Determination Of Saponin And Various Chemical Compounds In Camellia Sinensis And Genus Ilex.

Sensus Technical Note (SEN-TN-0027) 05/22/2009

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ABSTRACT

Saponin content was determined using an HPLC method. Since saponin contains significant antioxidant capacity, determination of saponin content in various tea products would be important due to its potential value. Saponin content was highest in yaupon holly (*Ilex vomitoria*) compared to the teas from *Camellia sinensis*.

INTRODUCTION

Saponins are glycosides found in many plant and it is well-known as a foaming agent. The materials containing saponin have been historically used as soap but the use of saponin is widely expanded in food industry due to its antioxidant capacity. Saponins are generally present in a root of plant but several studies have reported saponins are also found in plant leaves including tea leaves. Thus, in this study, saponin content in currently utilized teas such as green and black teas was compared with potential saponin sources such as yaupon holly and yerba mate.

MATERIALS AND METHODS

Materials:
Five teas (1. Green tea, 2. Black tea, 3. Yaupon holly (naturally contains caffeine), 4. Yaupon holly (naturally caffeine free), 5. Yerba mate were used in this study.

Tea preparation:
Dried tea leaves were finely ground with a mortar and a pestle and brewed with hot water (90°C) for 10 min. The obtained tea extract was divided into 3 groups containing 20 mL of infusion and treated with 3 mL of chloridiric acid (32% hydrochloric acid solution) in view to yield an acid concentration of 4 mol/L prior to hydrolysis for 2 hours. The saponin fraction was extracted with an equal volume of chloroform using separatory funnel. This extraction was performed three times. The sapogenins (saponin aglycone) was obtained with 10 mL of methanol after evaporated to dryness.

Methods:

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1. Total Phenolics (TP) (also known as total soluble Phenolics) was determined using Folin-Ciocalteau assay as early described by Swain and Hillis (1959) and expressed as gallic acid equivalent.

2. Turbidity of each tea liquor was measured using 2100P turbidimeter (Hach Company) at room temperature.

3. The saponin extract from each tea infusion was diluted 3-fold using deionized water and filtered through a 0.45 µm PTFE filter (Whatman, Clifton, NJ) prior to injection. Polyphenolic separations was conducted on a Agilent 1200 HPLC system using a UV-Vis detector with a Dionex 250 x 4.6 mm Acclaim 120-C_{18} column run at 0.8 mL/min. The pH of 100 % H_2O was adjusted with pH 2.4 using *o*-phosphoric acid for mobile phase and it was run for 30 min at 0.8 mL/min. Total saponin (sapogenin) in each infusions was detected and quantified at 280 nm against external standards of ursolic acid procured from Sigma Adrich (Sigma Chemical Co., St. Louis, MO).

4. Total liquor color was measured at 460nm using Genesis 6 spectometer (Thermo Fisher Inc.). The absorbance of each tea infusion was recorded and the color value was calculated as described by Obanda et al., (2004).

5. Tea creaming. Each aliquot was transferred into four 15 mL eppendorf tubes and held in cold storage (4 °C) to induce tea cream formation in the absence of light. After 12 hour incubation, the infusions in eppendorf tubes were immediately centrifuged at 3500 r.p.m. for 30 min to remove tea cream. The supernatant was removed for phytochemical analysis whereas the tea cream sediment was carefully moved to a pre-weighed aluminum dish by washing with two 5 mL aliquots of distilled water and dried for 12 hrs at 85 °C. The dried tea cream in the aluminum weighting dishes from the oven was carefully weighed and the amount of tea cream formed was determined by calculating the difference between total solids in the initial infusion and the dried tea cream described by Nagalashmi et al. (1984).

6. The amount of soluble protein present in tea was determined using Biuret assay as early described by Gornall et al. (1949). Filtered and diluted tea infusions were mixed with an equal volume of Biuret reagent against a standard curve and allow to react for 20 min for absorbance reading at 540 nm. Properly diluted tea samples (1 mL/4 mL) in test tubes in duplicated was moved to another test tubes and 4 mL of the Biuret reagent (Biuret reagent TS (USP) test solution. Ricca Chemical Company, Arlington, TX) was added to each test tube. After through mixing via vortex, the samples were placed on the bench top for 20 min at room temperature. The absorbance values were measured using spectrometer (Genesis 6 UV-Vis spectrometer. The amount of protein was obtained by computation of the absorbance of the tea samples against standard curve (concentration vs. absorbance). The standard curve was prepared by serial dilutions of 10, 8, 6, 4, 2, and 1 mg/mL (Bovine serum albumin/water, w/v) and blank (0 mg/mL) was applied first for the spectrometer before measuring the absorbance of samples.

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RESULTS AND DISCUSSION

Polyphenolic content was highest in yerba mate followed by yaupon holly w/o caffeine and green tea when determined by Folin-Ciocalteau assay. Even though tea catechins in green tea or theaflavins in black tea are known as stronger antioxidant compound, the concentration of chlorogenic acid that is the most prevalent phenolic compound in yaupon holly and yerba mate was higher in concentration. Total phenolic content was displayed in Table 1.

Liquor color of black tea was darkest followed by green tea. Even though polyphenolic content in yaupon holly and yerba mate was higher, color intensity is higher in black tea and green tea. The color value was illustrated in Table 1.

Protein level was slightly higher in green tea compared to other teas but the amount of protein and polyphenolic level was not directly proportional to tea creaming. Tea cream was formed in yaupon holly w/ caffeine at the highest concentration indicating that caffeine contributed to tea creaming (Table 1).

Saponin content was highest in order of yaupon holly w/ caffeine, yaupon holly w/o caffeine, yerba mate, black tea, and green tea (Table 1). As previously determined, yaupon holly contained higher saponin content than the teas from genus *Camellia*.

Figure 1 displays the chromatogram of standard (ursolic acid) and saponins detected by HPLC at 280 nm.

Table 1. Total phenolics, liquor color, protein, tea cream, turbidity, and saponin content in each saponin containing teas.

	Green tea	Black tea	Yaupon holly w/ caffeine	Yaupon holly w/o caffeine	Yerba maté
Total phenolics (g/L)	1.61	1.41	1.53	1.70	2.02
Liquor Color	216	430	178	101	129
Protein (mg/L)	931.48	962.96	885.19	885.19	888.89
Tea Cream (mg/L)	269.70	179.50	303.60	263.55	299.15
Turbidity (Day 0/1, NTU)	15.5/14.3	65.4/78.2	38.8/39.4	22.2/33.5	43.1/64.3
Saponin (mg/L)	75.09	77.32	91.23	92.62	87.61

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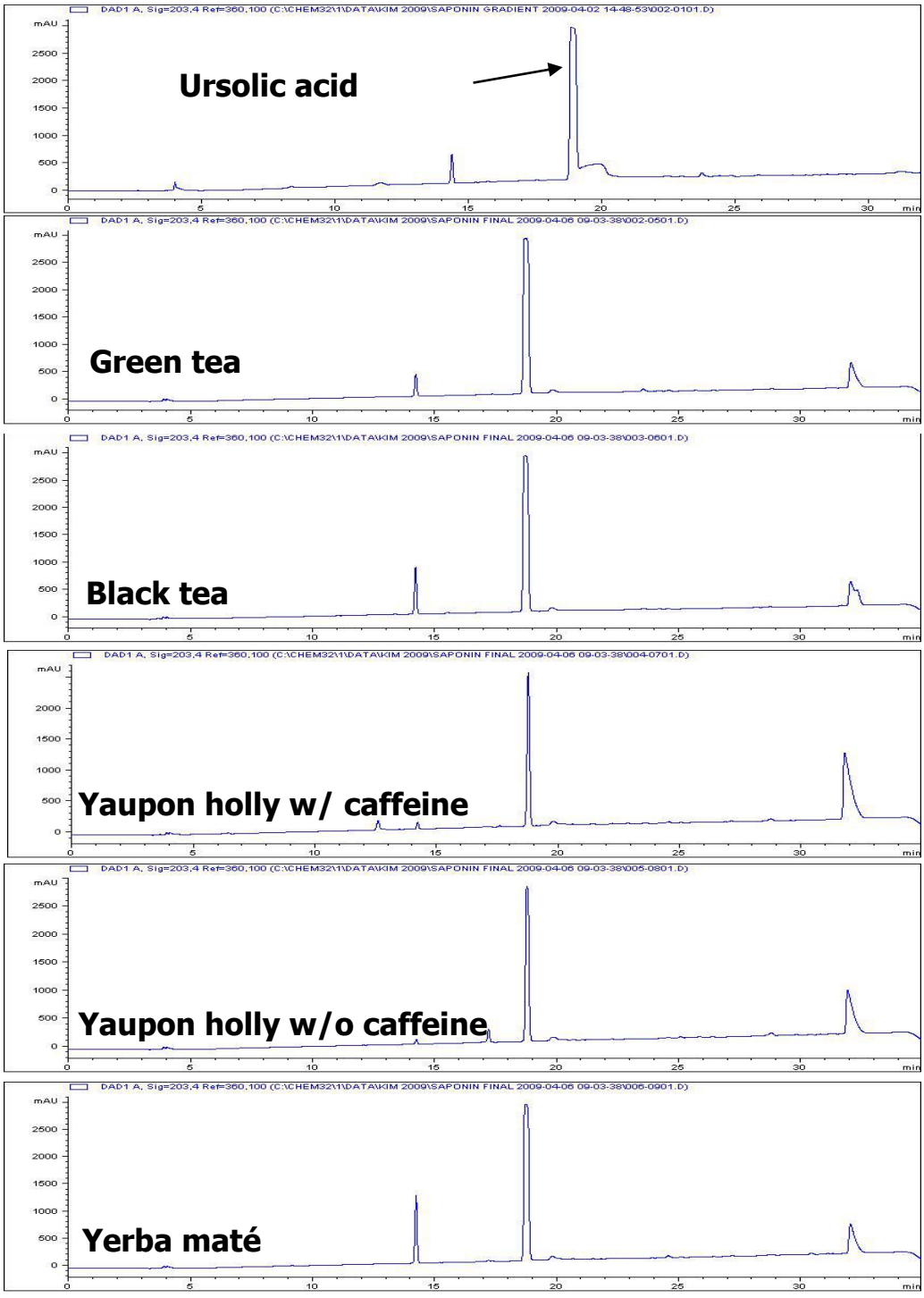

Figure 1. Chromatograms of 5 teas containing saponins. All the peaks in chromatogram represent saponin and the concentrations were expressed as Ursolic acid equivalent.

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