

ABSTRACT

SIMPSON, JOY ANNA. Antioxidant Properties of Peanut Plant Leaves and Roots and Contribution of Specific Phenolic Compounds to Antioxidant Capacity. (Under the direction of Dr. T. H. Sanders).

The peanut seeds represent less than 40 % of the total biomass of the peanut plant. Currently, peanut plants are left in the field after harvest or baled for animal feed. The research presented here was the second step in identifying bioactive compounds from peanut plants that may be the source for value added products for the peanut industry. The objective of this work was to determine the antioxidant capacity and total phenolic content for peanut leaves and roots, and to identify and quantify specific phenolic compounds contributing to the total antioxidant activity. Peanut leaves and roots were collected from a North Carolina (NC) research farm. Additional roots were collected from a Texas (TX) farm. Plant parts were freeze dried and extracted with methanol and aqueous methanol.

Antioxidant activity of the extracts was tested using ORAC and DPPH assays. Total phenolics were measured using the Folin-Ciocalteu method. Chlorophyll was tested with the ORAC assay to dispel concern for potential chlorophyll antioxidant activity. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used for separation and preliminary identification of compounds contributing to the total antioxidant capacity of the peanut leaves and roots. HPLC coupled with Mass Spectrometry (MS) was utilized for identification and quantification of individual phenolic compounds in the peanut plant parts. The identified phenolic compounds were assayed individually and as a quantitative mixture with both antioxidant assays to determine the contribution of the individual compounds to the total antioxidant activity previously determined for peanut plant extracts.

NC leaves had the highest antioxidant capacity and total phenolic content, followed by NC roots and TX roots. Aqueous methanol extracted more antioxidant compounds from peanut plants than methanol, which was displayed by significantly higher antioxidant capacities and total phenolic content. The total phenolic content was highly correlated with the antioxidant activity of the ORAC and DPPH suggesting that phenolic compounds may contribute to the antioxidant activity. Compounds identified in the peanut leaves and roots were *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, catechin, epicatechin, and gallic acid. Epigallocatechin gallate was identified in NC leaves and NC roots, while resveratrol was identified in NC roots and TX roots only. Individual assays of the phenolic compounds identified indicated that they contributed approximately 40% of the total antioxidant activity in the ORAC assay, and 15% in the DPPH assay.

**ANTIOXIDANT PROPERTIES OF PEANUT PLANT LEAVES AND ROOTS AND
CONTRIBUTION OF SPECIFIC PHENOLIC COMPOUNDS TO ANTIOXIDANT
CAPACITY**

By

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INTRODUCTION

Phytochemicals are chemicals derived from plant sources. Plants contain hundreds of phytochemicals such as flavonoids and phenolic acids. Research indicates phytochemicals such as polyphenols have high antioxidant activity (Fukumoto and Mazza, 2000).

Free radicals are highly reactive and are generated in the body through normal cellular function (USDA, 2005). Free radicals are believed to cause lipid oxidation leading to cellular membrane damage (Nijveldt et al., 2001).

Antioxidant research is a key topic in both the medical and food industry today. Antioxidants protect the body from reactive species. Research investigating bioactive compounds from plant materials have inversely correlated to the intake of bioactive phytochemicals and disease. Other research has confirmed that peanut and peanut plant parts contain antioxidant compounds (Green, 2004).

Peanuts (*Arachis hypogaea* L.) are an excellent source of proteins, lipids, and minerals (USDA, 2000a). Recent interest in phytochemicals may provide a new use of peanut leaves and roots (Chen et al., 2002). The peanut is a member of the legume family, which is classified based on root nodulation (Vance, 2002). Compounds of interest from the peanut plant include phenolic compounds such as resveratrol and catechins. High levels of resveratrol have been identified in peanut roots (Chen et al., 2002). Resveratrol has been linked with the deceleration of age-related diseases (Soleas et al., 1997).

Current cultural and harvest practices of peanuts are not designed for utilization of the plant leaves and roots. Plants are either baled as animal feed (Golden, 2001) or left in the field after the peanuts are harvested. Bioactive compounds may be present in the peanut leaves and roots and could potentially create a value added agricultural by-product.

LITERATURE REVIEW

Peanut Origin and Production

Origin and History

Peanuts, *Arachis hypogaea* L., were first discovered as a food source in the Western Hemisphere, most likely South America (Woodruff, 1983). Spanish explorers have been credited with spreading peanuts throughout the new world; however, commercial production of peanuts did not start in the United States until about 1876 (Woodruff, 1983).

Economic Importance

Peanuts have been cultivated to grow in varying soils and temperature zones throughout the world. The United States produces approximately 10% of the world's peanut crop (Acquaah, 2005). In 2002, China was the top producer of peanuts (14 million tons), followed by India (7.6 million tons) and the United States (1.9 million tons) (USDA, 2004). The United States produces higher average yield per acre (2,800-3,000lb/acre) than the rest of the world (800-1,000 lb/acre) due to more efficient and sophisticated production technology (Acquaah, 2005). Nine states account for 99% of the US crop production. They are Georgia, Texas, Alabama, North Carolina, Florida, Oklahoma, Virginia, South Carolina, and New Mexico (Hammons, 1994).

Peanut Production

Peanut production started in the United States around 1876. The Civil War increased demand for the crop and production began in the southeastern United States (Acquaah, 2005). Until about 1920, peanut production was one of the most labor intensive crops in the United States (Woodruff, 1983). By 1964, peanuts in concentrated production areas were about 90% mechanized. Mechanization eased the workload, increased production, and improved efficiency

(Woodruff, 1983). Today, many smaller countries still produce peanuts using hand labor or animal drawn equipment (Hammons, 1994).

Peanuts are produced in regions with moderate rainfall, abundant sunlight, and average growing temperatures above 65°F (Acquaah, 2005). Most varieties require 120-160 days without frost and a growing season rainfall of 42-54 inches per annum. Peanuts are best grown in well-drained, calcium rich, sandy loam with pH between 6.5 and 7.2 (Acquaah, 2005).

Cultural Practices and Harvesting

Generally, peanut production entails tillage, planting, and harvesting. The growth of the peanut industry in the United States is related to increased output and efficiency of production (Woodruff, 1983). Mechanization increased the use of deep tillage and smooth preparation of the soil, broadcasting fertilizers, treating and planting seed, applying herbicides, light multi-row cultivation, digging, windrowing, and drying methods (Woodruff, 1983). Improvements in disease and insect control and peanut varieties also increased production and efficiency.

Peanut harvesting refers to the process of removing peanuts from the soil and separation of the peanuts from the foliage. Physiological maturity, soil type, soil moisture content, and weather dictate digging time. Typically, peanuts are dug when most of the pods are mature (Pattee and Young, 1982); however, there are conditions of disease, weather, and equipment that may override maturity based harvest decisions. In the United States, peanuts are cured or dried to reduce the moisture content for safe storage.

Peanut Foliage

The peanut plant, exclusive of the peanut fruit, accounts for approximately 40% of the peanut biomass. In 2002, the total peanut production was 3.32 billion pounds of peanuts (Womach, 2003). Based on this volume of production, peanut plant material (leaves, stems and

roots) for 2002 would be estimated at 221 million pounds. Currently, plants are either baled for animal feed or left in the field. While little research has been completed on the phytochemical composition of peanut leaves and roots; two studies reported high levels of resveratrol in peanut plant roots (Chen et al., 2002, Liu et al., 2003).

The perennial peanut plant (*Arachis glabrata* Benth) has been reported as having nutrition equal to alfalfa, which is a major source of livestock feed. The perennial plant differs from other varieties of peanuts in that it produces very few peanuts (French et al., 2006). Peanut plants are good pasture forage, excellent dry hay, and exceptional silage. Over the last few decades, prices of baled perennial peanut have steadily increased (French et al., 2006) ranging from \$100 to \$1,000 per acre depending on yields and variety (Golden, 2001).

Nutrition

Nutritional information in the USDA database (USDA, 2000b) indicates that peanuts are an excellent source of proteins, lipids, and minerals. A recent report stated that a daily serving of peanut products helps humans meet recommended nutrient requirements and that people who consume peanuts regularly tend to have increased levels of essential vitamins and minerals and leaner bodies (Fuhrman, 2005). Kris-Ehrt et al. (1999) and O'Byrne et al. (1997) studied the effects of diets rich in peanuts. These studies showed that peanut rich diets lowered LDL cholesterol, a significant risk factor for heart disease.

Researchers are interested in phytosterols, isoflavones, flavonoids, and phenolic acids from legume sources (Wardlaw et al., 2004). Peanuts contain bioactive flavonoids and plant sterols (Ahmed et al., 1982, Sobolev and Cole, 1999, Sanders et al., 2000, Awad et al., 2000). Ahmed et al. (1982) and Awad et al. (2000) investigated phytosterols in the peanut seed. Both studies reported that peanuts are a good source of β -sistosterol, a plant sterol believed to have

anticancer properties. Resveratrol has been identified in raw peanut seeds, roasted peanuts, peanut butter, and boiled peanuts (Sobolev and Cole, 1999, Sanders, et al., 2000, Huang et al., 2003). Resveratrol is a phytoalexin associated with reducing atherosclerosis and carcinogenesis (Soleas et al., 1997).

Resveratrol has also been isolated and identified in peanut plants (Chung et al. 2003, Wang et al., 2005). Their research suggested that resveratrol production within the plant is associated with increased stresses such as UV light and plant disease. Chen et al. (2002) confirmed resveratrol presence in the peanut plant root. They found the addition of peanut plant root powder inhibited oxidation when added to pork fat patties.

Peanut Plant Morphology

Taxonomy and Plant Morphology

Peanuts are classified in the Fabacea family, which are dicotyledonous herbs or shrubs having fruit that are legumes or loment, bearing nodules on the roots containing nitrogen fixing bacteria. Legumes include peanuts, beans, and peas (Hopkins, 2004). The peanut plant is unusual because it flowers above ground and fruit develop below ground. The plant is annual, with a central upright stem bearing branches that are prostrate to nearly erect and have compound leaves. Varieties in cultivation are grouped based on branching habit, branch length, and fruit location. Bunch peanuts have peanuts which are clustered about the base of the plant, while runner peanuts have fruit along the prostrate branches that spread out from the base of the plant.

Four major peanut market types are grown in the United States: spanish, runner, virginia, and valencia. Peanut cultivars have variations in flavor, oil content, size, shape, and disease resistance. The majority of peanuts sold in the shell are of the virginia type, along with some

valencias, which are selected for large size and light colored shells. Spanish peanuts are used mostly for peanut candy, salted nuts, and peanut butter. Runners are used to make peanut butter. (Acquaah, 2005).

Plant Metabolism

Plants utilize energy from the sun through a process called photosynthesis (Wardlaw et al., 2004). Energy from sugar produced by photosynthesis is utilized in many plant functions (Starr et al., 2004) and leaves are vital for plant metabolism because of photosynthesis.

Leaves are rich in photosynthetic pigments that capture sunlight. Pigments can be categorized into three classes: chlorophyll, phycobilin, and carotenoids (Prezelin and Nelson, 1997). Chlorophyll is an essential component of photosynthesis because it absorbs and transfers light. Chlorophyll has a structure of alternating single and double bonds which facilitates absorption of light energy (Figure 1).

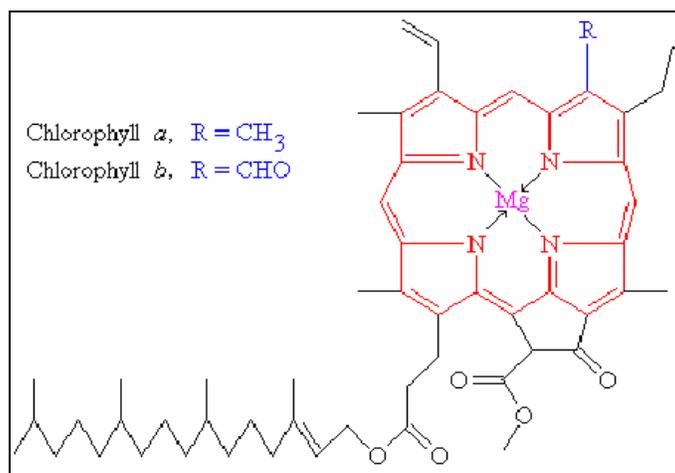


Figure 1: Structure of Chlorophyll a and b (May, 2005)

Carotenoids are pigments which can be split into two classes, xanthophylls and carotenes. The carotenoid family of pigments range in color from pale yellow to deep red. In plants, carotenoids absorb light, transfer energy to chlorophyll and serve as photoprotectants, or

antioxidants (Blankenship, 2002). Carotenoids are photoprotectants because they may reduce singlet oxygen to a less reactive state. Carotenoids are generally considered lipophilic (Wrolstad et al., 2005).

Energy produced by photosynthesis is in the form of adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), and sugars. ATP is an energy source in plants consisting of a nucleotide and ribose sugar with three phosphate groups (Lawlor, 2001). NADPH provides reducing energy for nutrient assimilation within the plant (Starr et al., 2004). Sugars are formed independent of light in the Calvin-Benson cycle (Farabee, 2001).

The fourth most abundant nutrient in plants is nitrogen. Nitrogen is necessary for the formation of proteins, nucleic acids and other plant metabolites. Acquisition and absorption of nitrogen by plants is vital for metabolism (Vance, 2002). Nitrogen is in abundance in the environment in the form of N_2 ($N\equiv N$). Atmospheric nitrogen cannot be utilized by plants, because they do not contain the enzyme able to breakdown the triple covalent bond of N_2 . Legumes have a symbiotic relationship with *Rhizobia* bacteria, which form root nodules and produce nitrogenase. Nitrogenase is the enzyme responsible for the reduction of nitrogen gas to ammonia. Legume plants actively utilize ammonia produced by the bacteria. Nitrogen fixation is more energy intensive than the integration of nitrate (NO_3^-) and ammonium (NH_4), which are other sources of nitrogen found in the environment (Sprent, 1994).

Nitrogen fixation is possible for legumes due to the interaction of the *Rhizobia* and roots. Roots of legumes produce chemoattracts that serve to attract *Rhizobia* towards the roots. Chemoattracts produced include: phenolic flavonoids and isoflavonoids, aromatic acids, amino acids, and dicarboxylic acids. Phenolic compounds within the roots are a nutrient source, promote microbial growth and aid in plant defense (Daroka and Phillips., 2002).

Flavonoids such as luteolin, hesperitin, daidzein, and apigenin isoflavonoids induce nodulation (Figure 2) (Vance, 2002). Isoflavonoids play a vital role in symbiosis and plant disease resistance. Frequent microbial infections induce the accumulation of certain isoflavonoids which act as antibiotics and are also known as phytoalexins. Antibiotic properties limit the growth of invading organisms (Vance, 2002).

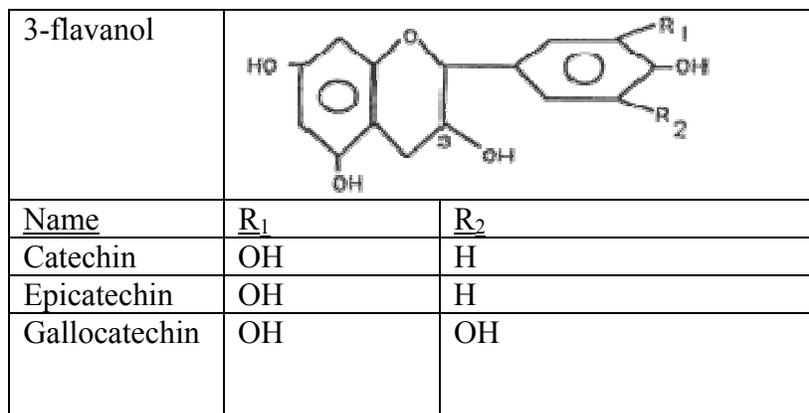


Figure 2: Phenolic Compounds: Flavonoids ($C_6.C_3.C_6$) three rings molecule, two of them aromatic connected by heterocyclic center ring and major 3-flavanol structures

Phytochemicals

Phytochemicals are natural bioactive compounds found in plant foods that function with nutrients and dietary fiber to protect against disease. Health effects of phytochemicals are not completely understood. Only well-designed long-term research can determine whether any of these chemicals will be useful for preventing any disease (Nijveldt et al., 2001). The most publicized phytochemicals that are believed to have antioxidant properties have been vitamin C, vitamin E, and beta-carotene which is converted into vitamin A by the body. In 1993, The New England Journal of Medicine published a study which found that people who took vitamin E supplements had fewer deaths from heart disease (Stampfer et al., 1993).

Pigments

Plant pigments are generally classified in the phytochemical category. There are three main categories of plant pigments: chlorophyll, carotenoids, and phycobilins. The plant utilizes pigments for photosynthesis as discussed earlier.

Chlorophyll

Chlorophyll is the most abundant phytochemical pigment in higher plants (Ferruzzi et al., 2002). It is responsible for the green color in the leaves and stem cells of the plant. There are three types of chlorophyll: a, b, and c. Chlorophyll a is found in all plants, algae and cyanobacteria, and is mainly responsible for photosynthesis. Chlorophyll b is found only in green algae and in plants, while chlorophyll c can be found only in aquatic photosynthetic organisms (Wrolstad et al., 2005).

Little is known about the potential or actual function of chlorophyll as an antimutagen or anticarcinogen (Negishi et al., 1997). Chlorophyll has been associated with antioxidant and disease preventative functions. Endo et al. (1985) reported that chlorophyll derivatives may act as chain breaking antioxidants, by donating electrons. Epidemiological studies indicate diets high in green raw vegetables may protect against cancer, but research needs to be completed correlating chlorophyll and cancer prevention (Vogel, 2005).

Phenolic Compounds

Phenolic compounds are a large, diverse group of secondary plant metabolites that are widespread in the plant kingdom and include phenolic acids, flavonoids, and tannins (Figure 3). Polyphenol compounds may accumulate as end-products from the shikimate pathway and acetate pathways (Carey, 2003). Phenolic acids include hydroxybenzoic acid and hydroxycinnamic acids which constitute the majority of phenolic acids found in plant tissue (Wrolstad et al., 2005).

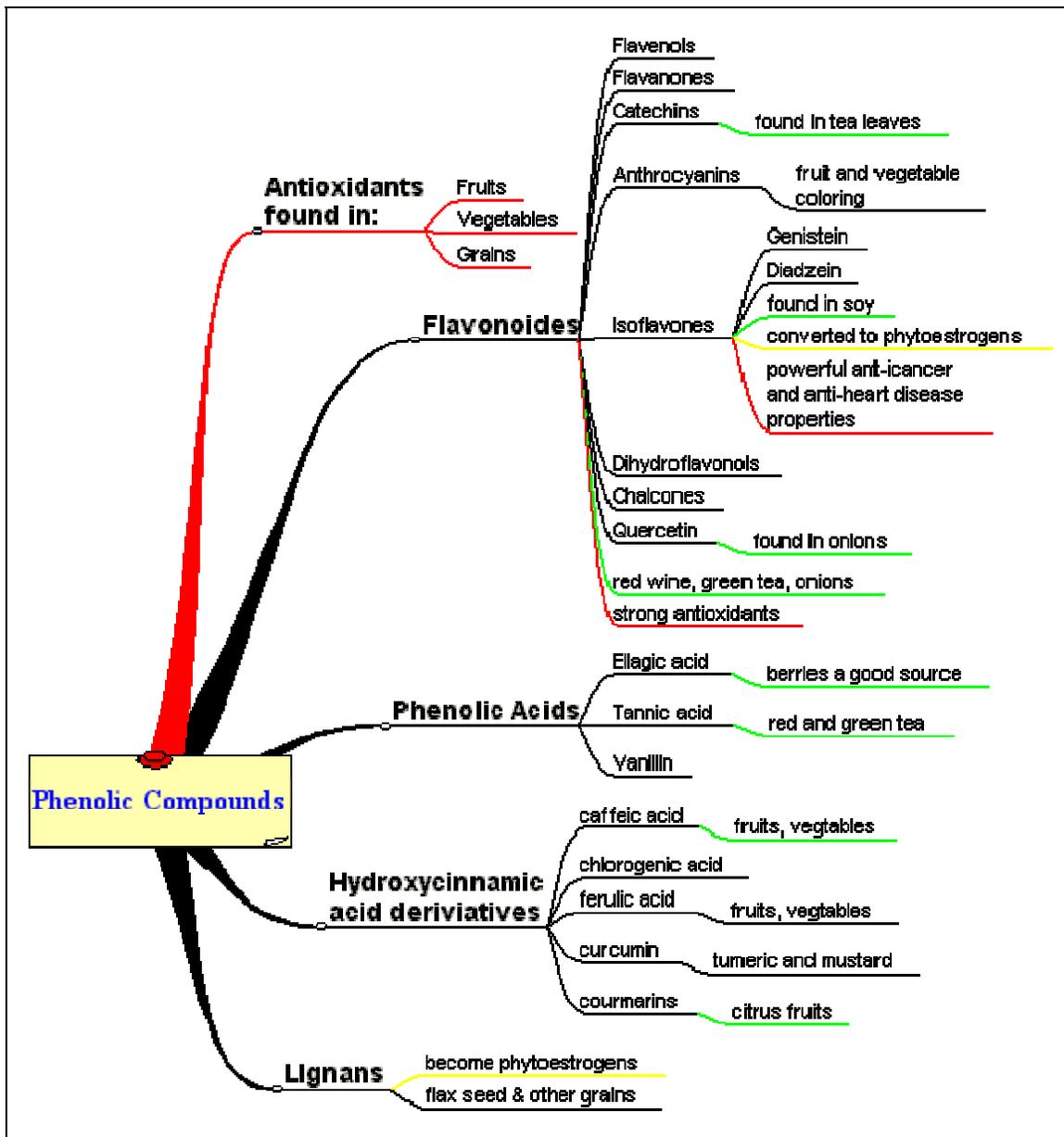
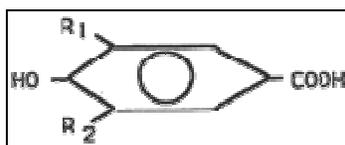


Figure 3: Phenolic Compounds (Novus, 2006)

Flavonoids are the most abundant of the plant phenolics. The structural basis for all flavonoids is a flavone nucleus. Flavonoid groups are classified based on hydroxylation of the nucleus and linked sugar. Tannins are phenolic compounds capable of tanning leather, or precipitating gelatin from solution. Phenolic acid groups include hydroxybenzoic acids (Figure

4) and hydroxycinnamic acids (Figure 5), which are derived from nonphenolic molecules benzoic and cinnamic acid, respectively (Harborne and Williams, 2000).

Research on phenolic compounds has increased significantly in the last decade. Currently, almost 6500 flavonoids are known (Harborne and Williams, 2000). Many foods that are considered as healthy contain relatively high levels of flavonoids (Escarpa and Gonzalez, 2001). An important phenomenon attributing to increase interest in phenolics is known as the “French paradox”. Frankel et al., (1993) credited antioxidant phenolics in red wine with a low coronary heart disease mortality rate in France.



<u>Compound</u>	<u>R₁</u>	<u>R₂</u>
<i>p</i> -Hydroxybenzoic acid	H	H
Vanillic acid	H	OCH ₃
Gallic acid	OH	OH
Syringic acid	OCH ₃	OCH ₃

Figure 4: Phenolic Compounds: Benzoic acid derivatives (C₆.C₁)



<u>Compound</u>	<u>R₁</u>	<u>R₂</u>
Cinnamic acid	H	H
<i>p</i> -Coumaric acid	H	OH
Caffeic acid	OH	OH
Ferulic acid	OCH ₃	OH

Figure 5: Phenolic Compounds: Cinnamic acid derivatives (C₆.C₃)

Phenolic compounds have specific health effects, even though they are non-nutritive compounds. A considerable amount of research has been conducted on the antioxidant activity

and scavenging activity of phenolic compounds (Escarpa and Gonzalez, 2001). Antioxidant properties of phenolic acids play a vital role in the stability of food products, as well as in the antioxidative defense mechanisms of biological systems (Macheix and Fleuriert, 1998). Plant polyphenols can act as reducing agents by donating hydrogen and quenching singlet oxygen. Flavanoids, particularly catechin, quercetin, kaempferol, and their glycosides have been found in teas and wine (Nijveldt et al., 2001). Common phenolic include caffeic acid, chlorogenic acid, resveratrol, catechin, epicatechin, and *p*-hydrobenzoic acid.

Caffeic acid

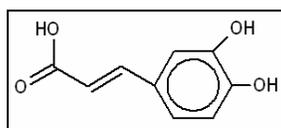


Figure 6: Structure of caffeic acid

Caffeic acid is a hydroxycinnamic acid commonly found in many fruits, vegetables, seasonings and beverages (Figure 6). Caffeic acid is an antioxidant in vitro and may contribute to the prevention of cardiovascular disease (Olthof et al., 2001). Olthof et al. (2001) found that caffeic acid is absorbed by the body. Caffeic acid has been reported at a high level in fresh raspberries and pears (Escarpa and Gonzalez, 2001). Caffeic acid appears to contribute to the resistance of roots to microbial infections, fungi, and insects (Harrison et al., 2003).

Chlorogenic acid

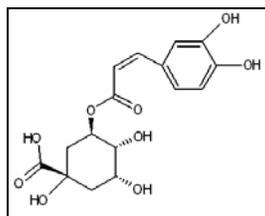


Figure 7: Structure of chlorogenic acid

Chlorogenic acid is an ester of caffeic acid and quinic acid (Figure 7). Chlorogenic acid is a major phenolic compound in coffee and has been isolated from the leaves and fruits of plants (Escarpa and Gonzalez, 2001). Chlorogenic acid is an antioxidant in vitro, which may contribute to reduction of diseases (Olthof et al., 2001). Like caffeic acid, Olthof et al. (2001) found that chlorogenic acid is absorbed by the body.

Resveratrol

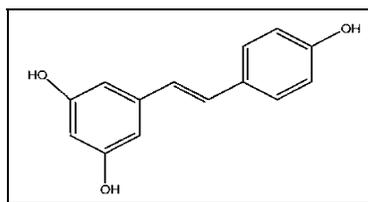


Figure 8: Structure of resveratrol

Resveratrol is a flavonoid which is more specifically classified as a hydroxystilbene (Figure 8). Resveratrol is produced by the plant in response to some stress such as fungal invasion. Many health effects, such as anti-cancer, anti-viral, neuroprotective, anti-inflammatory have been attributed to resveratrol. It is found in a variety of plants including the skins of certain red grapes, peanuts, blueberries, and some pines (King et al., 2006). The amount of resveratrol in food substances varies considerably. Red wine contains approximately 0.6-8.0 $\mu\text{g/mL}$ resveratrol, while peanuts have a range of 0.02-1.79 $\mu\text{g/gram}$ (Sanders, et al., 2000). Resveratrol has been reported at high and variable levels in peanut plant roots (Chen et al. 2002, Liu et al. 2003). Cultivar, growing season, weather, and peanut maturity have been shown to affect resveratrol concentration in peanuts (Sobolev and Cole, 1999; Sanders et al., 2000; Chen et al., 2002). Resveratrol levels vary among different part of the peanut. Higher concentrations of resveratrol have been found in the seed coat and shell than in the embryos and kernels (Sobolev

and Cole, 1999; Sanders, et al., 2000). Also, boiled peanuts had a higher concentration of resveratrol, followed by peanut butter, and roasted peanuts (Sobolev and Cole, 1999).

Catechin

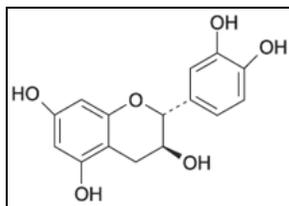


Figure 9: Structure of catechin

Catechins are classified as proanthocyanidins (Figure 9). Catechin has been deemed a powerful antioxidant because it is easily oxidized (Nijveldt et al., 2001). Common sources of catechin include white tea, green tea, chocolate and apples (Escarpa and Gonzalez, 2001).

Epicatechin

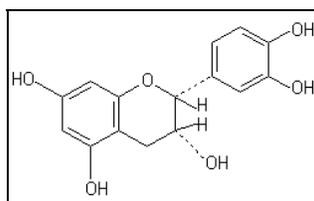


Figure 10: Structure of epicatechin

Epicatechin is a flavanol, which has been classified as an antioxidant (Figure 10). High concentrations of epicatechin have been found in cocoa, particularly dark chocolate (Mayer and Stern, 2003). Epicatechin concentration in cocoa may contribute to the higher antioxidant activity compared to green tea and wine (Lee, et al. 2003).

p-hydroxybenzoic acid

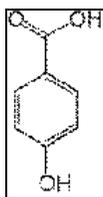


Figure 11: Structure of *p*-hydroxybenzoic acid

p-hydroxybenzoic acid (Figure 11) is a phenolic derivative of benzoic acid. It is primarily known as the basis for the preparation of its esters, which are used as preservatives in cosmetics. One study indicated *p*-hydroxybenzoic acid acts as an antioxidant against peroxy radicals (Yeh and Yen, 2003).

Ferulic Acid

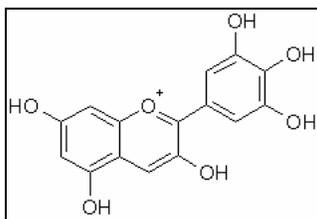


Figure 12: Structure of ferulic acid

Ferulic acid is a phenolic acid belonging to the hydroxycinnamic group (Figure 12). Ferulic acid is found in the leaves and seeds of many plants including whole wheat, oats, coffee, apples, peanuts, and pineapples (Escarpa and Gonzalez, 2001). Ferulic acid is an antioxidant which neutralizes free radicals (Graf, 1992). Ferulic acid seems to reduce the risk of many types of cancer (Mori et al., 1999). One study showed that both dietary caffeic acid and ferulic acid may play a key role in defending against carcinogenesis (Kuenzig et al., 1984).

Oxidation

Free Radicals

Free radicals are highly reactive, unstable compounds containing an unpaired electron (Lee et al., 2003). Electrons are most stable in pairs, therefore free radicals attach to or receive hydrogen ions from molecules with low bond dissociation energy. Molecules with lower bond dissociation energy include unsaturated fatty acids and phenolic acids. Damage caused by free radicals includes the change in structures of cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins (Machlin and Bendich, 1987).

Oxidation

Oxidation is the process in which electrons are removed from atoms or pairs of atoms (Fennema, 1996). The oxidized molecule loses an electron, while the reduced molecule receives an electron. Oxidation is a concern because some reactions become uncoupled and free radicals are formed (Lee et al., 2003).

Reactive oxygen species (ROS) are oxygen-centered free radicals (Pietta, 2000). Experimental evidence has suggested that there are six major reactive oxygen species (ROS) responsible for oxidative damage (Huang et al., 2005). Living cells have a natural defense composed of enzymatic antioxidants that change ROS to harmless species. No enzymatic antioxidant is known to scavenge ROO^\cdot , HO^\cdot , $^1\text{O}_2$, and ONOO^\cdot . Defense against these ROS relies on nonenzymatic antioxidants such as vitamins C and E and many phytochemicals (Huang et al., 2005).

In the body, ROS function in energy production, regulation of cell growth, and synthesis of compounds (Pietta, 2000). ROS reactions are believed to accelerate aging and lead to cancer and heart disease. Cellular damage is believed to be a product of lipid oxidation due to damage to the cellular membranes (Nijveldt et al., 2001).

Autoxidation of lipids advances through free radical mechanisms. Oxidation of lipids concerns the food industry because it causes off flavors, which leads to a decrease in shelf life and overall product quality. Lipid oxidation can also decrease nutritional value of foods (Fennema, 1996). The rate of lipid oxidation is dependent upon fatty acid composition, oxygen concentration, temperature, surface area, moisture, antioxidants, and radiant energy. Autoxidation is initiated by the removal of hydrogen from a fatty acid (RH). The result is formation of an alkyl radical (R^\cdot) and hydrogen radical (H^\cdot). Inhibition includes the basic steps

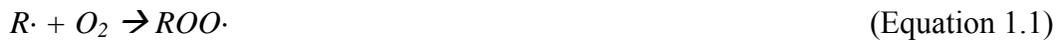
listed below, assuming one antioxidant scavenges two radicals and oxygen is in large excess (Fennema, 1996).

Initiation



Production of the alkyl radical is thermodynamically complex, so initiation begins with a catalyst such as hydroperoxide decomposition, light, metal ions or heat (Fennema, 1996). Alkyl radicals are highly reactive and will interact with atmospheric oxygen (O₂) to form unstable peroxy radicals in the propagation step.

Propagation



Peroxy radicals are highly unstable and may withdraw hydrogen atoms from other fatty acids (RH) to produce hydroperoxides (ROOH) and new alkyl radicals. Propagation continues until termination begins.

Termination



As radical concentration increases, the alkyl free radicals begin reacting with themselves and other peroxy free radicals (ROO·) to form free fatty acid dimers (RR or ROOR) and hydroperoxides. Hydroperoxides are easily degraded into acids, aldehydes, alcohols, carbonyls, and ketones, all of which cause off flavors in foods generally called rancidity (Schmidt, 2000).

Antioxidants

The Institute of Medicine of the National Academies defines antioxidants as “a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans” (Panel on Dietary Antioxidants, 2000). Antioxidants can scavenge reactive oxygen species to stop radical chain reactions or inhibit the radical production (Huang et al., 2005).

Inhibition



Antioxidants can be classified into two types: preventative and chain-breaking. Preventative antioxidants chelate metals and/or decompose peroxides in order to stall the initiation of free radicals before oxidation (Fennema, 1996). Citric acid, ethylenediaminetetraacetic acid (EDTA), and polyphosphates are common preventative antioxidants because they chelate metal ions. Research on antioxidants has been focused on the action of free radical acceptors because food systems are very complex and it is nearly impossible to remove all trace metals and peroxides (Huang et al., 2005).

The mechanism of antioxidants in the autoxidation cycle is molecule dependant. Chain breaking antioxidants have been classified by two pathways: hydrogen atom transfer (HAT) and single electron transfer (ET) (Huang et al., 2005). For HAT, the free radical acceptor reacts with the peroxy radical in the propagation stage. Flavonoids and phenolic compounds fall into this classification because they donate hydrogen ions while maintaining a stable structure (Huang et al., 2005). During the hydrogen atom transfer a radical formed during oxidation is quenched via donation of a hydrogen atom by an aromatic ring antioxidant (ArOH). A radical chain reaction

inhibitor is commonly regarded as an antioxidant and is the most widely studied antioxidant mechanism (Wright et al., 2001).

Hydrogen Atom Transfer



The aromatic radicals remain stable by aromatic ring stabilization and a hydroperoxide is the product.

Another antioxidant pathway is single electron transfer (ET). As an antioxidant is added to a system, the antioxidant inhibits propagation of the peroxy radical by hydrogen atom transfer, and then will follow a single electron transfer pathway for antioxidant activity (Wright et al., 2001).

Single Electron Transfer



Single electron transfer involves the antioxidant molecule donating an electron to stabilize the peroxy radical. This process creates a peroxy radical with a negative charge due to an over abundance of electrons on the oxygen. A positively charged aromatic free radical is available to donate a proton (H⁺) to quench the charged hydroperoxide. The single electron transfer pathway is very dependent upon solvent due to stabilization of charged species (Wright et al., 2001).

Antioxidants are used in food products to delay or inhibit the oxidation process maximizing product shelf life and quality. Antioxidants are primarily added to foods in combination with synergists like ascorbic, tartic, and phosphoric acids to increase efficiency. The number of synergist and natural compounds used as antioxidant ingredients is limited, even though there are at least 8,000 phytochemical compounds that have antioxidant properties

(Pietta, 2000). The wide number of phytochemicals implies that plants may contain many unidentified antioxidant compounds.

Prooxidants

Prooxidants can be defined as components that lower the activation energy for the initiation of lipid oxidation (Labuza, 1971). Krinsky (1998) also determined that radical initiators may be classified as pro-oxidants. A compound may display both antioxidant and prooxidant properties depending on concentrations and experimental conditions. Aruoma (1990) noted that lipid antioxidants act as prooxidants in nonlipid systems.

Antioxidant Methodology

Extractions

Extraction is a very common laboratory procedure used when isolating or purifying compounds. Solid-liquid, liquid-liquid, and acid-base extractions are used in organic chemistry. A wide range of isolation and extraction techniques for plants and plant materials have been detailed in published literature. Extraction methods are selected based on preserving the integrity of possible activity of extracted compounds. Wet extraction, also known as solid-liquid extraction is the use of solid material in direct contact with a liquid solvent (Houghton and Raman, 1998). During plant extractions, organic solvents are used and diffuse into the solid material which solubilizes compounds of similar polarity. The composition and nature of the solvent used will determine the types of compounds extracted. Organic solvents include polar solvents such as water, methanol, and acetone and nonpolar solvents such as dichloromethane and hexane.

Antioxidant Assays

Numerous methods have been developed to measure the antioxidant capacity of compounds and extracts. Antioxidant methods may focus on scavenging of oxygen and hydroxyl radicals, reduction of lipid peroxy radicals, chelation of metal ions, or inhibition of lipid peroxidation. Antioxidant methods generally focus on one mechanism of the action. A number of methods verify the capacity of an antioxidant to scavenge free radicals generated by the system, for instance the Oxygen Radical Absorbance Capacity (ORAC) (Cao and Prior, 1999), Total Reducing Ability of Plasma (TRAP) (Ghiselli, et al., 1995), and Trolox Equivalent Antioxidant Capacity (TEAC) (Miller et al., 1993). Methods such as DPPH• (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995) and ABTS• (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) (Re et al., 1999) measure the capacity of antioxidants to scavenge free radicals. Other methods have been established to evaluate antioxidants by quantifying lipid peroxidation products, for instance malonaldehyde by Thiobarbituric Acid Reactive Substances (TBARS) (Plumb et al., 1996) and volatile organic acid decomposition products in the Oxidative Stability Index (OSI) (Firestone, 1992).

Oxygen Radical Absorbance Capacity

The ORAC method was adapted from the work by Cao and colleagues (1993) utilizing the fluorescent capacity of β -phycoerythrin and the inhibition of deterioration by antioxidant compounds after addition of 2,2'-azobis(2-amidino-propane) (AAPH). Ou et al. (2001) reported that β -phycoerythrin reacted with polyphenols, which are commonly known for their antioxidant properties and demonstrated inconsistency between sample repetitions. Ou and his colleagues changed the fluorescent indicator to fluorescein (FL), which does not interact with the sample. The original ORAC assay was performed in a water-based buffer solution; therefore, the use of

lipophilic extracts resulted in erroneous data. In order to test lipophilic extracts, 7% (w/v) randomly methylated β -cyclodextrin (RMCD) in acetone:water (1:1) was incorporated. Compounds or extracts were suspended into the RMCD solution, which increased the solubility of the antioxidants into the ORAC buffer solution (Huang et al., 2002). Initial development of the ORAC assay was performed on the COBAS FARA II analyzer (Roche Diagnostic Systems, Inc, Branchburg, NJ), which Davalos et al. (2004) adapted for a 96-well fluorescent plate reader.

The ORAC assay involves the deterioration of fluorescein, a synthetic non-protein probe, by free radicals. AAPH is an azo compound (R-N \equiv N-R), which undergoes spontaneous decomposition. Dinitrogen (N₂) and two molecules of AAPH• radical (Equation 3.0) are generated at a constant rate upon AAPH disintegration.



Unstable fatty acid radicals (R•) quickly react with atmospheric oxygen (O₂) to form peroxy radicals (ROO•). The loss of fluorescent intensity of fluorescein indicates oxidation by peroxy radicals. Addition of antioxidants inhibits propagation of the peroxy radical (ROO•) and thus, reducing the loss of fluorescent intensity over time. The phenoxy radical (ArO•) may react with the fluorescein to inhibit deterioration, but reacts more with peroxy radicals (ROO•) that deteriorate fluorescein (Ou et al., 2002).

2,2-Diphenyl-1-picrahydrazyl (DPPH)

The 2,2-diphenyl-1-picrahydrazyl (DPPH) assay is a simple method developed for the analysis of antioxidant capacity (Huang et al., 2005). The DPPH method is used to determine the capacity of a sample to donate hydrogen and/or electrons to quench DPPH radicals. Quenching and preventing radical propagation via hydrogen atom transfer is mimicked in the 2,2-diphenyl-1-picrahydrazyl (DPPH) assay. As the free radical is quenched, the color of the

DPPH solution changes from a deep purple to a light yellow and absorbance at 515 nm decreases (Brand-Williams et al., 1995). Figure 13, shown below, is an illustration of the structure of the DPPH and reduction by an antioxidant.

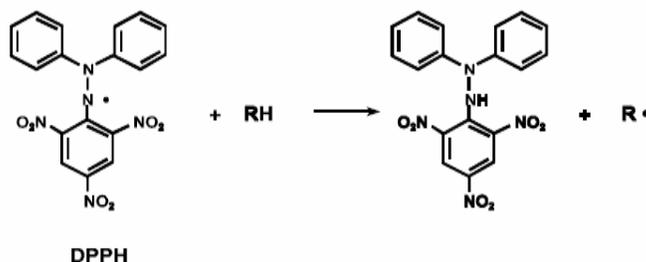


Figure 13: Structure of DPPH and reduction by an antioxidant (Prakash, 2001)

Brand-Williams et al. (1995) found that certain antioxidant compounds elicited different reaction kinetics with DPPH•. In the study, antioxidants such as BHT and protocatechuic acid did not reach steady state, or the reaction endpoint, until three and two hours respectively, whereas with compounds like ascorbic acid, isoascorbic acid, and isoeugenol steady state was achieved within one minute. At steady state, the DPPH• reaction has been shown to have a stoichiometric correlation with the quantity of antioxidant present. Caffeic acid, gentistic acid and gallic acid exhibited the highest antiradical activity with stoichiometry of 4.54, 5.6, and 6.25 reduced DPPH• molecules per molecule of antioxidant, respectively, while one molecule of phenol, ascorbic acid, α -tocopherol, and BHT reduced <1, 1.85, 2, and 2.63 molecules of DPPH•, respectively (Brand-Williams et al., 1995).

According to Brand-Williams et al. (1995), there are different ways to explain the efficiencies of monophenolic compounds in reducing one DPPH radical. One mechanism involves the delocalization of an electron onto the para-substituted group of the molecule prior to the donation of hydrogen to reduce DPPH radicals. Another pathway involves the dimerization between two phenoxy radicals in which two hydroxyl groups would be regenerated through an

intramolecular transfer of H•, consequently reacting further with DPPH radicals. Both pathways depend on molecular structure of the antioxidants in regards to stability and reaction potential.

Total Phenolics

The total phenolic method was first adapted to assay food products by Singleton and Rossi (1965). The assay consists of a colorimetric method based on the chemical reduction of the reagent, Folin-Ciocalteu (Wroslad et al., 2005). Total phenolics methodology consists of the addition of Folin-Ciocalteu reagent to a sample held in the dark for two hours, followed by measurement of the absorption. Folin-Ciocalteu reagent contains metal oxide which is reduced by phenols, with a light absorption maximum at 765 nm. Intensity of the light absorption is directly proportional to the concentration of phenols (Wroslad et al., 2005). A standard curve is generated by plotting the concentration of a standard phenolic compound, in this case gallic acid, versus absorption (Figure 14). Results are then calculated as Gallic Acid Equivalents (GAE) of a sample using the standard curve. Gallic acid is used as the reference compound because it is only present in small amounts in the plant material, and it is a stable, pure, inexpensive substance (Wroslad et al., 2005). Gallic acid standard solutions have been determined to lose less than 5% of their value over two weeks when refrigerated (Waterhouse, 2006).

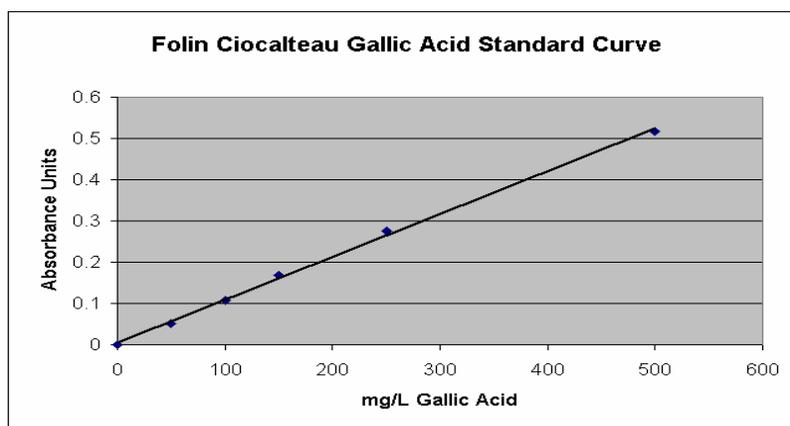


Figure 14: Typical Gallic Acid Standard Curve for Total Phenols Assay (Wroslad et al., 2005)

Instrumental Methods

Chromatography

Chromatography is a process in which a chemical mixture is separated into components based on differences between solutes (Sherma and Fried, 2003). Chromatography is widely used to separate individual components of a mixture of related substances. Compound separation is based upon different components of the mixture traveling at different speeds due to affinity for the medium, solubility in the solvent, and molecular weight (Rouessac and Rouessac, 2000). One theory of chromatographic separation is retention separation, where retention is a measure of the distance at which a substance moves in a chromatographic system (Sherma and Fried, 2003). For systems such as thin layer chromatography, retention is measured as the retention factor, R_f , as defined in Equation 4.0).

$$R_f = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent}} \quad (\text{Equation 4.0})$$

In systems where the compounds are eluted with an eluant, the retention is determined as retention time R_t or t_R , the time between injection and detection. Retention of a compound may differ between experiments and laboratories due to variations of the eluant, stationary phase, temperature, and setup. Standard compounds must be tested under identical conditions in order for valid comparisons to be made (Wagner and Bladt, 2001). Separation techniques include liquid-solid chromatography, liquid-liquid chromatography, gas-liquid chromatography, gas-solid chromatography, and supercritical fluid chromatography.

Thin Layer Chromatography

Thin-layer chromatography (TLC) may be used to optimize the solvent system for a given separation problem. In TLC, the stationary phase, a special finely ground material, usually silica gel or alumina, is coated on a glass plate as a thin layer (~0.25 mm). The mobile phase is

composed of a solvent or solvent mixture. TLC is usually conducted in a closed chamber (Wagner and Bladt, 2001). The solvent, or mobile phase, travels up the stationary phase by capillary action, moving the components of the sample at various rates due to differing degrees of interaction with the solvent (Fried, 1996). Non-polar compounds will travel to the top of the plate because the compounds will dissolve into non-polar solvents and will not interact with the polar stationary phase. Components may be tentatively identified by comparing the distances traveled with those of known reference materials in the same system (Fried, 1996).

Visualization of compounds on the TLC plates is common in order to determine the location of specific compounds on the plate as spots of color, due to their natural color or a reaction with reagents (Fried, 1996). Chen and Chen (1992) used TLC to separate carotenoids and chlorophylls in flowering plants that live in water (convolvulus). Bailey and Chen (1988) developed a method to prepare neoxanthin and violaxanthin standards from saponified spinach extracts.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is a form of liquid chromatography used to separate compounds dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Gradient elution is one type of HPLC system that varies the mobile phase composition during analysis (Lough and Wainer, 1996). The gradient separates compounds as a function of how well the analyte is mobilized by the changing solvent. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. HPLC is advantageous over other chromatography techniques due to exacting resolution, ease of use, and short analysis time (Lough and Wainer, 1996). Detection of phenolic compounds by photodiode array detectors has been based on

absorption measurements at characteristic wavelengths (Robards and Antolovich, 1997). Flavonoids characteristically have two absorption bands; where Band I has a maximum absorption in the range of 300 to 550 nm and Band II has a maximum absorption in the range of 240 to 285 nm (Wrolstad et al., 2005).

Liquid Chromatography-Mass Spectrometry

Numerous analytical methods are available to analyze the composition of phenolic compounds by liquid chromatography mass spectrophotometry (LC-MS). Liquid chromatography-mass spectrometry (LC-MS) is a technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. LC-MS is used to identify and verify the structures of components that have already been separated by HPLC. LC-MS is widely used for numerous purposes due to high sensitivity and specificity (Kasimu et al., 1998). The LC-MS is used for detection and identification of chemicals in complex mixtures.

Mass spectrometry (MS) is an analytical technique used to measure the mass-to-charge ratio of ions. This technique is widely used for identifying unknown compounds and is based on the fact that different chemicals have different masses (McLafferty and Turecek, 1993). The mass spectrometer generates a mass spectrum, which is a plot of intensity versus mass-to-charge ratio. The mass spectrum for a given sample will be a pattern representing the distribution of molecules by mass (Harborne, 2000).

REFERENCES

1. Acquaaah, G. (2005). Principles of Crop Production: Theory, Techniques, and Technology. New Jersey: Pearson Education Inc.
2. Ahmed, E., Young, C. (1982). Composition, quality, and flavor of peanuts. In Pattee, H., Young, C. (eds.) Peanut Science and Technology. Yoakum, Texas: American Peanut Research and Education Society.
3. Aruoma, O., Evan, P., Kaur, H., Sutcliffe, L., Halliwell, B. (1990). An evaluation of the antioxidant activity and potential pro-oxidant properties of food additives Trolox C, vitamin E, and probucol. *Free Radical Res. Comm.* 10, 143-157.
4. Awad, A., Chan, K., Downie, A., Fink, C. (2000). Peanuts as a source of β - sitosterol, as sterol with anticancer properties. *Nutrition and Cancer*, 36(2), 238-241.
5. Awika, J., Rooney, L., Wu, X., Prior, R., Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Sorghum products. *J. Agric. Food Chem.*, 51, 6657-6662.
6. Bailey, C., Chen, B. (1988). Simultaneous separation and identification of carotenoids and chlorophylls in turf Bermuda grass by high performance liquid chromatography. *J. Chromatogr.*, 445, 396-400.
7. Benbrook, B. (2005). Elevating Antioxidant Level in Foods through Organic Farming and Food Processing. *Organic Center State of Science Review*. January.
8. Blankenship, R. (2002). Molecular Mechanisms of Photosynthesis. Oxford UK: Blackwell Science Ltd.
9. Brand-Williams, W., Cuvelier, M., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28, 25-30.
10. Buratti, S., Pellegrini, N., Brenna, O., Mannino, S. (2001). Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. *J. Agric. Food Chem.*, 49, 5136-5141.
11. Carey, F. (2003). Organic Chemistry 5th Edition. New York: McGraw-Hill Co.
12. Cao, G., Alessio, H., Cutler, R. (1993). The measurement of oxygen radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* 14 (3), 303-311.
13. Cao, G., Prior, R. (1999). Oxygen-radical absorbance capacity in biological samples. *Methods Enzymol.* 299, 50-62.

14. Chen, B., Chen, Y. (1992). Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. *Food Chemistry*, 45, 129-134.
15. Chen, R., Wu, P., Chiou, R. (2002). Peanut roots as a source of resveratrol. *J. Agric. Food Chem.* 50, 1665-1667.
16. Chung, I., Park, M., Chun, J., Yun, S. (2003). Resveratrol accumulation and resveratrol synthase gene expression in response to abiotic stresses and hormones in peanut plants. *Plant Science*, 164, 103-109.
17. Dakora, F., Phillips, D. (2002). Root exudates as mediators of mineral acquisition in low nutrient environments. *Plants and Soil*, 245, 35-47.
18. Davalos, A., Gomez-Cordoves, C., & Bartolome, B. (2004). Extending the applicability of the oxygen radical absorbance capacity (ORAC-Flourescein) assay. *J. Agric. Food Chem.*, 52, 48-54.
19. Endo, Y., Usuki, R., Kandena, T. (1985). Antioxidant effects of chlorophyll and pheophytin on the autooxidation of oils. *Journal of the American Oil Chemists Society*, 62, 1375-1390.
20. Escarpa, A., Gonzalez, M. (2001). An overview of analytical chemistry of phenolic compounds in foods. *Critical Reviews in Analytical Chemistry*, 31(2), 57-139.
21. Farabee, M. (2001). Cellular Metabolism. Retrieved June 1, 2006:
<http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookGlyc.html>.
22. Fennema, O. ed. (1996). Food Chemistry. New York: Marcel Dekker.
23. Ferruzzi, M., Bohm, V., Courtney, P., Schwartz, S. (2002). Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assay. *J. Food Science Food Chemistry and Toxicology*, 67, 2589-2595.
24. Firestone, D. (ed.). (1992). Official Methods and Recommended Practices of the American Oil Chemists' Society. Champaign, IL: AOCS. Method Cd 12b-92.
25. Frankel, E., Kanner, J., German, J., Parks, E., Kinsella, J. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, 341(8843), 454-457.
26. French, E., Prine, G., Blount, A. (2006). Perennial Peanut: An Alternative Forage of Growing Importance. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

27. Fried, F. (1996). Practical Thin-Layer Chromatography: A Multidisciplinary Approach. Boca Raton: CRC Press, Inc.
28. Fuhrman, E. (2005). Good for you – naturally: nuts not only taste good in products, but they are also good for you. *Candy Industry*, 170(1), 52-55.
29. Fukumoto, L., Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.*, 48, 3597-3604.
30. Ghiselli, A., Serafini, M., Maiani, G., Azzini, E., & Ferro-Luzzi, A. (1995). A fluorescence-based method for measuring total plasma antioxidant capability. *Free Rad. Biol. Med.* 18, 29-36.
31. Golden, P. (2001). Perennial Profit. *Farm Progress*, Sept., 12-14.
32. Graf, E. (1992). Antioxidant potential of ferulic acid. *Free Rad. Biol. Med.*, 13(4), 435-448.
33. Green, R. (2004). Antioxidant Activity in Peanut Plant Tissues. Master's Thesis submitted to NCSU Department of Food Science.
34. Hammons, R. (1994). The Origin and History of the Groundnut. In J. Smart (Ed.). The Groundnut Crop – A Scientific Basis for Improvement. London, UK: Chapman & Hall.
35. Harborne, J., Williams, C. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55(6), 481-504.
36. Harrison, H., Peterson, J., Snook, M., Bohac, J., Jackson, D. (2003). Quantitation and potential biological activity of caffeic Acid in sweet potato [ipomoea Batatas (L.) LAM.] storage roots. *J. Agric. Food Chem.* 51(10):2943-2948.
37. Hopkins, W., Huner, N. (2004). Introduction to Plant Physiology 3rd Ed. Hoboken, NJ: John Wiley & Sons, Inc.
38. Houghton, P., Raman, A. (1998). Laboratory Handbook for the Fractionation of Natural Extracts 1st ed. New York, NY: Chapman & Hall.
39. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E. (2002) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* 50, 1815-1821.
40. Huang, D., Ou, B., Prior, R. (2005). The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53, 1841-185.

41. Huang, S., Yen, G., Chang, L., Yen, W., Duh, P. (2003). Identification of an Antioxidant, Ethyl Protocatechuate in Peanut Seed Testa. *J. Agric. Food Chem.* 51, 2380-2383.
42. Kasimu, R., Tanaka, K., Tezuka, Y., Gong, Z., Li, J., Basnet, P., Namba, T., Kadota, S. (1998). Comparative study of seventeen *Salvia* plants: aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. *Chem. Pharm. Bull.*, 46(3), 500-504.
43. King, R., Bomser, J., Min, D. (2006). Bioactivity of Resveratrol. *Comprehensive Reviews in Food Science and Food Safety*. In Press.
44. Krinsky, N. (1998). The antioxidant and biological properties of the carotenoids. *Ann. NY Acad. Sci.*, 854, 443 -7.
45. Kris-Etherton, P., Pearson T., & Wan, Y. (1999). High-monounsaturated fatty acids diets lower both plasma cholesterol and triacylglycerol concentrations. *Amer. J. of Clin. Nutr.* 70, 1009-1015.
46. Kuenzig, W., Chan, J., Norkus, E., Holowaschenko, H., Newmark, H., Mergens, W., Conney, A. (1984). Caffeic and ferulic acid as blockers of nitrosamine formation. *Carcinogenesis*, 3(5), 309-313.
47. Labuza, T.P. (1971). *Kinetics and Lipid Oxidation in Foods*. CRC. Critical Reviews in Food Technology.
48. Lawlor, D. (2001). Photosynthesis. Hertfordshire, UK: IACR-Rothamsted.
49. Lee, K., Kim, Y., Lee, H., Lee, C. (2003). Cocoa Has More phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J. Agric. Food Chem.*, 51 (25), 7292 -7295.
50. Liu, C., Chiou, Y., Wang, J., Chiou, K., Robin, Y. (2003). Comparative characterization of peanuts grown by aquatic floating cultivation and field cultivation for seed and resveratrol production. *J. Agric. Food Chem.* 51: 1582-1585.
51. Lough, W., Wainer, I. (1996). High Performance Liquid Chromatography: Fundamental Principles and Practice. Glasgow: Chapman & Hall.
52. Macheix, J., Fleuriet, A. (1998). Flavonoids in health and disease. Marcel Dekker Inc, New York.
53. Machlin, L., Bendich, A. (1987). Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J.* 1, 441-445.
54. May, P. (2005). Chlorophyll. Retrieved June 1, 2006.
http://www.chm.bris.ac.uk/motm/chlorophyll/chlorophyll_h.htm

55. Mayer, J., Stern, F. (2003). USDA Database for the Flavonoid Content of Selected Foods. USDA-ARS Beltsville Human Nutrition Research Center, Beltsville, Maryland.
56. McLafferty, F., Turecek, F. (1993). Interpretation of Mass Spectra. University Science Books. 4th edition.
57. Miller, N., Rice-Evans, C., Davies, M., Gopinathan, V., Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* 84, 407-412.
58. Mori, H., Kawabata, K., Yoshimi, N., Tanaka, T., Murakami, T., Okada, T., Murai, H. (1999). Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. *Anticancer Res.* 19(5A), 3775-8.
59. Nijveldt, R., Nood, E., Hoorn, D., Boelens, P., Norren, K., Leeuwen, P. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Amer. J. Clin. Nutr.*, 74, 418-425.
60. Negishi, T., Haruki, R., Hayatsu, H. (1997). Antigenotoxic activity of natural chlorophylls. *Fund. Mol. Mech. Mutag.*, 376: 97-100.
61. Novus Research (2006). Phenolic Compounds in Plants. Gilbert, Arizona. Retrieved October 14, 2006: <http://www.organicashitaba.com/pc.html>.
62. O'Byrne, D., Knauff, D., Shireman, R. (1997). Low-fat monounsaturated diets containing high-oleic peanuts improve serum lipoprotein profiles. *Lipids*, 32, 687-695.
63. Olthof M., Hollman P., Katan M. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr.*, 131(1), 66-71.
64. Ou, B., Hampsch-Woodill, M., Prior, R. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.*, 49, 4610-4626.
65. Ou, B., Huang, D., Wampsch-Woodill, M., Flanagan, J., Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) assays: A comparative study. *J. Agric. Food Chem.*, 50, 3122-3128.
66. Panel on dietary antioxidants and related compounds, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board Vitamin C, Vitamin E, Selenium, and β -Carotene and other Carotenoids: Overview, Antioxidant Definition, and Relationship to Chronic Disease. (2000). In Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids; National Academy of Science: Washington D.C., 35-37.

67. Pattee, H., Young, C. (1982). Peanut Science and Technology. American Peanut Research and Education Society, Inc.: Yoakum, Texas.
68. Pietta, P. (2000). Flavonoids as antioxidants. *J. Nat. Prod.*, 62, 1035-1042.
69. Plumb, G., Lambert, N., Chambers, S., Wanigatunga, S., Heaney, R., Plumb, J.A., Aruoma, O., Halliwell, B., Williamson, G. (1996). Are extracts of purified glucosinolates from cruciferous vegetables antioxidants? *Free Rad. Res.*, 25, 75-86.
70. Prakash, A. (2001). Antioxidant Activity. *Medallion Laboratories Analytical Progress*, 19, 2.
71. Prezelin, B., Nelson, N. (1997). The formation of ATP and reducing power in the light. In Dennis, D., Turpin, D., Lefebvre, D., and Lyzell, D., eds., Plant Metabolism 2nd ed. Essex, England: Addison Wesley Longman, 274-285.
72. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.*, 26, 1231-1237.
73. Robards, K., Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids. *Anal.*, 122, 11R-34R.
74. Rouessac, F., Rouessac, A. (2000). Chemical Analysis: Modern Instrumentation Methods and Techniques. West Sussex, England: Wiley.
75. Sanders, T., McMichael, R., Hendrix, K. (2000). Occurrence of Resveratrol in edible peanuts. *J. Agric. Food Chem.*, 48 (4), 1243 -1246, 2000.
76. Schmidt, K. (2000). Lipids: Functional Properties. In Christen, G., and Smith, J. (Eds.), Food Chemistry: Principles and Applications. West Sacramento, CA: Science Technology System.
77. Sherma, J., Fried, B. (2003). Handbook of Thin Layer Chromatography. Boca Raton, FL: CRC, Inc.
78. Sobolev, V., Cole, R. (1999). trans-Resveratrol content in commercial peanuts and peanut products. *J. Agr. Food Chem.*, 47, 1435-1439.
79. Soleas, G., Diamondis, E., Goldberg, D. (1997). Resveratrol: A molecule whose time has come? And gone? *Clin. Biochem.*, 30, 91-113.
80. Sprent, J. (1994). Nitrogen Fixation. In J. Smart (Ed.), The Groundnut Crop – A Scientific Basis for Improvement. London, UK: Chapman & Hall.

81. Stampfer, M., Hennekens, C., Manson, J., Colditz, G., Rosner, B. and Willett, W. (1993). Vitamin E consumption and the risk of coronary disease in women. *NE J. of Med.* 328, 1444-1449.
82. Starr, C., Taggart, R. (2004). Biology: The Unity and Diversity of Life, 3rd Ed. Belmont, CA: Thomson.
83. US Department of Agriculture (USDA), Agricultural Research Service & Iowa State University. (2000a). USDA-Iowa State University Database on the Nutrient Content of Foods, Release 1.3—2002. Retrieved April 18, 2005 from the Nutrient Data Laboratory Home Page on the World Wide Web: <http://www.nal.usda.gov/fnic/foodcomp>.
84. USDA Center for Nutrition Policy and Promotion. (2000b). Nutrition Insights: The Role of Nuts in a Healthy Diet. Washington, DC. Retrieved April 24, 2005. www.usda.gov/cnpp
85. USDA, FAS, Production Estimates and Crop Assessment Division (2004). Peanut area, yield, and production. April, 2005. Table 13.
86. USDA, Natural Resource Conservation Service, Plants Database (2005). Retrieved August 21, 2005 from Plants Database on the World Wide Web: http://plants.usda.gov/cgi_bin/topics.cgi?earl=plant_profile.cgi&symbol=ARHY
87. Vance, C. (2002). Root-Bacteria interactions: Symbiotic N₂ fixation. In Waisel, Y., Eshel, A., & Kafkafi, U. (Editors), Plant Roots, The Hidden Half 3rd ed. Rev. and Exp. New York, NY: Marcel Dekker, Inc., 839-868.
88. Vogel, J., Jonker-Termont, D., Lieshout, E., Katan, M.B., Meer, R. (2005). Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. *Carcinogen.*, 26(2), 387-393.
89. Wagner, H., Blatt, Sabine (2001). Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin: Springer-Verlag.
90. Wardlaw, G. Hampl, J., DiSilvestro, R. (2004). Perspectives in Nutrition. Boston: McGraw Hill.
91. Wang, K., Lai, Y., Chang, J., Ko, T., Shyu, S., Chiou, R. (2005). Germination of peanut kernels to enhance Resveratrol biosynthesis and prepare sprouts as a functional vegetable. *J. Agric. Food Chem.*, 53, 242-246.
92. Waterhouse, A. (2006). Folin-Ciocalteu Micro Method for Total Phenol in Wine. Retrieved June 1, 2006: <http://waterhouse.ucdavis.edu/phenol/folinmicro.htm>

93. Wright, J., Johnson, E., Carpenter, D. (2001). Predicting the activity of phenolic antioxidants: theoretical method, analysis of constituent effects, and application to major families of antioxidants. *JACS*, 123, 1173-1183.
94. Woodruff, J. (1983). Peanuts: Production, Processing, and Products 3rd Ed. Westport, CT: AVI Publishing Co.
95. Womach, R. (2003). Comparing Quota Buyout Payments for Peanuts and Tobacco. CRS Report for Congress. Retrieved October 14, 2006:
<http://216.239.51.104/search?q=cache:XtsJbqk5TgJ:www.nationalaglawcenter.org/aset/crs/RS21642.pdf+estimated+peanut+production+in+united+states+per+year&hl=en&gl=us&ct=clnk&cd=1>
96. Wrolstad, R., Acree, T., Decker, E., Penner, Reid, D., Schwartz, S., Shoemaker, C., Smith, D., Sporns, P (2005). Handbook of Food Analytical Chemistry. John Wiley & Sons: Hoboken, NY.
97. Yeh, C., Yen G. (2003). Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxidant activity. *J. Agric. Food Chem.*, 51(5), 1474 – 1479.

CHAPTER 2

Contribution of Specific Phenolic Compounds to the Total Antioxidant Activity of Peanut Leaves and Roots

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are an economically important crop for the United States, especially for the southeast. The United States produces approximately 10% of the world's peanut crop (Acquaah, 2005). Nine states in the United States account for 99% of the US crop production. Those states are: Georgia, Texas, Alabama, North Carolina, Florida, Oklahoma, Virginia, South Carolina, and New Mexico (Acquaah, 2005). Peanuts are an excellent source of nutritional compounds.

The peanut plant accounts for approximately 40% of the peanut biomass. Peanut leaves and roots can be considered a byproduct of peanut production and have low economic value (French et al., 2006). Currently, plants are either baled for high protein animal feed or left in the field.

Little research has been completed on the phytochemical composition of peanut leaves and roots. Two studies reported high levels of resveratrol in peanut plant roots (Chen et al. 2002, Liu et al. 2003). Chen et al. (2002) reported different levels of resveratrol in peanut plant roots from different growing seasons. Phytochemical research has increased over the past ten years; because, phytochemicals are natural bioactive compounds found in plant foods which are believed to protect against disease. Numerous studies have associated phytochemicals, especially phenolic compounds, with a decrease in cardiovascular disease, cancer, and other degenerative diseases (Wrolstad et al., 2005). At least 8,000 phytochemical compounds have demonstrated antioxidant properties (Pietta, 2000). Total phenolics of food products were first assayed by Singleton and Rossi (1965).

Two commonly used procedures are the Oxygen Radical Absorbance Capacity (ORAC) (Cao et al., 1993) assay which verifies the capacity of an antioxidant to scavenge free radicals

generated by the system, and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995) which measure the capability of the antioxidant compound to scavenge stable free radicals.

Green (2004) determined the antioxidant ability for peanut leaves, roots, and stems. Sampling date, drying method, extraction methodology, and extraction technique were variables analyzed for all three peanut plant parts. The highest antioxidant activity level was found in the leaves, followed by the roots, and low activity was determined for the stems. Methanol extracts exhibited the highest antioxidant activity. Green's (2004) work was an initial study to determine whether peanut plants contain bioactive compounds that provide the basis for value added products.

The objectives of this study were to determine the antioxidant capacity and total phenolic content of peanut leaves and roots extracted in methanol and to compare extraction solvent systems of methanol versus aqueous methanol. Additional objectives included identification of phenolic compounds in peanut leaves and roots, and determination of the specific antioxidant activity contribution of these compounds.

MATERIALS AND METHODS

Plant Samples

Peanut plants (*Arachis hypogaea* L.) were collected from the Upper Coastal Plain Research Station in Rocky Mount, NC on October 2, 2005. Peanut roots (var FlavoRunner 458) were collected from a farm in Seminole, Texas on October 19, 2005 in order to compare root antioxidant activity and phenolic composition from two different locations. Peanut plant parts were separated and rinsed with water to remove all visible soil before storage at -45°C in separate sealed plastic containers.

Peanut plant parts were removed from cold storage and placed into a Virtis Freeze Dryer (Model No. FFD-40-WS, Gardiner, NY). The refrigeration condenser was set at -15.5°C . The shelf temperature was set at 15.5°C for the first 24 hours, 38°C for the second 24 hour period, and 57°C for the final 24 hour period of drying. Samples were ground in a Krups 3 oz. household coffee mill (Krups North America, Closter, NJ) to a coarse powder. The peanut plant powder was then stored in screw capped glass jars at -20°C until extraction.

Extraction

Three 5 grams of dried ground peanut plant parts were measured into 50 mL conical tubes and extracted 3 times with 15 ml of either methanol or 80/20 (v/v) methanol/water. Conical tubes containing plant powder and 15 ml of solvent were rocked on a Thermolyne Vari Mix Test Tube Mixer (Barnstead International, Dubuque, Iowa) at 15 rpms for 30 minutes. The tubes were then transferred to a Damon/IEC International Centrifuge (Needham Hts., Massachusetts) and centrifuged at 1075 rpms for 20 minutes. The supernatant was pipetted to amber vials, and the final extraction amount was 45 ml. For the lipophilic ORAC extracts,

hexane was used as the extraction solvent. Extracts were stored in amber vials which were sealed with Teflon lined screw-caps and held at $-20\text{ }^{\circ}\text{C}$ until further analysis.

Chlorophyll Content Measurement

Chlorophyll content was determined according to methods in the Handbook for Food Analytical Chemistry (Wrolstad et al, 2005). Half a gram of plant powder was ground using a mortar and pestle with 3 ml of methanol, the mortar was rinsed with 1.5 ml methanol, and the final volume was adjusted to 5 ml with methanol. Chlorophyll extracts were then transferred to a Damon/IEC International Centrifuge and centrifuged at 1075 rpms for 5 minutes. Two hundred μL of the supernatant was transferred to a 96 microwell plate and absorbance was read using a SAFIRE plate reader with the Magellan V 4.00 software (TECAN, Raleigh, NC). Absorbance was determined at 665 nm and 652 nm. Absorbance values were used to calculate the concentration of chlorophyll a (C_a) and b (C_b) from equations based on the Lambert-Beer law and absorbance coefficients taken from the literature (Wrolstad, 2005):

$$C_a (\mu\text{g/ml}) = 16.72 A_{665} - 9.16 A_{652} \quad (\text{Equation 1.0})$$

$$C_b (\mu\text{g/ml}) = 34.09 A_{652} - 15.28 A_{665} \quad (\text{Equation 1.1})$$

Oxygen Radical Absorbance Capacity Assay

The ORAC procedure was adapted from Cao et al. (1993), and Prior et al. (2003) with fluorescence measurements made by the Safire monochromator based microplate reader equipped with a Magellan V4-W reader software (Tecan USA, Raleigh NC). The samples were loaded on a Costar polystyrene flat-bottom translucent 96 microwell plate (Corning, Acton, Massachusetts) (Huang et al., 2002). The concentration of solvents used equaled that of Prior et al. (2003), with the exception of the samples which had to be diluted. The reaction was carried out in 75mM phosphate buffer (pH 7.4), which was prepared weekly. Fluorescein (Reidel-de

Haen, Milwaukee, WI) solution stock was stored at 4°C for no longer than one week as 15 mL aliquots which were diluted to the final concentration of 70 nM daily. 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) (Sigma, St. Louis, MO) was prepared daily. For the lipophilic extracts, a solution of 7% randomly methylated cyclohexane (RMCD) (Sigma, St. Louis, MO) was used as a blank, dilution solvent, and Trolox solvent (Huang et al., 2002a).

The total volume of liquid in the microwell was 200µL. Sample extracts and standards, both at 20µL, were added to the well followed by 120µL of fluorescein. The plate containing only the samples and fluorescein was incubated in the SAFIRE for 15 minutes at 37°C ± 2°C, before 60µL of the AAPH solution was added. Prior to beginning measurements, a high-intensity three second linear shaking was performed by the SAFIRE followed by one second of settling time. Data points were acquired over eighty minutes as relative fluorescent units (RFU) ranging from 0-60000 RFU using the Magellan V 4.00 software (TECAN, Raleigh, NC) and exported into Microsoft Excel for further analysis. Measurement parameters were set to fluorescent excitation at 485 nm and fluorescent emission at 520 nm for the SAFIRE.

The antioxidant activity, or ORAC, is expressed in Trolox Equivalents by using a standard curve calculated from Trolox standards 3.125, 6.25, 12, 25, 50 µM well concentrations. The Trolox standard curve describes the change in the area under the curve (AUC) with concentration. The AUC of the sample is calculated as:

$$\text{AUC} = (0.5 + f_5 / f_4 + f_6 / f_4 + f_7 / f_4 \dots + f_i / f_4)] \times \text{CT} \quad (\text{Equation 5.0})$$

Where f_4 is the initial fluorescence reading at cycle 4, and is the fluorescence reading at cycle i , and CT is the cycle time in minutes. The AUC is then expressed as µM Trolox Equivalents/gram of sample by way of the standard curve linear regression:

$$\text{AUC} = (\mu\text{M Trolox}) \times (m) + b \quad (\text{Equation 6.0})$$

The net area under the curve was obtained by subtracting the blank value from that of the sample or standard. The net area under the curve value is then input into the $y = mx + b$ equation format. The m and b signify the slope and intercept respectively from the standard curve results. Each sample was analyzed in triplicate. Antioxidant activity was reported in μM Trolox Equivalents per gram of dry powder.

DPPH Radical Scavenging Assay

The method of Brand-Williams et al. (1995) was adapted for testing the radical scavenging of the extracts using the stable free radical 2,2-diphenyl-1-picrahydrazyl (DPPH \bullet) (Sigma, St. Louis, MO). All spectrophotometric data were acquired using absorbance measurements made by the Safire monochromator based microplate reader equipped with a Magellan V4-W reader software (Tecan USA, Raleigh NC). The samples were loaded on Costar polystyrene flat-bottom translucent 96-well plate (Corning, Acton, Massachusetts).

Extracts and standards were diluted with ethanol. A 0.1 ml aliquot of the diluted sample was added to 3.9 ml of DPPH solution (6×10^{-5} M). Absorbance was measured at 515 nm each minute for 180 minutes. Once the absorbance reached a plateau, the reaction was complete. The reaction time of 60 minutes was used for the DPPH analysis. The blank was 95% ethanol. Trolox concentrations of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mM were used as the standards. Each sample was analyzed in triplicate. Antioxidant activity was reported in μM Trolox Equivalents per gram of dry powder.

Total Phenolic Assay

Total phenolics were measured by the method of Folin-Ciocalteu as adapted by Singleton et al. (1999) in triplicate. Gallic acid calibration standards were prepared at 50, 100, 250, and 500 mg/liter concentrations. Five hundred microliters of extract and standards were

diluted with distilled water to a volume of 5.0 ml. Half a milliliter of Folin-Ciocalteu reagent (Fisher Scientific, Fairlawn, NJ) was added to extracts or standards and allowed to react at room temperature for 3 minutes. One milliliter of 1 N sodium carbonate was added and the mixture was incubated in the dark, at room temperature for an hour. Absorbance was measured at 725 nm with the Safire monochromator based microplate reader equipped with a Magellan V4-W reader software (Tecan USA, Raleigh, NC).

Thin Layer Chromatography

Peanut plant powder extracted with aqueous methanol was fractionated by thin layer chromatography in duplicate for pigment separation and to determine the location of antioxidant compounds based on solvent differences. The mobile phase used was 70:25:10:5 (v/v/v/v) hexane/acetone/chloroform/methanol (Chen and Chen, 1992). Five hundred microliters of extract was spotted in a line on the chromatography plate. The mobile phase was added to the TLC chamber thirty minutes prior to addition of the plate, in order to for the chamber to reach equilibrium. Visualization of phenolic compounds was done utilizing a method developed by Pratt and Miller (1984). Equal volumes of ferric chloride (1%) and potassium chloride (1%) (Sigma, St. Louis, MO) were mixed, resulting in an orange-brown solution. TLC plates were sprayed with the solution. Phenolic compounds were visualized as a blue color, which developed upon reduction of the visualization mixture.

HPLC-DAD Analyses

Three replicates of powder samples from each of the plant parts extracted with aqueous methanol were analyzed using a Thermo Quest HPLC system (San Jose, CA) equipped with a P2000 binary pump, UV 6000 LP photodiode array detector (DAD), A5 3000 autosampler and Thermo Quest 4.1 chromatography software. Separation was achieved using a Phenomenex

Synergi Polar Reverse Phase 4 μ 4.6 x 250 column (Phenomenex, Torrance, CA). The analysis was monitored at 255 and 326 nm and the absorption spectra of the analyzed compounds were recorded between 200 and 600 nm. Solvent A was 0.1% Formic acid and 100% methanol was employed as solvent B. A gradient was used starting at 95% A and 5% B, then decreased to 75% A in 50 minutes, and then increased to 95% A in 5 minutes and re-equilibrated for 10 minutes. The operating conditions were set to an injection volume of 20 μ l, flow rate of 1 ml per minute and a column temperature of 35°C. The UV spectra of the different compounds were recorded with the diode array detector. Identification of phenolic compounds was tentatively based on retention time and UV spectra maxima absorption curves as compared to phenolic standard compounds which were prepared at 1 mM concentration for HPLC analysis.

HPLC-MS Analyses

Three replicates of powder for each of the plant parts extracted with aqueous methanol were analyzed using HPLC-MS. Resveratrol, catechins, and other phenolics were analyzed with differing HPLC conditions. For phenolics, samples were filtered through 0.02 μ m Anotop 10 membrane filters (Whatman Corp., Clifton, NJ), before injection onto a Thermo Finnegan Surveyor HPLC system (San Jose, CA) interfaced with a LQC Duo mass spectrometer. The HPLC was equipped with a quaternary gradient pump, photo diode array detector, autosampler, and Xcalibur chromatography software (Version 3.1, Thermo Electron Corp.). Separation was achieved using a Phenomenex Synergi Polar Reverse Phase 4 μ , 80 Å, 250 mm x 2.0 mm column (Phenomenex, Torrance, CA). Analysis was monitored at 280 and 325 nm and the absorption spectra of the analyzed compounds were recorded between 200 and 600 nm. Solvent A was 0.1% Formic acid in acetonitrile and 0.1% Formic acid in H₂O was employed as solvent B. A gradient was used starting at 5% A and 95% B initially, then A was increased to 95% in 30

minutes, held five minutes then decreased to 5% A in 1 minute and re-equilibrated at 5% A for 5 minutes. The operating conditions were set to an injection volume of 10 μl , flow rate of 200 μl per minute and a column temperature of 30°C. Electrospray Ionization (ESI) MS was completed in the negative mode, with the exception of ferulic acid analysis which was analyzed in the positive mode. Spray voltage was set to 4.5 kV, capillary voltage was 14V, and the capillary temperature was 200°C. The lens positions and offsets were optimized for each compound analyzed. Nitrogen was used as the sheath gas at a flow rate of 60 arbitrary units, helium was used as the auxiliary gas at 10 arbitrary units.

Extracts for resveratrol analysis were prepared as described by Sanders et al. (2000). Aqueous methanol extracts were injected onto a Thermo Finnegan Surveyor HPLC system (San Jose, CA) equipped with a quaternary pump, diode array detector (DAD), and autosampler. Separation was achieved using a Phenomenex Synergi Hydro-RP 4 μ , 80 Å, 150 mm x 2.0 mm column (Phenomenex, Torrance, CA). Analysis was monitored at 280 and 305 nm and the absorption spectra of the analyzed compounds were recorded between 200 and 600 nm. One hundred percent methanol was used as solvent A and 5% methanol in H₂O was employed as solvent B. Isocratic HPLC was employed with 40% solvent A and 60% solvent B. Operating conditions were set to an injection volume of 10 μl , flow rate of 150 μl per minute and column oven temperature of 30°C. Electrospray Ionization (ESI) MS was completed in negative mode with a spray voltage of 4.5 kV, capillary voltage was 29V, capillary temperature was 200°C, and tube lens offset 30V. Nitrogen was used as the sheath gas at a flow rate of 60 arbitrary units, helium was used as the auxiliary gas at 10 arbitrary units.

For catechin analysis, aqueous methanol extracts were filtered through 0.02 μm Anotop 10 membrane filters (Whatman Corp., Clifton, NJ), before injection into a Thermo Finnegan

to be a statistically significant difference using the SAS Statistical Analysis System v8.1 (SAS Institute Inc., Cary, NC).

RESULTS and DISCUSSION

Determination of Antioxidant Properties of Peanut Parts Extracted in Methanol

Oxygen Radical Absorbance Capacity Assay

Antioxidant capacity values from the ORAC assay are calculated based on a Trolox standard curve (Figure 1). Initial determination of the antioxidant capacity for peanut leaves and roots was determined for methanol extracts (Figure 2), based on previous work in this laboratory (Green 2004). It was concluded that methanol was the most efficient of the extraction solvents tested for antioxidant activity optimization for peanut plant parts.

The mean total ORAC for NC peanut leaves, NC peanut roots and TX peanut roots extracted with methanol was 163.1, 124.5 and 89.92 μM Trolox equivalents per gram dry weight plant powder, respectively.

ORAC for peanut leaves and roots are comparable to other fruits and vegetables. Iceberg lettuce has an ORAC of 288.6 μM TE/gram dry weight (Wu et al, 2004). Ou et al. (2002) concluded that spinach has an ORAC of 154.0 μM TE/gram dry weight. Wu et al. (2004) determined antioxidant activities for other common foods including cashews and avocado. Cashews have ORAC of 16.7 μM TE/g dry weight and avocados have ORAC of 79.0 μM TE/g dry weight. Prior et al. (2003) determined the ORAC for pine nuts as 8.4 μM TE/g dry weight, 1260.5 μM TE/g dry weight for sorghum bran, and 444.3 μM TE/g dry weight for strawberries. Peanut leaves and roots have an ORAC higher than cashews, avocados, and pine nuts, but lower than strawberries and sorghum bran.

Ou et al. (2002) determined that broccoli had nearly a ten-fold difference in ORAC values among varieties, green peppers had nearly a six-fold difference and spinach had a two-fold difference. Antioxidant capacity variation among the same vegetable can be explained by

variety, location, and harvest season (Ou et al., 2002). Wu et al. (2004) also explained antioxidant activity discrepancies between samples based on production location. Numerical differences in antioxidant activity between leaves and roots extracted with methanol were determined. Differences in production location are significant for NC root and TX root for total antioxidant activity as determined by the ORAC assay ($p < 0.05$), which indicated that NC roots have a higher ORAC than TX roots. Green (2004) concluded that peanut leaves had the highest activity, followed by the roots, and similar results were found in this study.

For the ORAC assay, total antioxidant activity is the sum of the hydrophilic activity plus the lipophilic activity. The hydrophilic ORAC assay primarily measures the activity of compounds such as phenolics and anthocyanins, while the lipophilic assay assesses the activity of carotenoids and tocopherols. The hydrophilic portion of the total antioxidant activity of peanut leaves and roots extracted with methanol accounts for more than 90% of the total antioxidant activity, which is consistent with other food products as reported by Wu et al. (2005). Wu et al. (2005) reported the hydrophilic fraction contributed 85% of the total antioxidant activity for various fruits and vegetables. Kurilich et al. (2002) extracted lipophilic components from broccoli and determined the total antioxidant contribution to be approximately 15% of the total antioxidant capacity. The lipophilic fraction for both the peanut leaves and roots accounted for less than 10% of the total antioxidant capacity. The results for peanut leaves and roots are similar to ORAC reported for other food commodities (Wu et al., 2005; Kurilich et al., 2002).

DPPH Radical Scavenging Assay

Antioxidant activity of peanut leaves and roots extracted in methanol was assessed using the DPPH radical scavenging assay. The total antioxidant activity for NC peanut leaves, NC peanut roots and TX peanut roots extracted with methanol was 38.6, 23.4 and 21.0 μM Trolox

equivalents per gram dry weight plant powder, respectively (Figure 3). Harish et al. (2005) extracted root antioxidants from an endangered shrub, *Decalepis hamiltonii* Wight & Arn., and determined that methanol maximized antioxidant activity as compared to hexane, chloroform, and ethyl acetate. NC roots and TX roots have similar radical scavenging capabilities, since no significant difference was determined ($p>0.05$). NC leaves had the highest antioxidant capacity in the DPPH assay, which may be due to the presence of compounds responsible for photosynthesis. DPPH antioxidant capacity values for sweet potato leaves and onion leaves were higher than antioxidant capacity than sweet potatoes, onions, and other vegetables (Chu et al., 2005).

Peanut leaves had higher ORAC and DPPH values than both roots. Leaves are the site of synthesis of many compounds for plant functions. Xanthophylls, for example, are oxygenated carotenoids that may have antioxidant capacity and are present only in the leaves (Wrolstad, 2005). Roots contain less pigment compounds and more flavonoid compounds, which may have less antioxidant capacity especially if flavonoid glycoside compounds are extracted.

Chlorophyll Content and ORAC

Chlorophyll content and antioxidant activity was measured to determine if chlorophyll contributes to the antioxidant activity of NC leaves extracted in methanol. Chlorophyll derivatives have been analyzed as an antioxidant and pro-oxidant in previous studies (Endo et al. 1985). NC leaves had a total chlorophyll content of 8.1 milligrams per gram dry weight of leaf powder, which is similar in chlorophyll content to other leaves and green foods. Buratti et al. (2001) determined that green peppers have one fourth of the chlorophyll content compared with spinach leaves. Beech leaves have 6.2 to 12.0 milligrams chlorophyll per gram dry weight, Hornbeam leaves have a chlorophyll content ranging from 8.2 to 19.1 milligrams per gram dry

weight, and Poplar leaves contain 8.0 to 12.4 milligrams per gram dry weight (Wrolstad et al., 2005).

Chlorophyll b isolated from spinach was purchased from Sigma (St. Louis, Missouri) and tested in triplicate for antioxidant activity using the ORAC assay. Chlorophyll had an antioxidant activity of 415.6 ± 16.6 μM Trolox Equivalents per gram chlorophyll. Since the chlorophyll content of NC leaves was determined previously as 8.1 milligrams per gram of leaf powder, the overall contribution of chlorophyll to the total antioxidant activity was 3.4 ± 0.1 μM Trolox Equivalents per gram of leaf powder. Total contribution of chlorophyll was calculated as 2% of the total antioxidant activity of peanut leaves extracted in methanol, and was determined to not be a major contributor to the ORAC antioxidant activity of peanut leaves.

Potential bioactivity of dietary chlorophyll derivatives have been suggested and support a potential role for chlorophyll as a phytochemical (Ferruzzi et al., 2002). Reddy et al. (2005) considered chlorophyll a lipophilic pigment, which had a higher bioavailability than hydrophilic pigments when tested against tumor cell growth inhibition. Pigments tested for synergism had a reduced negative impact on biological activities (Reddy et al., 2005).

Total Phenolics Assay

The total phenolic assay is a measurement of the reduction of the reagent by phenolic groups (Huang et al., 2005). NC leaves had the highest total phenolics content of 75.2 milligrams gallic acid/100 grams dry weight (GAE) (Figure 4), followed by the NC roots with 59.9 GAE and TX roots with 29.0 GAE.

Phenolic content in plant products can vary considerably. Varieties of nectarines, peaches, and plums contained ranges of total phenolics from 13.8-140.3, 17.2-120.2 and 22.0-318.0 GAE dry weight (Gil et al., 2003). Liu et al. (2002) also reported the total phenolic

content for raspberries ranged from 359.0 to 513.0 mg gallic acid/100 grams fresh weight. Berries are considered a high source of phenolic compounds. Ehlenfeldt and Prior (2001) determined total phenolic content of blueberry fruit equal to 1.8 mg gallic acid/gram fresh weight, while leaves from the same plant had a total phenolic of 44.8 mg gallic acid/gram fresh weight. These studies indicate plant materials, such as fruits and vegetables, can have varying total phenolic contents based on variety. Phenolic compounds are secondary metabolites, so levels of production in the plant are dependent upon environmental factors such as stress, nutrients, weather, and other growing conditions (Benbrook, 2005). Total phenolics determined in the peanut plant are comparable to common food products and other plant sources.

Comparison of Methanol versus Aqueous Methanol (80%) on Antioxidant Properties of Peanut Leaves and Roots

Oxygen Radical Absorbance Capacity

Methanol was the initial extraction solvent selected for the analysis of peanut plant antioxidant properties based on previous work (Green, 2004). Literature suggested the use of aqueous methanol would optimize the extraction of phenolic compounds (Yen et al., 1996; Fukumoto and Mazza, 2000).

The hydrophilic ORAC of the peanut leaves and roots extracted in both methanol (M) and 80% aqueous methanol (A) was determined (Figure 5). Plant parts extracted in aqueous methanol had a significantly higher activity than the corresponding plant part extracted in methanol ($p > 0.05$). NC leaves extracted with aqueous methanol had an activity of 261.6 μM Trolox Equivalents per gram dry weight plant powder, while leaves extracted in methanol had an activity of 150.9 μM Trolox Equivalents per gram dry weight plant powder. NC roots extracted with aqueous methanol had an activity of 165.9 μM Trolox Equivalents per gram dry weight plant powder as compared to the methanol extracted NC roots with 116.2 μM Trolox Equivalents per gram dry weight plant powder. TX roots extracted with aqueous methanol had an activity of 121.0 μM Trolox Equivalents per gram dry weight plant powder, and the methanol extracted TX roots had an activity of 80.6 μM Trolox Equivalents per gram dry weight plant powder.

Siddhuraja and Beecker (2003) determined that 80% aqueous methanol was the most efficient solvent for extracting antioxidant containing phenolic constituents from drumstick tree leaves. Yen et al. (1996) also found that an 80% aqueous methanol extract contained the highest phenolic content for mulberry leaves. Aqueous methanol extracts of peanut leaves and roots had significantly higher ORAC antioxidant activities as compared with extracts with methanol ($p < 0.05$).

Solvent polarity affects the type of compounds extracted. Both water and methanol are polar solvents. Phenolic compounds are polar, although the degree of polarity is dependent upon structure. Methanol extracts compounds with functional groups such as acids and alcohols from plant tissue, because acids and alcohols are able to form hydrogen bonds with methanol due to the presence of hydroxyl groups (Carey, 2003). Water is more polar than methanol and extracts other compounds containing functional groups such as ketones and aldehydes, which lack hydroxyl groups and may not be extracted with methanol (Carey, 2003). From this, it would be expected that the extracts would contain compounds capable of hydrogen donation capable of quenching free radicals.

DPPH Radical Scavenger Assay

Peanut leaves and roots extracted with aqueous methanol had a significantly higher antioxidant capacity with the DPPH assay, than the corresponding leaves and roots extracted with methanol ($p < 0.05$). NC leaves extracted in aqueous methanol had a mean antioxidant capacity of 91.6 μM Trolox Equivalents per gram dry weight plant powder; the NC leaves extracted in methanol had an activity of 38.6 μM Trolox Equivalents per gram dry weight plant powder (Figure 3). NC roots extracted with aqueous methanol had an capacity of 27.4 μM Trolox Equivalents per gram dry weight plant powder and the roots extracted with methanol had an activity of 23.4 μM Trolox Equivalents per gram dry weight plant powder. TX roots extracted in aqueous methanol had an activity of 25.3 μM Trolox Equivalents per gram dry weight plant powder, and TX roots extracted with methanol extract had an activity of 21.0 μM Trolox Equivalents per gram dry weight plant powder. Antioxidant activity determined by the DPPH method followed the same trend as the ORAC assay due to the extraction of compounds with ketone and aldehyde functional groups by the addition of water to the solvent system.

Total Phenolics Assay

Peanut leaves and roots extracted with aqueous methanol contained significantly higher total phenolics, than the corresponding leaves and roots extracted with methanol ($p < 0.05$). NC leaves extracted in aqueous methanol had 138.0 gallic acid equivalents (GAE), while methanol extracted leaves 75.2 GAE (Figure 4). NC roots extracted with aqueous methanol had a mean phenolics content of 77.7 GAE and the roots extracted with methanol had a mean content of 59.9 GAE. TX roots extracted in aqueous methanol had 50.7 GAE, and Texas roots extracted with methanol extract had a total phenolics content of 29.0 GAE. Total phenolics content followed a similar trend as both antioxidant assays. Extracting in aqueous methanol increased the phenolics content as compared with pure methanol. The results are consistent with previous research findings (Yen et al., 1996; Fukumoto and Mazza, 2000; Siddhuraja and Beecker, 2003).

Addition of water to methanol allows compounds that are able to bond with the oxygen molecule in water to be extracted. Water is able to form hydrogen bonds with phenolic compounds that may not have numerous hydroxyl groups, therefore extracting more phenolic compounds than methanol alone (Carey, 2003). Water can also extract compounds such as sugar, so phenolic compounds that are bound to sugar may be extracted with the addition of water. From this, it would be expected that the extracts would contain compounds capable of quenching DPPH radicals.

Correlations

Correlations between ORAC and DPPH using aqueous methanol extracted samples were determined as positive. The coefficient of determination (R^2) value between the ORAC and DPPH procedure was 0.8759 (Figure 6). Awika et al. (2003) positively correlated ORAC and DPPH antioxidant capacity for sorghum and found R^2 equal to 0.97. The high correlation

demonstrates the peanut plant parts had comparable radical scavenging capabilities in both antioxidant capacity assays.

The total phenolics content correlated to the DPPH assay. Total phenolics versus DPPH resulted in an R^2 value of 0.9063 (Figure 7). Blueberry analysis resulted in an excellent correlation between DPPH and total phenolics (Huang et al., 2002). Huang et al. (2005) concluded this correlation is due to similar reaction electron transfer mechanisms between the assays.

Total ORAC values correlated with total phenolics, R^2 equaled 0.9113 (Figure 8). The hydrophilic fraction of the ORAC assay was highly correlated to the total phenolics content, where R^2 equaled 0.937 (Figure 9). Huang et al. (2002) found an excellent correlation between total phenolic content and ORAC antioxidant capacity values for grape seeds and skins. Spinach leaves display a similar trend between antioxidant capacity and total phenolics (Kuti and Konuru, 2004). Peanut leaves and roots were similar to previous research where total phenolics and ORAC have been positively correlated.

Awika et al. (2003) positively correlated both antioxidant assays, ORAC and DPPH, with total phenolics results. Awika et al. (2003) concluded these results confirm that phenols are largely responsible for the antioxidant activity of sorghums. The high correlation between antioxidant capacity and total phenolic content for peanut leaves and roots suggests phenolic compounds contribute significantly to the total antioxidant capacity of the plant parts measured with the both assays. The ORAC assay measures chain breaking antioxidant capacity versus peroxy radicals, while the DPPH assay measures ability to quench DPPH radicals.

Identification and Quantification of Phenolic Antioxidants and Their Contribution to Total Antioxidant Activity of Peanut Leaves and Roots

Thin Layer Chromatography – Pigment Separation

Fractions were generated using a pigment separation mobile phase on TLC and antioxidant activity of aqueous methanol fractions was determined using the ORAC assay (Figures 10, 11, and 12). Fractions were taken in 2 cm bands if colored bands were not present. If colored bands were present, each color was taken as a separate fraction. A control was taken from the top of a blank plate with the solvent system and above the solvent front to determine solvent or plate effects on antioxidant activity.

The separation fractions from NC leaves had mean ORAC ranging from 17.3 μM Trolox Equivalents per gram dry weight plant powder to 57.0 μM Trolox Equivalents per gram dry weight plant powder. The fraction with the highest activity had a light yellow color. The first and last fraction did not exhibit any color upon re-suspension and other fractions were either green or blue. Chen and Chen (1992) separated pigments from a flowering plant (*Ipomoea aquatica*) utilizing the same solvent system. Separated pigments were then tentatively identified with R_f values as xanthophylls (Chen and Chen, 1992).

Fractions with green pigments did not have high antioxidant capacity values; which tends to confirm that chlorophyll is not a major antioxidant in peanut leaves. The sum of ORAC from all individual fractions was 235.6 μM Trolox Equivalents per gram dry weight plant powder. The ORAC for NC leaves extracted in aqueous methanol was 261.6 μM Trolox Equivalents per gram dry weight plant powder. Antioxidant values for the fractions had similar values, with the exception of the fraction with the highest activity. These results indicated antioxidant compounds are present throughout the leaves.

The TLC fractions of NC root had ORAC from 27.8 μM Trolox Equivalents per gram dry weight plant powder to 75.6 μM Trolox Equivalents per gram dry weight plant powder (Figure 12). Re-suspended fractions from root samples were clear. The fraction with the highest antioxidant capacity was the band at the solvent front with a $R_f = 0.8570$. For this TLC system, the stationary phase is very non-polar, while the mobile phase is composed of solvents with various polarities. Results indicate compounds in the NC roots with the highest antioxidant capacity have the highest polarity. The antioxidant compound may be an aromatic hydrocarbon with few substitutions, such as an organic acid. Other fractions had numerically similar antioxidant activities which suggests the presence of antioxidants throughout the root extract with varying polarities. The ORAC for NC roots extracted in aqueous methanol was determined as 165.9 μM Trolox Equivalents per gram dry weight plant powder. Total antioxidant activity from the NC root fractions summed up to 287.0 μM Trolox Equivalents per gram dry weight plant powder.

The TX root fraction (Figure 12) ORAC ranged from 32.8 μM Trolox Equivalents per gram dry weight plant powder to 42.7 μM Trolox Equivalents per gram dry weight plant powder. The fraction with the highest activity had a retention factor of 0.4290. Based on polarity of the stationary and mobile phases, compounds with higher antioxidant capacity had moderate polarity and may be aromatic hydrocarbons with several substitutions, such as catechin (Wrolstad et al., 2005). The ORAC of TX roots extracted in aqueous methanol was determined as 127.0 μM Trolox Equivalents per gram dry weight plant powder, while the ORAC determined for the TX root fractions summed up to 222.8 μM Trolox Equivalents per gram dry weight plant powder. Results suggest either competition between compounds, bonding of compounds, or the presence of prooxidants.

Data generated from the TLC results indicates differences in composition for NC roots and TX roots. Many phenolic compounds are end products of the shikimic acid pathway, which converts simple carbohydrate to the aromatic amino acids (Carey, 2003). The biosynthesis of these natural products is greatly influenced by a number of factors, such as location, weather conditions, and harvest period (Ou et al., 2002). North Carolina and Texas differ in growing conditions, more specifically soil and climate. Soil taxonomy in Rocky Mount, North Carolina is most likely ultisols, while Seminole, Texas soil is aridisol (USDA, 2006). The climate in Texas is more arid and hotter than in North Carolina. Less water and higher temperatures cause more stress, and result in the production of defense compounds, such as polyphenols. The production of defense compounds may decrease production of other phenolic compounds. Wu et al. (2004) also concluded a number of factors, including location, affect the level of secondary metabolites found in plants. For the peanut root, production location affected antioxidant activity, total phenolic content, and location of activity fractions when separated with TLC.

High Performance Liquid Chromatography

HPLC retention time and UV-Vis spectra data were used for tentative identification of phenolic compounds in peanut plant parts (Figure 13, Table 1). Sample peaks were identified by matching against retention time and UV-Vis data of twenty known phenolic standards under the same chromatographic conditions.

Three major peaks were tentatively identified by the HPLC work for NC leaves extracted with aqueous methanol (Figure 16). Compounds tentatively identified are labeled a, b, and c and are caffeic acid, chlorogenic acid, and epicatechin respectively. Compounds identified in NC roots were epicatechin, caffeic acid, ferulic acid, and resveratrol, and compounds that may be present in TX roots were caffeic acid, chlorogenic acid, and epicatechin.

HPLC–Mass Spectrometry

Major peaks were separated, identified, and quantified using HPLC-MS (Table 2). Phenolic acids, catechins, and tannins were selected for investigation by the HPLC-MS systems based upon literature and HPLC results. Chlorogenic acid, caffeic acid, ferulic acid, and *p*-hydroxybenzoic acid are phenolic acids and are widespread throughout the plant kingdom (Wrolstad et al., 2005). Catechins include catechin, epicatechin, and catechin gallates, and sources include white tea, green tea, chocolate and apples (Escarpa and Gonzalez, 2001). Catechins are believed to be powerful antioxidants (Nijveldt et al., 2001). Resveratrol has been identified in peanut products and plants (Sovolev and Cole, 1999; Sanders et. al., 2000; Chen et. al., 2002).

Caffeic acid, chlorogenic acid, ferulic acid, *p*-hydroxybenzoic acid, catechin, epicatechin, epigallocatechin gallate, gallic acid, gallic acid gallate, and resveratrol were quantified in the peanut plant extractions. From compounds identified in the NC Leaves, epicatechin and gallic acid gallate were present at the highest concentrations. Both the NC and TX Roots had a high quantity of gallic acid gallate. Catechin compounds have been identified as contributors to the antioxidant activity of tea, chocolate, wine and grape products. Chardonnay seeds contain 4175 µg/g epicatechin (Escarpa and Gonzalez, 2001), dried green tea leaves have 7195 µg/g and dark chocolate has 415 µg/g epicatechin (USDA, 2003). Peanut leaves have a lower epicatechin content than chardonnay seed, but higher than black tea and dark chocolate. NC roots have lower amounts of epicatechin than chardonnay seeds, black tea, and dark chocolate. While TX roots have less epicatechin than chardonnay seed, almost equal to dark chocolate, and more than black tea leaves. Gallic acid gallate levels have not been previously reported. Resveratrol was not identified in the peanut leaves, but was quantified for roots in both locations. Red wine

contains approximately 0.6-8.0 $\mu\text{g/mL}$ resveratrol, while peanuts have a range of 0.02-1.79 $\mu\text{g/g}$ (Sanders, et al., 2000). NC leaves had 7.1 $\mu\text{g/g}$, while TX roots had 20.8. Both root samples had higher amounts of resveratrol than reported previously in peanuts. NC leaves did not contain resveratrol, because resveratrol is produced by the plant as a defense response to an invasion. Both root samples had higher amounts of resveratrol than reported previously in peanuts. Epigallocatechin gallate was identified in NC Leaves and NC Roots. Epigallocatechin gallate has not been identified in chocolate and grapes, but has been isolated from green and black tea at 82949 $\mu\text{g/g}$ and 11993 $\mu\text{g/g}$ respectively (USDA, 2003).

Antioxidant Activity of Phenolic Compounds

Phenolic compounds identified by HPLC-MS were analyzed using ORAC and DPPH assays to determine individual antioxidant capacities. All compounds were evaluated at 1 mM concentration and antioxidant activities were calculated to milligrams for comparison with the peanut plant extracts (Figures 14 and 15).

Catechin had the highest ORAC of 61.2 μM Trolox Equivalents per milligram. The activity of the phenolic compounds decreased in the following order catechin > 2-hydroxybenzoic acid > ferulic acid > epicatechin > caffeic acid > resveratrol > epigallocatechin gallate > chlorogenic acid > gallic acid > gallic acid.

The hydrogen atom transfer reaction mechanism of the ORAC assay measures chain-breaking antioxidant ability (Huang et al., 2005). Quenching of radicals by phenolic compounds is dependant upon the structure of the antioxidant. Catechin was determined as the best chain breaking antioxidant by the ORAC assay. The A ring of catechin and epicatechin have no double bond, which allows the addition of two more atoms of hydrogen, which may have the ability to quench free radicals. Other catechin compounds identified in the peanut plant were

epicatechin gallate and galocatechin gallate. Epicatechin gallate and galocatechin gallate had lower activity than catechin and epicatechin, which is attributed to the addition of the gallate group on the 3 position of the A ring. For the phenolic acids, *p*-hydrobenzoic acid had the highest antioxidant activity, followed by ferulic acid, caffeic acid, and chlorogenic acid. The overall trend for phenolic acid antioxidant capability may be attributed to position of and type of functional groups. *p*-hydroxybenzoic acid has carboxyl group at the 4 position, while ferulic acid has a methoxy group at the 3 position, and hydroxyl group at the 4 position. The addition of the carboxyl group increases the chain breaking activity of the phenolic acid. Caffeic acid has hydroxyl groups at the 3 and 4 position, as well as propenoic acid at the 2 position. The addition of propenoic acid appears to reduce antioxidant activity in the ORAC assay. Chlorogenic acid is an ester of caffeic acid, and the esterification reduces antioxidant activity.

Epigallocatechin elicited the highest antioxidant capacity with 8.1 μ M Trolox Equivalents per gram for the DPPH assay. Phenolic activities were determined as follows epigallocatechin gallate > caffeic acid > catechin > epicatechin > chlorogenic acid > galocatechin gallate > resveratrol > ferulic acid > *p*-hydroxybenzoic acid.

The antioxidant mechanism of the DPPH assay is the reduction of DPPH radicals (Huang et al., 2005), which is dependant upon the structure of the antioxidant. From the mechanism of the assay and results, it is concluded that epigallocatechin gallate has the highest antioxidant activity due to the presence of the gallate group at the 3 position. The difference between epigallocatechin gallate and galocatechin gallate is between their sterical structures, which seem to play an important role in their abilities to scavenge free radicals. Other catechin compounds identified in peanut leaves and roots are catechin and epicatechin. Epicatechin and catechin have no double bond in their A ring, which allows the addition of two additional atoms of hydrogen.

The additional hydrogen compounds on epicatechin and catechin are believed to have the ability to reduce free radicals. Caffeic acid was determined to have the second highest antioxidant capacity using the DPPH assay. Other phenolic compounds, chlorogenic acid, ferulic acid, and *p*-hydroxybenzoic acid had DPPH radical reducing ability. Caffeic acid has hydroxyl groups at the 3 and 4 position as well as propenoic acid at the 2 position. The high antioxidant ability is attributed to the two hydroxyl groups in the ortho position. Chlorogenic acid has lower antioxidant activity than caffeic acid, due to its esterification. Ferulic acid has a single hydroxyl group and a highly conjugated side chain. *p*-hydroxybenzoic acid does not have a conjugated side chain and only one hydroxyl group. Results suggests antioxidant capacity using the DPPH assay increases with the number of hydroxyl groups in the main aromatic ring (the A ring for flavonoids), and type of functional group substitutions.

Contribution of Identified Phenolic Compounds to Antioxidant Activity

The calculated contribution of the quantified phenolic compounds to the total antioxidant capacity of the peanut leaves and roots extracted with aqueous methanol is shown in Tables 3, 4, and 5. Contribution was calculated as the percent of the whole antioxidant activity of the peanut plant extracts. For NC Leaves, the calculated contribution of the identified phenolic compounds was 47% of the total ORAC; whereas, the phenolic compounds contribute 14% to the total antioxidant activity from the DPPH method. Phenolic compounds contribute 22% of the ORAC and 14% of the DPPH antioxidant activity for the NC Roots. Phenolic compounds account for 44% of the ORAC activity and 17% of the DPPH activity for TX Roots.

Actual Antioxidant Activity versus Calculated Antioxidant Contribution

Phenolic compounds identified were quantitatively mixed and analyzed with both antioxidant assays antioxidant capacity. Phenolic standards were prepared at 1 mM

concentrations and mixed as determined by HPLC-MS data. Antioxidant capacity of the phenolic standard mixtures was measured by the ORAC and DPPH assays (Figures 16 and 17). The calculated antioxidant activity is the sum of antioxidant activity for individual phenolic compounds as determined with the ORAC and DPPH assay.

Mix 1 was had the highest ORAC with 94.9 μM Trolox Equivalents per gram. The calculated ORAC of phenolic compounds in Mix 1 was 122.5 μM Trolox Equivalents per gram. Actual versus calculated antioxidant activities for the compounds in Mix 1 were similar. The additional phenolic standard mixtures exhibited higher antioxidant capacity values than calculated. Mix 2 had an activity equal to 66.8 μM Trolox Equivalents per gram, as compared to the calculated value from the sum of the phenolic standards of 29.6 μM Trolox Equivalents per gram. ORAC of the Mix 3 was 56.0 μM Trolox Equivalents per gram, and the calculated value for the phenolic standard compounds was 38.7 μM Trolox Equivalents per gram.

DPPH values for the phenolic standard mixture were highest for Mix 1. Mix 1 had an antioxidant activity of 10.9, followed by Mix 2 at 3.4, and Mix 3 with 2.6 μM Trolox Equivalents per gram. The calculated antioxidant capacity for phenolic compounds identified in Mix 2 was 12.5 μM Trolox Equivalents per gram. Calculated antioxidant activity for Mix 2 had an activity equal to 3.7 μM Trolox Equivalents per gram, and the calculated value from the quantified phenolic standards for Mix 3 was 4.2 μM Trolox Equivalents per gram. Actual versus calculated antioxidant capacities for the quantified compounds in Mix 1 and Mix 2 were numerically similar, while the calculated value for Mix 3 was higher than the actual activity of the standard phenolic mixture.

CONCLUSION

The results obtained in this study demonstrate that methanol and aqueous methanol extracts of peanut leaves and roots have the ability to quench free radicals and prevent peroxides from oxidizing fluorescein. The chromatography results confirm peanut leaves and roots contain phenolic compounds. Extractions utilizing aqueous methanol more efficiently extracted phenolic compounds, which may also be correlated to an increase in antioxidant activity with the addition of water. Chlorophyll does not contribute considerably to the antioxidant activity of peanut leaves. Peanut roots grown in various regions of the country (North Carolina versus Texas) have different antioxidant activities and total phenolic content. Growing conditions such as soil type and climate are attributed to varying levels of specific antioxidant compounds in North Carolina roots as compared to Texas roots. Specific phenolic compounds do not account for all of the antioxidant activity in peanut leaves and roots, they include: caffeic acid, chlorogenic acid, ferulic acid, *p*-hydroxybenzoic acid, catechin, epicatechin, epicatechin gallate, gallic acid, gallic acid gallate, and resveratrol. Phenolic compounds have different antioxidant abilities based on the antioxidant assay used. Reduction and quenching of free radicals by phenolic compounds is structure dependent. The total antioxidant activity attributed to the specific phenolic compounds does not equal the sum of the individual compounds due to interactions between phenolic compounds when mixed.

Future studies should focus on identification of additional antioxidant compounds in peanut leaves and roots. Further studies should be designed to analyze the variability of antioxidant compounds in peanut plants of different peanut cultivars. Research is also needed to determine which lipophilic antioxidants are present. Future investigations may be needed to determine whether current peanut production practices can continue or if alternative harvesting

equipment or practices need to be developed in order to maximize bioactive compounds extracted from the peanut plant parts.

REFERENCES

1. Acquaaah, G. (2005). Principles of Crop Production: Theory, Techniques, and Technology. New Jersey: Pearson Education Inc.
2. Awika, J., Rooney, L., Wu, X., Prior, R., Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Sorghum products. *J. Agric. Food Chem.*, 51, 6657-6662.
3. Benbrook, B. (2005). Elevating Antioxidant Level in Foods through Organic Farming and Food Processing. *Organic Center State of Science Review*. January.
4. Brand-Williams, W., Cuvelier, M., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28, 25-30.
5. Buratti, S., Pellegrini, N., Brenna, O., Mannino, S. (2001). Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. *J. Agric. Food Chem.*, 49, 5136-5141.
6. Carey, F. (2003). Organic Chemistry 5th Edition. New York: McGraw-Hill Co.
7. Cao, G., Alessio, H., Cutler, R. (1993). The measurement of oxygen radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* 14 (3), 303-311.
8. Chen, B., Chen, Y. (1992). Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. *Food Chemistry*. 45, 129-134.
9. Chen, R., Wu, P., Chiou, R. (2002). Peanut roots as a source of resveratrol. *J. Agric. Food Chem.* 50, 1665-1667.
10. Chu, Y., Chang, C., Hsu, H. (2000). Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.*, 80, 561-566.
11. Davalos, A., Gomez-Cordoves, C., & Bartolome, B. (2004). Extending the applicability of the oxygen radical absorbance capacity (ORAC-Flourescein) assay. *J. Agric. Food Chem.*, 52, 48-54.
12. Ehlenfeldt, M., Prior, R. (2001). Oxygen radical absorbance (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *J. Agric. Food Chem.*, 49, 2222-2227.
13. Endo, Y., Usuki, R., Kandena, T. (1985). Antioxidant effects of chlorophyll and pheophytin on the autooxidation of oils. *Journal of the American Oil Chemists Society*, 62, 1375-1390.

14. Escarpa, A., Gonzalez, M. (2001). An overview of analytical chemistry of phenolic compounds in foods. *Critical Reviews in Analytical Chemistry*, 31(2), 57-139.
15. Ferruzzi, M., Bohm, V., Courtney, P., Schwartz, S. (2002). Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assay. *J. Food Science Food Chemistry and Toxicology*, 67, 2589-2595.
16. French, E., Prine, G., Blount, A. (2006). Perennial Peanut: An Alternative Forage of Growing Importance. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.
17. Fukumoto, L., Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.*, 48, 3597-3604.
18. Gil, M., Tomas-Barberan, F., Hess-Pierce, B., Kader, A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.*, 50, 4976-4982.
19. Green, R. (2004). Antioxidant Activity in Peanut Plant Tissues. Master's Thesis submitted to NCSU Department of Food Science.
20. Harish, R., Divakar, S., Srivastava, A., Shivanandappa, T. (2005). Isolation of antioxidant compounds from the methanolic extract of the roots of *Decalepis hamiltonii* (Wight and Arn.). *J. Agric. Food Chem.*, 53, 7709-7714.
21. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E. (2002a) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* 50, 1815-1821.
22. Huang, D., Ou, B., Hampsch-Woodill, M, Flanagan, J., Prior, R. (2002b). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* 50, 4437-4444.
23. Huang, D., Ou, B., Prior, R. (2005). The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 53, 1841-185.
24. Kurilich, A., Jefferey, E., Juvik, J., Wallig, M., Klein, B. (2002). Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using oxygen radical absorbance capacity (ORAC) assay. *J. Agric. Food Chem.*, 50, 5053-5057.
25. Kuti, J., Konuru, H. (2004). Antioxidant capacity and phenolic content in leaf extracts of tree spinach (*Cnidoscolus* spp.). *J. Agric. Food Chem.*, 52, 117-121.

26. Liu, C., Chiou, Y., Wang, J., Chiou, K., Robin, Y. (2003). Comparative characterization of peanuts grown by aquatic floating cultivation and field cultivation for seed and resveratrol production. *J. Agric. Food Chem.* 51: 1582-1585.
27. Nijveldt, R., Nood, E., Hoorn, D., Boelens, P., Norren, K., Leeuwen, P. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Amer. J. Clin. Nutr.*, 74, 418-425.
28. Ou, B., Huang, D., Wampsch-Woodill, M., Flanagan, J., Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) assays: A comparative study. *J. Agric. Food Chem.*, 50, 3122-3128.
29. Pratt, D., Miller, E. (1984). A flavonoid antioxidant in Spanish peanuts. *J. Am. Oil Chem. Society*, 61, 1064-1067.
30. Prior, R., Hoang, H., Gu, L., Wu, X., Baccioca, M., Howard, L., Hampsch-Woodill, M.; Huang, D., Ou, B., Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC_{FL})) of plasma and other biological and food samples. *J. Agric. Food Chem.* 51, 3273-3279.
31. Reddy, M., Alexander-Lindo, R., Nair, M. (2005). Relative inhibition of lipid peroxidation, cyclooxygenase, enzymes, and human tumor cell proliferation by natural foods colors. *J. Agric. Food Chem.*, 53, 9268-9273.
32. Sanders, T., McMichael, R., Hendrix, K. (2000). Occurrence of Resveratrol in edible peanuts. *J. Agric. Food Chem.*, 48 (4), 1243 -1246, 2000.
33. Siddhuraju, P., Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of Drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.*, 57, 2144-2155.
34. Singleton, V., Rossi, J. (1965). Colormetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *J. Food Processing and Pres.* 18, 75-84.
35. Singleton, V., Orthofer, R., Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidant means of Folin-Ciocalteu reagent. *Meth. Enzy.*, 299, 152-178.
36. Sobolev, V., Cole, R. (1999). *trans*-Resveratrol content in commercial peanuts and peanut products. *J. Agr. Food Chem.*, 47, 1435-1439.
37. USDA, Natural Resource Conservation Service, Soils Database (2006). Retrieved November 25, 2006 from Soil Order Map of United States. http://www.cei.psu.edu/soiltool/semtool_phase2.html

38. Wrolstad, R., Acree, T., Decker, E., Penner, Reid, D., Schwartz, S., Shoemaker, C., Smith, D., Sporns, P (2005). Handbook of Food Analytical Chemistry. John Wiley & Sons: Hoboken, NY.
39. Wu, X., Beecher, G., Holden, J., Haytowitz, D., Gebhardt, S., Prior, R. (2004a). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* 52, 4026-4037.
40. Wu, X., Gu, L., Holden, J., Haytowitz, D., Gebhardt, S., Beecher, G., Prior, R. (2004b). Development of a database for total antioxidant capacity of foods: A preliminary study. *J. Food Comp. Anal.*
41. Yen, G., Wu, S., Duh, P. (1996). Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *J. Agric. Food Chem.*, 44, 1678-1690.

FIGURES

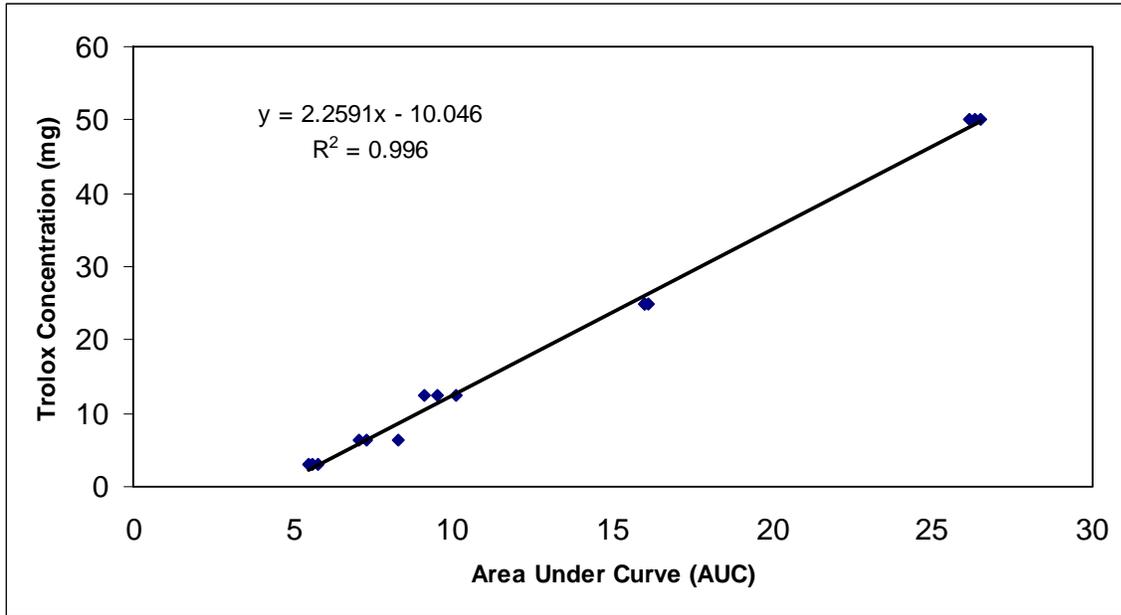


Figure 1: Trolox Standard Curve for ORAC Assay

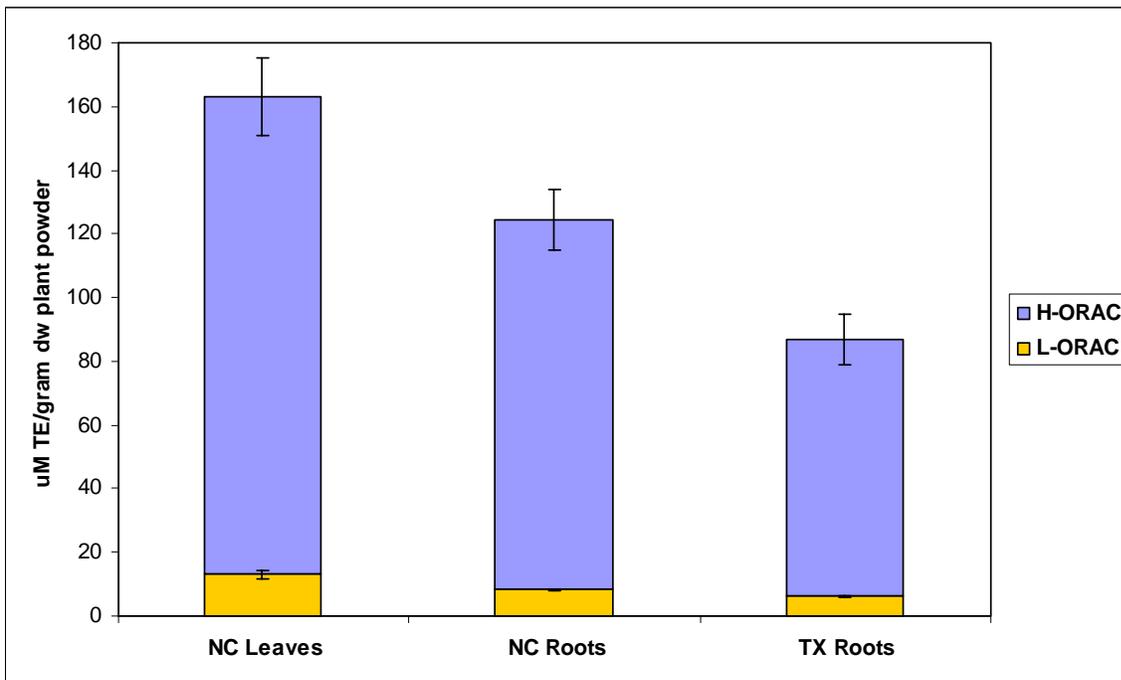


Figure 2: Total ORAC Values (Lipophilic-ORAC Value plus Hydrophilic-ORAC Value) for NC Leaves, NC Roots, and TX Roots Extracted with Methanol; Bars indicate standard deviation

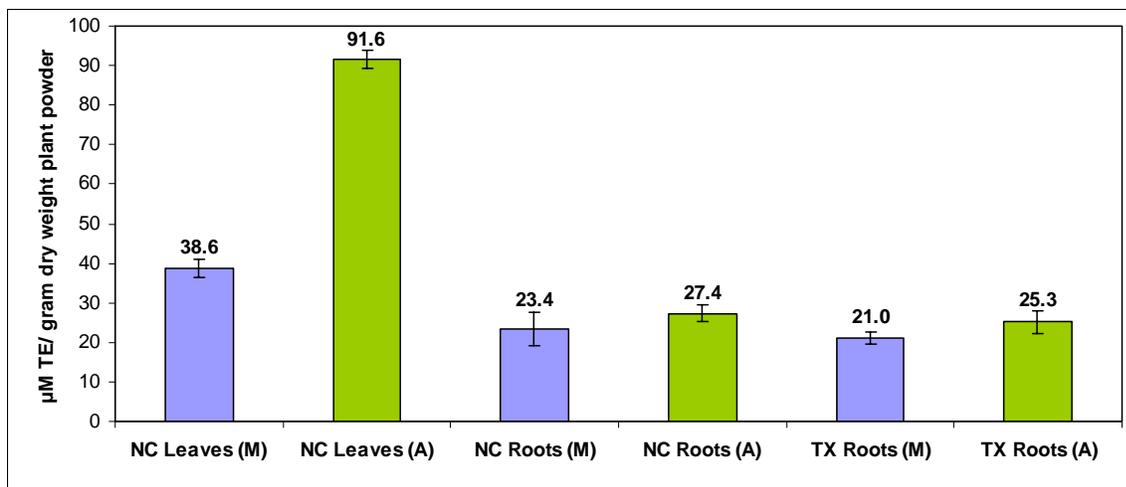


Figure 3: DPPH Values for NC Leaves, NC Roots and TX Roots Extracted in Methanol (M) and 80% Aqueous Methanol (A); Bars indicate standard deviations

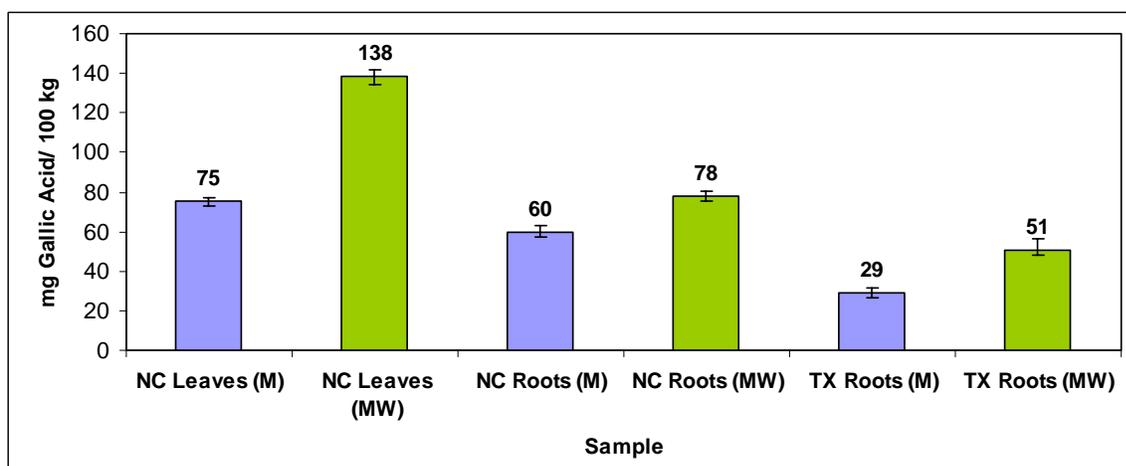


Figure 4: Total Phenolics for NC Leaves, NC Roots and TX Roots Extracted in Methanol (M) and 80% Aqueous Methanol (A); Bars indicate standard deviations

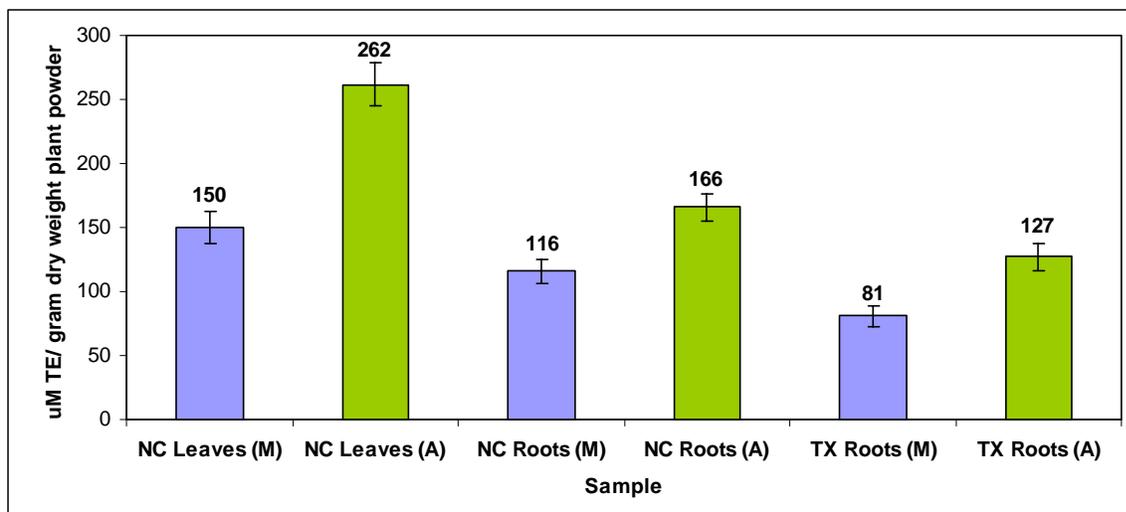


Figure 5: H-ORAC Values for NC Leaves, NC Roots and TX Roots Extracted in Methanol (M) and 80% Aqueous Methanol (A); Bars indicate standard deviations

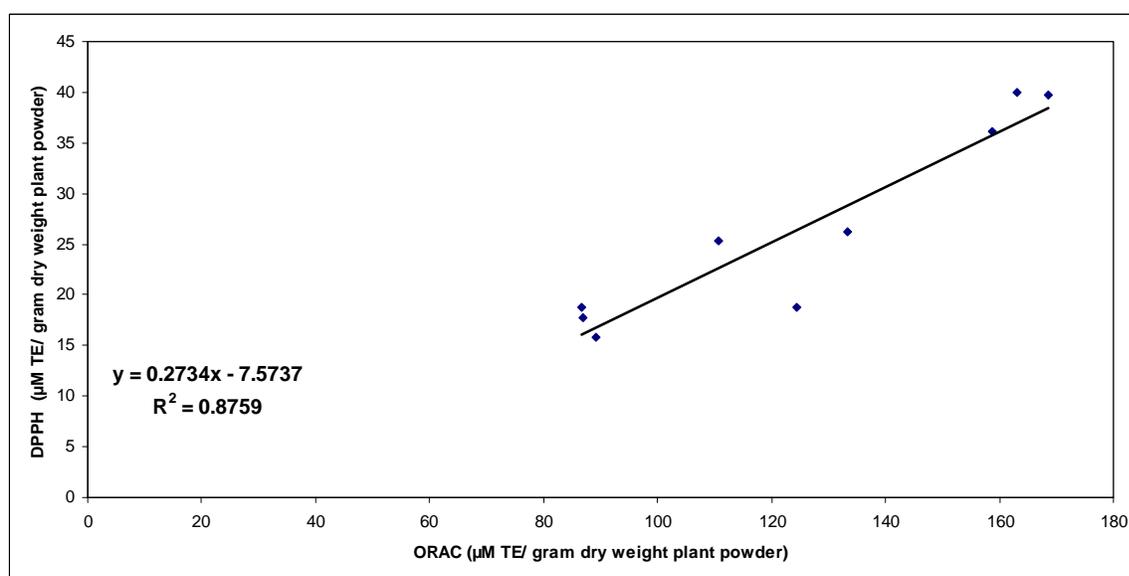


Figure 6: Correlation between ORAC and DPPH Values for Peanut Leaves and Roots Extracted with Aqueous Methanol

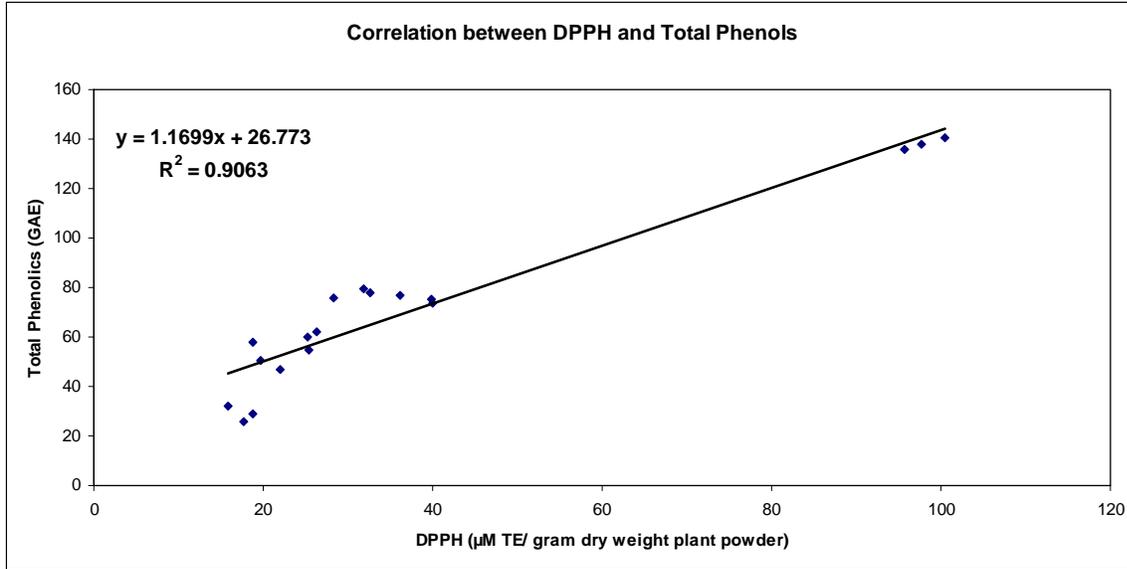


Figure 7: Correlation between DPPH and Total Phenolic Values for Peanut Leaves and Roots Extracted with Aqueous Methanol

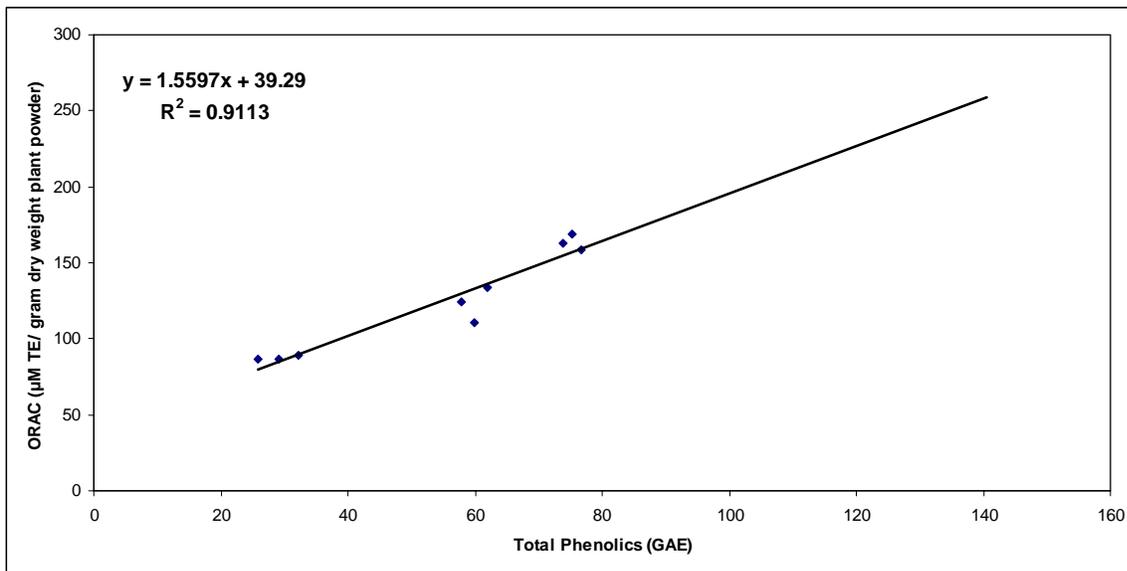


Figure 8: Correlation between T-ORAC and Total Phenolic Values for Peanut Leaves and Roots Extracted with Aqueous Methanol

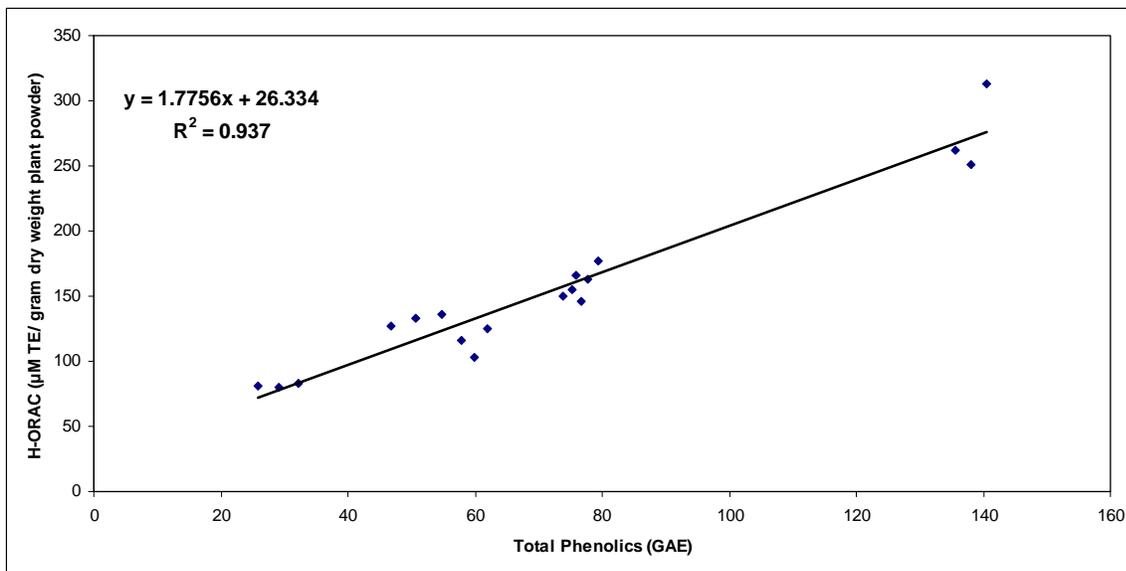


Figure 9: Correlation between ORAC and Total Phenolic Values for Peanut Leaves and Roots Extracted with Aqueous Methanol

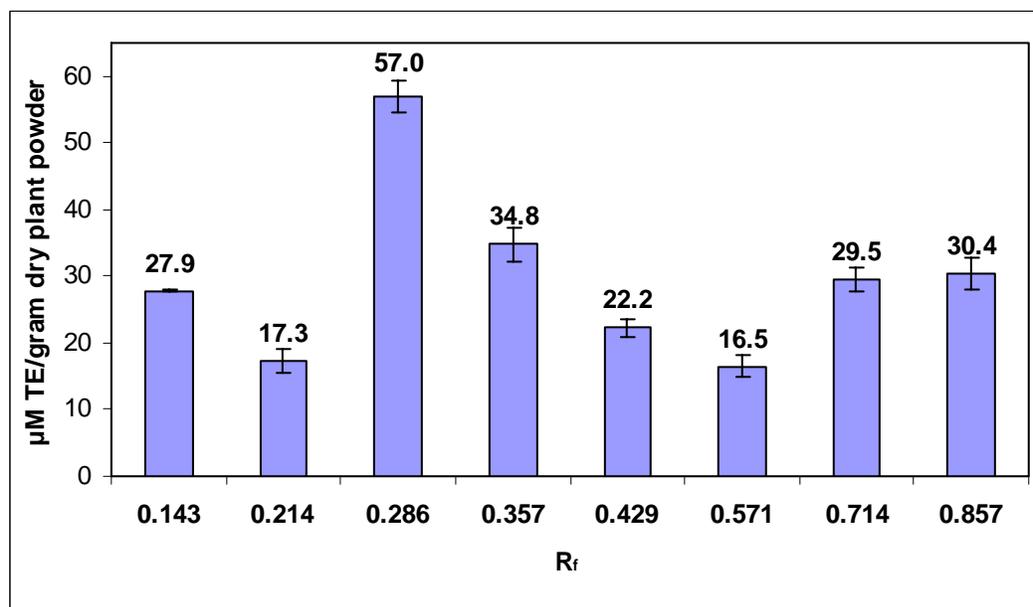


Figure 10: H-ORAC Values for Fractions Generated from TLC of NC Leaves, R_f is retention factor, Bars indicate standard deviations

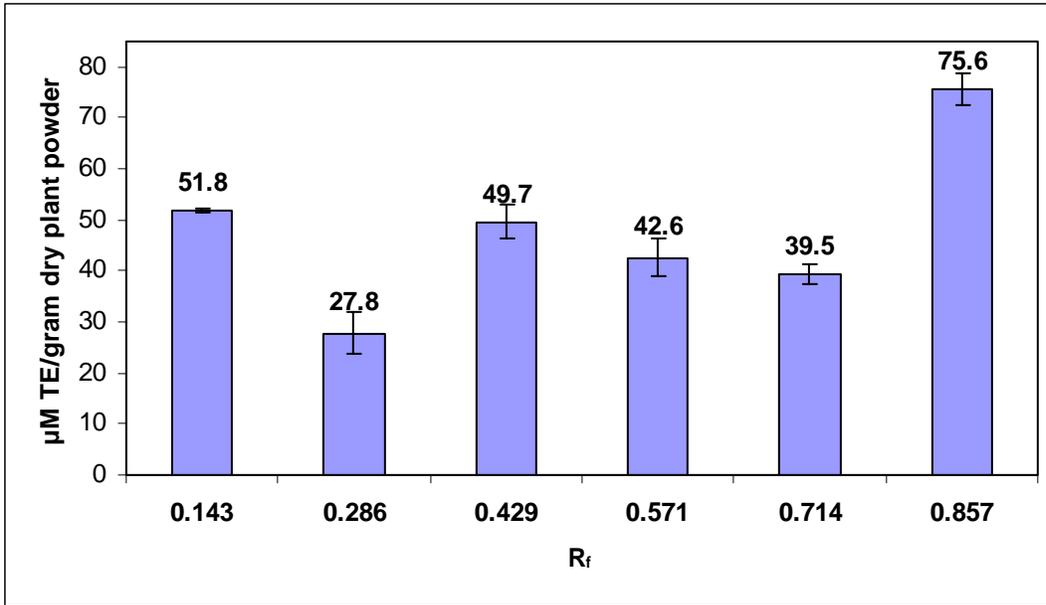


Figure 11: H-ORAC Values for Fractions Generated from TLC of NC Roots, R_f is retention factor, Bars indicate standard deviations

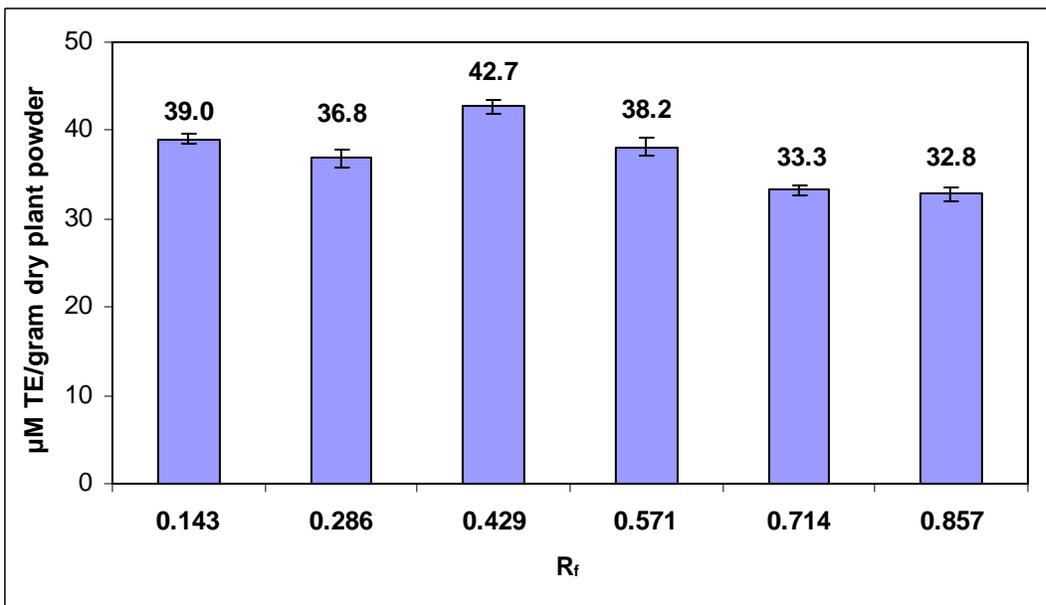


Figure 12: H-ORAC Values for Fractions Generated from TLC of TX Roots, R_f is retention factor, Bars indicate standard deviations

Table 1: HPLC Data for NC Leaves, NC Roots, and TX Roots Extracted with Aqueous Methanol, RT indicates R_T , $UV\lambda_{max}$ is UV-Vis spectra data

Compound	R_T (min)	R_T (min) standard	$UV\lambda_{max}$
NC Leaves			
Caffeic Acid	40.533	40.433	216
Chlorogenic Acid	44.050	44.117	250, 322
Epicatechin	48.300	48.217	227, 274
NC Root			
Caffeic Acid	40.533	40.433	216
Epicatechin	48.300	48.217	227, 274
Ferulic Acid	65.933	66.017	254, 313
Resveratrol	85.783	85.817	218, 310
TX Root			
Caffeic Acid	40.383	40.433	216
Chlorogenic Acid	44.133	44.117	250, 322
Epicatechin	48.300	48.217	227, 274

Table 2: Quantity of Phenolic Compounds as Determined by HPLC-MS for NC Leaves, NC Roots, and TX Roots Extracted with Aqueous Methanol

LC-MS Data	NC Leaves ($\mu\text{g} / \text{g}$)	NC Root ($\mu\text{g} / \text{g}$)	TX Root ($\mu\text{g} / \text{g}$)
<i>p</i> -Hydroxybenzoic acid	3.2	5.3	2.0
Caffeic acid	11.8	2.3	2.3
Catechin	118.1	116.8	134.4
Chlorogenic acid	206.5	4.4	36.4
Epicatechin	2346.9	217.8	410.8
Epigallocatechin gallate	255.3	18.2	0.0
Ferulic Acid	60.8	59.9	65.7
Gallic acid	1909.7	1488.0	1453.4
Resveratrol	0.0	7.1	20.8

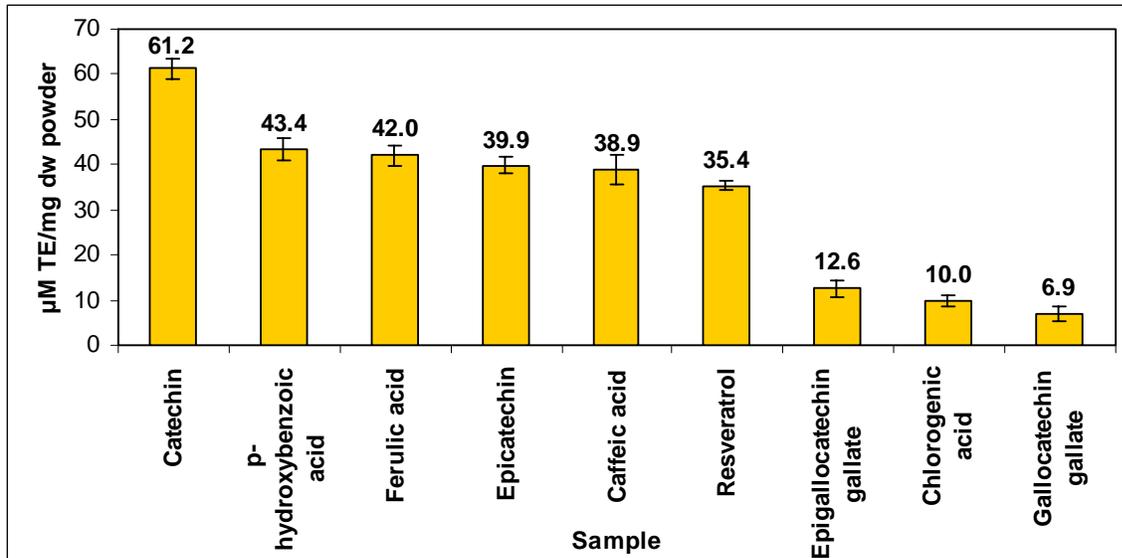


Figure 13: H-ORAC Values for Phenolic Compounds Identified in NC Leaves, NC Roots, and TX Roots, Values are calculated as μM Trolox Equivalents/mg dry weight

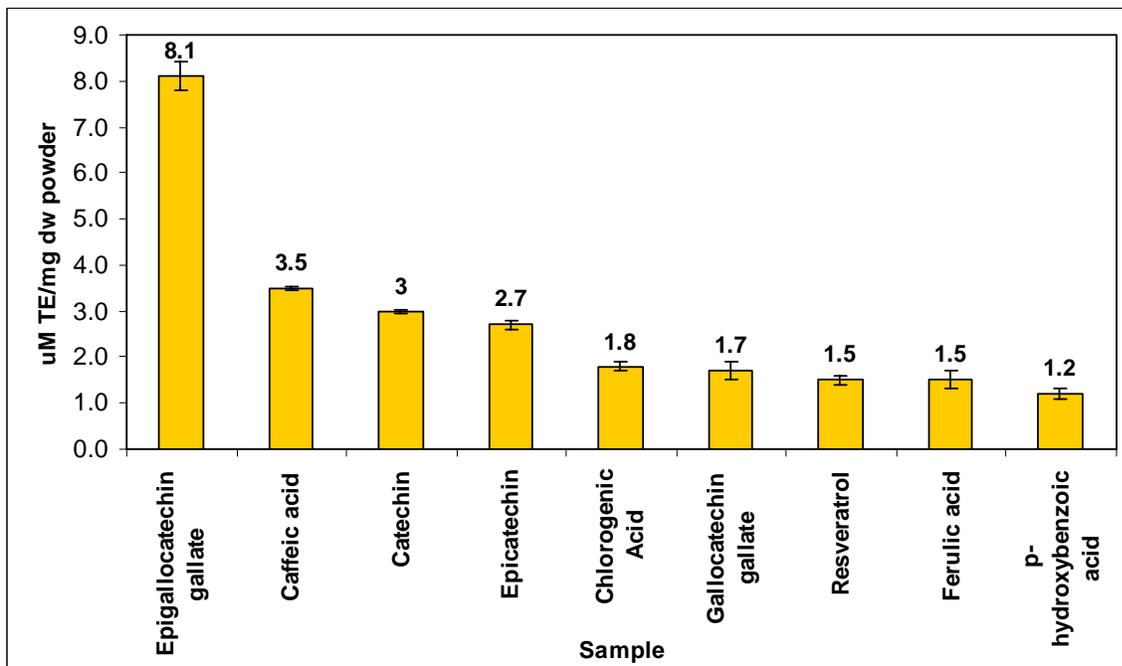


Figure 14: DPPH Values for Phenolic Compounds Identified in Peanut Plant Samples, Values are calculated as μM Trolox Equivalents/mg dry weight, Bars indicated standard deviations

Table 3: Calculated Contribution of Identified Phenolic Compounds to NC Leaves Antioxidant Activity as a Percentage of Total Activity

Leaves	% ORAC	% DPPH
<i>p</i> -hydroxybenzoic acid	0.05	0.00
Caffeic Acid	0.18	0.04
Catechin	2.76	0.39
Chlorogenic Acid	0.79	0.40
Epicatechin	35.74	6.89
Epigallocatechin gallate	1.23	2.25
Ferulic Acid	0.97	0.10
Gallocatechin gallate	5.03	3.53
Resveratrol	0.00	0.00
Total % Activity	46.75	13.60

Table 4: Calculated Contribution of Identified Phenolic Compounds to NC Roots Antioxidant Activity as a Percentage of Total Activity

NC Roots	% ORAC	% DPPH
<i>p</i> -hydroxybenzoic acid	0.17	0.03
Caffeic Acid	0.07	1.30
Catechin	5.33	0.03
Chlorogenic Acid	0.03	2.18
Epicatechin	6.49	0.55
Epigallocatechin gallate	0.17	0.33
Ferulic Acid	1.88	9.37
Gallocatechin gallate	7.66	0.04
Resveratrol	0.19	0.00
Total % Activity	21.99	13.82

Table 5: Calculated Contribution of Identified Phenolic Compounds to TX Roots Antioxidant Activity as a Percentage of Total Activity

TX Roots	% ORAC	% DPPH
<i>p</i> -hydroxybenzoic acid	0.10	0.01
Caffeic Acid	0.10	0.03
Catechin	9.45	1.61
Chlorogenic Acid	0.42	0.26
Epicatechin	18.84	4.44
Epigallocatechin gallate	0.00	0.00
Ferulic Acid	3.17	0.39
Gallocatechin gallate	11.53	9.88
Resveratrol	0.85	0.12
Total % Activity	44.46	16.76

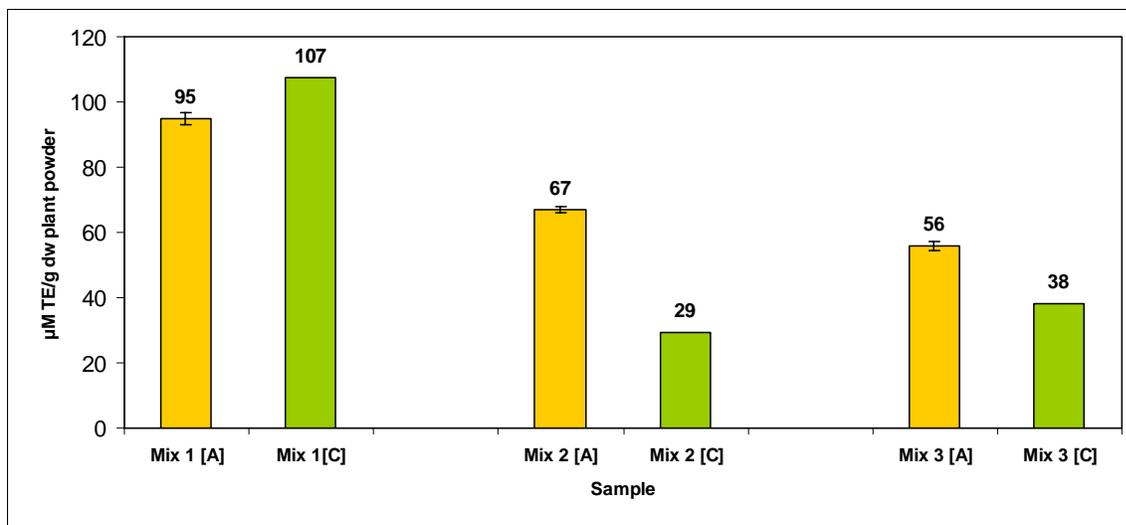


Figure 15: ORAC Values for Phenolic Standard Mixture [A] and Calculated Total Activity Based on HPLC-MS Results for the Sum of Identified Individual Phenolic Compounds [C], Results calculated to μM Trolox Equivalents per g dry weight

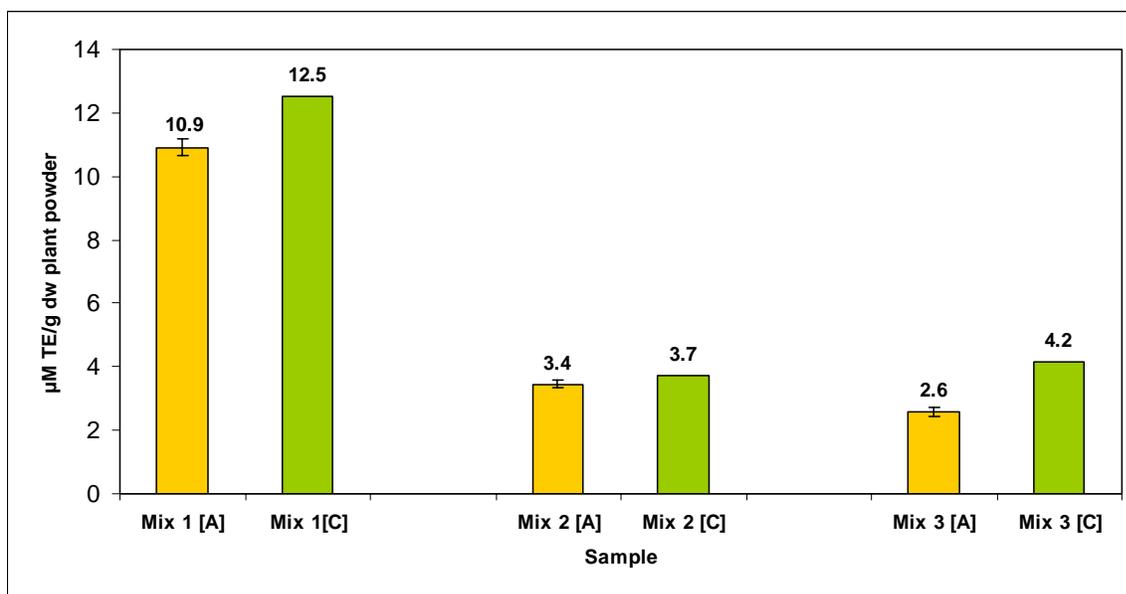


Figure 16: DPPH Values for Phenolic Standard Mixture [A] and Calculated Total Activity Based on HPLC-MS Results for the Sum of Identified Individual Phenolic Compounds [C], Results calculated to μM Trolox Equivalents per g dry weight