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Phenolic Content and Antioxidant Capacity of American Persimmon *(Diospyros virginiana* L.) Teas

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Abstract

Regular consumption of green tea, a rich source of phenolic compounds, has been linked to various health benefits. Since most green tea on the market in the U.S. is imported, there have been concerns for contamination with heavy metals and pesticides. Leaves and other plant parts of various species such as American persimmon (*D. virginiana* L.), which are native to the eastern U.S., have been similarly used to make beverages in the past. Unfortunately, the health benefits of these teas have not been studied. The objectives of this study were to examine phenolic content and antioxidant capacity of American persimmon infusions (tea) made from the leaves of different cultivars. Leaves from five cultivars of American persimmon were harvested in May of 2012. Folin-Ciocalteu assay was performed to determine phenolic content of teas. The phenolic content of green tea was 209.7, and that of American persimmon teas ranged from 136.8 to 166.2 in mg of gallic acid equivalent per ml. The Ferric Reducing Antioxidant Power assay was performed to determine antioxidant capacity, revealing that the antioxidant capacity of the persimmon tea was roughly a half of that of green tea. Teas made from American persimmon leaves are a caffeine-free healthy alternative to regular or green tea.

Tea, *Camellia sinensis*, especially green tea, has long been claimed to be helpful for prevention of hypercholesterolemia, arthero-sclerosis, Parkinson's disease, Alzheimer's disease, and other aging-related disorders (Zaveri, 2006). Along with, *C. sinensis,* various plant species have been used as a source for teas, and some have been reported to be beneficial to human health.

Despite ever increasing tea consumption in U.S., domestic tea production is a very limited endeavor. Currently, there is only one tea plantation in South Carolina and the magnitude of tea production is very small compared to the amount consumed in the U.S. This may be partly due to the fact that tea plants require specific cultural conditions. They are also prone to various disease and insect problems aside from the climatic requirement. Because of the significant shortage of, and possible indifference to tea cultivation in this country, the vast majority of tea consumed in the U.S. is imported (Meeberg, 1992).

As is often the case with imported foodstuffs, there is always a concern for food safety. In the case of tea, residual pesticide and mineral contamination are of particular concern. For example, one study revealed polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, chlorinated pesticides and polynuclear aromatic hydrocarbons (Fielder et al., 2002) in tea leaves. Similarly, a relatively high concentration of heavy metal elements, including lead (Jin et al., 2005a, 2005b; Han et al., 2006; Han et al., 2007), chromium (Seenivasan et al., 2008), and copper (Jin et al., 2008), have been detected in different kinds of tea. Furthermore, tea is also known to contain concentrations of aluminum, fluoride and oxalate, which may pose a potential health threat to some

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consumers (Flaten, 2002). Therefore, it is advisable to seek an alternative with equal or greater health benefits and fewer undesirable characteristics.

American persimmon (*Diospyros virginiana* L.) is a native species that is found throughout the eastern half of the U.S. and Canada, ranging from New England to Florida and west to Kansas, Oklahoma and Texas (NRCS, 2013). This tree grows wild, but has been cultivated for its fruit and wood by Native Americans. However, this species is most commonly grown for fruit, which is high in vitamin C. The unripe fruit is noted for its astringency, but the ripe fruit may be eaten raw, cooked or dried. Additionally, tea can be made from the leaves, and the roasted leaves were used as a coffee substitute during the Civil War (Lee and Gordon, 1993).

The Asian counterpart of the American persimmon, the Asian persimmon (*Diospyros kaki* Thun.) has been extensively studied for its medicinal and health ameliorating properties. Asian persimmon fruit is particularly rich in vitamin C, carotenoids and polyphenols (Giordani et al., 2011), all of which are considered powerful antioxidants that protect against free-radicals and prevent the risk of cardiovascular disease, diabetes and cancer (Georgé et al., 2011). The antioxidant activity of persimmons has been chemically assessed by determining the radical scavenging activity through various chemiluminescent assays, including the 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) method, or 2,2′-azino-bis (3-ethyl-benzoathiazoline-6-sulfonic acid) diammonium salt (ABTS), the measuring of ferric reducing antioxidant power (FRAP) and low-density-lipoprotein (LDL) oxidation. It has been suggested that proanthocyanidins found in persimmon may reduce blood pressure and platelet aggregation and therefore exert a beneficial effect on coronary diseases (Giordani et al., 2011).

In addition to fruit, leaves of the Asian persimmon have been used for human consumption (Kotani et al., 2000, Sakanaka et al., 2005, Weijian et al., 2005, Lee et al., 2006). The most notable example would be persimmon teas. Similar to fruit, persimmon leaves are rich in phenolic constituents, particularly tannins (Weijan et al., 2005), and persimmon tea has health promoting properties, including inhibiting development of dermatitis (Kotani et al., 2000); improving the lipid profile of rats fed a high-fat diet (Weijan et al., 2005); and reduction of hydrogen peroxide-induced injury of NG108-15 cells. While there is a recorded use of American persimmon leaves for tea (Lee et al., 2006), and Asian persimmon teas have been studied, it appears that an investigation on health benefits of American persimmon tea has not been conducted.

While the medicinal and health promoting properties of Asian relatives have been studied, very little information is presently available for American persimmon on its health benefits and medicinal components. As the Asian species is known for health promoting properties, it is natural to hypothesize that American counterparts may also possess health beneficial properties. A chemical characterization of compounds found in teas of these plants may lead to further investigation on health amelioration with underutilized common species found in many parts of Kentucky and the surrounding region. Thus a thorough and detailed investigation of the properties of American persimmon tea could lead to a wider usage of these teas.

Materials and methods

Samples. Leaves from five American persimmon cultivars 'Early Golden', 'Evelyn' (Orleans, KY), 'Evelyn' (Upton, KY), 'John Rick', 'Valeene Beauty', and 'Yates' with three replicates were collected from a commercial orchard in Orleans, IN. Additionally, leaves of 'Evelyn' with three replicates were collected from two nurseries. Locations, and a list of cultivars with descriptions are depicted in Table 1. Lipton® Green Tea was purchased to compare its phenolic content and antioxidant capacity to those of persimmon teas.

Table 1. Cultivars of American persimmon used. Compiled from Reich (2004) and Raymond (2006).

Cultivar	Description
'Early Golden'	Origin: Alton, Illinois in the late 1800's. Probably the oldest cultivar known.
'Evelyn'	Origin: North Tonowonda, NY.
'John Rick'	Origin: A seedling of 'Killen,' which was a seedling of 'Early Golden'
'Valeene Beauty'	Origin: Bred by James Claypool and released by Don Compton. A seedling of 'Lena' x 'Early Golden.' Reddish leaf color when leaves emerge and expand.
'Yates'	Origin: Southern Indiana. Probably same as 'Juhl.'

Fresh young leaves from 50 shoots were first weighed and thoroughly washed to remove debris, insects, etc. Excess moisture was removed with paper towels, and leaves were placed in a Ziploc® Zip'n Steam® Microwave Cooking bag. Leaves were microwaved for 30 sec/50 g of samples in a 750w Whippoorwill counter top microwave. Leaves were then roasted on an electric skillet (Hamilton Beach, Southern Pines, NC) at 400˚F, immediately after removal from the bag.

Preparation of teas. American persimmon tea was prepared in the same manner previously described for green tea (Chandra and de Mejia, 2004). After boiling 140 ml of double distilled water (DDH_2O), 1.4 mg of roasted American persimmon leaves were added and brewed for 5 min. with heat. The tea was left to cool down for another 5 min, and then vacuum filtered through fiberglass microfiber filter paper (Whatman, Piscataway, NJ).

Measurement of phenolic content. The amount of soluble phenolic content was quantified by a modified protocol for 96 well plates (Dicko et al., 2002). To each well of the 96-well plate, 10 ml of either DDH_2O, standard, or sample was added, followed by dispensing of 25 ml Folin-Ciocalteau reagent (Sigma, St. Louis). After 10 min. incubation, 25 ml of 20% (w/v) Na_2CO_3 was added to each well. Immediately after addition of Na_2CO_3, 140 ml of DDH_2O was added to the wells. The final volume of the reaction mixture in each well was 200 ml. Absorbance of the mixture was measured at 760 nm with a microplate reader (Infinite®200 Pro, Tecan, Raleigh, NC) and analyzed with i-Control™. Kinetics of the reaction were observed for two hrs. to determine the total PC, expressed in g of gallic acid equivalent per ml of tea (mg GAE/ml).

Ferric reducing antioxidant power (FRAP) assay. Antioxidant capacity of the teas was quantified by a modified ferric reducing antioxidant power (FRAP) assay for 96 well plates (Firuzi et al., 2005). Working FRAP solution was freshly made by mixing 15 ml of acetate buffer (300 mM) and 1.5 ml ea. of TPTZ (10 mM) and ferric chloride solution (20 mM). Both acetate buffer and FRAP solution were warmed to 37ºC prior to adding to the well. In each well, 25 ml of either standard or sample of different concentrations was be dispensed, and the equal amount of solvents used to dissolve standards was used as blank. Plates was incubated after adding 175 ml of FRAP solution. Absorbance of the mixture was measured at 595 nm. Temperature was kept at 37ºC for the whole period of experiments, and kinetics of the reaction were observed for two hrs. Antioxidant power is then expressed in mol of Trolox equivalent (mM TE).

Data analysis. Gallic acid and Trolox equivalent values of teas were obtained by using the equations for these standard curves. The equation and the value were obtained after plotting absorbance readings. Results were analyzed using one-way ANOVA followed by Student's least significant difference test with the general linear model (LSD, $P < 0.05$), and correlation coefficients between phenolic content and antioxidant capacity was determined. All statistical analyses were performed using the statistical software package CoStat Version 6.400 (CoHort, Pacific Grove, CA).

Results and Discussion

This is the first study to report a comparison of the phenolic and antioxidant capacity of American persimmon and *C. sinensis* teas. Phenolic content of green tea was significantly higher than that of American persimmon teas (209.7 mg GAE/ml) (Table 2). Phenolic content of American persimmon teas ranged from 136.8 to 166.2 mg GAE/ml, approximately 65.2% to 79.2% of green tea tested in this study. Of American persimmon cultivars tested, the tea made of 'John Rick' leaves had the greatest phenolic content. 'Valeene Beauty' had the second highest phenolic content, followed by 'Evelyn,' 'Yates,' and 'Early Golden.' The current finding suggests that there is a great deal of diversity in the amounts of phenolics contained in leaves of different cultivars examined in this study.

Similarly, antioxidant capacity of green tea was significantly higher than that of persimmon teas at 1015.9 mM TE (Table 2). Antioxidant capacity of American persimmon was roughly half of green tea's, ranging from 577.5 to 437.2 mol TE/g FW. Similar to phenolic content result, teas made from 'John Rick' had the greatest antioxidant capacity (575.5 mM), followed by 'Valeene Beauty' (500.6 mM TE). However, there was no statistically significant difference among cultivar samples with the exception of 'John Rick' in antioxidant capacity.

Leaves of 'Evelyn' were collected at both Upton, KY and Orleans, IN sites. This was the only cultivar available in this study with triplicate trees at both sites. Phenolic content of 'Evelyn' teas were very similar for both Orleans (149.0 mg GAE/ml) and Upton (146.4 mg GAE/ml) samples. Similarly, a small difference in antioxidant capacity was observed for 'Evelyn' samples collected in Orleans (495.6 mM TE) and Upton (437.2 mM TE), but it was not statistically significant. This may be due to a relative proximity between the two sites (app. 160 km), but it appears that differences in location had little effect on phenolic content and antioxidant capacity of teas made of this cultivar.

Of all cultivars tested, three were genetically related. 'Early Golden' is probably the oldest cultivar available, and has sired other well-known cultivars. 'John Rick' is a seedling of 'Killen,' which is a seedling of 'Early Golden.' In addition, 'Valeene Beauty' is a seedling of 'Lena' ('Mitchellena') and 'Early Golden' originated in Claypool breeding (Raymond, 2006). Despite their genetic relatedness, phenolic content and antioxidant of these cultivars, especially, 'John Rick' was significantly different from 'Early Golden.'

The foliage of 'Valeene Beauty' has a reddish tinge when leaves emerge in spring, indicating the presence of phenolics such as anthocyanins. This cultivar also seems more

Table 2. Phenolic content and antioxidant capacity of American persimmon teas

Species	Cultivar	Phenolic content (mg GAE/ml)	Antioxidant capacity (mM TE)
Camellia sinensis	NA	209.7 a	1015.9 a
Diospyrus virginiana	'Early Golden'	136.8 e[z]	474.4 c
	'Evelyn' (Orleans)	149.0 cd	495.6 c
	'Evelyn (Upton)	146.4 cd	437.2 c
	'John Rick'	166.2 b	577.5 b
	'Valeene Beauty'	156.8 c	500.6 c
	'Yates'	143.9 d	495.2 c

[z] Means followed by the same letters are not significantly different within the same column (P<0.05)

prone to diseases than others based on visual observation. As is known, plants produce phenolics and other secondary metabolites in response to both abiotic and biotic stress, including fungal infection (Latouche, 2013). Elicitation by fungal pathogens, along with anthocyanins in leaves, might have also contributed to the higher amount of phenolic found in 'Valeene Beauty' teas.

In conclusion, while teas made of American persimmon had lower phenolic content and antioxidant capacity, consumption of such tea may be beneficial to human health. The phenolic content and antioxidant capacity of American persimmon tea is comparable to more commercially available black tea. In one report, phenolic content and antioxidant capacity of black tea were reported to be approximately 75.2% and 54.8% of green tea respectively (Lee and Lee, 2002). In this study, phenolic content and antioxidant capacity of American persimmon tea was 68.3~77.8% and 39.3~51.7%. Furthermore, Yerba mate (*Ilex paraguariensis* A. St. Hil.) or Ardisia tea (*Ardisia compressa* Kunth.), and other teas known for their chemopreventitive properties also have lower phenolic content and antioxidant capacity compared to those of green tea (Chandra and de Mejia, 2004).

Aside from health benefits, teas made from American persimmon may prove to be a valuable alternative as people become more aware of importance in local food production. In spite of local abundance, American persimmon is relatively unexploited as a foodstuff. Likewise, production of teas with its leaves may provide an additional income source for wildcrafters or source limited farmers. Finally, consumers may prefer safer alternatives such as American persimmon teas due to lack of caffeine or contaminants such as pesticides and heavy metals that have been reported in commercially available teas.

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