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Comparison of Proanthocyanidins in Commercial Antioxidants: Grape Seed and Pine Bark Extracts

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The major constituents in grape seed and pine bark extracts are proanthocyanidins. To evaluate material available to consumers, select lots were analyzed using high-performance liquid chromatography, gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), gel permeation chromatography (GPC), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Atmospheric pressure chemical ionization (APCI) LC/MS was used to identify monomers, dimers, and trimers present. GC/MS analyses led to the identification of ethyl esters of hexadecanoic acid, linoleic acid, and oleic acid, as well as smaller phenolic and terpene components. The GPC molecular weight (MW) distribution indicated components ranging from ~162 to ~5500 MW (pine bark less than 1180 MW and grape seed ~1180 to ~5000 MW). MALDI-TOF MS analyses showed that pine bark did not contain oligomers with odd numbers of gallate units and grape seed contained oligomers with both odd and even numbers of gallate. Reflectron MALDI-TOF MS identified oligomers up to a pentamer and heptamer, and linear MALDI-TOF MS showed a mass range nearly double that of reflectron analyses.

KEYWORDS: Grape seed extract; pine bark extract; proanthocyanidin; catechin; epicatechin; HPLC; LC/MS; GC/MS; GPC; MALDI-TOF MS

INTRODUCTION

Polyphenols are ubiquitous compounds found in most fruits and vegetables and are associated with their beneficial dietary effects, including reduced rates of cancer and cardiovascular disease. Polymeric and oligomeric proanthocyanidins, also called condensed tannins, are one type of polyphenol and consist of chains of flavan-3-ol units, (+)-catechin (**1**), and (−)-epicatechin (**2**), linked through C4−C6 and C4−C8 interflavan bonds (**Figure 1**). In addition, plants produce oligomeric proanthocyanidins coupled with gallic acid (**3**), forming gallate esters, and/or sugar molecules, forming glycosides (*1*, *2*).

Oligomeric proanthocyanidins are associated with a number of biological activities, most of which are attributed to their antioxidant capabilities. Two popular commercial sources of oligomeric proanthocyanidins are grape seed extract and pine bark extract, which are found in a variety of commercial products ranging from capsules to sports drinks and cosmetics. Grape seed and pine bark extracts are reported to have potent antioxidant activities and are free radical scavengers (*3*−*8*). They have been reported to have radioprotective (*9*−*11*), anticancer (*12*, *13*), and cardioprotective properties (*14*−*21*) and are used in the treatment of circulatory disorders (*22*−*24*), eye disorders (*25*, *26*), and inflammation (*27*−*30*), as well as other conditions (*31*−*33*). In addition to the evidence provided by the amelioration of various conditions during clinical trials, metabolism studies indicated that oligomeric proanthocyanidins are absorbed into the blood stream (*34*, *35*).

While the characterization of grape seed extracts from a variety of sources has been reported (*36*−*45*), most of these analyses were conducted with noncommercial extracts, which had been fractionated using size exclusion chromatography. According to these reports, oligomeric proanthocyanidins are the only chemical constituents present in grape seed extract, which also contains monomeric catechin and epicatechin in nearly equal amounts. Oligomeric proanthocyanidins, up to the undecamer containing one gallate ester, were identified in grape seed extract using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, *38*). Using electrospray mass spectrometry, single-, double-, and triple-charged ions were observed, which corresponded to grape seed extract oligomeric proanthocyanidins containing up to 28 degrees of polymerization (*43*). In contrast, except for Pycnogenol, a proprietary pine bark extract, little has been reported

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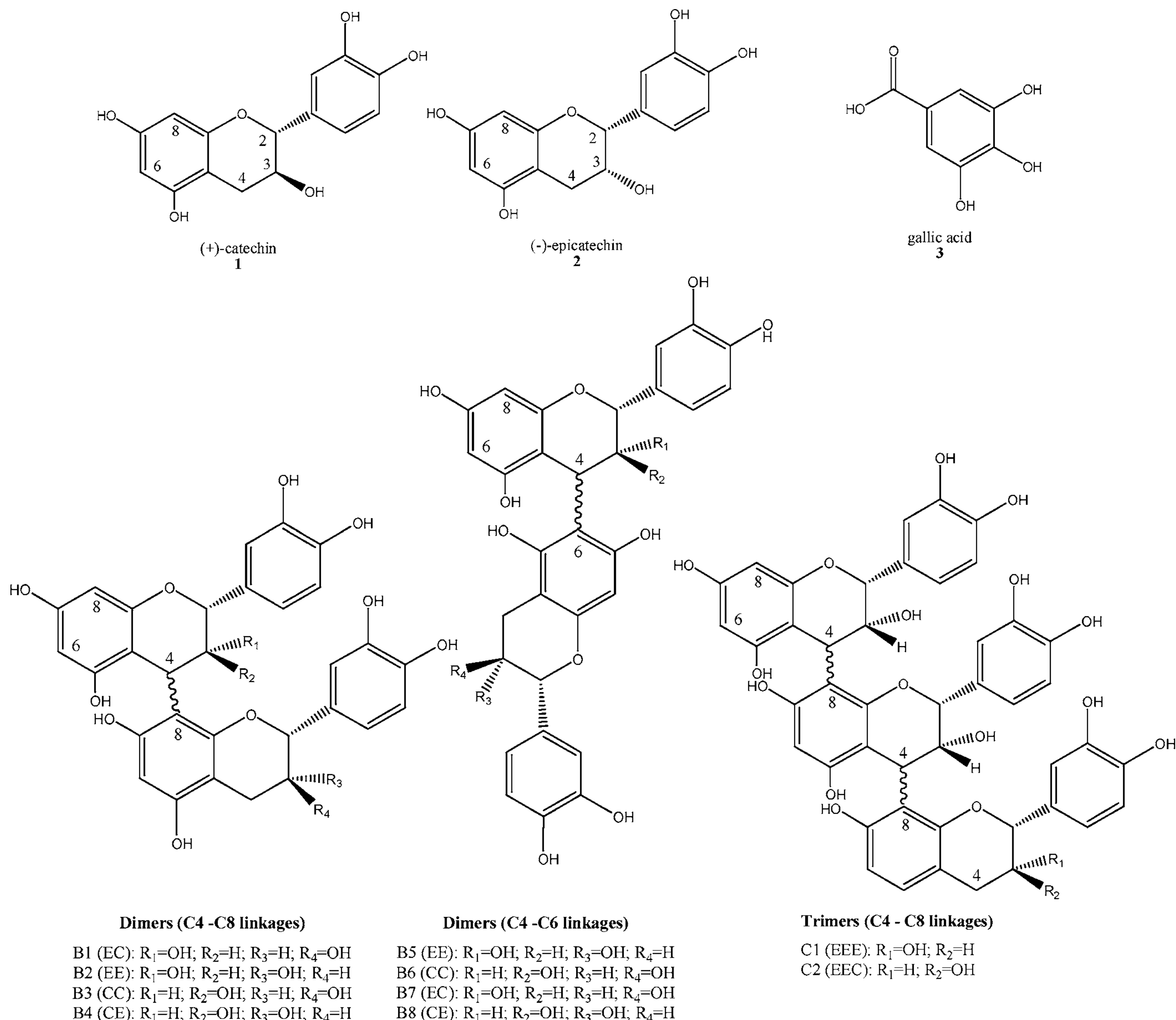

Figure 1. Proposed structures of proanthocyanidin monomers [(+)-catechin (**1**) and (–)-epicatechin (**2**)], gallic acid (**3**), dimers, and trimers.

on the chemical composition of pine bark extract (*8*). Pycnogenol, prepared exclusively from the bark of the French maritime pine of Landes de Gascogne, has been reported to contain free phenolic acids (i.e., benzoic acid and cinnamic acid derivatives) along with their glucosides; oligomeric proanthocyanidins; catechin as the predominant monomer with only trace amounts of epicatechin; and taxifolin, a flavon-3-ol, and its glucoside (*17*, *31*, *45*). While Pycnogenol has been reported to contain oligomeric proanthocyanidins with chain lengths between two and 12 monomeric units (*45*), a more complete description of its oligomeric proanthocyanidins was not found in the literature.

Because of widespread use, grape seed and pine bark extracts are being considered for toxicological evaluation by the National Institute of Environmental Health Sciences (NIEHS). Because characterization of any test article is required prior to initiation of any valid toxicological or efficacy study, a thorough chemical characterization of pine bark and grape seed extract available to consumers was undertaken. This report is the first direct chemical comparison of oligomeric proanthocyanidins obtained from commercial pine bark and grape seed extract sources. In an effort to identify both oligomeric proanthocyanidins and other constituents, analyses were performed using high-performance liquid chromatography (HPLC)/UV, liquid chromatography/mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS), gel permeation chromatography (GPC), and MALDI-TOF MS. The developed HPLC method was transferred directly to atmospheric pressure chemical ionization (APCI) LC/MS, which was used to identify monomers, dimers, and trimers and their gallates present in the extracts. GC/MS analyses were used to identify volatile constituents. GPC was used to determine molecular weight distribution profiles, and MALDI-TOF MS analyses were used to characterize larger molecular mass proanthocyanidins.

MATERIALS AND METHODS

Chemicals and Raw Materials. (+)-Catechin, (−)-epicatechin, (−)-epicatechin gallate, 2,5-dihydroxybenzoic acid, and α-cyano-4-hydroxycinnamic acid were purchased from Sigma (St. Louis, MO). 3-Indoleacrylic acid was purchased from Fluka (Buchs, Switzerland). HPLC-grade solvents (acetone, methanol, acetonitrile, and tetrahydrofuran) and trifluoroacetic acid were purchased from VWR (West Chester, PA). Ethanol (USP-grade, 200 proof) was obtained from AAPER (Shelbyville, KY). ACS-grade formic acid was purchased from Aldrich (Milwaukee, WI). ACS-grade phosphoric acid was purchased from

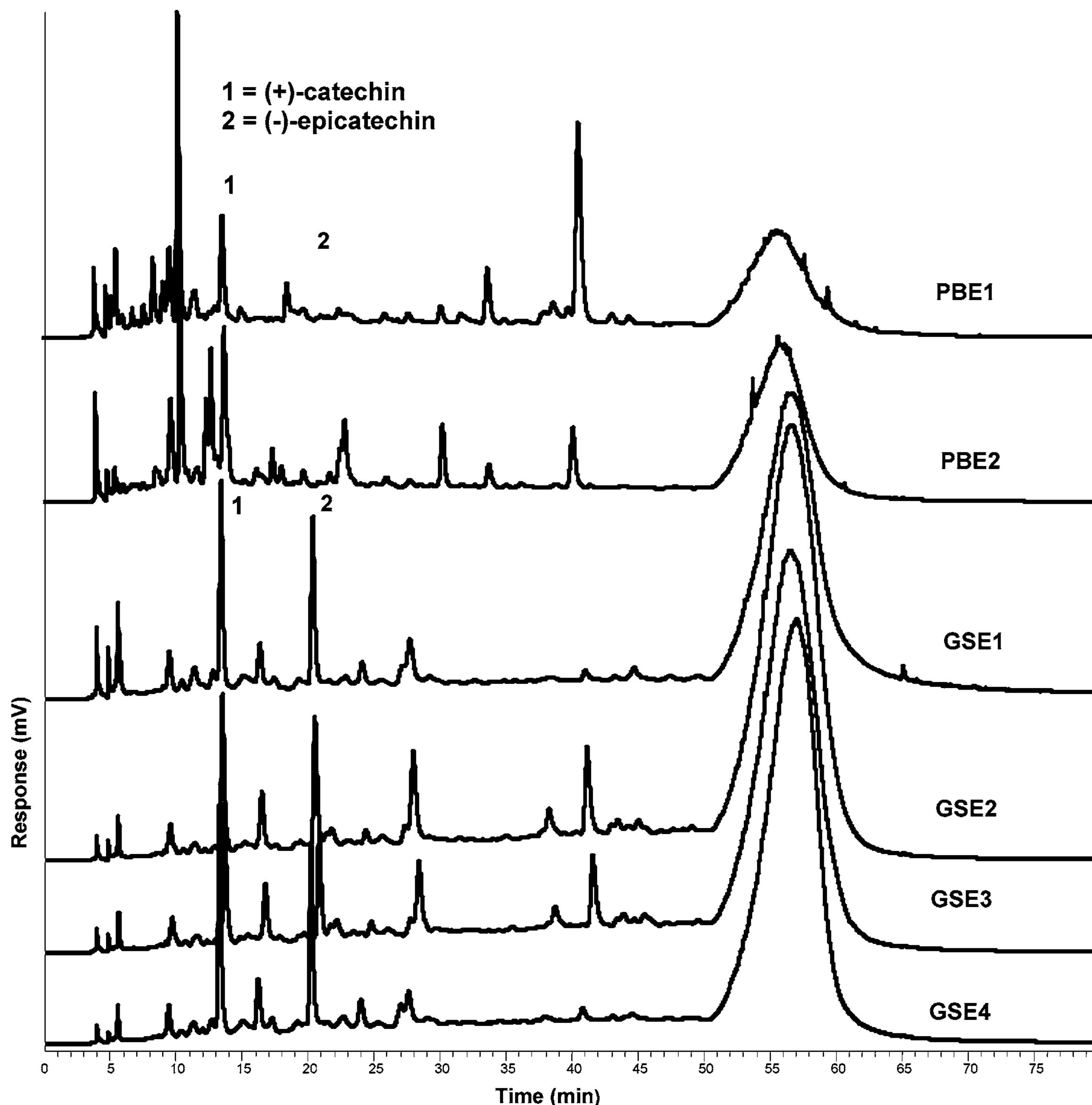

Figure 2. Representative HPLC/UV chromatograms of commercial grape seed (GSE) and pine bark extracts (PBE), prepared at ∼5 mg/mL in methanol/water (1:1, v/v).

Mallinckrodt (Phillipsburg, NJ). HPLC-grade water (18 MΩ-cm) was obtained from an in-house Labconco Water ProPS system (Kansas City, MO). MALDI-TOF MS calibration standards were purchased from Applied Biosystems (Foster City, CA). GPC polystyrene molecular weight standards were obtained from Phenomenex (Torrance, CA). Two lots of pine bark (*Pinus pinaster* and *Pinus massoniana*) extract (PBE1 and PBE2) were obtained from two commercial suppliers. Four lots of grape seed (*Vitis vinifera*) extract (GSE1, GSE2, GSE3, and GSE4) were obtained from three commercial suppliers. One lot of grape seed extract (GSE1) was obtained as a 50 mg formulated capsule, while the other materials were obtained in bulk. All grape seed and pine bark extracts were stored protected from light at ambient temperature.

HPLC/UV Analysis. HPLC analysis of extracts was performed using a 2690 Separations Module (Waters, Milford, MA) equipped with a 2487 UV dual absorbance detector (278 nm) and a TurboChrom data system (Perkin-Elmer, Norwalk, CT) with a 250 mm × 4.6 mm i.d., 5 μm, Supelcosil LC18 column (Supelco, Bellefonte, PA). Mobile phase A was 0.3% phosphoric acid in water, and mobile phase B was acetonitrile. Alternatively, 0.3% trifluoroacetic acid or 0.3% formic acid was used as mobile phase A, with no changes observed in the chromatographic separation, thus indicating that the method could be directly transferred to LC/MS. The linear gradient elution was used as follows: 10−15% B over 45 min; 15−60% B over 15 min, hold for 20 min; 60 to 10% B over 1 min; column equilibration at 10% B for 20 min. The flow rate was 0.7 mL/min, and the data were collected for 80 min. The column temperature was ambient, with an injection volume of 20 μL.

Standard solutions of catechin (29.7 − 986.4 μg/mL) and epicatechin (29.9 − 904.2 μg/mL) were prepared in methanol/water (1:1, v/v) to determine monomer linearity. To determine extraction method linearity, PBE1 and GSE2 solutions were prepared in methanol/water (1:1, v/v) at ∼0.05−25 mg/mL. Six solutions of PBE1 and six solutions of GSE2 were prepared in methanol/water (1:1, v/v) at ∼5 mg/mL to determine reproducibilty of the method. For chromatographic comparisons, each pine bark and grape seed extract sample was prepared in methanol/water (1:1, v/v) at ∼5 mg/mL.

LC/MS Analysis. An LC/MS system consisted of a Waters 2795 separations module and a 2487 UV detector interfaced with a Quattro tandem triple quadrupole mass spectrometer (Micromass, Milford, MA). The previously described HPLC parameters were used, with 0.3% formic acid in water as mobile phase A and UV (278 nm) and MS detection (both positive and negative APCI modes). Conditions for mass spectrometric detection in APCI mode were as follows: mass range scanned, 100−1700 amu; source temperature, 120 °C; desolvation temperature, 550 °C; cone voltage, 25 V; scan time, 3 s; and desolvation gas, 118 L/h. Mass spectrometric data were processed using MassLynx

Table 1. Components Identified in Pine Bark and Grape Seed Extract Samples by LC/MS

approximate retention time from UV (min)	RRT[a]	mass (amu)	observed masses	proposed structure[b]	PBE1	PBE2	GSE1	GSE2	GSE3	GSE4
10.38	0.70[c]	578	M + H = 579 M + Na = 601 M + K = 617	B dimer	X[d]	X	X	X	X	X
11.19	0.75[e]	618	M + H = 619	taxifolin gallate glucoside (or an isomer)	X	X				
12.41	0.84	578	M + H = 579 M + Na = 601 M + K = 617	B dimer	X	X	X	X	X	X
13.42	0.90	618	M + H = 619 M + Na = 641 M + K = 657	taxifolin gallate glucoside (or an isomer)		X				
14.08	0.95	866	M + H = 867 M + Na = 889 M + K = 905 dimer − H = 577 dimer + H = 579	trimer	X	X	X	X	X	X
14.84	1.00	290	M + H = 291 M + H + 2 = 293	catechin[f]	X	X	X	X	X	X
15.59	1.05	456	M + H = 457 M + H + 2 = 459 M + H + 2Na = 503 M + H + Na + K = 519	taxifolin gallate	X	X				
18.64	1.26	578	M + H = 579 M + Na = 601 M + K = 617	B dimer			X	X	X	X
23.59	1.59	290	M + H = 291 M + H + 2 = 293	epicatechin[f]			X	X	X	X
25.05	1.69	730	M + H = 731 M + Na = 753 M + K = 769	dimer monogallate		X				
25.59	1.72	730	M + H = 731 M + Na = 753 M + K = 769	dimer monogallate				X	X	
26.82	1.81	886	M + H = 867 M + Na = 889 M + K = 905 dimer − H = 577 dimer + H = 579	trimer		X				X
28.68	1.93	886	M + H = 867 M + Na = 889 M + K = 905 dimer − H = 577 dimer + H = 579 dimer + K = 617	trimer			X	X	X	X
32.54	2.19	578	M + H = 579 M + Na = 601 M + K = 617	B dimer						X
32.90	2.22[e]	730	M + H = 731 M + Na = 753 M + K = 769	dimer monogallate			X	X	X	X
33.96	2.29	578	M + H = 579	B dimer				X	X	
35.23	2.37	578	M + H = 579 M + Na = 601 M + K = 617	B dimer			X	X	X	X
46.86	3.16[e]	882	M + H = 883 M + Na = 905 M + K = 921	dimer digallate				X	X	

[a] Relative retention time; relative to catechin. [b] Because catechin and epicatechin have the same mass, only the number of monomer units in each component could be proposed; the chemical connectivity of the monomers in each component is not proposed. [c] Corresponds with a marker in both GSE and PBE. [d] Boxes with X = compound is proposed as present in the sample. [e] Corresponds with a marker in either GSE or PBE. [f] Supported through analysis of standard solutions.

software, version 3.4. Catechin and epicatechin standards were prepared at ∼100 μg/mL in methanol/water (1:1, v/v). Pine bark and grape seed extract samples were prepared in methanol/water (1:1, v/v) at ∼5 mg/mL.

GC/MS Analysis. The GC/MS system was comprised of a TRACE GC gas chromatograph (Thermo Finnigan, San Jose, CA) with a Thermo Finnigan A200S autosampler interfaced with a Thermo Finnigan TRACE MS mass spectrometer. GC/MS analyses of the

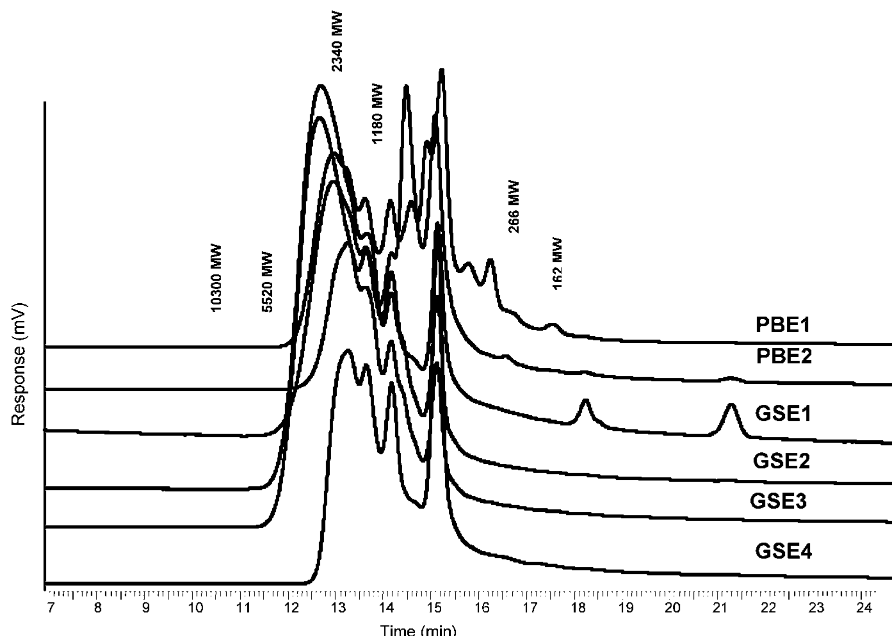

Figure 3. Representative GPC chromatograms of commercial grape seed and pine bark extracts, prepared at ∼5 mg/mL in tetrahydrofuran.

sample and standard solutions were conducted using a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-5 column (J&W Scientific, Palo Alto, CA). Gas chromatographic operating conditions were as follows: injection mode, splitless; injection temperature, 270 °C; injection volume, 1 μL; carrier gas, helium; oven temperature, 40 °C for 4 min then programmed at 5 °C/min to 300 °C and held for 5 min. The mass spectrometer operating conditions were as follows: mode, positive electron ionization (EI+); mass range scanned, 35−550 amu; source temperature, 250 °C; voltage, 70 V; scan time, 0.5 s; and transfer line temperature, 250 °C. Mass spectral data were processed using Thermo Finnigan Xcalibur software, version 1.2. Components were identified using the NIST/EPA/NIH mass spectral library, version 1.7. Pine bark and grape seed extract samples were prepared in acetone at ∼5 mg/mL. Catechin and epicatechin standard solutions were prepared at ∼100 μg/mL in acetone.

GPC Analysis. GPC analysis was performed using the previously described HPLC system, with UV detection at 280 nm. A 300 × 7.8 mm i.d., 5 μ Phenogel 500 Å column and a 300 × 7.8 mm i.d., 5 μ Phenogel 100 Å column (Phenomenex) were connected in series. Separation took place with the columns at ambient temperature, using an isocratic mobile phase of tetrahydrofuran (1.0 mL/min) and an injection volume of 15 μL. Polystyrene molecular weight standards, ranging from 162 − 10,300 MW, were prepared in tetrahydrofuran at ∼ 0.4 mg/mL. Sample solutions were prepared in tetrahydrofuran at ∼ 5 mg/mL.

MALDI-TOF MS Analysis. Reflectron and linear mode MALDI-TOF mass spectral data were acquired on an Applied Biosystems Voyager DE-STR system equipped with delayed extraction and a nitrogen laser at 337 nm. The positive reflectron mode spectra parameters were as follows: accelerating voltage, 20 kV; grid voltage, 65%; mirror voltage ratio, 1.12; extraction delay time, 225 ns; number of laser shots, 100/spectrum; laser intensity, 2400; and mass range, 100−5000 MW. The samples analyzed using reflectron mode were internally calibrated using 2,5-dihydroxybenzoic acid matrix masses and known sample masses at 1178 MW. Developmental analyses included sample preparation at different concentrations (1, 5, and 10 mg/mL) in methanol/water (1:1, v/v), methanol, and tetrahydrofuran. The investigated matrix systems (2,5-dihydroxybenzoic acid or 3-indoleacrylic acid) were prepared at 20 mg/mL in the same solvents as the samples. For reported reflectron mode data, pine bark and grape seed extract samples were prepared in methanol at ∼5 mg/mL and mixed (1:1, v/v) with 2,5-dihydroxybenzoic acid (∼20 mg/mL in methanol) just prior to analysis.

The positive linear mode spectra parameters were as follows: accelerating voltage, 20 kV; grid voltage, 93%; extraction delay time, 250 ns; number of laser shots, 50/spectrum; laser intensity, 2500; and mass range, 500−10000 MW. The samples analyzed using linear mode were externally calibrated using peptide masses, including ACTH (clip 1−17, MW = 2094.3 MW). The investigated matrix systems (2,5-dihydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid) were prepared at ∼20 mg/mL in methanol. For reported linear mode analyses, pine bark and grape seed extract samples were prepared in methanol at ∼10 mg/mL and mixed (1:1, v/v) with 2,5-dihydroxybenzoic acid (∼20 mg/mL in methanol) just prior to analysis.

RESULTS AND DISCUSSION

HPLC/UV Analysis. A reversed-phase HPLC method was developed to chromatographically "fingerprint" pine bark and grape seed extracts and to identify oligomeric proanthocyanidin monomers, dimers, and trimers. While several reversed-phase HPLC methods (*36*, *37*, *40*, *41*) have been reported for grape seed extract oligomeric proanthocyanidins, there was only one reported pine bark extract method (*8*). HPLC method development work focused on developing a single analysis that could be directly transferred to LC/MS for the identification of components and marker compounds that could definitively distinguish pine bark from grape seed extract.

A number of alcohol/water and acetonitrile/water mixtures, with and without the addition of acid, were investigated as diluent and extraction solvents. It was determined that the use of acetonitrile/water as the sample diluent led to both peak fronting and peak tailing of catechin and epicatechin. Subsequent investigations proved that methanol/water (1:1, v/v) was the most suitable solvent for dilution of pine bark and grape seed extracts. Chromatograms are presented in **Figure 2**. Catechin and epicatechin standard solutions were found to be linear (r > 0.9999) over a concentration range of ∼30−990 μg/mL. To evaluate extraction method linearity, seven PBE1 marker components (relative retention times 0.60, 0.70, 0.76, 1.00, 1.36, 2.71, and 3.24) and seven GSE2 marker components (relative retention times 0.69, 1.00, 1.17, 1.58, 2.20, 3.17, and 3.42) were monitored relative to the catechin present and found to be linear (r ≥ 0.999) over a concentration range of ∼0.2−20 mg/mL. The same seven marker components were also used to monitor method precision, using six replicates of PBE1 and GSE2 at ∼5 mg/mL (see Supporting Information, Supplement 1).

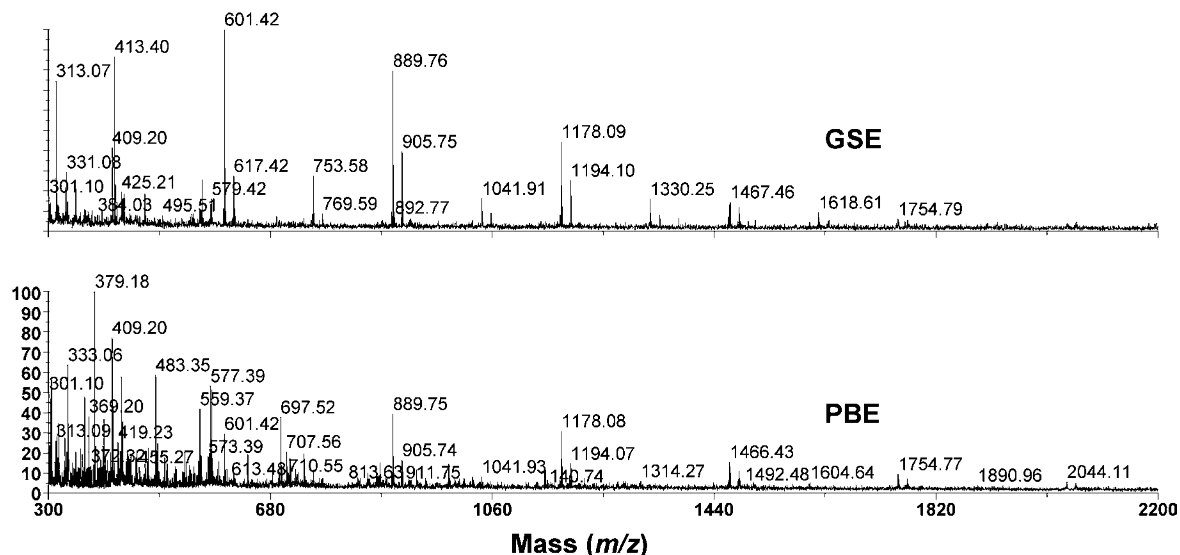

Figure 4. Representative reflectron-mode MALDI-TOF mass spectra of commercial grape seed and pine bark extracts.

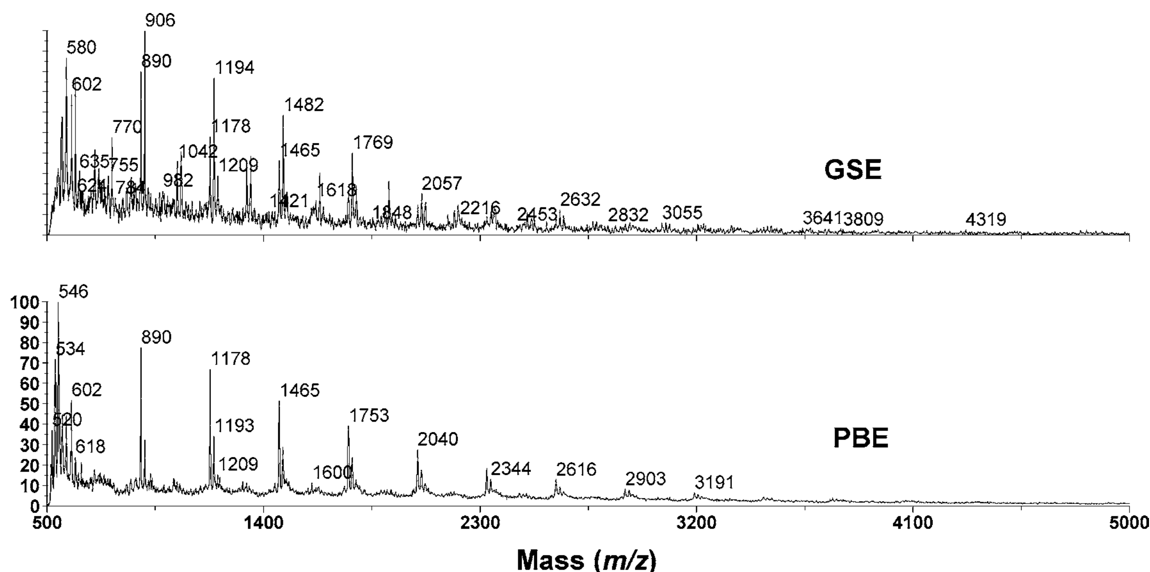

Figure 5. Representative linear-mode MALDI-TOF mass spectra of commercial grape seed and pine bark extracts.

LC/MS Analysis. LC/MS analyses of pine bark and grape seed extracts were used to identify monomers, dimers, trimers, and their gallates in HPLC chromatograms. The sample solutions [~5 mg/mL in methanol/water (1:1, v/v)] were analyzed using positive mode APCI LC/MS. For most components, the sodium and potassium adducts were observed in addition to the M + H ions. Only catechin and epicatechin were identified using negative mode APCI LC/MS. Nine components present in PBE were tentatively identified, while 14 components were tentatively identified in grape seed extract. As shown in **Table 1**, many of the identified components were isomers; the chemical connectivity of the different isomers was not determined.

GC/MS Analysis. GC/MS analyses evaluated volatile components in pine bark and grape seed extracts. Samples, prepared in acetone at ~5 mg/mL, were analyzed using positive electron impact ionization GC/MS. Catechin and epicatechin standard solutions were analyzed along with the samples. Six major peaks not attributed to the solvent were observed in the total ion chromatograms of pine bark extract. Four peaks were identified as 1,8-terpenediol, 2-methoxy-4-vinylphenol, 1-(3-ethoxy-4-hydroxyphenyl)ethanone, and cholesterol. Only 2-methoxy-4-vinylphenol was common to both pine bark extract lots. Five major peaks not attributed to the solvent were observed in the total ion chromatograms of grape seed extract. The three identified grape seed extract peaks, ethyl esters of hexadecanoic acid, linoleic acid, and oleic acid, were observed only in GSE1. The lack of peaks corresponding to catechin and epicatechin in any of the prepared sample or standard solutions was most likely due to lack of volatility of these highly oxygenated compounds.

GPC Analysis. GPC provided pine bark and grape seed molecular weight (MW) distribution profiles. Molecular weight data for the samples (~5 mg/mL in tetrahydrofuran) were obtained through comparison of polystyrene molecular mass standards. Components of up to ~5500 MW were observed (**Figure 3**). The grape seed extract molecular weight distributions were similar to literature reports (*40*), while no reports were found for GPC analysis of pine bark extract. The molecular weight distributions of PBE1 and PBE2 were significantly different, with PBE1 containing more high molecular weight oligomeric proanthocyanidins. In both samples, the majority of components was less than 1180 MW, with a second group of components near 2340 MW. In contrast to pine bark, grape seed extract contained higher molecular weight components, most ranging from ~1180 to ~5000 MW. Grape seed extract con-

tained a definitive dimer peak (~600 MW) followed by a sharp reduction in components in the range of ~600 to ~800 MW. These were followed by an increase in components from ~1500 to ~4000 MW. In comparison to the other grape seed extracts, GSE4 contained fewer higher molecular weight oligomeric proanthocyanidins, with the largest oligomeric proanthocyanidins less than 2340 MW. Low molecular weight peaks were observed in GSE1 but were probably due to excipients in the formulation.

MALDI-TOF MS Analysis. Reflectron- and linear-mode MALDI-TOF MS analyses were performed to identify oligomeric proanthocyanidins present in pine bark and grape seed extracts. MALDI-TOF MS analysis was used to determine the number of catechin and/or epicatechin units and the number of gallate units present in oligomeric proanthocyanidins. Because catechin and epicatechin are stereoisomers, it was impossible to determine the exact structure of the monomer constituents in each oligomeric proanthocyanidin. MALDI-TOF MS analyses also did not provide information regarding the molecular connectivity of the monomer units present.

2,5-Dihydroxybenzoic acid and 3-indoleacrylic acid, used in previous grape seed extract work (*38*, *39*), were investigated as MALDI matrices for analyses conducted in the positive-ion reflectron mode. Several sample concentrations (~1−10 mg/mL) and solvent systems were evaluated, including tetrahydrofuran, methanol/water (1:1, v/v), and methanol. For positive ion spectra acquired in the reflectron mode, the highest masses were observed using 2,5-dihydroxybenzoic acid as the MALDI matrix (~20 mg/mL in methanol) mixed (1:1, v/v) with pine bark and grape seed extracts (~5 mg/mL in methanol).

For positive-ion linear mode MALDI-TOF MS analysis, 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid were investigated. α-Cyano-4-hydroxycinnamic acid was found to be unsuitable for linear-mode MALDI-TOF MS analysis of pine bark and grape seed extracts, since neither low nor high molecular mass oligomeric proanthocyanidins were detected. As with reflectron-mode analyses, the largest masses observed during linear-mode MALDI-TOF MS analyses were obtained using 2,5-dihydroxybenzoic acid as the MALDI matrix (~20 mg/mL in methanol) mixed (1:1, v/v) with pine bark and grape seed extract samples (~5 mg/mL in methanol).

As previously reported (*38*, *39*, *44*), only the sodium adducts of oligomeric proanthocyanidins were observed during MALDI-TOF MS analyses. Even though there were similarities between the observed spectra of the pine bark and grape seed extract samples, there were also some significant differences. Other than the dimer monogallate observed in PBE1, the pine bark extract spectra did not contain oligomers odd (e.g, one, three, or five) number of gallate units. Oligomers with even and odd numbers of gallate units were observed in the grape seed extract spectra. Using reflectron mode, oligomeric proanthocyanidins up to a pentamer with two gallate units were observed in grape seed extract. Oligomeric proanthocyanidins up to a heptamer were observed in pine bark extract. The observed mass range was nearly doubled using linear-mode MALDI-TOF MS. Oligomeric proanthocyanidins up to a dodecamer trigallate were observed in grape seed extract, while a tridecamer was observed in pine bark extract. The observed grape seed extract results correlated with those reported in the literature (*38*, *39*, *44*), which indicated the presence of oligomeric proanthocyanidins up to the undecamer monogallate. No literature reports of MALDI-TOF MS analysis of pine bark extract were found. Representative reflectron- and linear-mode mass spectra are presented in **Figures 4** and **5**, respectively, and the data are summarized in the Supporting Information (Supplement 2). It must be noted that ion intensities do not correlate to the concentration of a component in the sample (*44*), which makes quantitation using MALDI-TOF MS problematic. However, in addition to identification of pine bark and grape seed extract constituents, MALDI-TOF MS analyses may be useful for semiquantitative "fingerprinting," especially with regard to evaluation of sample stability.

While pine bark and grape seed extracts are marketed as oligomeric proanthocyanidins, which suggests that they contain the same constituents, there were significant differences observed in the chemical composition of the commercially available materials. The results of our work indicate that MALDI-TOF MS analyses provided the best chemical definition of oligomeric proanthocyanidins present in pine bark and grape seed extract. It must be noted, however, that MALDI-TOF MS detection of high molecular mass oligomeric proanthocyanidins is less sensitive than the detection of lower molecular mass oligomeric proanthocyanidins (*44*). Therefore, MALDI-TOF MS analyses cannot be used to quantitate all oligomeric proanthocyanidins. In contrast, HPLC and GPC analyses were extremely useful for "fingerprinting" the commercially available material and highlighted differences in extracts of the same or different species. The results of this study lead to a better understanding of the chemical composition of widely used commercially available oligomeric proanthocyanidins, and the developed methods may be useful in verification of label claims of extract products.

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Supporting Information Available: Data obtained by HPLC/UV and MALDI-TOF MS analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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