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Determination of the Predominant Catechins in *Acacia catechu* by Liquid Chromatography/Electrospray Ionization−Mass Spectrometry

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A high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) method under selected ion monitoring mode (SIM) was developed to quantitate the predominant catechins, catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate, in the medicinal plant catechu (*Acacia catechu*). Other major secondary products including caffeine, flavanol dimers, and flavonol glycosides were also identified by their molecular ion peaks and fragmentation peaks using LC/MS and LC/MS/MS. For the investigated ion concentration ranges of catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate, good linearities ($r^2 > 0.99$) were obtained for each calibration curve. Validation for this method showed an accuracy ranging from 1.06 to 11.76%, and the precision (relative standard deviation) varied between 1.60 and 9.36% for these four analytes. This is the first quantitative determination of all predominant catechins in catechu heartwood and leaves.

KEYWORDS: Catechu; khail; cutch tree; *Acacia catechu*; catechins; LC/ESI-MS

INTRODUCTION

Acacia catechu commonly known as catechu, Khail, or Cutch tree is native to Southern Asia and widely distributed in India and Burma (*1*). Khail is a multipurpose leguminous tree providing wood, gum, tannin, and dye and is also used for reforestation and land reclamation due to its wide adaptability and rapid growth rate (*2*, *3*). The wood of *A. catechu* is hard and is used in making furniture and agriculture implements (*4*, *5*). Khair gum is a pale, yellow, mucilaginous gum exuded from the tree, yielding one of the best natural substitutes for gum arabic (*3*). The plant extract has been used as an astringent and for itching, indigestion, and inflammations (*1*, *6*). The heartwood extract of catechu is a traditional Chinese medicine called "Ercha" and is used in the treatment of cough and dysentery, as well as topically for skin ulceration and lesions (*7*). Catechu is also known to be used as a component of the betel leaf chewed in India for the local treatment of otitis and otorrhoea (*8*).

Previous studies have reported on the isolation and identification of several chemical constituents from the heartwood, bark, roots, and leaves; the major components are catechins, caffeine, flavonol glycosides, and other phenolic compounds (*9*−*14*). Of the dietary flavonoids examined, catechins, naturally occurring flavan-3-ols, such as (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, and (−)-epigallocatechin-3-*O*-gallate (**Figure 1**), exhibit strong antioxidant activities that are generally associated with free radical scavenging and metal chelation properties (*15*−*18*). These natural products have also been reported as anticarcinogenic (*19*−*21*), antimutagenic (*22*), and cardioprotective agents (*23*, *24*). An inhibitory effect to mouse IV allergy was recently reported by Suzuki et al. from tea catechins by determining the mouse ear-swelling ratios (*25*). Abe et al. found that green tea gallocatechins potently and selectively inhibited rat squalene epoxidase, a rate-limiting enzyme of cholesterol biogenesis (*26*).

In *A. catechu*, catechins occur with many additional UV-absorbing phenolic compounds, making their chromatographic separation and detection difficult. The only reference available for the high-performance liquid chromatography (HPLC) quantitative analysis of catechins from catechu was the liquid chromatography/ultraviolet quantification of two catechins components (+)-catechin and (−)-epicatechin found in a traditional medicine extract of catechu (*7*). In this study, we procured botanically authenticated catechu heartwood and leaves plus a

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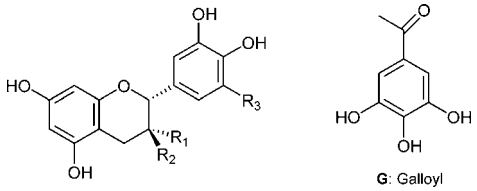

Figure 1. Catechin (**1**): $R_1 = H$, $R_2 = OH$, and $R_3 = H$. Epicatechin (**2**): $R_1 = OH$, $R_2 = H$, and $R_3 = H$. Gallocatechin (**3**): $R_1 = H$, $R_2 = OH$, and $R_3 = OH$. Epigallocatechin (**4**): $R_1 = OH$, $R_2 = H$, and $R_3 = OH$. Catechin-3-*O*-gallate (**5**): $R_1 = H$, $R_2 = O$**G**, and $R_3 = H$. Epicatechin-3-*O*-gallate (**6**): $R_1 = O$**G**, $R_2 = H$, and $R_3 = H$. Gallocatechin-3-*O*-gallate (**7**): $R_1 = H$, $R_2 = O$**G**, and $R_3 = OH$. Epigallocatechin-3-*O*-gallate (**8**): $R_1 = O$**G**, $R_2 = H$, and $R_3 = OH$.

commercially available catechu product, catechu resin chunks, to quantitate the individual catechins. By using LC/electrospray ionization−mass spectrometry (ESI-MS) and LC/tandem MS (MS/MS), catechin and epicatechin in catechu heartwood and resin chunks and catechin, epicatechin, epigallocatechin-3-*O*-gallate, and epicatechin-3-*O*-gallate in catechu leaves were selectively detected and quantified. In addition to these flavan-3-ols, other major components were identified or tentatively identified.

MATERIALS AND METHODS

Materials. Standard compounds, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-*O*-gallate, and (−)-epigallocatechin-3-*O*-gallate, and the internal standard (IS) glycitein were purchased from Sigma Chemical Co. (St. Louis, MO). The solvents methanol (MeOH) and acetonitrile (MeCN) used in this research were HPLC-grade and purchased from Fisher Scientific Co. (Fair Lawn, NJ). HPLC-grade water (18 MΩ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA) and was used for preparing all solutions. Formic acid was purchased from Acros Organics (NJ). The catechu leaves were collected from Yunnan province (P. R. China) and authenticated by the botanical taxonomist, Dr. Yonghong Yang, Yunnan Agricultural University (China). The catechu heartwood sample was obtained as dry wood powder from Shree Baidyanath Ayurved Bhawan Pvt. Ltd. (India). The catechu resin chunks (extract from catechu heartwood) were purchased as a commercial product of Chinese traditional medicine from 13 Moons store (Johnson City, NY). Both of these catechu heartwood samples and catechu resin chunks were authenticated microscopically by Dr. Qingli Wu in our research team, and the description and identification of catechu (Ercha) were further confirmed using Chinese Pharmacopoeia (1995 edition, part I). Product samples were also deposited in our botanical products library for future reference. A green tea sample used as a comparative control was procured from P. R. Bigelow Inc. (Fairfield, CT).

Equipment. HPLC separation was performed on a 250 mm × 4.6 mm i.d., 5 μm, Polaris Amide C18 column (Varian Inc., Palo Alto, CA), 1100 Series LC/MSD system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, quaternary pump system, diode array, multiple wavelength detector, thermostated column compartment, degasser, MSD trap with an ESI source, and HP ChemStation software. Bruker Daltonics 4.1 and DataAnalysis 4.1 software were used for LC/ESI-MS and LC/MS/MS experiments.

Calibration Standards and Quality Control (QC) Samples. The stock solution was prepared by dissolving the appropriate amounts of ∼5.0 mg of each standard, catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate, in 7 mL of diluent (0.1% formic acid water and MeOH, 3:7) with sonication for 15 min. The final volume of each solution was then diluted to 10 mL with the diluent at room temperature. Calibration standards were prepared by diluting the stock solution with the diluent and then spiking the same amount of IS glycitein. The calibration curves were established on 10 data points covering a concentration range for catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate. QC samples were prepared by diluting the separated stock solutions with diluent and then spiking the same amount of known ISs.

Sample Preparation. All catechu and green tea samples were finely ground with a coffee grinder. About 100 mg of powder was accurately weighed from each sample and placed into a 100 mL volumetric flask, and ∼70 mL of diluent (0.1% formic acid water and MeOH, 3:7) was added. The sample was sonicated for 20 min (only for 70% MeOH extraction) and allowed to cool to room temperature and then filled to the full volume with the diluent. The extract was transferred to a centrifuge tube and centrifuged at 12000 rpm for 2 min to obtain a clear solution and filtered through a 0.45 μm filter. The injection volume was 20 μL. The recovery test was performed by the addition of known quantities of standards corresponding approximately to 100% of the expected values in the original sample and then together extraction according to the same extraction method described above.

LC/MS Conditions for Identification. The mobile phase for chromatographic separation consisted of solvent A (0.1% formic acid in water, v/v) and B (0.1% formic acid in MeCN, v/v) in gradient. The total running time was 50 min. The gradients were 0 min, 90% solvent A; 10 min, 80% solvent A; 30 min, 70% solvent A; and 40 min, 80% solvent A. Prior to the next injection, the column was equilibrated for 10 min with 10% B. The flow rate was set to 1.0 mL/min with the column compartment maintained at 25 °C, and the injection volume was 20 μL. The eluent was monitored by ESI-MS under positive ion mode, and the sample was scanned from *m*/*z* 120 to 2200. ESI was conducted by using a needle voltage of 3.5 kV under an optimum collision energy level of 20%. The identity of flavonoids was based on their molecular ions in a total ion chromatogram (TIC) and fragment ions in auto MS/MS spectra (threshold, 30000). MS/MS was conducted in the positive ion mode and under the collision energy level of 20%. Helium was used as the nebulizer at 70 psi, and desolvation was achieved by using high-purity nitrogen (99.999%) heated to 350 °C at a flow rate of 12 L/min.

LC/MS Conditions for Quantification. HPLC conditions were the same as described. SIM mode (selected ion monitoring) was applied and protonated molecular ions were isolated for analytes of catechin, epicatechin, epigallocatechin-3-*O*-gallate, and epicatechin-3-*O*-gallate, respectively. The mass spectrometer was set into four time segments: 0−13 min for catechin and epicatechin; 13−18.5 min for epigallocatechin-3-*O*-gallate; 18.5−30 min for epicatechin-3-*O*-gallate; and 30−50 min for glycitein (IS). The scan range was set from *m*/*z* 200 to 500, and the isolation width was 1.0 *m*/*z*. Other MS parameters were the same as described for qualitative identification. An IS glycitein was added to the analytes before injection, and a plot of the peak area ratio (analyte/IS) vs analyte concentration for calibration standards resulted in equations of $y = 0.0279x + 0.0052$ ($r^2 = 0.999$) for catechin; $y = 0.0358x + 0.0049$ ($r^2 = 0.997$) for epicatechin; $y = 0.0073x - 0.0007$ ($r^2 = 0.998$) for epigallocatechin-3-*O*-gallate; and $y = 0.005x - 0.0014$ ($r^2 = 0.998$) for epicatechin-3-*O*-gallate. The concentrations of the QC and catechu samples were calculated from these linear equations. All analyses were carried out in triplicate.

RESULTS AND DISCUSSION

Characterization of the Major Components in Catechu by LC/MS and LC/MS/MS Method. Positive LC/MS and the subsequent fragmentation of the predominant positive ions in auto MS/MS were used to obtain information about the molecular weights (**Figure 2**), the molecular masses of conjugates, and the masses of the sugar moieties bound to the aglycones. Whenever possible, the authentic standards and literature were applied to support the identification of peaks. The retro Diels−Alder (RDA) fragmentation ion can serve as a characteristic fingerprint for the presence of catechins in complex matrices (*27*). $[M + H\text{-galloyl} + H - H_2O]^+$ is also a general fragmentation pattern observed for all catechin gallates and gallocatechin gallates (*27*). Because epicatechin is an epimer of catechin, its mass spectrum is identical to that of catechin. The positive full scan LC/MS analysis produced peaks for

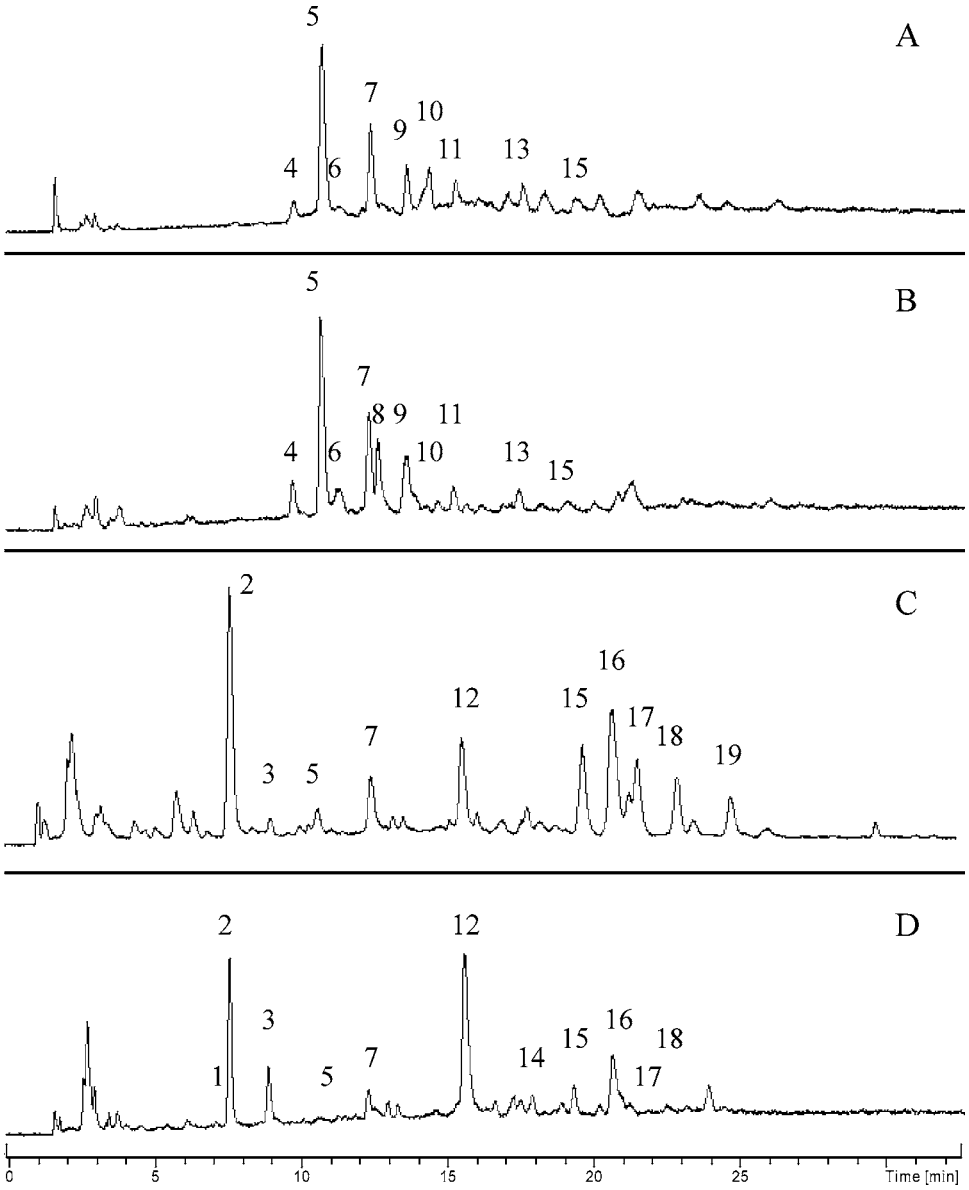

Figure 2. TIC of catechu heartwood (**A**), catechu resin chunks (**B**), catechu leaves (**C**), and green tea leaves (**D**) scanned from *m*/*z* 120 to 2200. For identities, see **Table 1**.

catechin (peak 5) and epicatechin (peak 7) matching the molecular ions at *m*/*z* at 291 under a 20% collision energy level (**Figure 3**). In addition, in the second MS stage, the characteristic RDA fragmentations at *m*/*z* 139 further confirmed the structures. The identities of epicatechin-3-*O*-gallate (peak 16) and epigallocatechin-3-*O*-gallate (peak 12) in catechu were confirmed on the basis of molecular ions at *m*/*z* 443 for epicatechin-3-*O*-gallate and 459 for epigallocatechin-3-*O*-gallate and the fragmentation $[M + H\text{-galloyl} + H - H_2O]^+$ at *m*/*z* 273 for epicatechin-3-*O*-gallate and *m*/*z* 289 for epigallocatechin-3-*O*-gallate.

Full scan LC/MS produced two series of flavanol dimers with molecular ions at *m*/*z* 579 (peaks 4 and 6) and *m*/*z* 581 (peaks 9−11, and 13), respectively. The dimers with molecular ion peaks at *m*/*z* 579 appear to be procyanidin dimers on the basis of catechin-like RDA fragmentation $[M + H - 152]^+$ at *m*/*z* 427 and a catechin-like monomer fragmentation at *m*/*z* 291 in MS/MS (**Table 1**) (*11*, *28*, *29*). The characterization of chalcan-flavan type dimers was based on the molecular ion peaks at *m*/*z* 581, the RDA fragmentation at *m*/*z* 429, the catechin-like monomer fragmentation at 291, and the previous structural elucidations (*30*−*32*).

The identification of flavonol glycosides was performed according to the MS spectra and the identities of flavonoids from catechu on previous studies (**Table 1**) (*9*−*11*). Peak 15 seems to be a quercetin hexose-deoxyhexoside based on positive molecular ion at *m*/*z* 611 in LC/MS, which fragmented to ions at *m*/*z* 465 (loss of a deoxyhexose) and 303 (loss of a hexose-deoxyhexose) in MS/MS. The molecular ion at *m*/*z* 465 and the fragmentation at *m*/*z* 303 for peak 17 indicated the presence of quercetin hexoside. Peak 18 showed a positive molecular ion at *m*/*z* 595, which fragmented to ions at *m*/*z* 449 (loss of a deoxyhexose) and 287 (loss of a hexose-deoxyhexose), suggesting the presence of kaempferol hexose-deoxyhexoside. The identification of peak 19 as kaempferol hexoside was based on the molecular ion at *m*/*z* 449 and the fragmentation at *m*/*z* 287 (loss of hexose).

Quantification of the Catechins by LC/MS Method. To generate more molecular ions and prevent further fragmentation, different collision energy levels were evaluated, and an optimal energy level of 20% was selected (*27*). Under SIM mode, protonated molecular ions $[M + H]^+$ were isolated for individual compounds catechin and epicatechin at *m*/*z* 291, epigallocat-

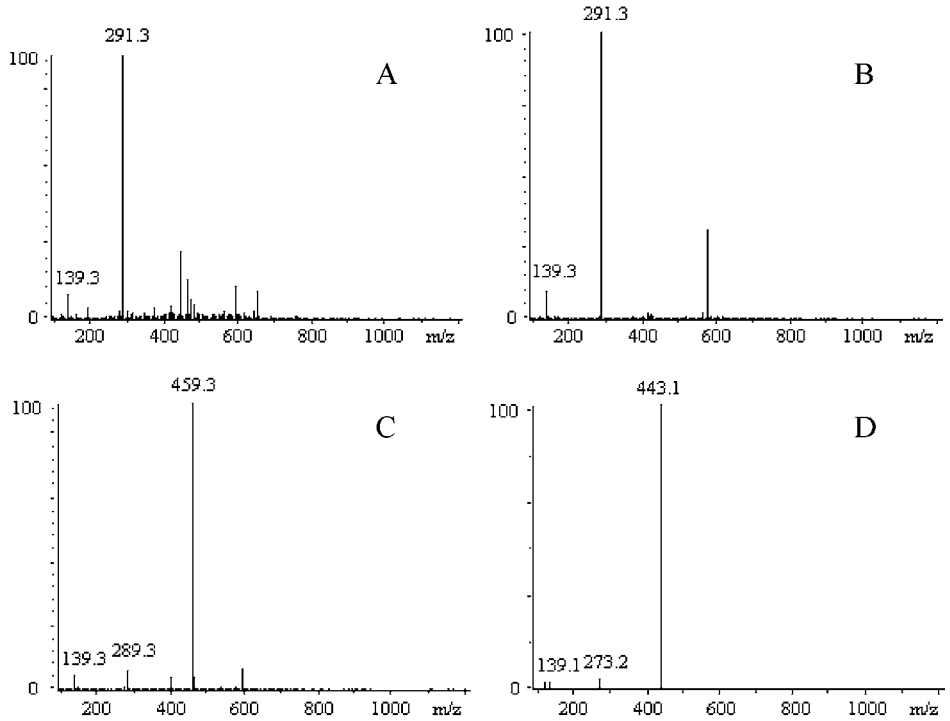

Figure 3. Representative positive ESI mass spectra obtained from the LC/MS TICs of catechu leaf extract in **Figure 2**. (**A**) Catechin (peak 5 in **Figure 2**), (**B**) epicatechin (peak 7 in **Figure 2**), (**C**) epigallocatechin-3-*O*-gallate (peak 12 in **Figure 2**), and (**D**) epicatechin-3-*O*-gallate (peak 16 in **Figure 2**).

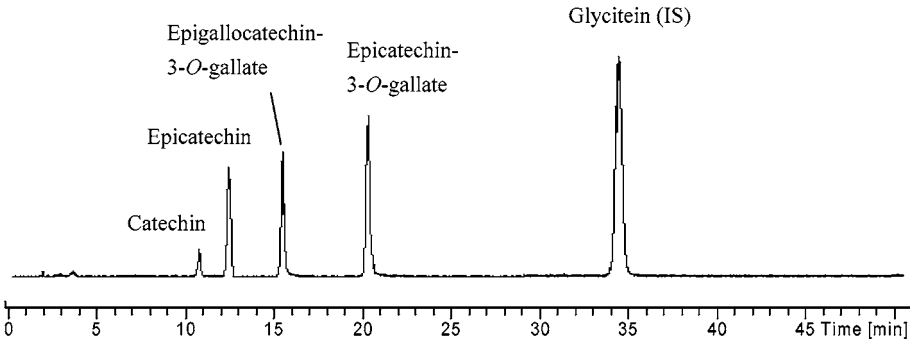

Figure 4. Representative SIM chromatogram from catechu leaf extract set with four time segments: 0–13 min at *m*/*z* 291 (catechin and epicatechin), 13–18.5 min at *m*/*z* 459 (epigallocatechin-3-*O*-gallate), 18.5–30 min at *m*/*z* 443 (epicatechin-3-*O*-gallate), and 30–50 min at *m*/*z* 285 (glycitein).

Table 1. Peak Assignment for the LC/MS and LC/MS/MS Analysis of Catechu and Green Tea

peak no.	t_R (min)	$[M + H]^+$ MS (*m*/*z*)	fragment ion MS/MS (*m*/*z*)	tentative identification
1	7.0	307	289, 139	(–)-gallocatechin
2	7.5	195	138	caffeine (std)[a]
3	8.8	307	289, 139	(–)-epigallocatechin (std)[a]
4, 6	9.7, 11.2	579	427, 409, 291	procyanidin dimers
5	10.6	291	273, 139	(+)-catechin (std)[a]
7	12.1	291	273, 139	(–)-epicatechin (std)[a]
8	12.5	329	279	unknown compound
9–11, 13	13.4, 14.3, 15.1, 17.2	581	563, 429, 411, 291	chalcan-flavan dimers
12	15.4	459	289, 139	(–)-epigallocatechin-3-*O*-gallate (std)[a]
14	17.7	459	289, 139	(–)-gallocatechin-3-*O*-gallate
15	19.1	611	465, 303	quercetin hexose-deoxyhexoside
16	20.4	443	273, 139	(–)-epicatechin-3-*O*-gallate (std)[a]
17	20.9	465	303	quercetin hexoside
18	22.7	595	449, 287	kaempferol hexose-deoxyhexoside
19	24.3	449	287	kaempferol hexoside

[a] As compared with the standard.

echin-3-*O*-gallate at *m*/*z* 459, and epicatechin-3-*O*-gallate at *m*/*z* 443. Glycitein, a type of flavone, was used as an IS in the analytes. The selective ion chromatograms of catechins were successfully performed within 50 min (**Figure 4**). Lower limits of quantification for catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate were established by analyzing a set of working solutions across 10 different concentrations. The linearity of the calibration curves was found to be 0.32−41 μg/mL for catechin, 0.34−43.8 μg/mL for epicatechin, 2.55−81.6 μg/mL for epicatechin-3-*O*-gallate, and 1.88−60 μg/mL for epigallocatechin-3-*O*-gallate. On the basis of the calibration equations, the contents of catechin and epicatechin in catechu heartwood as well as resin chunks were quantitated, and the concentrations of catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate in catechu leaves were calculated (**Table 2**).

Validation of LC/MS Method with Selected Ion Chromatogram. The precision (relative standard deviation, RSD) and accuracy (deviation from nominal concentration) were assessed by analyzing the QC samples, catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate, by lower limit of quantification, low QC, medium QC, and high QC ($n = 6$). Results showed an accuracy ranging from 1.06 to 11.76%, and the precision (RSD) varied between 1.60 and 9.36% for these four analytes. The recovery test was validated

Table 2. Quantification of the Predominant Catechins in Catechu and Catechu Products (mg/g)

	catechin[a]	epicatechin[a]	epigallocatechin-3-*O*-gallate[a]	epicatechin-3-*O*-gallate[a]
catechu heartwood	20.00 ± 0.32	4.63 ± 0.12	ND[b]	ND[b]
catechu resin chunks	74.76 ± 1.07	14.14 ± 0.42	ND[b]	ND[b]
catechu leaves	1.07 ± 0.05	4.09 ± 0.22	9.10 ± 0.47	20.45 ± 0.58

[a] Mean value ± SD in triplicate. [b] Not detectable.

by spiking the catechu leaf sample with a known concentration of epicatechin and epicatechin-3-*O*-gallate, and the resulting combination was subjected to the entire extraction and analytical sequence. The recovery rates were calculated as 95.2 and 97.0% for epicatechin and epicatechin-3-*O*-gallate, respectively. These validation studies showed that the recommended method is reliable and sensitive for the quantification of catechins in *A. catechu*.

Distribution of Catechins and Other Flavonols in *A. catechu*. Catechu was found to be a rich source for catechins that accumulate in both the heartwood and the leaves. Catechin and epicatechin are the dominant secondary metabolites in catechu heartwood with 2.46% of the weight of dry wood and 8.89% of the resin chunks (heartwood extract), thus exhibiting both a simple and a high concentration catechins profile. In contrast, catechin, epicatechin, epicatechin-3-*O*-gallate, and epigallocatechin-3-*O*-gallate were the major flavan-3-ol components found in catechu leaves. As compared to catechu heartwood, gallated catechins are the major form according to catechin concentrations in leaves. Some low levels of flavanol dimers are present in catechu wood, such as procyanidin dimers and chalcan-flavan dimers, whereas few dimers were found in leaves. We observed a similar chemical profile in catechu heartwood to that of catechu resin chunks, indicating that the qualitative composition can be characteristic of catechu products. In addition to catechins, the presence of several flavonol glycosides was also found in both catechu wood and leaves.

Comparison of Catechu Leaves and Green Tea Leaves. Green tea is one of the plants that is well-studied and recognized as an abundant source of catechins. The total catechins content in green tea ranges from 9 to 117 mg/g of dry weight of green tea leaves, varietal-, environmental-, and processing-dependent (*33*). This is within the same range that we now report for the catechins in catechu leaves. The chemical profiles of the catechins in catechu leaves and green tea were compared using the same method as described, indicating many similarities between both plant species relative to catechins accumulation. Seven catechins were found in green tea including epigallocatechin-3-*O*-gallate, epigallocatechin, epicatechin-3-*O*-gallate, epicatechin, catechin, gallocatechin, and gallocatechin-3-*O*-gallate, of which the last three catechins were in trace amounts. In comparison, the catechu leaves contain five catechins, epicatechin-3-*O*-gallate, epigallocatechin-3-*O*-gallate, epicatechin, catechin, and a trace amount of epigallocatechin. Both species accumulated a high concentration of gallated catechins. Epigallocatechin-3-*O*-gallate and epicatechin-3-*O*-gallate in green tea were quantified as 19.20 ± 0.75 and 8.87 ± 0.19 mg/g, respectively. Epigallocatechin-3-*O*-gallate is the highest catechin form in green tea, whereas epicatechin-3-*O*-gallate (20.45 ± 0.58 mg/g) was the highest in catechu leaves. In addition to the catechins, some flavonols, such as quercetin glycosides and kaempferol glycosides, were also present in both green tea and catechu leaves. These findings show that catechu may have potential as an alternative source of natural catechins.

LITERATURE CITED

(1) Naik, G. H.; Priyadarsini, K. I.; Satav, J. G.; Banavalikar, M. M.; Sohoni, D. P.; Biyani, M. K.; Mohan, H. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry* **2003**, *63*, 97−104.

(2) Kaur, K.; Verma, B.; Kant, U. Plant obtained from the Khair tree (*Acacia catechu* Willd.) using mature nodal segments. *Plant Cell Rep.* **1998**, *17*, 427−429.

(3) Rout, G. R.; Samantary, S.; Das, P. Somatic embryogenesis and plant regeneration from callus culture of *Acacia catechu*, a multipurpose leguminous tree. *Plant Cell, Tissue Organ Cult.* **1995**, *42*, 283−285.

(4) Kaur, K.; Kant, U. Clonal propagation of *Acacia catechu* Willd. by shoot tip culture. *Plant Growth Regul.* **2000**, *31*, 143−145.

(5) Singhal, P. C.; Joshi, L. D. Role of gum arabica and gum catechu in glycemia and cholesterolemia. *Curr. Sci.* **1984**, *53*, 91.

(6) Yadava, R. N.; Sodhi, S. A new flavone glycoside: 5,7,3′,4′-Tetrahydroxy-3-methoxy flavone-7-*O*-*β*-D-galactopyranosyl-(1→4)-*O*-*β*-D-glycopyranoside from the stem of *Acacia catechu* Willd. *J. Asian Nat. Prod. Res.* **2002**, *4*, 11−15.

(7) Wang, G.; Yu, J.; Tian, J.; Zhang, J. Analysis of chemical composition of catechu, quantitative determination of catechin and epicatechin by RP-HPLC. *Yaowu Fenxi Zazhi (China)* **1999**, *19*, 88−90.

(8) Yang, N.; Krishna, K.; Wang, P.; Jong, D.; Wu, L.; Lin, J. Effects of aqueous extract of "Betel quid" and its constituents on testosterone production by dispersed mouse interstitial cells. *Am. J. Chin. Med.* **2004**, *32*, 705−715.

(9) Sharma, P.; Dayal, R.; Ayyar, K. S. Chemical constituents of *Acacia catechu* leaves. *J. Indian Chem. Soc.* **1997**, *74*, 60.

(10) Sharma, P.; Dayal, R.; Ayyar, K. S. Acylglucosterols from *Acacia catechu*. *J. Med. Aromat. Plant Sci.* **1999**, *21*, 1002−1005.

(11) Deshpande, V. H.; Patil, A. D. Flavonoids of *Acacia catechu* heartwood. *Indian J. Chem.* **1981**, *20B*, 628.

(12) Azad, A. K.; Ogiyama, K.; Sassa, T. Isolation of (+)-catechin and a new polyphenolic compound in *Bengal catechu*. *J. Wood Sci.* **2001**, *47*, 406−409.

(13) Yadav, R. N. A novel flavone glycoside from the stem of *Acacia catechu* Willd. *J. Inst. Chem. (India)* **2001**, *73*, 104−108.

(14) Agarwal, A.; Soni, P. L. Structure of the oligosaccharides isolated from *Acacia catechu* (Khail) gum polysaccharide. *Indian J. Chem.* **1988**, *27B*, 55−58.

(15) Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549−1552.

(16) Arpentine, G.; Fernandez, Y.; Bourzeix, M.; Mitjavila, S. Relation between the structure of a series of procyanidins and their radical superoxide scavenger capacity. *Process Group Polyphenols* **1992**, *16*, 237−240.

(17) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratol. *Lancet* **1993**, *341*, 1103−1104.

(18) Hu, J. P.; Calomme, M.; Lasure, A.; De Bruyne, T.; Pieters, L.; Vlietinck, A.; Vanden Berge, D. A. Structue-activity relationship of flavonoids with superoxide scavenging activity. *Biol. Trace Elem. Res.* **1995**, *47*, 327−331.

(19) Liu, L.; Castonguay, A. Inhibition of the metabolism and genotoxicity of 4-(methylnitroamino)-1-butanone (NNK) in rat hepatocytes by (+)-catechin. *Carcinogenesis* **1991**, *12*, 1203−1208.

(20) Blot, W. J.; Chow, W. H.; McLaughlin, J. K. Tea and cancer: A review of the epidemiological evidence. *Eur. J. Cancer* **1996**, *5*, 425−438.

(21) Khokhar, S.; Venema, D.; Hollman, P. C. H.; Dekker, M.; Jongen, W. A RP-HPLC method for the determination of tea catechins. *Cancer Lett.* **1997**, *114*, 171−172.

(22) Liviero, L.; Puglisi, P.; Morazzoni, P.; Bombardelli, E. Antimutagenic activity of procyanidins from *Vitis vinifera*. *Fitoterapia* **1994**, *65*, 203−209.

(23) Terencio, M. C.; Sanz, M. J.; Paya, M. Antihypertensive action of a procyanidin glycoside from *Rhamnus lycioides*. *J. Ethnopharmacol.* **1991**, *31*, 109−114.
(24) Meunier, M. T.; Villie, F.; Jonadet, M.; Bastide, J.; Bastide, P. Inhibition of angiotensin I converting enzyme by flavanolic compounds: *In-vitro* and *in-vivo* studies. *Planta Med.* **1987**, *53*, 12−15.
(25) Suzuki, M.; Yoshino, K.; Maeda-Yamamoto, M.; Miyase, T.; Sano, M. Inhibitory effects of tea catechins and *O*-methylated derivatives of (−)-epigallocatechin-3-*O*-gallate on mouse type IV allergy. *J. Agric. Food Chem.* **2000**, *48*, 5649−5653.
(26) Abe, I.; Seki, T.; Umehara, K.; Miyase, T.; Niroshi, N.; Sakakibara, J.; Ono, T. Green tea polyphenols: Novel and potent inhibitors of squalene epoxidase. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 767−771.
(27) Wu, Q.; Wang, M.; Simon, J. E. Determination of proanthocyanidins in grape products by liquid chromatography/mass spectrometric detection under low collision energy. *Anal. Chem.* **2003**, *75*, 2440−2444.
(28) Maatta-Riihinen, K. R.; Kamal-Eldin, A.; Torronen, A. R. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family Rosaceae). *J. Agric. Food Chem.* **2004**, *52*, 6178−6187.
(29) Nelson, B. C.; Sharpless, K. E. Quantification of the predominant monomeric catechins in baking chocolate standard reference material by LC/APCI-MS. *J. Agric. Food Chem.* **2003**, *51*, 531−537.
(30) Nonaka, G.; Nishioka, I. Novel biflavonoids, chalcan-flavan dimers form Gambir. *Chem. Pharm. Bull.* **1980**, *28*, 3145−3149.
(31) Bajadurai, K. U.; Nayudamma, Y. The tannins of babul bark. *Aust. J. Chem.* **1964**, *17*, 803−809.
(32) Santhanam, P. S.; Ghosh, D.; Nayudamma, Y. Phenolic constituents of babul bark. *Leather Sci.* **1965**, *12*, 179−183.
(33) Dalluge, J. J.; Nelson, B. C. Determination of tea catechins. *J. Chromatogr. A* **2000**, *881*, 411−424.

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