. Individually, they accounted for 1%-23% of total variation for all the measured parameters. Since CO112F2-2 was high in most of the measured parameters and was stable across environments, this genotype would be an ideal parent for use in breeding programs aimed at increasing antioxidant content in potato. Further research is needed to investigate the effects of individual environmental parameters, such as temperature, rainfall, irrigation, solar radiation, temperature stress etc. on AOA, TP, TC, phenolic and carotenoid composition.

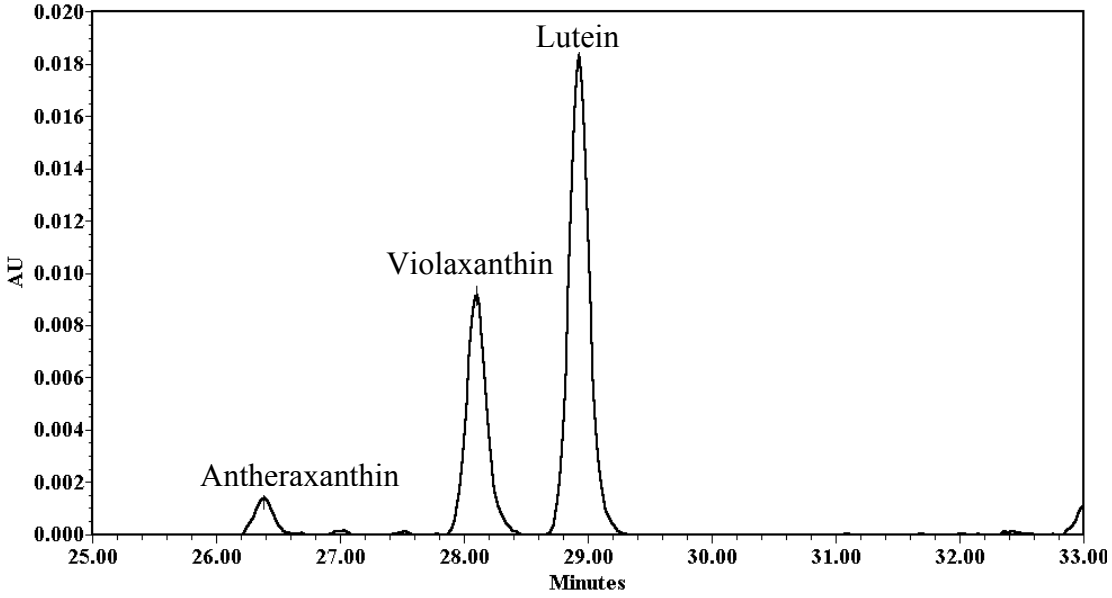

Figure 3.3. HPLC chromatogram of specialty potato genotype ATTX98493-1R/YR. Retention times on the x-axis and absorbance on the y-axis.

CHAPTER IV

ANTHOCYANIN FRACTION FROM POTATO EXTRACT IS CYTOTOXIC TO PROSTATE CANCER CELLS THROUGH ACTIVATION OF CASPASE-DEPENDENT AND CASPASE-INDEPENDENT PATHWAYS

Introduction

Prostate cancer is the third leading cause of cancer deaths in men in the US, and it is estimated that there will be 234,460 new cases and 27,350 additional deaths in 2006 due to prostate cancer (*182*). Epidemiological studies have shown that diets rich in fruits and vegetables have preventive effects on chronic diseases including prostate cancer (*140, 183, 184*). The preventive effects associated with vegetable consumption can be attributed in part to their content of antioxidant polyphenols, because oxidative stress plays an important role in tumor development (*76, 185*). Chronic inflammation, uncontrolled proliferation of prostatic epithelial cells, and suppression of apoptosis plays crucial role in prostate cancer promotion and progression (*70, 186*). Polyphenols also exhibit antiinflamatory, antiproliferative, and proapoptotic properties in addition to antioxidant activity, suggesting their role as chemopreventive agents (*187, 188*).

A number of studies have examined the antiproliferative, and proapoptotic properties of polyphenols such as phenolic acids and anthocyanins. Phenolic anti-proliferative mechanisms include elevation of p27 and suppression of cyclin D1 (CD1). CD1 over expression and reduction in p27 are most common molecular aberrations in many human cancers. In addition more than 47 % of metastatic prostate cancer patients showed homozygous deletion of the gene region coding for p27(*108, 109*). Caffeic acid

phenyl ester suppressed proliferation of HO-1 (human melanoma cells), GBM-18 (human glioblastoma) (*189*). Anthocyanins blocked the G0-G1 cell cycle in HT-29 cells through increased p27 levels (*190*). Delphinidin also showed increased p27 and decreased CD1 levels in blocking G0/G1 phase (*106*). Proapoptotic mechanisms of phenolics can be caspase-dependent or caspase-independent. Intrinsic caspase-dependent apoptosis involves activation of caspases 9, 3 and cleavage of PARP (*126, 129*). Caspase-independent apoptosis is through activation of AIF (apoptosis inducing factor) or Endo G (endonuclease G) (*129*). Induction of apoptosis by phenolic acids in HL-60 leukemia cells was caspase-dependent (*191*). Phenolics induced HT-116 colon cancer cell death through both caspase-dependent and –independent pathways through activation of caspase-3 and AIF respectively (*192*). The chemopreventive mechanisms of anthocyanins also include activation of mitogen activated protein kinases such as JNK (c-Jun NH2-terminal Kinase) and Erk (extra-cellular signal regulated kinase), which in turn activate proapoptotic factors like Bax, bid etc. through phosphorylation (*146*).

Specialty (colored) potato (*Solanum tuberosum* L.) contains significant levels of phenolic acids and anthocyanins (*63, 154*) and there is high consumption of this vegetable, crop with approximately 135 pounds per capita in the US. Earlier studies (*35, 193-195*) have focused on identification and quantification of polyphenols and their antioxidant properties in potatoes. Antioxidant polyphenols exhibited preventive effects against prostate cancer, but the antiproliferative and proapoptotic properties of potato polyphenols against prostate cancer and the molecular mechanisms involved in this process are not clearly understood.

In this study, we have investigated the effects of specialty potato extracts and their fractions on PC-3 (androgen independent) and LNCaP (androgen dependent) prostate cancer cells. Polyphenols from four specialty potato cultivars, CO112F2-2, PATX99P32-2, ATTX98462-3 and ATTX98491-3, were used. The CO112F2-2 extracts showed potential growth inhibition, and extracts were then fractionated into anthocyanin, phenolic acid and organic acid fractions. The anthocyanin fraction showed potent anti-proliferative properties and increased the level of the cyclin-dependent kinase inhibitor p27 in both LNCaP and PC-3 cell lines. The anthocyanin fraction induced apoptosis in both LNCaP and PC-3 cells, however, the effects were cell-context dependent. In PC-3 cells, the anthocyanin fraction induced caspase-independent apoptosis associated with nuclear translocation of endonuclease G (Endo G) and apoptosis-inducing factor (AIF), whereas both caspase-dependent and independent pathways were induced in LNCaP cells. These findings suggest that the cytotoxic effects involve induction of apoptosis rather than necrosis through caspase dependent and independent pathways. In addition, pretreatment of cells with JNK and MAPK inhibitors blocked apoptotic pathways in both cell lines.

Materials and Methods

Chemicals

The 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), Folin Ciocalteu reagent (FCR), DMSO and N-acetyl cysteine were purchased from Fisher Scientific (Pittsburgh, PA). Ethanol, methanol and ethyl acetate were purchased from VWR International (Bristol, CT). Trolox and chlorogenic acid were obtained from Sigma (St. Louis, MO),

and malvidin was purchased from the Indofine Chemical Company (Hillsborough, NJ). The 4-(3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate (WST-1), cell death detection ELISA kit, and In situ cell death detection-POD kit (TUNEL assay) were obtained from Roche Applied Sciences (Indianapolis, IN). Antibodies for p27, cyclin D1, pc-jun, p-erk, c-jun, erk, Grp-78, AIF and, bax, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), endo-G was from Prosci Inc. (Poway, CA). Cleaved PARP, cleaved capases 3, 9 and cytochrome C were obtained from Cell Signaling Technology (Beverly, MA). Western lightning™ chemiluminiscence reagents were from PerkinElmer Life Sciences (Boston, MA).

Selection and Sample Preparation and Fractionation

Four different specialty potato selections (CO112F2-2P/P, ATTX98462-3RY/Y, PATX99P32-2RY/YR, ATTX98491-3YR/Y) were grown near McCook, TX. Tubers from each selection were harvested and transported at ambient temperature to Texas A&M University, College Station. Uniformly sized tubers without any defects were washed, diced into 0.5 cm cubes and freeze-dried. These freeze-dried samples were stored at -20^{o} C for further analysis.

Freed-dried tuber samples (0.5 g) were homogenized with 15 ml of 85:15 ethanol:water using an Ultra Turrax Tissumizer T25 (Cincinnati, OH) (30,000 x g) and stored for 12 - 15 h at -20^{o} C. Samples collected after centrifugation were concentrated and diluted to 5 ml using 0.01% aqueous HCl (whole extract). The whole extract was passed through the C-18 Sep-Pak cartridges (Waters, MA) preconditioned with 0.01% acidified methanol to absorb phenolics and anthocyanins (*196, 197*). Sugars and organic

acids were eluted from the column with 0.01% aqueous HCl, and collected as the organic acid fraction (OAF). Phenolics other than anthocyanins were collected by eluting the column with ethyl acetate (phenolic acid fraction - PF), and the anthocyanin fraction (AF) was obtained by eluting the column with 0.01% methanolic HCl. The fractions were concentrated (under nitrogen gas) and reconstituted with either alcohol or DMSO.

Antioxidant Activity

Antioxidant activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH is a stable free radical which reacts with oxidants and exhibits decreased absorbance at 515 nm (*159*). Aliquots of the column fractions (150 µl) were incubated with 2850 µl of diluted DPPH solution for 24 h at 25^{o} C, and the absorbance was measured at 515 nm. The blank or negative control contained all the reaction reagents except the sample. Trolox (6-hydroxy-2, 3, 7, 8-tetramethylchroman-2-carboxylic acid) was used as positive control. The results were expressed as µg of Trolox equivalents per gram fresh weight (µg Teq/gfw).

Total Phenolic Content

The total phenolic content was measured spectrometrically using the Folin-Ciocalteu colorimetric method (*162*)**.** The sample (150 µl) was diluted with 2.4 ml water and incubated with 150 µl of 0.25 N Folin-Ciocalteu reagent and 300 µl of 1N Na_2CO_3 for 2 h with shaking at 25^{o} C. The absorbance was recorded at 725 nm, and results were expressed as µg of chlorogenic acid equivalents per gfw (µg CGA Eq/gfw). The phenolic

composition of the fractions was identified using HPLC with authentic reference standards.

Cell Lines

Human prostate carcinoma cell lines PC-3 (androgen-independent) and LNCaP (androgen-dependent) were obtained from the American Type Culture Collection (Manassas, VA), and cells were maintained at 37^0 C in 5% CO_2 jacketed incubator in RPMI 1640 (Sigma; St. Louis, MO) supplemented with 2.38 g/L HEPES 2.0 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 4.5 g/L glucose, 100 ml/L FBS, and 10 mL/L antibiotic antimycotic solution (Sigma).

Cell Proliferation Assay

Cells were plated at a density of $2x10^4$ /well in 96 well plates. After 24 h, media was replaced with DMEM F-12 media containing 2.5% charcoal-stripped serum and ethanol extracts of four different specialty potato varieties were tested at different concentrations. Every 24 h, cell proliferation was measured using the WST assay which required preincubation of cells in media for 4 h with the tetrazolium salt WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) (10 µl/well) followed by measuring absorbance at 450 nm. Percent cell proliferation was calculated based on control absorbance (100%). Results were expressed as means ± SE for at least three separate experiments for each treatment group.

Cell proliferation was also determined using a Z1 Coulter Counter. Cells were plated at a density of $2.5x10^4$ cells/well in 12-well plates, and after 24 h, DMEM F-12 media containing 2.5% charcoal-stripped Fetal Bovine Serum (FBS) was used and cells

were treated with DMSO (solvent control) and different concentrations of the whole potato extracts or fractions. Fresh media along with compounds was added every 48 h. Cells were counted after 24, 48, and 72 h using a Z1 Coulter Counter. Each experiment was carried out in triplicate, and results were expressed as means ± SE.

Fluorescence-Activated Cell Sorting Analysis

LNCap and PC-3 cells were plated at a density of $15x10^5$ cells/100 mm plate, and after 24 h treatment with either the vehicle (DMSO) or whole potato extracts (5 or 10 μg/ml), cells were trypsinized and centrifuged. The pellet was resuspended with 1 ml of PI staining buffer containing 4 mM sodium citrate, 0.1% Triton X-100, 50 μg/ml propidium iodide, and 200 μg/ml Rnase, and incubated for 10 min at 37° C in the dark and the final concentration of sodium chloride was adjusted to 0.15 M. Cells were analyzed using FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA), and CellQuest (Becton Dickinson Immunocytometry Systems) acquisition software. Results were reported as percent cells in each phase of the cell cycle.

Apoptosis

DNA fragmentation was measured using an enzyme-linked immunosorbent assay (Cell Death Detection ELISA; Roche Diagnostics, Indianapolis, IN) in both LNCaP and PC-3 cell lines. Cells were seeded in a 48 well plate at a density of $2.5x10^4$ cells/well. Cells were incubated for 24 h with whole potato extract or fractions, then scraped and pelleted by centrifugation. Cells were then lysed and diluted to $1x10^4$ cells/ml with incubation buffer, and the color development was measured according to the

manufacturer's manual. The supernatant from the pelleted cells was used to measure cytotoxicity using an LDH cytotoxicity detection kit.

TUNEL Assay

Cells ($4x10^4$) were seeded in four chambered glass slides, and after treatment for 12 h the i*n situ* cell death detection POD kit was used for the TUNEL assay according to the instruction manual protocol for fixed cells. After the incubation of cells with POD and DAB substrate, apoptotic DNA fragmentation was detected by visualizing labeled DNA using a light microscope. Slides incubated without TdT served as a negative control, and slides treated with 1000U DNAse I/ml for 10 min before TdT exposure served as a positive control. The percentage of apoptotic cells was calculated by counting the stained cells in 12 fields, each containing 50 cells.

Western Blot Analysis

LNCaP and PC-3 cells were seeded at a density of $1.5x10^5$ cells/ml in DMEM F-12 media with 2.5% charcoal-stripped FBS for 24 h. Cells were treated with DMSO or the potato extract or fraction for 24 and 48 h. Protein was extracted into high salt buffer (50 mM HEPES, 0.5 M NaCl, 1.5mM $MgCl_2$, 1 mM EGTA, 10% (v/v) glycerol, 1% (v/v) Triton-X-100) containing 1% Proteinase Inhibitor Cocktail (Sigma-Aldrich), and the amounts of protein were measured using the Bradford reagent. After boiling the sample for 3 min at 100^o C, 60 μg of protein was loaded per lane on acrylamide gel (10% higher molecular weight proteins, and 12%, 15% for lower molecular weight proteins) and subjected to SDS-PAGE at 120 V for 3 to 4 h. Proteins were transferred by

wet blotting onto 0.2µm PVDF membrane (Polyvinylidene difluoride; Bio Rad, CA). Membranes were blocked using 5% milk-TBST (10 mM Tris-HCl, 150 mM NaCl (pH 8.0), 0.05% Triton X-100 and 5% non-fat dry milk) for 30 min and incubated with primary antibody either in fresh 5% milk-TBST or in 3% BSA-TBST overnight at 4^{o} C with gentle shaking. The concentrations of the primary antibodies ranged from 1:250 to 1:5000. After washing with TBST for 10 min, the membrane was incubated with the secondary antibody (1:5000) in 5% milk-TBST for 90 min. The membrane was washed for 10 min and incubated with chemiluminescence substrate (PerkinElmer Life Sciences) for 1.0 min and exposed to X-ray film.

Table 4.1. Antioxidant activity and the total phenolic content of extracts from the specialty potato cultivars

Cultivar	Antioxidant activity (µg Teq/gfw) [a]	Total phenolic content (µg CGAeq/gfw) [b]
CO112F2-2	561.8*	769.8*
ATTX98462-3	322.7	314.7
PATX99P32-2	553.4*	757.9*
ATTX98491-3	410.3	577.5

[a] Micrograms of trolox equivalents per gram fresh weight.
[b] Micrograms of chlorogenic acid equivalents per gram fresh weight.
* significant ($p<0.05$)

Results

Ethanol Extract of the Freeze Dried CO112F2-2 Cultivar Showed High Antioxidant Activity and Total Phenolic Content

Previous studies indicated that potato extracts contain several classes of phytochemicals that exhibit anticancer activity, including phenolic acids, flavonoids, anthocyanins and carotenoids (*63*). Many of the phenolic components also exhibit antioxidant activity. **Table 4.1** summarizes the phenolic content and antioxidant activity of extracts from the four specialty potato cultivars used in this study. The total phenolic content was estimated using the Folin-Ciocalteu reagent, and the rank order for the phenolic content of these extracts was CO112F2-2 ≈ PATX99P32-2 > ATTX98491-3 > ATTX98462-3. The antioxidant activity of the extracts determined using the DPPH assay showed that their relative activities were CO112F2-2 ≈ PATX99P32-2 > ATTX98491-3 > ATTX98462-3. These results show that there was a correlation between the antioxidant activitys and phenolic content of the extracts, and this was similar to a previous report showing a positive correlation between antioxidant activity and phenolic content of potato extracts (*63*).

The CO112F2-2 Cultivar Extract Inhibited Prostate Cancer Cell Growth

Uncontrolled cell proliferation plays an important role in prostate cancer progression. Inhibition of cell proliferation is one of the potential targets of prostate cancer chemoprevention. So, the potential growth inhibitory effects of the extracts were investigated using androgen receptor (AR) – positive LNCaP and AR – negative PC-3

LNCaP

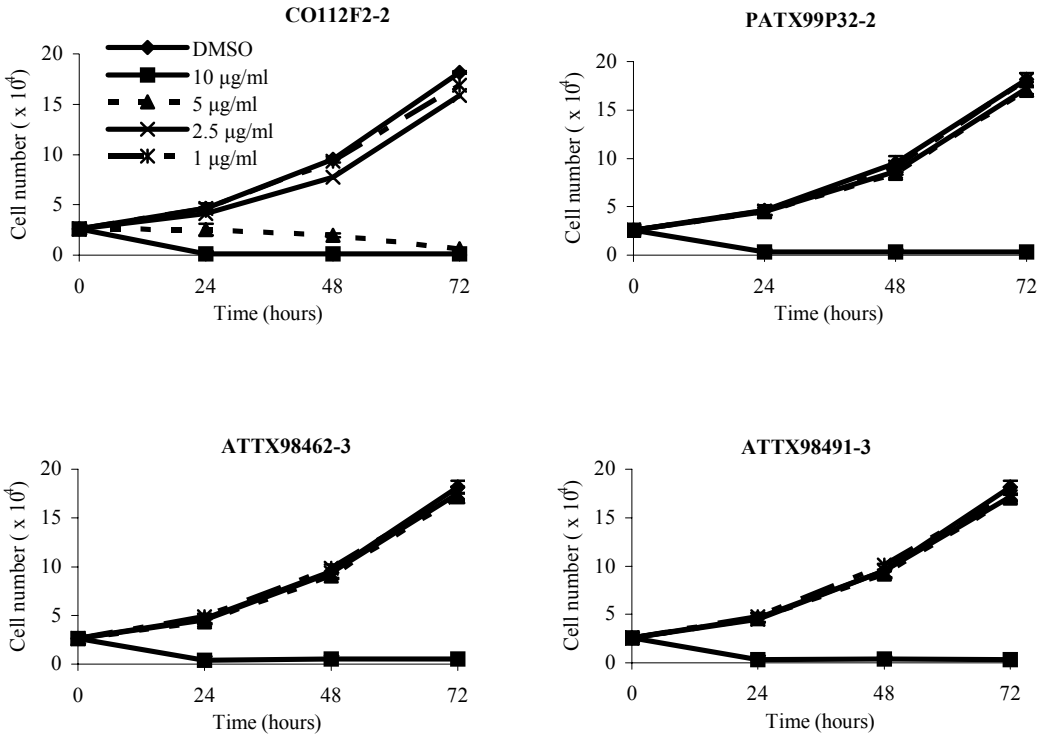

Figure 4.1. CO112F2-2 cultivar extract was the potent inhibitor of LNCaP cell growth. Growth inhibition of LNCaP cells treated with potato extracts from cultivars CO112F2-2, PATX99P32-2, ATTX98462-3 and ATTX98491-3 at 1 – 10 µg CGAeq /ml for 72 h and cell numbers were determined using a Coulter counter. Results are expressed as means ± SE for three experiments at each time point.

PC-3

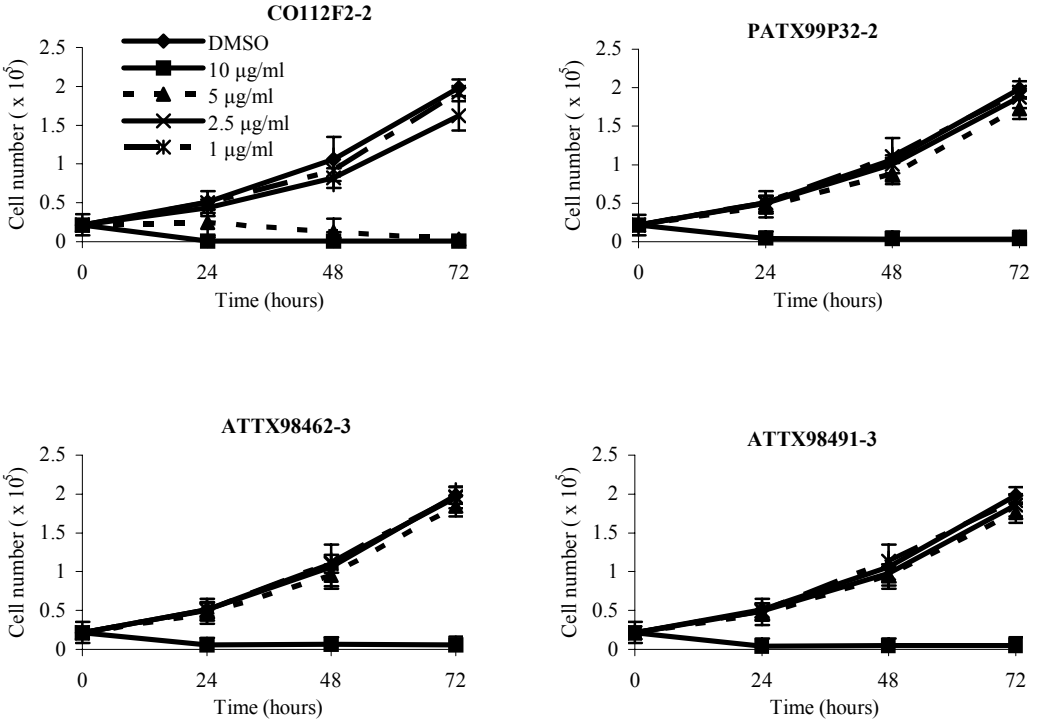

Figure 4.2. CO112F2-2 cultivar extract was the potent inhibitor of PC-3 cell growth. Growth inhibition of PC-3 cells treated with potato extracts from cultivars CO112F2-2, PATX99P32-2, ATTX98462-3 and ATTX98491-3 at 1 – 10 µg CGAeq /ml for 72 h and cell numbers were determined using a Coulter counter. Results are expressed as means ± SE for three experiments at each time point.

prostate cancer cell lines. **Figure 4.1** illustrates the effects of different concentrations of the extracts (expressed as μg of chlorogenic acid equivalents/ml) on proliferation of LNCaP cells, and the results show that the IC50 values for the CO112F2-2 extract (2.5 – 5 μg/ml) were lower than that observed for the other extracts where IC50 values were 5 – 10 μg/ml. A comparable cell proliferation study was carried out in PC-3 cells, and the results (**Figure 4.2**) were similar to those observed in LNCaP cells and the extracts from the CO112F2-2 cultivar were the most potent inhibitors of cell proliferation. Results using the WST assay in LNCaP and PC-3 cells (**Figure 4.3**) were similar to those observed for cell proliferation, with the CO112F2-2 extract the most potent.

LNCaP and PC-3 cells were grown in 2.5% charcoal-stripped serum for 24 h and treated with DMSO (solvent control), 5 and 10 μg/ml CO112F2-2 cultivar extract. The percentage distribution of cells in the G_0-G_1, S and G_2/M phases was determined by FACS analysis after 24 h (**Figure 4.3**). Both concentrations of the extract decreased the percentage of LNCaP cells in the S phase and increased the percentage in G_0-G_1. In contrast, even 10 μg/ml did not alter the percentage distribution of cells in the G_0-G_1, S or G_2-M phases in PC-3 cells, indicating cell context dependent differences in the effects of these extracts on G_0-G_1 – S phase cell cycle progression (**Figure 4.3**).

Anthocyanin Fraction Inhibited Prostate Cancer Cell Growth by Blocking G_0/G_1-S Phase Progression and Upregulating p27 Protein Levels

In order to identify the active fraction, extracts from the CO112F2-2 cultivar were fractionated into organic acid (OAF), phenolic acid (PF) and anthocyanin fractions (AF) using cartridges as described

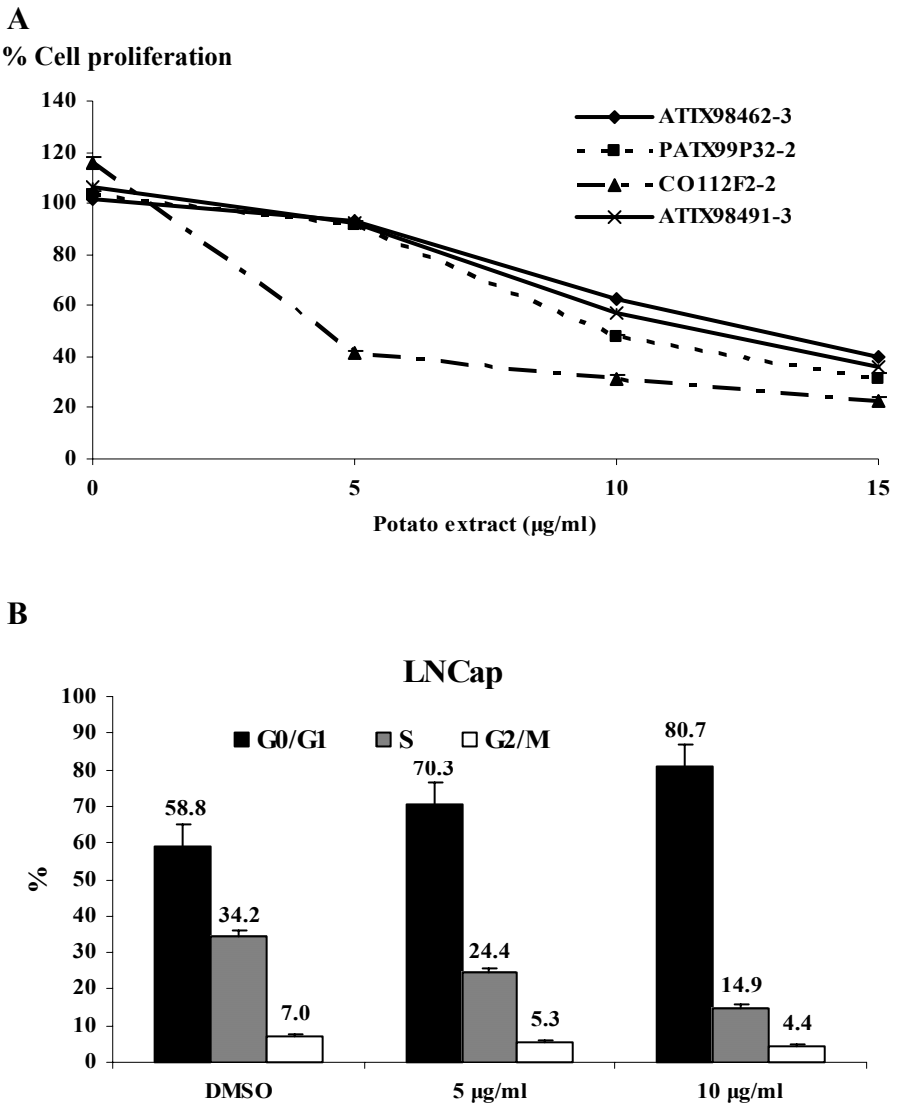

Figure 4.3. Growth inhibition of PC-3 cells and FACS analysis. A. CO112F2-2 Inhibited PC-3 cell proliferation. Cell growth was measured by the WST assay after treatment with potato extracts for 24 h as described in the Materials and Methods. Results are expressed as means ± SE for experiment determinations at each time point. LNCaP (B) and PC-3 (C) cells were treated for 24 h with 5, 10 µg/ml potato extract (CO112F2-2) and analyzed by FACS analysis as described in Materials and Methods. Results are presented as means ± SE for three replicate experiments for each treatment group.

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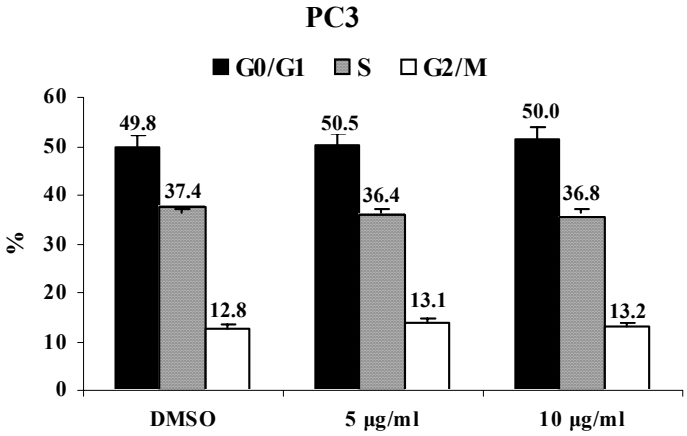

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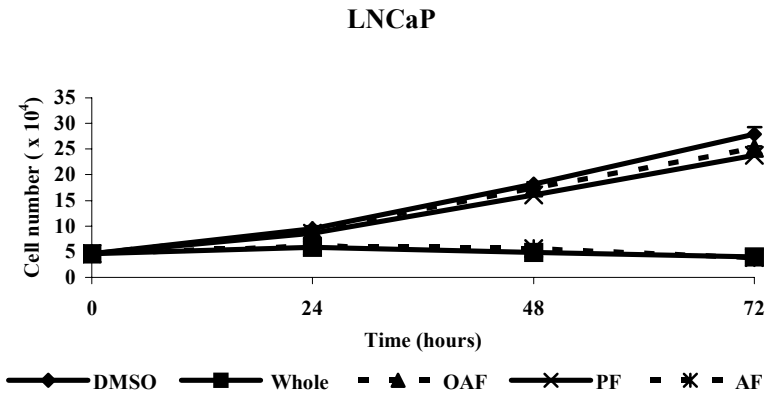

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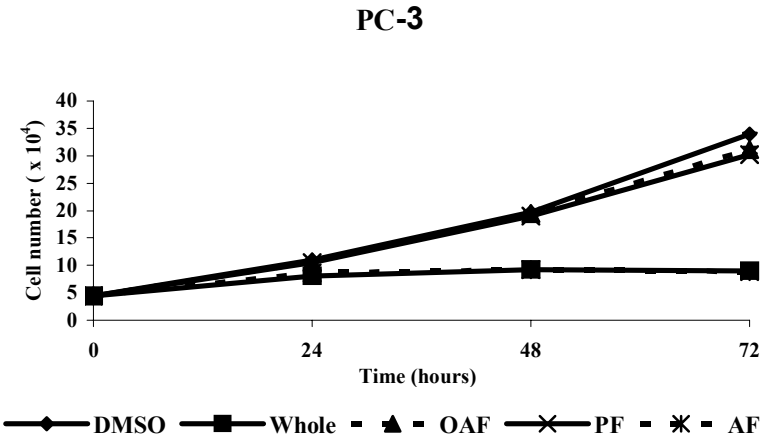
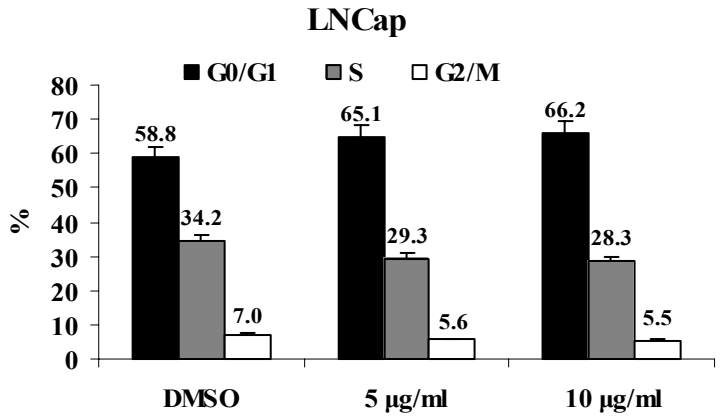


Figure 4.4. Cell growth inhibition and FACS analysis. LNCaP (A) and PC-3 (B) cells were treated with potato extract, organic acid fraction (OAF), phenolic fraction (PF) and anthocyanin fraction (AF) of potato cultivar CO112F2-2 at 5 μg/ml for 72 h. Cell number was determined using a Coulter counter. Results are expressed as means ± SE for three experiments. LNCaP (B) and PC-3 (C) cells were treated for 24 h with 5, 10 μg/ml anthocyanin fraction and analyzed by FACS analysis as described in Materials and Methods. Results are presented as means ± SE for a single experiment.

C



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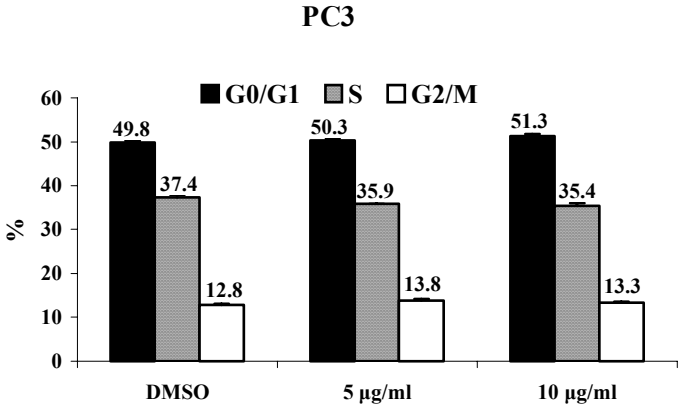

Figure 4.4. Continued.

in the Materials and Methods and previous studies (*196, 197*). **Figure 4.4** illustrates the effects of different fractions on proliferation of LNCaP and PC-3 cells. The effects of the OAF and PF were not significantly different from the solvent control as inhibitors of cell proliferation, even after treatment for 72 h. In contrast, IC50 values for the AF were 2.5 – 5 μg/ml, indicating that the anthocyanin fraction was the most active component of the potato extracts for inhibition of LNCaP and PC-3 cell proliferation.

FACS analysis, which measures the DNA content of the cell, was also used to determine the distribution of both LNCaP and PC-3 cell lines in the G_0-G_1, S and G_2-M phases after treatment of the cells with 5 or 10 μg/ml anthocyanin fraction for 24 h. The anthocyanin fraction significantly increased the percentage of LNCaP cells in the G_0-G_1 phase and decreased in the S phase. However, as observed with the whole cell extract (**Figure 4.3**), the AF did not affect G_0-G_1 - S phase progression in PC-3 cells.

Inhibition of G_0-G_1/S progression has been attributed to over-expression of p27 (cyclin dependent kinase inhibitor) and inhibition of cyclin D1 (*106*). The effects of whole potato extracts and the AF on cyclin D1 and P27 protein expression were investigated in LNCaP and PC-3 cells. The results show that the extract and AF considerably increased p27 protein levels in both LNCaP and PC-3 cell lines after treatment for 6 - 24 or 18 – 24 h respectively (**Figure 4.5**). In contrast, none of the treatments affected levels of cyclin D1 protein.

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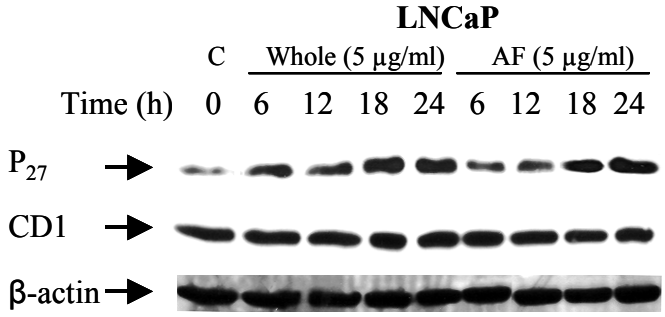

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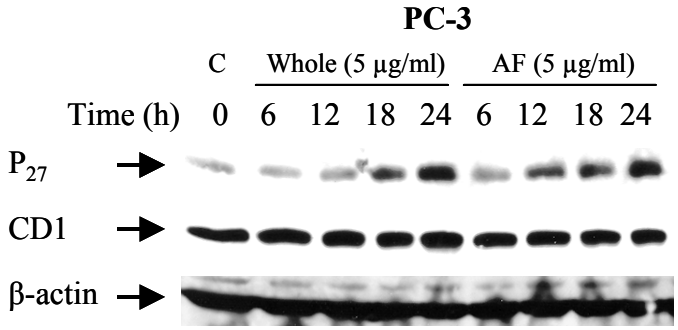

Figure 4.5. Potato extract and anthocyanin fraction increased p27 protein levels in prostate cancer cells. LNCaP (A) or PC-3 (B) cells were treated with 5 µg/ml potato extract or anthocyanin fraction for 6, 12, 18 and 24 h, and whole cell lysates were analyzed by western blotting as indicated in the Materials and Methods. P27 protein levels increased in both LNCaP and PC-3 cell lines after treatment for 6 - 24 or 18 – 24 h, respectively. CD 1 protein levels were similar in all the treatments. Similar results were observed in duplicate experiments.

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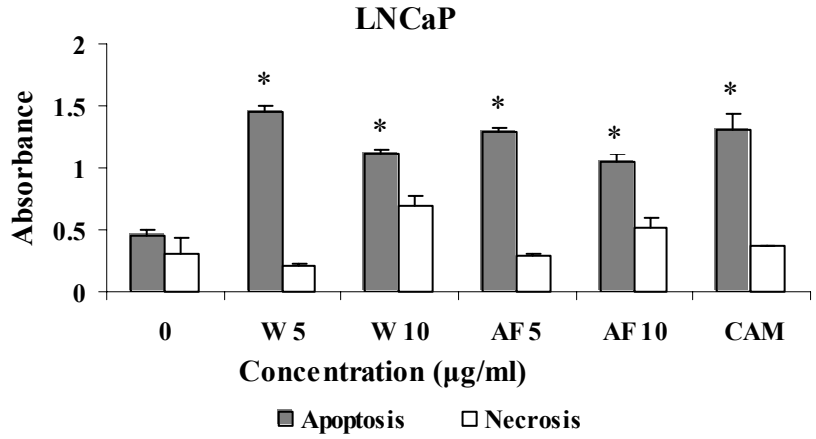

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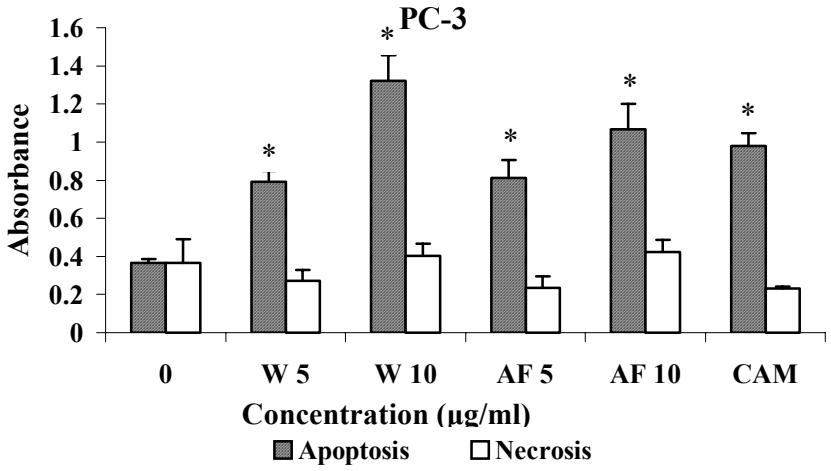
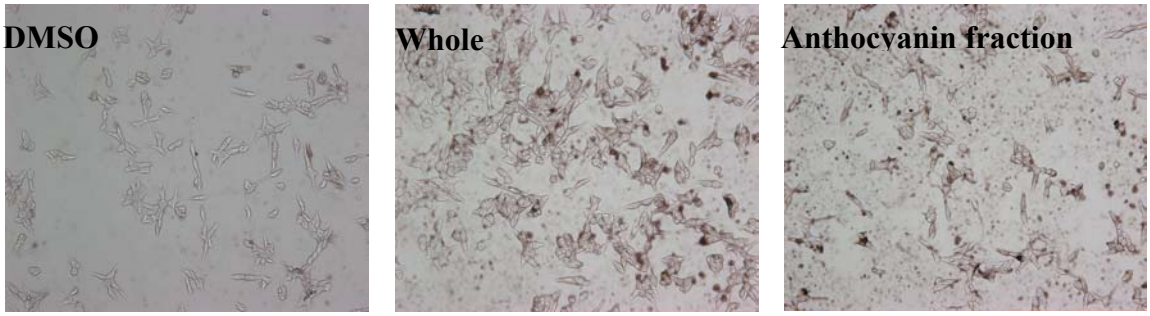


Figure 4.6. Potato extract and anthocyanin fraction (AF) induced apoptosis in LNCaP (A) and PC-3 (B) cells. LNCaP and PC-3 cells were treated with potato extract and AF at 5, 10 µg/ml for 24 h. Absobance is a measure of apoptosis (Cell death detection ELISA kit) and necrosis (LDH assay). Results are presented as means means ± SE of three independent experiments. Significant (Fishers LSD at $p < 0.05$) induction of apoptosis by treatment groups is indicated by (*).

Potato Extract and Anthocyanin Fraction Induced Apoptosis in Prostate Cancer Cells

The potato extracts and anthocyanin fraction (AF) inhibited cell proliferation. Dead cells were observed at higher concentrations or after prolonged treatment (72 h). Therefore, we further investigated whether the observed cell death is due to apoptosis or necrosis in a series of assays. The effects of the potato extract or AF (5 or 10 μg/ml) on prostate cancer cells were investigated using a cell death detection ELISA kit that detects cytoplasmic mono and oligo nucleosomes (**Figure 4.6**). The potato extract and AF significantly induced apoptosis in both cell lines compared to untreated cells, and the apoptotic responses were similar to those observed for camptothecin (CAM), a known apoptotic inducing agent. The TUNEL assay was used to confirm the induction of apoptosis by the potato extract and AF. The characteristic brown staining characteristic of DNA fragmentation was observed (**Figure 4.7A**), and quantification of these results showed (**Figure 4.7B**) significant induction of apoptosis in LNCaP and PC-3 cells after treatment with 5 μg/ml extract and AF.

A



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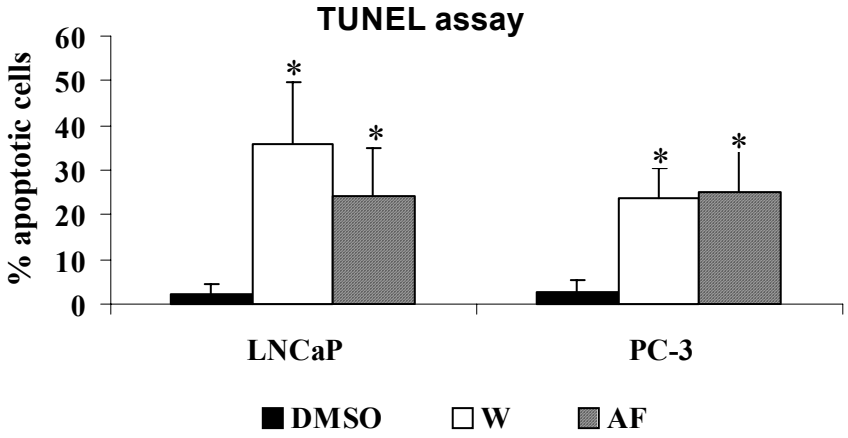

Figure 4.7. TUNEL assay. LNCaP and PC-3 cells were treated with potato extract and anthocyanin fraction for 12 h and analyzed for apoptosis using an In Situ cell death detection POD kit as described in Materials and Methods. A. Brown staining shows apoptotic cells observed under light microscope after adding POD and DAB substrate to treated LNCaP cells. B. Percent apoptotic cells were calculated based on stained cells in 12 fields, each field with 50 cells. W: potato extract (5 µg/ml), AF: Anthocyanin fraction (5 µg/ml); * represents significant difference against control (DMSO) as determined by Fishers LSD ($p < 0.05$).

Potato Extract and Anthocyanin Fraction Induced Both Caspase-dependent and Caspase-independent Apoptosis

The effects of potato extracts and the AF on caspase–dependent PARP cleavage and activation (cleavage) of caspases are illustrated in **Figure 4.8**. Cleaved PARP, cleaved caspase 3, and cleaved caspase 9 proteins were increased in LNCaP cells treated with potato extract or AF for 12, 18, and 24 h (**Figure 4.8A**). Bax protein levels were also induced, and these results confirm that the potato extracts and AF are potent inducers of apoptosis in LNCaP cells. In contrast, none of the treatments significantly affected the levels of cleaved PARP, caspase 3 and caspase 9 proteins (**Figure 4.8B**), whereas Bax protein levels significantly increased in PC-3 cells treated with the whole extract and AF.

Endo G and AIF are proapototic mitochondrial proteins that can be released from the mitochondria by apoptosis-inducing agents or conditions and translocate into nucleus (*198-202*). Both proteins are directly involved in DNA fragmentation and can be important mediators of caspase-independent cell death, although there are also reports that in some cells mitochondrial release of AIF and endonuclease G can be caspase-dependent (*198*). After treatment with the whole potato extract or AF nuclear AIF and endo G protein levels were increased in LNCaP cells (**Figure 4.9A**) and PC-3 cells (**Figure 4.9B**), demonstrating induction of caspase-independent apoptosis in both prostate cancer cell lines.

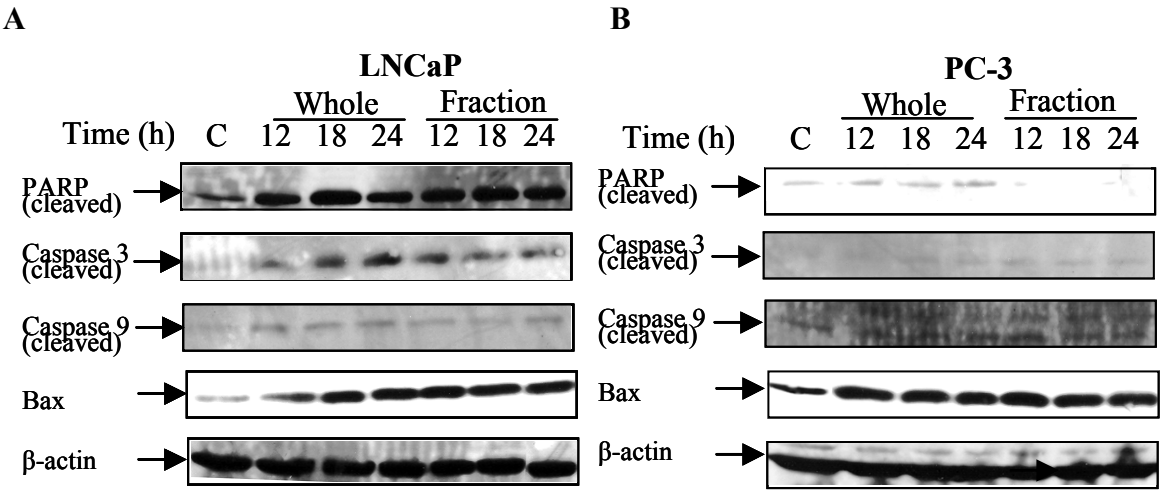

Figure 4.8. Potato extract and anthocyanin fraction caused caspase-dependent apoptosis in LNCaP cells by PARP cleavage and caspase activation. LNCaP (A) or PC-3 (B) cells were treated with whole extract (5 μg/ml), or the anthocyanin fraction (5 μg/ml) for 12, 18 and 24 h. Whole cell lysates were analyzed by western blotting as described in Materials and Methods. Similar results were obtained in duplicate experiments.

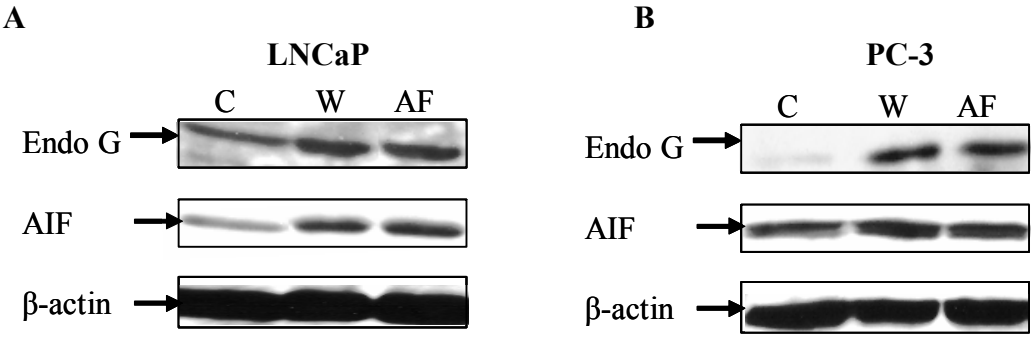

Figure 4.9. Potato extract and anthocyanin fraction increased nuclear Endo G and AIF levels in LNCaP and PC-3 cells. Both cells were treated with potato extract or anthocyanin fraction at 5 μg/ml for 24 h, and nuclear, cytoplasmic and mitochondrial proteins were separated using a mitochondria isolation kit coupled with cytoplasmic and nucleus isolation kits. The nuclear protein fraction was analyzed by western blot analysis as described in Materials and Methods. Similar results were observed in duplicate experiments.

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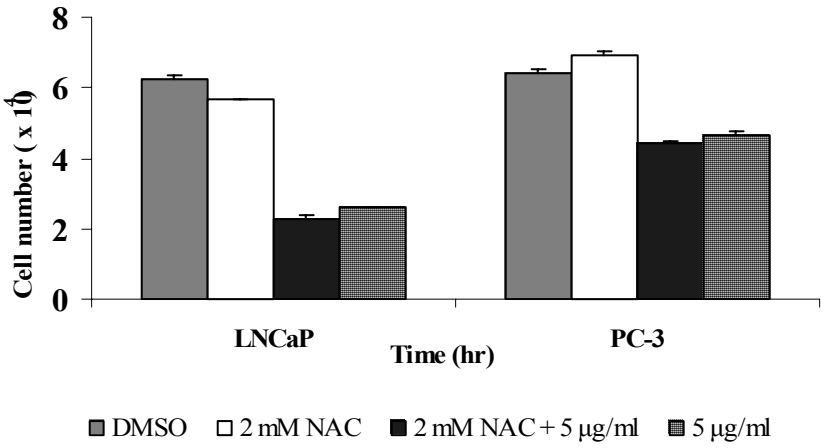
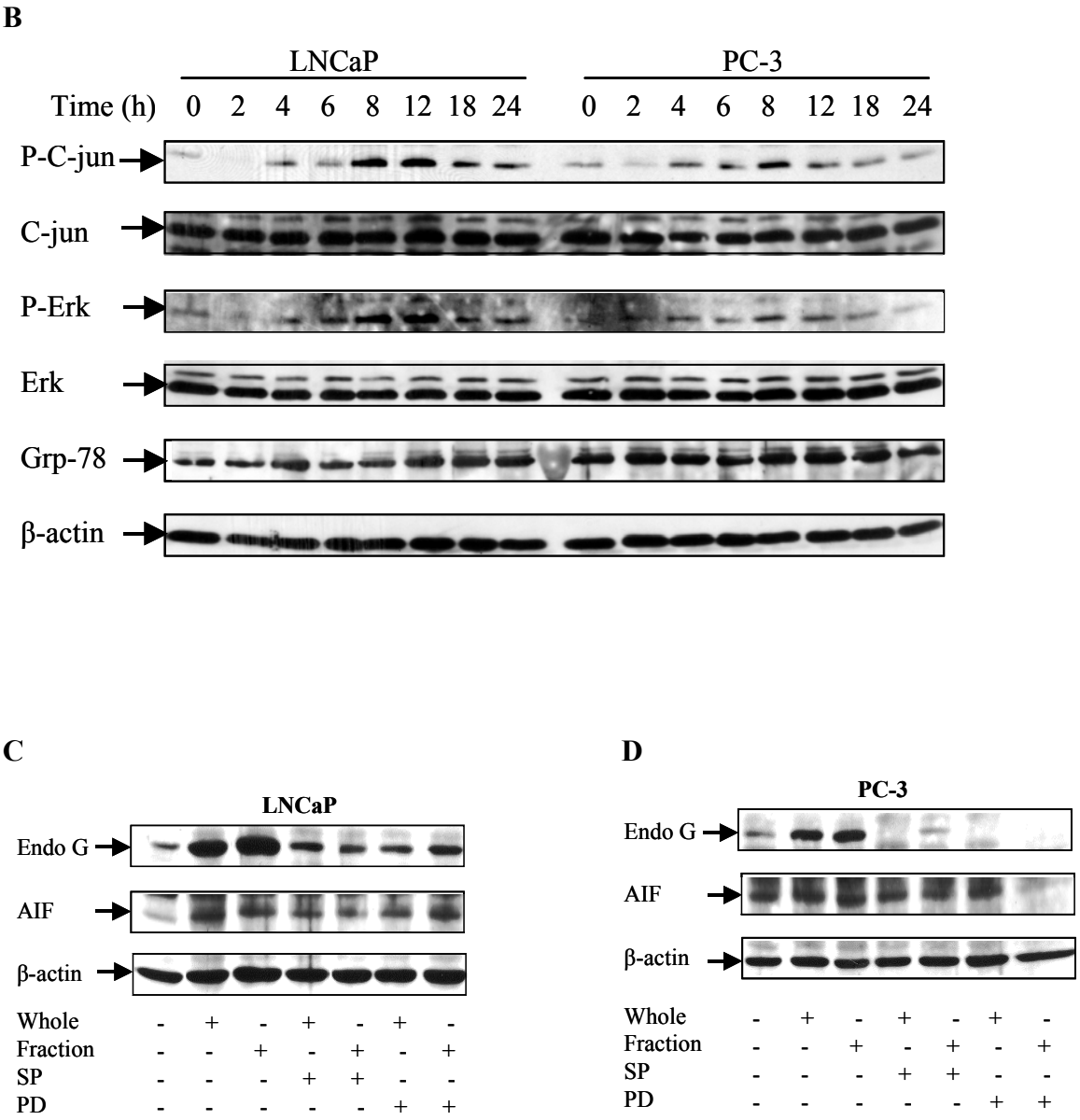


Figure 4.10. N-acetyl cysteine did not alter the anthocyanin fraction (AF)-induced responses in LNCaP and PC-3 cells, but the kinase inhibitors reversed these responses. A. Inhibition of cell growth. PC-3 and LNCaP cells were treated with 5 µg/ml of anthocyanin fraction for 72 h after pretreatment with NAC (2 mM) for 30 min. Cell numbers were analyzed using a Coulter counter. Results represent mean ± SE for duplicate experiment. B. Phosphorylation of kinases by potato extract or AF in LNCaP and PC-3 cells. Cells were treated with the extract/AF for 24 h and whole cell lysates were analyzed by western blot analysis at regular time intervals. Endo G nuclear translocation reversal by kinase inhibitors in LNCaP (C) and PC-3 (D) cells. Cells pretreated with SP (JNK inhibitor - 20µM) or PD (MAPK inhibitor - 20µM) for 30 min were treated with extract/AF at 5 µg/ml for 24 h and the nuclear fraction was analyzed by western blotting. Similar results were observed in duplicate experiments. E. PARP cleavage reversal in LNCaP (E) whole cell lysates. LNCaP cells were treated with 20 µM SP and PD for 30 min before treatment with the extract/AF for 24 h. Lysates were analyzed by western blot analysis.



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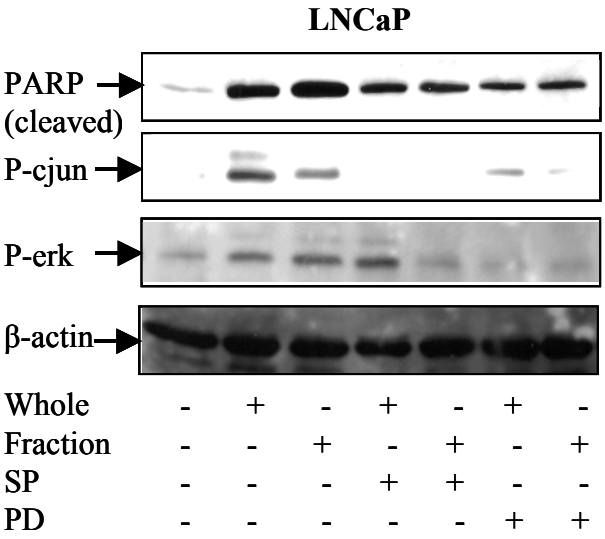

Figure 4.10. Continued.

Redox Status and ER Stress Did Not Affect Potato Extract and AF-induced Apoptosis

Anthocyanins are antioxidants and may modulate cellular redox reactions which can activate apoptosis (*203, 204*); therefore, we investigated the effects of a reactive oxygen species (ROS) scavenger, N-acetyl cysteine (NAC) as a potential inhibitor of extract /AF-induced apoptosis. NAC did not affect the growth inhibitory effects of the AF (**Figure 4.10A**) in PC-3 cells, suggesting that the induction of oxidative stress and/or redox cycling was not associated with the proapoptotic responses induced by AF. Previous studies show that induction of ER stress (*205, 206*) has also been linked to induction of apoptosis. Results summarized in **Figure 4.10B** show that an ER molecular chaperone glucose-regulated protein 78 (GRP 78), a prototypical marker of ER stress (*207, 208*) is not induced by AF in LNCaP or PC3 cells.

Potato Extract and AF Induced JNK and ERK Activation

Activation of kinase pathways such as JNK (*203, 209*) and MAPK (*146*) have been linked to induction of apoptosis. **Figure 4.10B** shows a time-dependent increase in both c-jun and Erk phosphorylation which is indicative of JNK and MAPK activation. Since both of these pathways have been previously associated with induction of apoptosis (*144, 146*), we further investigated the effects of JNK (SP600125) and MAPK (PD98059) inhibitors on induction of apoptosis by potato extracts and AF. The results in **Figures 4.10C and 4.10D** show that both PD and SP significantly decrease nuclear uptake of Endo G and AIF in LNCaP and PC-3 cells treated with the proapoptotic extract/fraction. Moreover, in LNCaP cells where the extract and AF strongly induce

PARP cleavage, co-treatment with PD or SP also inhibited this response (**Figure 4.10E**), further demonstrating the important role of the JNK and MAPK pathway on the proapoptotic activity of potato extracts and the AF in prostate cancer cells. We also investigated the effects of SP and PD on the antiproliferative activity of extracts and AF on growth of PC-3 (**Figure 4.11A**) and LNCaP (**Figure 4.11B**) cells. Although SP alone inhibited growth of PC-3 cells, the results show that both SP and PD significantly reversed the growth inhibitory effects of the potato extract and AF in both prostate cancer cell lines. These data demonstrate for the first time that the proapoptotic activity of potato extracts and the anthocyanin fraction is associated with activation of the JNK and MAPK signaling pathways which are necessary for induction of caspase – dependent apoptosis and nuclear uptake of the proapototic factors Endo G and AIF (caspase – independent).

Discussion

Several reports correlated intake of fruits and vegetables with decreased incidence of several cancers including prostate cancer (*210-216*). The health benefits and anticancer activities derived from consumption of vegetables and fruits are associated with different structural and functional classes of phytochemicals, many of which exhibit both chemopreventive and chemotherapeutic activity. Not surprisingly, natural products and their synthetic analogs are major sources for development of pharmaceuticals (*217*).

We have investigated the growth inhibitory/proapoptotic effects of extracts from various potato cultivars using AR-positive and AR-negative LNCaP and PC-3 prostate

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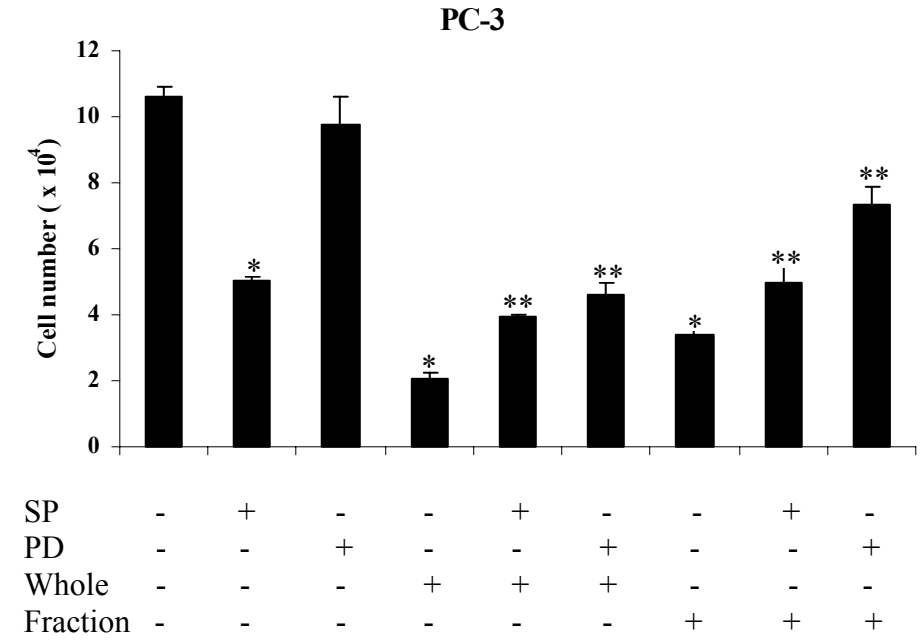

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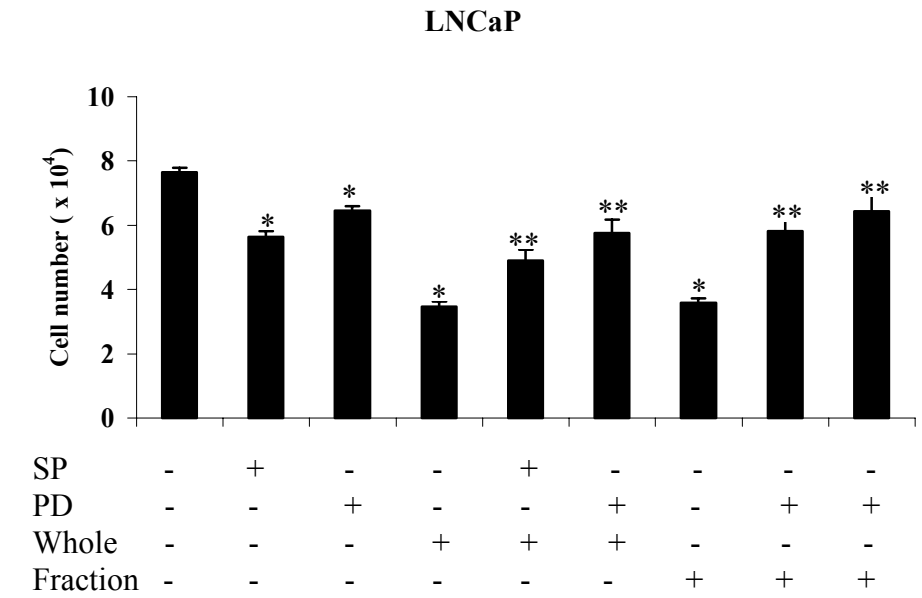


Figure 4.11. Kinase inhibitors reversed the extract or anthocyanin fraction (5 μg/ml)-induced cell growth inhibition in PC-3 (A) and LNCaP cells (B). SP or PD pretreated cells (30 min) were treated with extract or AF for 24 h and cell numbers were determined by a Coulter counter. Results are presented as mean ± SE for duplicate experiments. Significant ($p < 0.05$) inhibition by extracts or kinase inhibitors alone are indicated (*) and significantly increased cell numbers in cotreatment groups are also indicated (**).

cancer cell, respectively. Initial growth inhibition studies (**Figures 4.1-4.3**) clearly show that extracts from the CO112F2-2 cultivar were highly active. This extract also exhibited the highest total phenolic content and antioxidant activity among the cultivar extracts (**Table 4.1**); however, extracts of the PATX99P32-2 cultivar also exhibited comparable antioxidant activity and phenolic content, but were less active in the growth inhibition studies (**Figures 4.1-4.3**). Fractionation of the CO112F2-2 cultivar extracts showed that the anthocyanin fraction (AF) was the most active component and both the potato extract and AF inhibited growth of LNCaP and PC-3 cells and blocked G0/G-S phase progression only in the former cell line (**Figures 4.3** & **4.4**). Despite these differences, potato extract and AF induced the cyclin-dependent kinase inhibitor p27 in both cells (**Figure 4.5**).

The potato extract and AF inhibited growth of LNCaP and PC-3 cells, and after prolonged treatment (72 h and longer), there was a pronounced accumulation of dead cells. Studies in several laboratories have reported that anthocyanins and their glycosides induce death of many different cancer cell lines, and this has been linked to caspase-dependent apoptosis (*130, 144, 145, 183, 218-228*). Results of ELISA and TUNEL assays for apoptosis (**Figures 4.6** & **4.7**) demonstrate that the potato cultivar extract and AF also induced apoptosis in LNCaP and PC-3, and this is inconsistent with the proapototic effects of anthocyanins in other cancer cell lines (*130, 144, 145, 183, 218-228*). Moreover, the extract and AF induced PARP cleavage and activation of caspase 3 (cleavage) in LNCaP cells (**Figure 4.8A**), and these responses are typically observed for anthocyanin-induced caspase-dependent apoptosis in other cancer cell lines (*130, 144,*

145, 183, 218-228). In contrast, PARP cleavage and caspase 3 activation were not observed in AR-negative PC-3 cells (**Figure 4.8B**), demonstrating that anthocyanins differentially induced apoptosis in LNCaP (caspase –dependent) and PC-3 cells, whereas only a caspase-independent pathway appears to be activated in the latter cell line.

Since PC-3 cells did not show caspase-dependent cell death (**Figure 4.8A**) we further investigated the role of AIF and Endo G (caspase-independent factors) in mediating potato extract and AF-induced apoptosis in LNCaP and PC-3 cells (**Figure 4.9**). The results show that both potato extracts and AF induced nuclear uptake of Endo G and AIF in PC-3 and LNCaP cells and demonstrate for the first time that anthocyanins induce caspase-independent apoptosis in prostate cancer cells. Interestingly, the caspase-independent pathway is the major route for cell killing in PC-3 cells, whereas LNCaP cell death induced by the potato extract was both caspase-dependent and caspase-independent.

Previous studies showed that the anthocyanin delphinidin induced bax expression (*228*), and this was similar to potato extract/AF-induced up-regulation of bax observed in this study (**Figure 4.8**). Apoptosis induced by delphinidin in hepatoma cells was associated with induction of oxidative stress and was blocked by NAC and catalase (*228*). Whereas, NAC did not affect AF-induced effects on PC-3 and LNCaP cell survival, suggesting that oxidative stress was not a factor in mediating apoptosis in prostate cancer cells. However, several previous studies have suggested that one of the underlying proapoptotic mechanisms activated by anthocyanins in cancer cell lines involved modulation of kinases (*144, 218, 228*). For example, delphinidin induced

phosphorylation of JNK in hepatoma cells (*228*), malvidin decreased phospho-ERK but increased phosphorylation of p38 in gastric adenocarcinoma cells (*144*), and hibiscus anthocyanins (extract) also activated p38 in promyelocytic leukemia (HL-60) cells (*218*). All of these anthocyanins inhibited cancer cell growth and induced apoptosis, and in both gastric and leukemia cells the p38 inhibitior blocked anthocyanin-induced apoptosis (*144, 218*). The AF from potato extract induced phosphorylation of Erk (**Figure 4.10B**) and c-jun and both the JNK inhibitor SP6000125 and the MAPK inhibitor PD98056 blocked potato extract and/or AF-induced caspase-dependent apoptosis in LNCaP cells (**Figure 4.10E**) and caspase-independent Endo G and AIF nuclear translocation in PC-3 and LNCaP cells (**Figures 4.10C & 4.10D**). Thus, both apoptotic pathways were activated by the AF and extracts from potatoes, and these were dependent on "upstream" activation of the MAPK and JNK pathways which appeared to act cooperatively since inhibition of the either kinase was sufficient to block apoptosis.

In summary, this study has identified a specialty potato cultivar (CO112F-2) that contains phytochemicals in the anthocyanin fraction that inhibit LNCaP and PC-3 cell growth and induce apoptosis. Cell death pathways activated by the AF are associated with induction of both MAPK and JNK in both cell lines, and these kinases cooperatively induce caspase-independent cell death in both cell lines and also caspase-dependent apoptosis in LNCaP cells. This study demonstrates for the first time that AF/potato extract induce mitochondrial release and nuclear uptake of the proapoptotic Endo G and AIF proteins. This represents an important and hitherto undetected mechanism of anticancer action for these phytochemicals in prostate cancer cells, and

has not been reported in other cancer cell lines. Current studies are focused on identifying individual components of the AF responsible for the induction of cell death pathways in prostate and other cancer cell lines. This will facilitate further development of potato cultivars which overexpress specific phytochemicals and thereby provide enhanced chemopreventive activity against cancer.

CHAPTER V

POTATO FUNCTIONAL COMPOUNDS CHACONINE AND GALLIC ACID REDUCE CELL PROLIFERATION AND UPREGULATE APOPTOSIS IN PROSTATE CANCER CELLS

Introduction

Epidemiological studies have shown that diets rich in fruits and vegetables have preventive effects on chronic diseases including prostate cancer (*140, 183, 184*). The preventive effects associated with vegetable consumption can be attributed in part to their bioactive compounds (*76, 185*). Imbalance in the processes of cell proliferation, and apoptosis through increased prostatic epithelial cell proliferation and reduced cell death play an important role in prostate cancer promotion and progression (*94*).

A number of studies have examined the antiproliferative and proapoptotic effects of plant bioactive compounds including phenolic acids, flavonoids, anthocyanins and glycoalkaloids (*145, 229-236*). Phenolic acids inhibited the development of tongue neoplasms, and reduced the incidence colonic aberrant crypt foci and adenoma in stomach in F344 rats (*137*). Caffeic acid phenyl ester suppressed proliferation of HO-1 (human melanoma cells), GBM-18 (human glioblastoma) (*189*). Anthocyanins and their aglycones such as cyanidin, delphinidin, malvidin, pelargonidin and peonidin exhibit antiproliferative and proapototic properties in gastric adenocarcinoma, HT-29 and Caco-2 (colon cancer) and BAEC bovine aortic endothelial cells and also protect against esophageal cancer in rodents (*144, 146, 147, 183*). Glycoalkaloids showed

antiproliferative properties against human colon, liver, cervical, lymphoma and stomach cancer cells (ref).

Uncontrolled cell proliferation involves molecular aberrations such as CD1 overexpression and p27 (cyclin dependent kinase inhibitor) downregulation. An increase in p27 expression, reduction in CD1 and upregulation of apoptosis results in decreased tumor growth (*109*). Apoptosis can be induced by either caspase-dependent (intrinsic or extrinsic) or –independent pathways. Intrinsic caspase-dependent apoptosis, which involves activation of capases-3, -9 and cleavage of PARP, is the most common pathway through which many of the naturally occurring bioactive compounds induce apoptosis (*192, 237-240*).

In this study we have investigated the effects of different phenolic compounds (chlorogenic acid, caffeic acid, gallic acid, catechin, malvidin) and glycoalkaloids (chaconine and solanine) present in specialty potatoes on PC-3 (androgen independent) and LNCaP (androgen dependent) prostate cancer cells in order to identify the most active growth inhibiting compound. Chaconine and gallic acid showed potent antiproliferative properties and increased levels of the cyclin-dependent kinase inhibitor p27 in both LNCaP and PC-3 cell lines. Gallic acid decreased cyclin D1 levels in LnCap cells. Chaconine and gallic acid induced caspase-dependent apoptosis through PARP cleavage and activation of caspase-3 and phosphorylation of c-jun in LNCaP cells. Induction of apoptosis in PC-3 cells might be caspase-independent.

Materials and Methods

Chemicals

DMSO, chlorogenic acid, cafeic acid, gallic acid, catechin, α - solanine and α - chaconine were obtained from Sigma (St. Louis, MO) and malvidin was purchased from Indofine Chemical Company (Hillsborough, NJ). 4-(3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate (WST-1), cell death detection ELISA kit and In situ cell death detection-POD kit (TUNEL assay) were obtained from Roche Applied Sciences (Indianapolis, IN). Antibodies for p27, cyclin D1, pc-jun, p-erk, c-jun, erk, were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), cleaved PARP, cleaved capases 3, 9 and cytochrome C were obtained from Cell Signaling Technology (Beverly, MA). Western lightning™ chemiluminiscence reagents were from PerkinElmer Life Sciences (Boston, MA).

Cell Lines

Human prostate carcinoma cell lines PC-3 (androgen-independent) and LNCaP (androgen-dependent) were obtained from the American Type Culture Collection (Manassas, VA) and cells were maintained at 37^0 C in 5% CO_2 jacketed incubator in RPMI 1640 (Sigma; St. Louis, MO) supplemented with 2.38 g/L HEPES 2.0 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 4.5 g/L glucose, 100 ml/L FBS, and 10 mL/L antibiotic antimycotic solution (Sigma).

Cell Proliferation Assay

Cell proliferation was also determined using a Coulter counter. Cells were plated at a density of $2.5x10^4$ cells/well in 12-well plates and after 24 h, DMEM F-12 media

containing 2.5% charcoal-stripped FBS was used and cells were treated with DMSO (solvent control) and different concentrations of the compounds. Fresh media along with compounds was added every 48 h. Cells were counted after 24, 48, and 72 h using a Z1 Coulter Counter, each experiment was carried out in triplicate and results were expressed as means ± SE.

Apoptosis

DNA Fragmentation was measured using an Enzyme-linked immunosorbent assay (Cell Death Detection ELISA; Roche Diagnostics, Indianapolis, IN) in both LNCaP and PC-3 cell lines. Cells were seeded in a 48 well plate at a density of $2.5x10^4$ cells/well. Cells were incubated for 24 h with the compounds, then scraped and pelleted by centrifugation. Cells were then lysed and diluted to $1x10^4$ cells/ml with incubation buffer and the color development was measured according to the manufacturer's manual. The supernatant from the pelleted cells was used to measure cytotoxicity using an LDH cytotoxicity detection kit.

TUNEL Assay

Cells ($4x10^4$) were seeded in four chambered glass slides and after treatment for 12 h the In situ cell death detection POD kit was used for the TUNEL assay according to the instruction manual protocol for fixed cells. After the incubating cells with POD and DAB substrate, apoptotic DNA fragmentation was detected by visualizing labeled DNA using a light microscope. Slides incubated without TdT served as a negative control, and slides treated with 1000U DNAse I/ml for 10 min before TdT exposure

served as a positive control. The percentage of apoptotic cells were calculated by counting the stained cells in 12 fields each containing 50 cells.

Western Blot Analysis

LNCaP and PC-3 cells were seeded at a density of $1.5x10^5$ cells/ml in DMEM F-12 media with 2.5% charcoal-stripped FBS for 24 h. Cells were treated with DMSO or the compounds for 24 and 48 h. Protein was extracted into high salt buffer containing 1% proteinase inhibitor cocktail and protein levels were quantified using the Bradford reagent. Protein (60 μg) was loaded per lane on acrylamide gel (depending on the weight of the protein 10%, 12%, or 15% gel is used) after boiling the sample for 3 min at 100^o C and subjected to SDS-PAGE at 120 V for 3 to 4 h. Proteins were transferred by wet blotting onto 0.2 μm PVDF membrane (polyvinylidene difluoride; Bio Rad, CA). Membranes were blocked using 5% milk in TBST for 30 min and incubated with primary antibody either in fresh 5% milk in TBST or 3% BSA in TBST overnight at 4^o C with gentle shaking followed by secondary antibody. Concentrations of primary and secondary antibodies ranged from 1:250 to 1:5000 and 1:5000 respectively (1:250 for CD1, 1:500 for p27, Bax, c-jun, erk, p-c-jun, p-erk, AIF, 1:1000 for cleaved caspases, cleaved PARP, GRP78, endo G, and 1:5000 for β-actin). After incubating with chemiluminescence solution (PerkinElmer Life Sciences) for 1.0 min the membrane was washed for 10 min and exposed to X-ray film and developed.

Results

Among Specialty Potato Phenolic Acids and Flavanols, Gallic Acid Was the Most Potent Inhibitor of PC-3 and LnCap Cell Proliferation

Enhanced cell proliferation coupled with decreased cell death leads to cancer progression and compounds, which exhibit antiproliferative activity, may play a role in blocking tumor progression. The growth inhibitory effects of the phenolic acids and catechin were investigated using androgen receptor (AR) – positive LNCaP and AR – negative PC-3 prostate cancer cell lines in order to identify the most potent inhibitor. LNCaP and PC-3 cells were grown in 2.5% charcoal-stripped serum for 24 h and treated with DMSO (solvent control) and phenolic acids and catechin. **Figure 5.1** illustrates the effects of different concentrations chlorogenic acid, caffeic acid, gallic acid and catechin on proliferation of LNCaP cells and the results show that the IC50 values for gallic acid (10 – 25 μg/ml) was lower than the other phenolic acids and catechin where IC50 values were 50 – 100 μg/ml. The relative growth inhibitory potencies were gallic acid > caffeic acid > chlorogenic acid ≈ catechin. Similar cell proliferation study was also carried out in PC-3 cells and the results (**Figure 5.2**) were comparable to those observed in LNCaP cells with gallic acid as the most potent inhibitor of cell proliferation. The growth inhibitory effects of phenolic acids and catechin on PC-3 cells showed that their relative antiproliferative activities were in the order gallic acid > caffeic acid > chlorogenic acid > catechin.

LNCaP

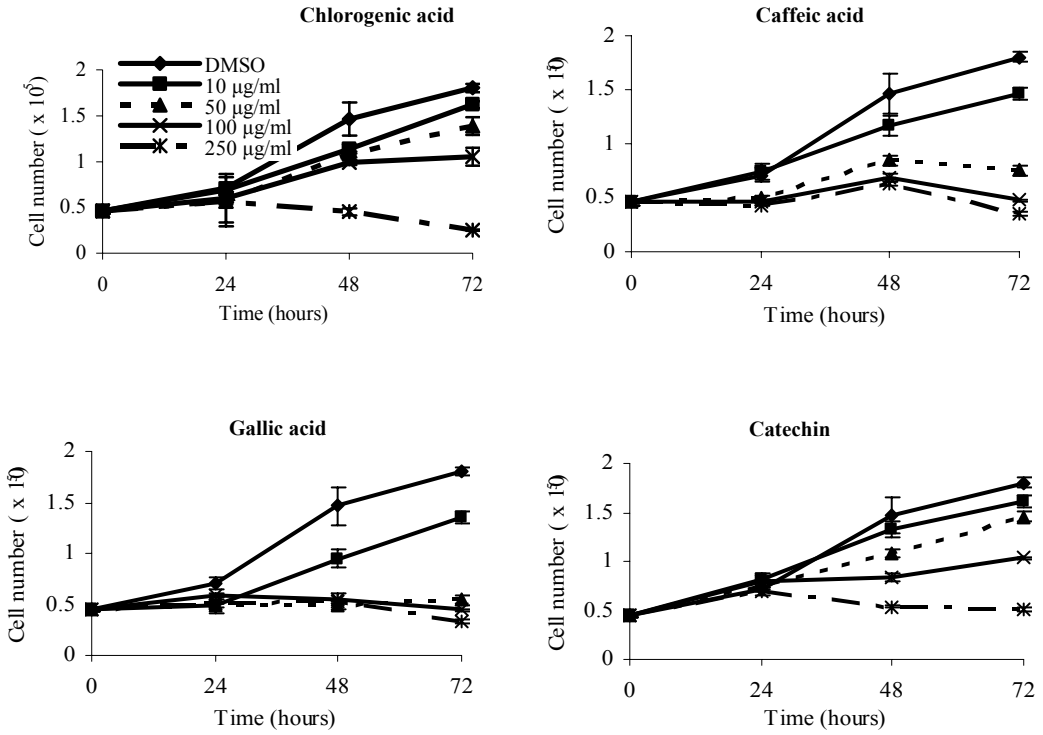


Figure 5.1. Inhibition of LNCaP cell growth by phenolic acids and catechin. LNCaP cells were treated with chlorogenic acid, caffeic acid, gallic acid (phenolic acids) and catechin (flavanol) at 10 – 250 µg /ml for 72 h and cell numbers were determined using a Coulter counter. Results are expressed as means ± SE for three experiments at each time point.

PC-3

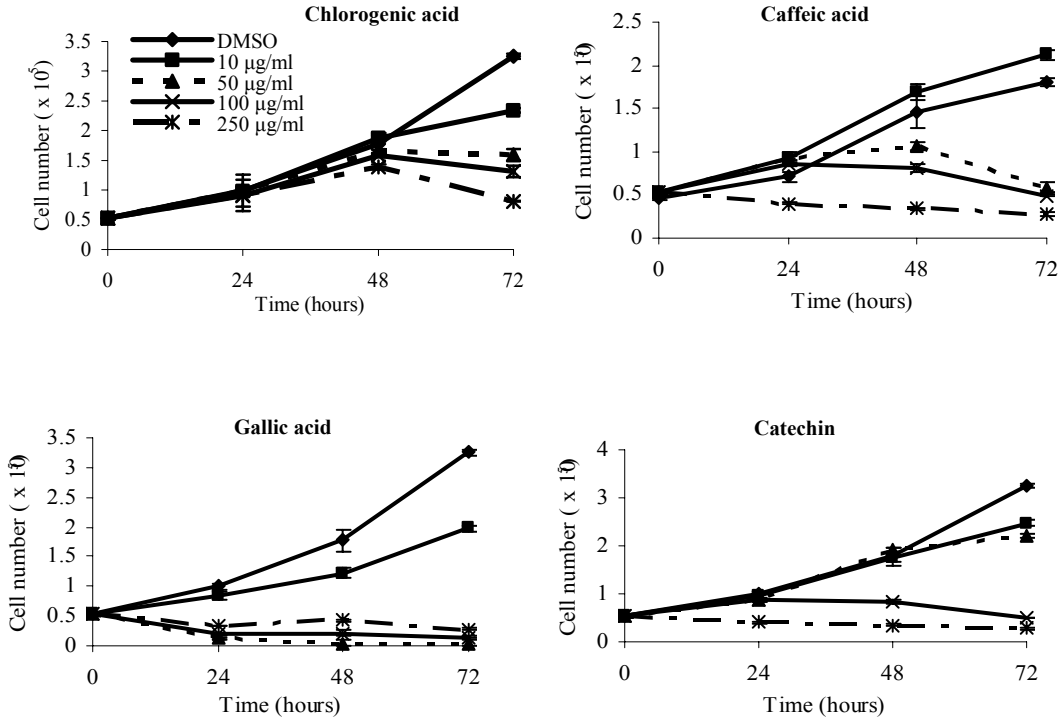
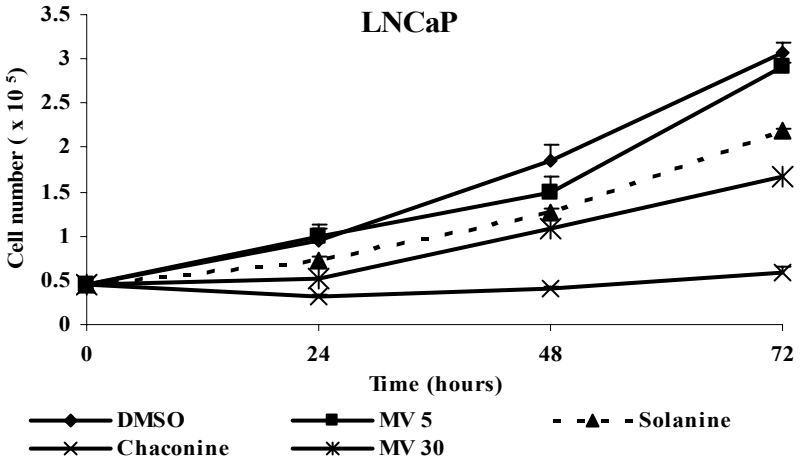


Figure 5.2. Inhibition of PC-3 cell growth by phenolic acids and catechin. PC-3 cells were treated with chlorogenic acid, caffeic acid, gallic acid (phenolic acids) and catechin (flavanol) at 10 – 250 µg /ml for 72 h and cell numbers were determined using a Coulter counter. Results are expressed as means ± SE for three experiments at each time point.

A



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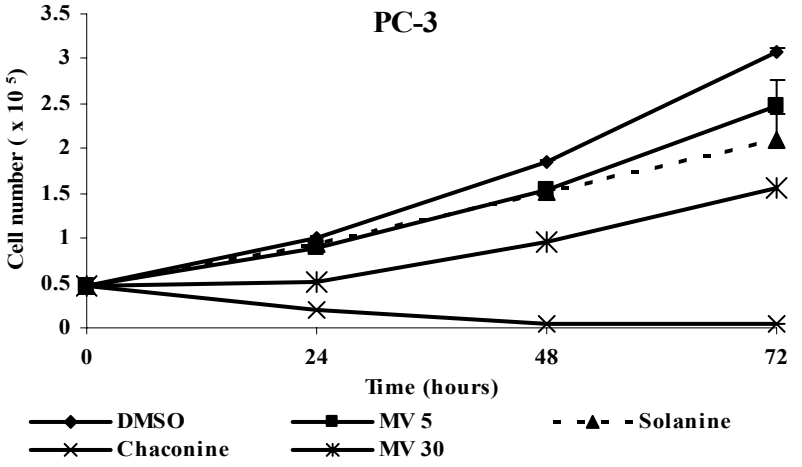


Figure 5.3. Alkaloids and malvidin as inhibitors of prostate cancer cell proliferation. LNCaP (A) and PC-3 (B) cells were treated with malvidin at 5 and 30 μg/ml, and solanine and chaconine at 5 μg/ml for 72 h. Cells number was determined using a Coulter counter. Results are expressed as means ± SE for three experiments.

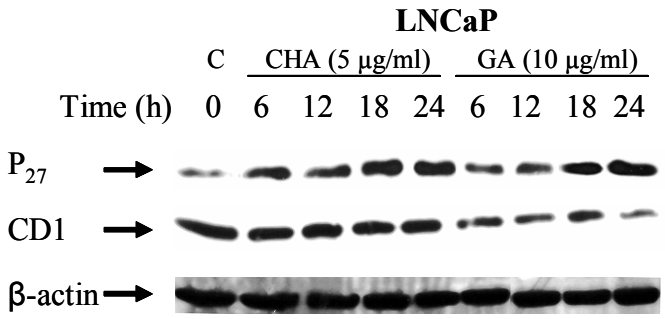
Chaconine Inhibited the Cell Proliferation of LNCaP and PC-3 Cells

The growth inhibitory effects of other bioactive compounds present in specialty potatoes such as anthocyanins and glycoalkaloids were also investigated in LNCaP and PC-3 cells. **Figure 5.3** illustrates the growth inhibitory effects of malvidin, α – solanine and α – chaconine at different concentrations on LNCaP and PC-3 cells. All three compounds exhibited antiproliferative activity and IC50 values were lower for α – chaconine (2.5 – 5 μg/ml) than α – solanine or malvidin indicating that of the potato bioactive compounds α – chaconine was the most active inhibitor of LNCaP and PC-3 cell proliferation.

Chaconine and Gallic Acid Modulate p27 and CD1 Protein Levels in LNCaP and PC-3 Cells

P27 and cyclin D1 (CD1) are important cell cycle proteins and CD1 overexpression and decreased p27 levels are common molecular aberrations in many cancers including prostate cancer, which leads to enhanced cell proliferation. The effects of chaconine and gallic acid on CD1 and p27 protein expression were investigated in LNCaP and PC-3 cells and chaconine and gallic acid increased p27 protein levels in LNCaP and PC-3 cells after treatment for 6 - 24 or 18 – 24 h respectively (**Figure 5.4**). Chaconine did not affect levels of cyclin D1 protein in these cells, whereas gallic acid significantly decreased CD1 in LNCaP cells.

A



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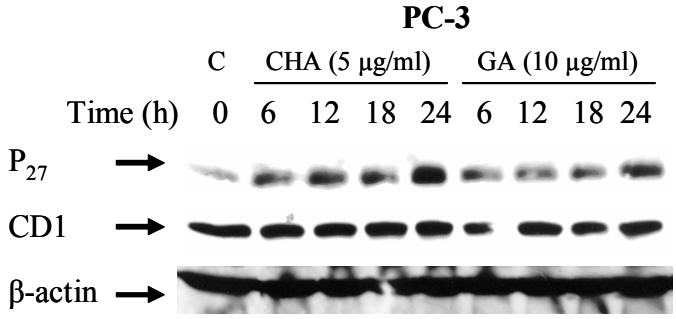


Figure 5.4. Effects of chaconine and gallic acid on CD1 and p27 expression in prostate cancer cells. LNCaP (A) or PC-3 (B) cells were treated with chaconine (5 µg/ml) and gallic acid (10 µg/ml) for 6, 12, 18 and 24 h and whole cell lysates were analyzed by western blotting as indicated in the Materials and Methods. Similar results were observed in duplicate experiments.

A

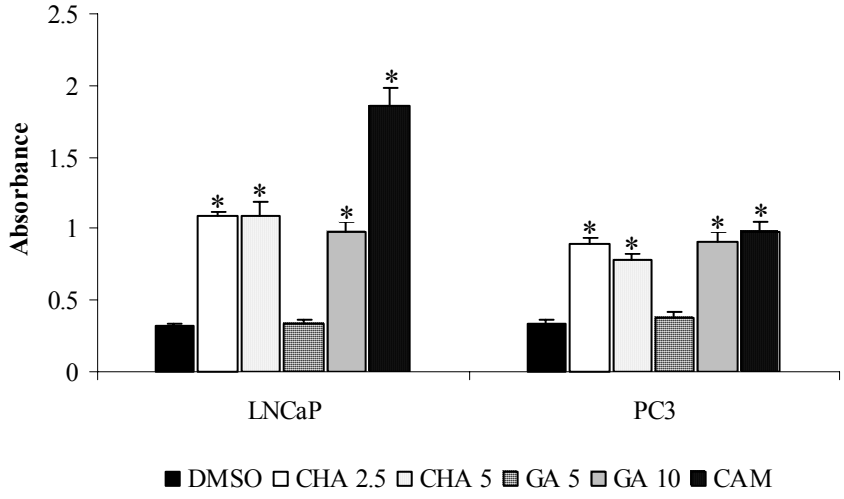
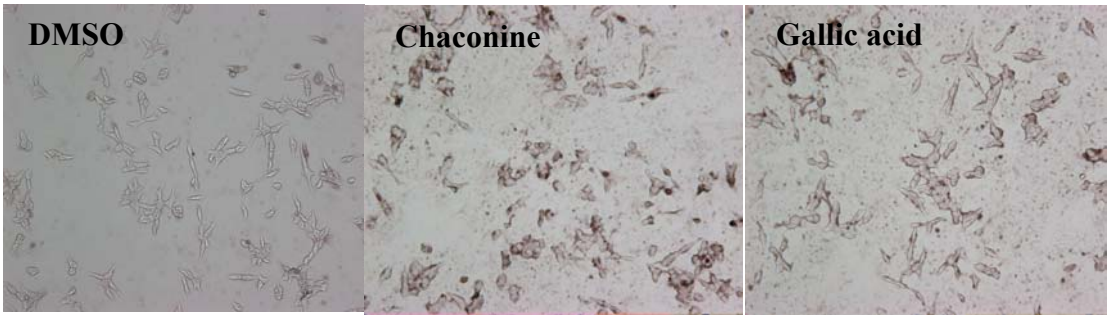


Figure 5.5. Chaconine and gallic acid (AF) induced apoptosis in LNCaP (A) and PC-3 (B) cells. LNCaP and PC-3 cells were treated with 2.5, 5 µg/ml chaconine (CHA) and 5, 10 µg/ml gallic acid (GA) for 24 h. Absobance is a measure of apoptosis (Cell death detection ELISA kit). Results are presented as means means ± SE of three independent experiments. Significant (Fishers LSD at $p < 0.05$) induction of apoptosis by treatment groups is indicated by (*).

Chaconine and Gallic Acid Induce Apoptosis in Prostate Cancer Cells

Cancer cells are characterized by increased cell proliferation and decreased apoptosis or programmed cell death. Chaconine and gallic acid inhibit cell proliferation, and higher concentrations for prolonged time periods caused cell death. A cell death detection ELISA kit was used to investigate the cell death pathways induced by chaconine (2.5 or 5 µg/ml) and gallic acid (5 or 10 µg/ml). The results show that chaconine and gallic acid (10 µg/ml) significantly induced apoptosis in both cell lines compared to untreated cells and the apoptotic responses were similar to those observed for camptothecin (CAM) a known apoptotic inducing agent (**Figure 5.5**). The TUNEL assay was also used to confirm that chaconine and gallic acid induced apoptosis in LNCaP and PC-3 cells. The TUNEL assay results in brown staining of apoptotic nuclei and this is characteristic apoptotic DNA fragmentation (**Figure 5.6A**). Quantification of TUNEL assay results showed (**Figure 5.6B**) significant induction of apoptosis in LNCaP and PC-3 cells after treatment with 2.5 and 10 µg/ml chaconine and gallic aicd respectively.

A



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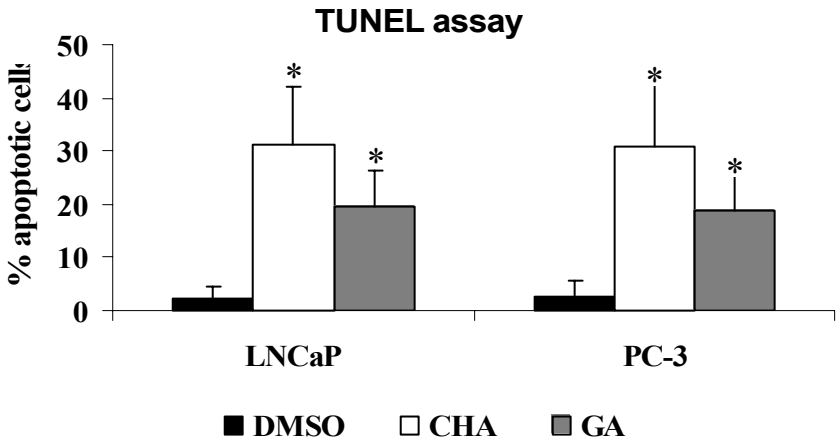

Figure 5.6. Brown staining of apoptotic nuclei -TUNEL assay. LNCaP and PC-3 cells were treated with chaconine and gallic acid for 12 h and analyzed for apoptosis using an In Situ cell death detection POD kit as described in Materials and Methods. A. Brown staining shows apoptotic cells observed under light microscope after adding POD and DAB substrate to LNCaP cells. B. Chaconine and gallic acid increased % apoptotic cells in LNCaP and PC-3 Cells. Percent apoptotic cells were calculated based on stained cells in 12 fields, each field with 50cells. CHA: Chaconine (2.5 µg/ml), GA: gallic acid (10 µg/ml); * represents significant difference against control (DMSO) as determined by Fishers LSD ($p < 0.05$).

Chaconine and Gallic Acid Induced Apoptosis Through Caspase-3 Activation in LNCaP Cells

Induction of apoptosis can be either caspase-dependent or caspase-independent. The effects of chaconine and gallic acid on caspase – dependent PARP cleavage and activation (cleavage) of caspases are illustrated in **Figure 5.7**. Chaconine and gallic acid increased PARP, caspase 3, and caspase 9 cleavage proteins in LNCaP cells treated for 12 and 18 h (**Figure 5.7A**). These results confirm that the chaconine and gallic acid are potent inducers of apoptosis in LNCaP cells. In contrast none of the treatments significantly affected levels of cleaved PARP, caspase 3 and caspase 9 proteins (**Figure 5.7B**) in PC-3 cells.

Previous studies show that activation of kinase pathways such as JNK (*150, 209*) and MAPK (*146*) have also been linked to induction of apoptosis. **Figure 5.8** illustrates that chaconine and gallic acid induce a time dependent increase of both c-jun and Erk phosphorylation in LNCaP cells and this was of JNK and MAPK activation.

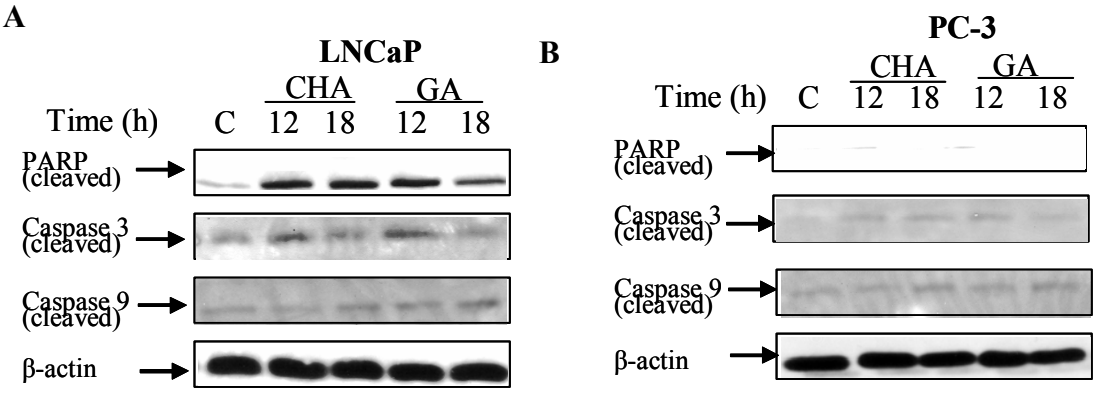

Figure 5.7. Modulation of apoptotic proteins in LNCaP (A) or PC-3 (B) cells. Both cell lines were treated with chaconine (2.5 µg/ml), or gallic acid (10 µg/ml) for 12 and 18 h. Whole cell lysates were analyzed by western blotting as described in Materials and Methods. Similar results were obtained in duplicate experiments.

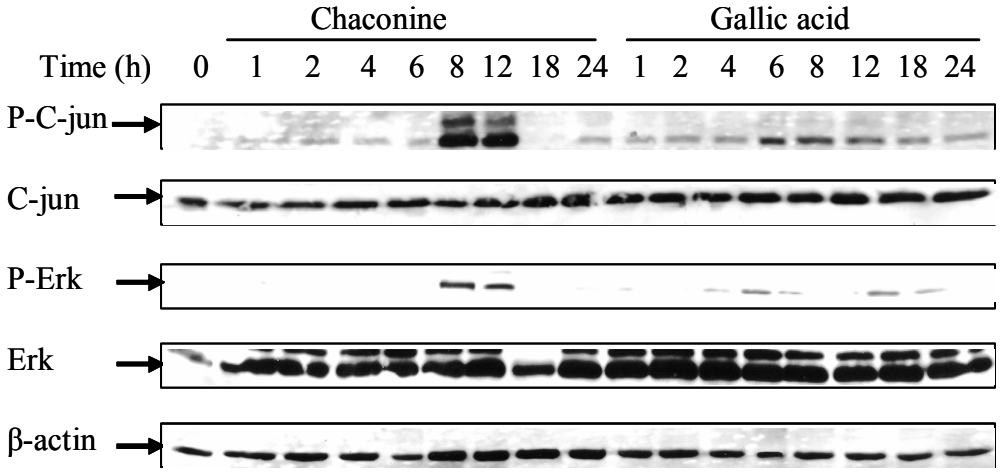

Figure 5.8. Chaconine and gallic acid caused time dependent increase in p-c-jun and p-erk levels in LNCaP cell lines. Cells were treated with the chaconine (5 µg/ml) and gallic acid (10 µg/ml) for different times up to 24 h and whole cell lysates were analyzed by western blot analysis.

Discussion

The health benefits and anticancer activities derived from consumption of vegetables and fruits are associated with different structural and functional classes of phytochemicals, many of which exhibit both chemopreventive and chemotherapeutic activity (*79, 241*).

In this study we have investigated the growth inhibitory effects of phenolic acids, catechin, malvidin and glycoalkaloids and the proapoptotic effects of chaconine and gallic acid in AR-positive and AR-negative LNCaP and PC-3 prostate cancer cells respectively. Growth inhibition studies (**Figures 5.1-5.3**) clearly show that chaconine and gallic acid were highly active and decreased LNCaP and PC-3 cell proliferation which was associated with induction of cyclin-dependent kinase inhibitor p27 in both cell lines (**figure 5.4**). However cyclin D1 regulation by gallic acid was cell context dependent. Gallic acid caused a time dependent decrease in CD1 levels in LNCaP cells but not in PC-3 cells and chaconine did not affect CD1 levels in either cell line.

Several studies have reported that phenolic acids and glycoalkaloids induce death of many different cancer cell lines and this has been linked to caspase-dependent apoptosis (*140, 192, 242*). Results of ELISA and TUNEL assays for apoptosis (**Figures 5.5** & **5.6**) demonstrate that chaconine and gallic acid also induce apoptosis in LNCaP and PC-3 cells, and this is in accordance with the proapototic effects of these compounds in other cancer cell lines (*140, 192, 242*). Chaconine and gallic acid induced PARP cleavage and activation of caspase 3 (cleavage) in LNCaP cells (**Figure 5.7A**) and these responses are typically observed for phenolic acid and glycoalkaloid-induced caspase-

dependent apoptosis in other cancer cell lines (*140, 192, 242*). In contrast, PARP cleavage and caspase 3 activation were not observed in AR-negative PC-3 cells (**Figure 5.7B**) demonstrating that the induction of apoptosis by these compounds is cell context dependent. Previous studies show that one of the underlying proapoptotic mechanisms activated by phenolics in cancer cell lines involved modulation of kinases (*242, 243*). Chaconine and gallic acid caused JNK and MAPK activation through phosphorylation of c-jun and erk.

In summary, this study has shown that chaconine and gallic acid are the most potent inhibitors of LNCaP and PC-3 cell growth compared to other phenolics and glycoalkaloids found in potato. Chaconine and gallic acid also increased p27 levels and growth inhibition of both prostate cancer cells. Chaconine and gallic acid induced apoptosis in both LNCaP and PC-3 cells however, these effects were associated with caspase-dependent and caspase-independent pathways respectively (**Figure 5.7**). Current studies are focused on chaconine and gallic acid induced caspase-independent cell death in PC-3 cells and mechanisms of these induced responses.

CHAPTER VI

SUMMARY

Antioxidant activity (AOA) of specialty (colored) potato selections from the Texas Potato Variety Development Program ranged from 157 µg trolox equivalents / gfw (µg TE/gfw) to 832 µg TE/gfw and 810 µg TE/gfw to 1622 µg TE/gfw using the DPPH and ABTS assays, respectively. Total phenolics (TP) ranged from 221µg chlorogenic acid equivalents / gfw (µg CGAE/gfw) to 1252 µg CGAE/gfw. Purple flesh selections had the highest AOA and TP followed by red flesh and yellow flesh selections. Selections with similar flesh color did not differ significantly in AOA and TP. Chlorogenic acid, gallic acid, catechin, caffeic acid, and malvidin-3-(p-coumaryl rutinoside)–5-galactoside were the major phenolics identified. Chlorogenic acid accounted for 50 – 70% of TP followed by catechin, gallic acid and caffeic acid. Chlorogenic acid contributed 28 – 45% to AOA followed by gallic acid, catechin and caffeic acid. Total carotenoid content ranged from 96 µg LE/100 gfw to 930 µg LE/100 gfw. Lutein and violaxanthin were the two major carotenoids identified.

Along with genotype, location also significantly affected AOA, TP, TC and phenolic composition of potatoes. Overall, CO112F2-2 had higher AOA, TP and TC. McCook had significantly higher AOA (29%), TP (7%) and CGA (20%) than Dalhart, and Dalhart had significantly higher TC (13%) than McCook. Year had a significant influence on TP and TC. Significant positive correlations were observed between AOA-TP, AOA-CGA and TP-CGA. The influence of genotype was highly significant for AOA, TP, TC, and phenolic composition and accounted for more than 55% of total

variation. Location, year and interaction effects were significant and accounted for only 1%-23% of total variation for all measured parameters.

Specialty potato selection CO112F-2 and the anthocyanin fraction (AF) inhibited LNCaP and PC-3 cell growth and induced apoptosis. Inhibition of cell growth by potato extract and AF involved blockage of G0/G1-S phase progression and activation of p27, a cyclin dependent kinase inhibitor. Cell death pathways activated by the AF are associated with induction of both JNK (c-Jun NH2-terminal Kinase) and Erk (extracellular signal regulated kinase) in both cell lines, and these kinases induced caspase-independent cell death in both cell lines and also caspase-dependent apoptosis in LNCaP cells. Caspase-dependent apoptosis in LNCaP cells was associated with cleavage of poly (ADP-ribose) polymerase (PARP), activation of caspase-3 and caspase-9. Extract and AF-induced caspase-independent apoptosis in both cell lines involved translocation of apoptosis inducing factor (AIF) and endonucleage G (endo G) from mitochondria to nucleus.

Among different phenolic acids, catechin, malvidin and glycoalkaloids, gallic acid and chaconine were the potent inhibitors of LNCaP and PC-3 cell growth. Chaconine and gallic acid induced apoptosis through activation of caspase-3 and cleavage of PARP in LNCaP cells. In both the cell types, activation of JNK and MAPK was associated with chaconine and gallic acid induced apoptosis.

This study demonstrates for the first time that AF/potato extract induce mitochondrial release and nuclear uptake of the proapoptotic Endo G and AIF proteins and this represents an important and hitherto undetected mechanism of anticancer action

for these phytochemicals in prostate cancer cells and has not been reported in other cancer cell lines.

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B.S. in Horticulture

Awards and Fellowships

- Who's Who Among Students in American Universities and Colleges for 2005-2006 at Texas A&M University
- Graduate Student Research and Presentation Grant – 2005, Office of Graduate Studies, TAMU
- AASIO –2005 Outstanding Graduate Student Award
- 1st place - Graduate Student Paper Competition – 2005, 89th Annual Meeting of Potato Association of America, Calgary, Alberta, Canada.
- National Potato Council Women's Auxiliary Scholarship, NPC - 2005
- The Margaret Plum Scholarship in recognition of outstanding accomplishments and scholastic achievements in Horticulture, Department of Horticultural Sciences, TAMU –2005,
- 1st place - Student Research Week Competition - 2005, Life Sciences, TAMU.
- 2nd place - Warren S. Barham Ph.D. Graduate Student Paper Competition – 2005, SR-ASHS Annual Meeting, Little Rock, Arkansas.
- Tom Slick Senior Graduate Fellow, TAMU - 2005
- Horticulture Graduate Council Scholarship, HGC - 2004
- Fellow of Graduate Teaching Academy, TAMU - 2004