Characterization and Antioxidative Properties of Condensed Tannins from the Mangrove Plant *Aegiceras corniculatum*

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ABSTRACT: Freeze-dried leaf, stem bark, and root bark powders of *Aegiceras corniculatum* were extracted with three different types of polar solvents: methanol, ethyl acetate, and water. The methanol extracts had the highest concentrations in total phenolics and extractable condensed tannins, followed by water and ethyl acetate extracts. Analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) suggested that condensed tannins from leaf, stem bark, and root bark contained prodelphinidins and procyanidins, with the predominance of prodelphinidins and high level of galloylation. Acid-catalyzed degradation in the presence of benzyl mercaptan indicated that gallocatechin, epigallocatechin, epigallocatechin-3-*O*-gallate, and epicatechin-3-*O*-gallate occurred as the terminal units and (epi)gallocatechin, (epi)gallocatechin-3-*O*-gallate, (epi)catechin, and (epi)catechin-3-*O*-gallate occurred as the extension units. The mean degrees of polymerization (mDP) of condensed tannins from leaf, stem bark, and root bark were 13.5, 7.4, and 12.3, respectively. The condensed tannins from leaf and stem bark exhibited a higher DPPH radical scavenging activity and ferric reducing/antioxidant power compared to that of synthetic antioxidant butylated hydroxyanisole (BHA). © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 2463–2472, 2012

Key words: *Aegiceras corniculatum*; condensed tannins; MALDI-TOF MS; degree of polymerization; antioxidant activity

INTRODUCTION

Condensed tannins are ubiquitous phenolic compounds, representing the second most abundant natural phenolics after lignin[1] and the fourth most widespread components in vascular plant tissues.[2] As natural antioxidants, the condensed tannins possess a broad spectrum of physiological properties, such as antioxidant activity,[3] antimicrobial effects,[4] anti-inflammatory properties,[5] application in cardiovascular diseases,[6] and antiallergy activity.[7]

The potential health benefits attributed to condensed tannins may be affected by their structures and particularly the degree of polymerization.[8,9] However, detailed information on these compounds profiles (especially with regard to most complex oligomeric structures) in most plants is currently lacking, and analysis of highly polymerized condensed tannins is not feasible, since the number of isomers increases with increasing degrees of polymerization.[10,11] Due to the complexity and diversity, condensed tannins are thus considered to be a final frontier of flavonoid research.[12]

Aegiceras corniculatum (L.) Blanco, a cryptoviviparous mangrove species, often grows at the seaward edge of the mangrove zone in China. According to previous studies, the extracts of *A. corniculatum* stem possess a pronounced antioxidant activity[13] and have been used as antiasthmatic, antidiabetic, antirheumatic, and anti-inflammatory products by the local community at coastal areas.[14,15] *A. corniculatum* leaves had a high phenolic content[16]; therefore, this plant might be a good candidate for further development as a nutraceutical or for its antioxidant remedies. In this study, we investigated the structures of condensed tannins from leaf, stem bark, and root bark of *A. corniculatum* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and reversed phase high-performance liquid chromatography (HPLC) analyses for the first time. In addition, the free radical scavenging capacities and ferric reducing/antioxidant powers of these condensed tannins were also discussed.

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EXPERIMENTAL

Chemicals and plant materials

The solvents methanol, acetone, and ethyl acetate were of analytical reagent (AR) purity grade. Trifluoroacetic acid (TFA) and acetonitrile were of HPLC grade. Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-*S*-triazine (TPTZ), ascorbic acid, butylated hydroxyanisole (BHA), 2,5-dihydroxybenzoic acid (DHB), cesium chloride, and benzyl mercaptan were purchased from Sigma (St. Louis, MO). Sephadex LH-20 was purchased from Amersham and HPLC standards were purchased from Sigma (St. Louis, MO). The leaf, stem bark, and root bark of *A. corniculatum* were collected from Zhangjiang River Estuary Mangrove National Natural Reserves (117°24′E, 23°55′N), Yunxiao, Fujian province, China, in December 2009 and immediately freeze-dried using a desktop freeze-dryer (FD-1, Beijing Detianyou Technology Development Co. China) at –56°C for 72 h and then ground to pass through the 40-mesh sieve. The freeze-dried powers of different parts of *A. corniculatum* were stored at −20°C prior to analysis.

Extraction and purification of condensed tannins

Freeze-dried leaf, stem bark, and root bark powders (5 g of each) were successively extracted with 50 mL of methanol, ethyl acetate, and distilled water at room temperature (26–28°C) and normal pressure for 24 h each separately. All the extracts were then centrifuged at 3500 × *g* for 15 min and collected. The same procedure was repeated three times. The organic solvents of the combined extracts were evaporated under reduced pressure, using a rotary evaporator at 38°C and the remaining water was then lyophilized. The freeze-dried extracts thus obtained were used directly for total phenolics and extractable condensed tannins estimation.

The dried crude methanol extracts of leaf, stem bark, and root bark were further processed through a Sephadex LH-20 column to obtain the respective purified tannins following a previous method described by Hagerman.[17] Briefly, the dried methanol extracts were re-dissolved in methanol–water (50:50, v/v), and then were applied to a Sephadex LH-20 column (30 × 2.5 cm i.d.). The column was washed with methanol–water (50:50, v/v) until the eluent turned colorless. The absorbed condensed tannins were then eluted with acetone–water (70:30, v/v; 500 mL). The acetone was removed under reduced pressure, using a rotary evaporator at 38°C and the remaining aqueous fractions were lyophilized to obtain the respective purified condensed tannins, which were further analyzed by MALDI-TOF mass spectrometry and thiolysis.

Extraction yield

The obtained freeze-dried extracts were weighed, and then the extraction yield was calculated according to the method of Zhang et al.[18] and expressed as the percentage of the weight of the crude extract to the raw material (5 g).

Determination of the amount of total phenolics and extractable condensed tannins

The established procedures[19] were used. The amount of total phenolics was determined using the Folin-Ciocalteu method.[20] Briefly, 0.2 mL aliquot of extract was added to a test tube containing 0.3 mL of distilled H_2O. Folin-Ciocalteu reagent (0.5 mL) and 20% Na_2CO_3 solution (2.5 mL) were added to the mixture and shaken. After incubation for 40 min at room temperature, the absorbance versus a blank was determined at 725 nm. The total phenolic concentrations of extracts were expressed as milligram gallic acid equivalents/gram extract.

The extractable condensed tannin concentration was assayed by the butanol–HCl method,[21] using purified leaf condensed tannins as the standard. All samples were analyzed in three replications.

MALDI-TOF MS analysis

The MALDI-TOF MS spectra were recorded on a Bruker Reflex III instrument (Germany). The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm, and the duration of the laser pulse was 3 ns. In the positive reflectron mode, an accelerating voltage of 20.0 kV and a reflectron voltage of 23.0 kV were used. 2,5-Dihydroxybenzoic acid (DHB, 10 mg/mL 30% acetone solution) was used as the matrix. The sample solutions (10 mg/mL 30% acetone solution) were mixed with the matrix solution at a volumetric ratio of 1:3. The mixture (1 μL) was spotted to the steel target. Amberlite IRP-64 cation-exchange resin (Sigma-Aldrich), equilibrated in deionized water, was used to deionize the analyte–matrix solution thrice. Cesium chloride (1.52 mg/mL) was mixed with the analyte–matrix solution (1:3, v/v) to promote the formation of a single type of ion adduct ($[M+Cs]^+$).[22]

Thiolysis and reversed phase HPLC of the condensed tannins

Thiolysis was carried out by a method based on that described by Gu et al.[23] with slight modifications. Briefly, the purified condensed tannins from leaf, stem bark, and root bark of *A. corniculatum* (5 mg/mL in methanol, 50 μL) were placed in a vial and to this was added hydrochloric acid in methanol (3.3:96.7, v/v; 50 μL) and benzyl mercaptan in

TABLE I
Extraction Yield, Total Phenolic and Extractable Condensed Tannin Concentrations of *A. corniculatum* Extracts

Solvents used for extraction	Samples	Extraction yield (%)	Total phenolics (mg/g extract)[a]	Extractable condensed tannins (mg/g extract)[b]
Methanol	Leaf	35.54 ± 1.79a	227.47 ± 2.54b	308.00 ± 13.50b
	Stem bark	29.61 ± 1.83b	334.71 ± 11.92a	374.06 ± 15.14a
	Root bark	37.18 ± 1.08a	152.97 ± 7.73c	207.82 ± 12.26c
Ethyl acetate	Leaf	4.51 ± 0.14a	78.01 ± 2.35b	38.38 ± 0.74b
	Stem bark	1.09 ± 0.07c	91.97 ± 1.34a	69.57 ± 4.32a
	Root bark	2.12 ± 0.20b	50.31 ± 5.34c	7.93 ± 2.13c
Distilled water	Leaf	29.83 ± 1.56c	156.09 ± 7.80b	200.94 ± 6.84b
	Stem bark	32.60 ± 1.70b	305.22 ± 8.49a	318.07 ± 11.98a
	Root bark	37.46 ± 1.23a	113.53 ± 5.17c	161.35 ± 3.81c

[a] Using gallic acid as the standard.
[b] Using purified leaf tannins as the standard.
Different letters in the same column show significant differences from each other at $P < 0.05$ level.

methanol (5:95, v/v; 100 μL). The solution was heated at 40°C for 30 min, and cooled to room temperature. The thiolysis reaction medium (20 μL) filtrated through a membrane filter with an aperture size of 0.45 μm was analyzed by reversed phase HPLC.

The high-performance liquid chromatograph was an Agilent 1200 system equipped with a diode array detector and a quaternary pump. The thiolysis medium was further analyzed using LC/MS (QTRAP 3200) with a Hypersil ODS column (4.6 mm × 250 mm, 5 μm) (China). Two solvents, namely A = 0.5% (v/v) TFA in aqueous and B = CH_3CN, were used. The gradient condition was: 0–45 min, 12–80% B (linear gradient); 45–50 min, 80–12% B (linear gradient). The column temperature was 25°C and the flow-rate was set at 1 mL/min. Detection was at a wavelength of 280 nm and the UV spectra were acquired between 200 and 600 nm. Degradation products were identified on chromatograms according to their relative retention times and LC/MS. The mean degree of polymerization (mDP) of the condensed tannins was calculated by comparing the peak areas, based on the following equation:

$$\mathrm{mDP} = 1 + \frac{\text{area under the curve of benzyl thioether derivative of flavan-3-ol units}}{\text{area under the curve of flavan-3-ol units}}$$

DPPH radical scavenging activity

The free radical scavenging activity of purified condensed tannins from leaf, stem bark, and root bark of *A. corniculatum* on the DPPH radical was measured according to the method described by Brand-Williams et al.,[24] with some modifications. A 0.1 mL of various concentrations of each freeze-dried sample at different concentrations (15.63, 31.25, 62.5, and 125 μg/mL dissolved in methanol) was added to 3.9 mL of DPPH solution (25 mg/L in methanol). An equal amount of methanol and DPPH served as control. After the mixture was shaken and allowed to stand at ambient temperature for 30 min, the absorbance at 517 nm was measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison. The capability to scavenge the DPPH radical was calculated by the following equation:

$$\text{DPPH scavenging effect}(\%) = [(A_1 - A_2)/A_1] \times 100$$

where A_1 = the absorbance of the control reaction; A_2 = the absorbance in the presence of the sample. BHA and ascorbic acid were used as standards.

Ferric reducing/antioxidant power (FRAP) assay

FRAP assay is a simple and reliable colorimetric method commonly used for measuring the total antioxidant capacity.[25] In brief, 3 mL of freshly prepared FRAP reagent was mixed with 0.1 mL of the purified condensed tannins (15.63, 31.25, 62.5, and 125 μg/mL dissolved in methanol) from leaf, stem bark, and root bark of *A. corniculatum* or methanol (for the reagent blank). The FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ made up in 40 mM hydrochloric acid. All the above three solutions

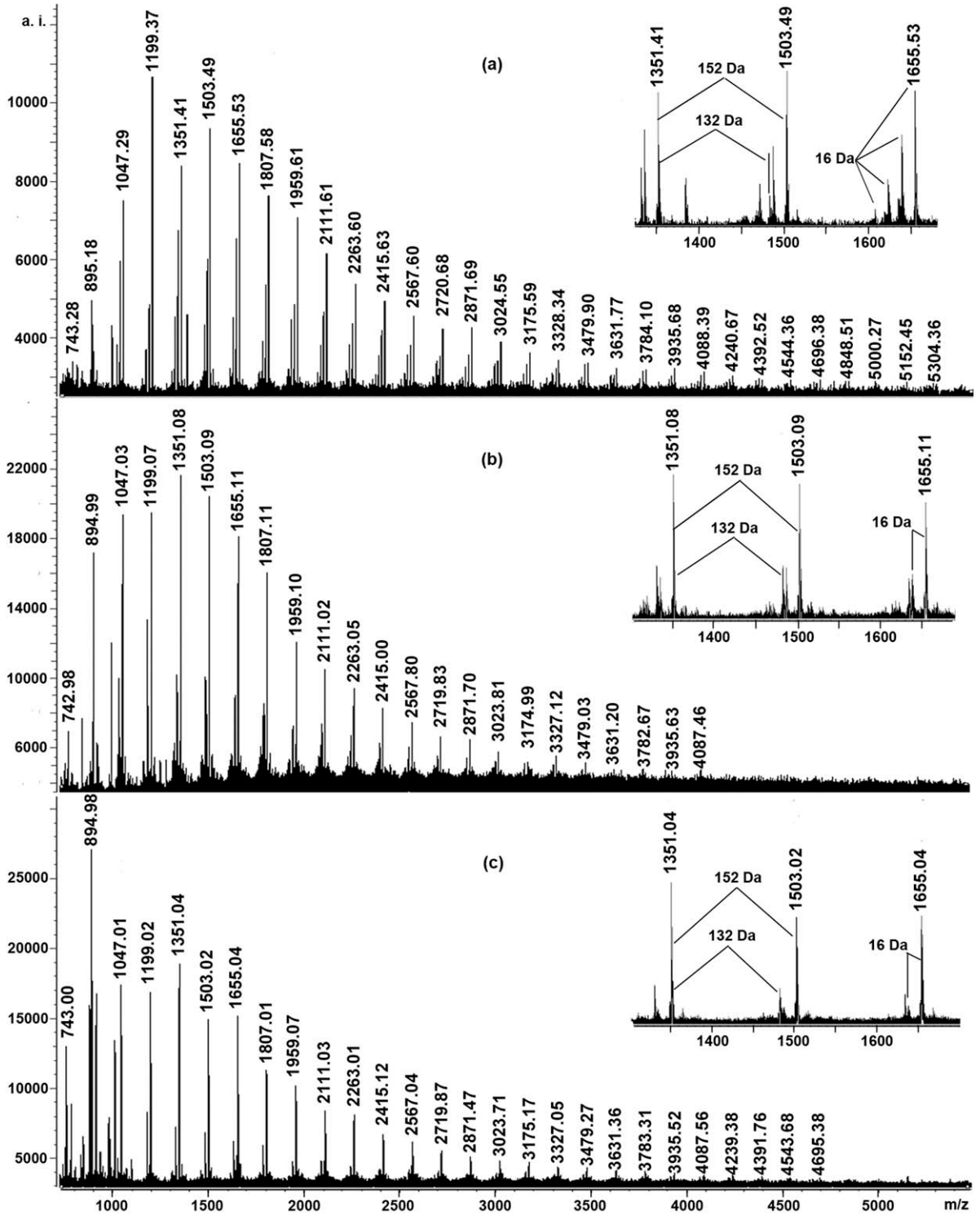

Figure 1 MALDI-TOF positive reflectron mode mass spectra of condensed tannins from leaf (a), stem bark (b), and root bark (c) of *A. corniculatum*.

were mixed together in the ratio of 25:2.5:2.5 (v/v/v). The absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 25°C for 10 min. The FRAP values, expressed in millimole ascorbic acid equivalents (AAE)/gram dried tannins, were derived from a standard curve.

Statistical analysis

All data were expressed as means ± standard deviation of three independent determinations. One-way analysis of variance (ANOVA) was used, and the differences were considered to be significant at

TABLE II
Observed and Calculated Masses of Condensed Tannins from Leaf, Stem Bark, and Root Bark of *A. corniculatum* by MALDI-TOF MS

					Observed $[M + Cs]^+$		
DP	n_1	n_2	n_3	Calculated $[M + Cs]^+$	Leaf	Stem bark	Root bark
2	0	2	0	743	743.28	742.98	743.00
2	0	2	1	895	895.18	894.99	894.98
3	2	1	0	1015	1014.76	—	1014.57
2	1	1	2	1031	1031.31	1031.04	1031.29
3	1	2	0	1031	1031.31	1031.04	1031.29
2	0	2	2	1047	1047.29	1047.03	1047.01
3	0	3	0	1047	1047.29	1047.03	1047.01
3	2	1	1	1167	1167.33	—	—
3	1	2	1	1183	1183.35	1183.09	1182.98
3	0	3	1	1199	1199.37	1199.07	1199.02
3	2	1	2	1319	1319.42	—	—
4	2	2	0	1319	1319.42	—	—
3	1	2	2	1335	1335.43	1335.09	1335.00
4	1	3	0	1335	1335.43	1335.09	1335.00
3	0	3	2	1351	1351.41	1351.08	1351.04
4	0	4	0	1351	1351.41	1351.08	1351.04
3	2	1	3	1471	1471.51	—	—
4	2	2	1	1471	1471.51	—	—
3	1	2	3	1487	1487.49	1487.13	1486.95
4	1	3	1	1487	1487.49	1487.13	1486.95
3	0	3	3	1503	1503.49	1503.09	1503.02
4	0	4	1	1503	1503.49	1503.09	1503.02
4	3	1	2	1607	1607.56	—	—
4	2	2	2	1623	1623.54	—	—
4	1	3	2	1639	1639.53	1639.09	1639.12
4	0	4	2	1655	1655.53	1655.11	1655.04
4	3	1	3	1759	1759.53	—	—
4	2	2	3	1775	1775.65	—	—
4	1	3	3	1791	1791.55	1791.12	1790.94
4	0	4	3	1807	1807.58	1807.11	1807.01
4	3	1	4	1911	1911.73	—	—
4	2	2	4	1927	1927.55	—	—
4	1	3	4	1943	1943.56	1943.08	1943.07
4	0	4	4	1959	1959.61	1959.10	1959.07

DP: degree of polymerization; n_1: number of catechin/epicatechin units; n_2: number of gallocatechin/epigallocatechin units; n_3: number of galloyl units; "—" means no observed peaks corresponding to the calculated ones.

$P<0.05$. All statistical analyses were performed with SPSS 13.0 for windows.

RESULTS AND DISCUSSION

Extraction yield, total phenolic concentration, and extractable condensed tannin concentration

Freeze-dried leaf, stem bark, and root bark powders were successively extracted with methanol, ethyl acetate, and distilled water. Ethyl acetate is usually used for extraction of flavonoid aglycones, while methanol and water are used for medium polar and polar compounds such as flavonoid glycoside, phenolic acids, polysaccharides, and sugars depending on their polarity.[26,27] Because of difference in polarity of extraction solvents, the solubility of phenolic compounds and the rate of mass transfer could be different.[28]

The lowest extraction yield, total phenolic and extractable condensed tannin concentrations were found in the ethyl acetate extract (Table I). Although the extraction yields of methanol and water extracts were the similar, the methanol extracts had significantly higher concentrations in total phenolics and extractable condensed tannins than the water extracts. Thus, the freeze-dried methanol extracts of leaf, stem bark, and root bark were further purified through a Sephadex LH-20 column to obtain the respective purified condensed tannins following a previous method described by Hagerman.[17]

MALDI-TOF MS analysis

MALDI-TOF MS has been widely used for characterizing synthetic and natural polymers such as condensed tannins.[29–32] It can distinguish between

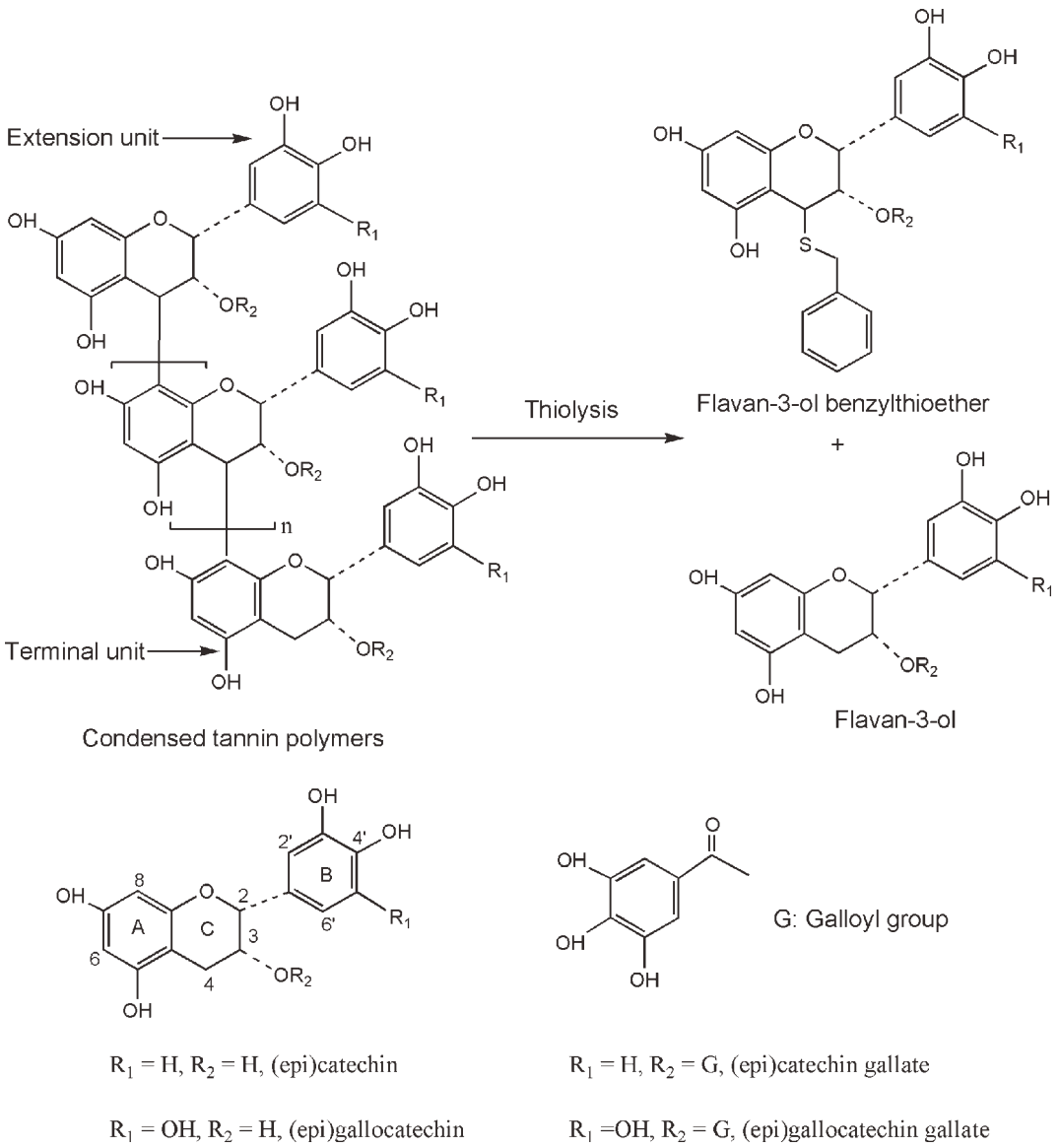

Figure 2 Chemical structure of flavan-3-ol monomer units and thiolysis reaction of condensed tannin polymers.

molecular weight differences due to procyanidin versus prodelphinidin subunits (Δ16 Da) or due to 3-*O*-galloylation (Δ152 Da).[33]

Figure 1 shows the MALDI-TOF mass spectra of the condensed tannins isolated from different parts of *A. corniculatum*, recorded as CS^+ adducts in the positive ion reflectron mode. The included magnification demonstrated the good resolution of the spectra. Condensed tannins from leaf, stem bark, and root bark were characterized by mass spectra with a series of peaks with distances of Δ152 Da, corresponding to the addition of one galloyl group at the heterocyclic C-ring as in (epi)gallocatechin gallate (Fig. 1). Another repeated pattern within each main set of peaks was signals separated by Δ16 Da difference (Fig. 1 and Table II). These masses could be explained by heteropolymers of repeating flavan-3-ol units being lack of an additional hydroxyl group (16 Da) at the 5′ position of the B-ring as (epi)catechin. In addition, each peak was always followed by mass signals at a distance of 132 Da in the spectra of the leaf, stem bark and root bark (Fig. 1), which was quasimolecular ions $[M + 2Cs - H]^+$ generated by simultaneous attachment of two Cs^+ and loss of a proton.[34]

On the basis of the report by Monagas et al.,[11] an equation was established to predict the mass distribution of the condensed tannins of leaf, stem bark, and root bark from *A. corniculatum*. The equation is $M = 306 + 304a + 288b + 152c + 133$, where M is calculated mass; 306, 304, 288, and 152 are the molecular weights of the terminal (epi)gallocatechin unit, the (epi)gallocatechin extending unit, the (epi)-catechin extending unit, and the galloyl ester, respectively; 133 is the weight of cesium; a and b are the degrees of polymerization contributed by the

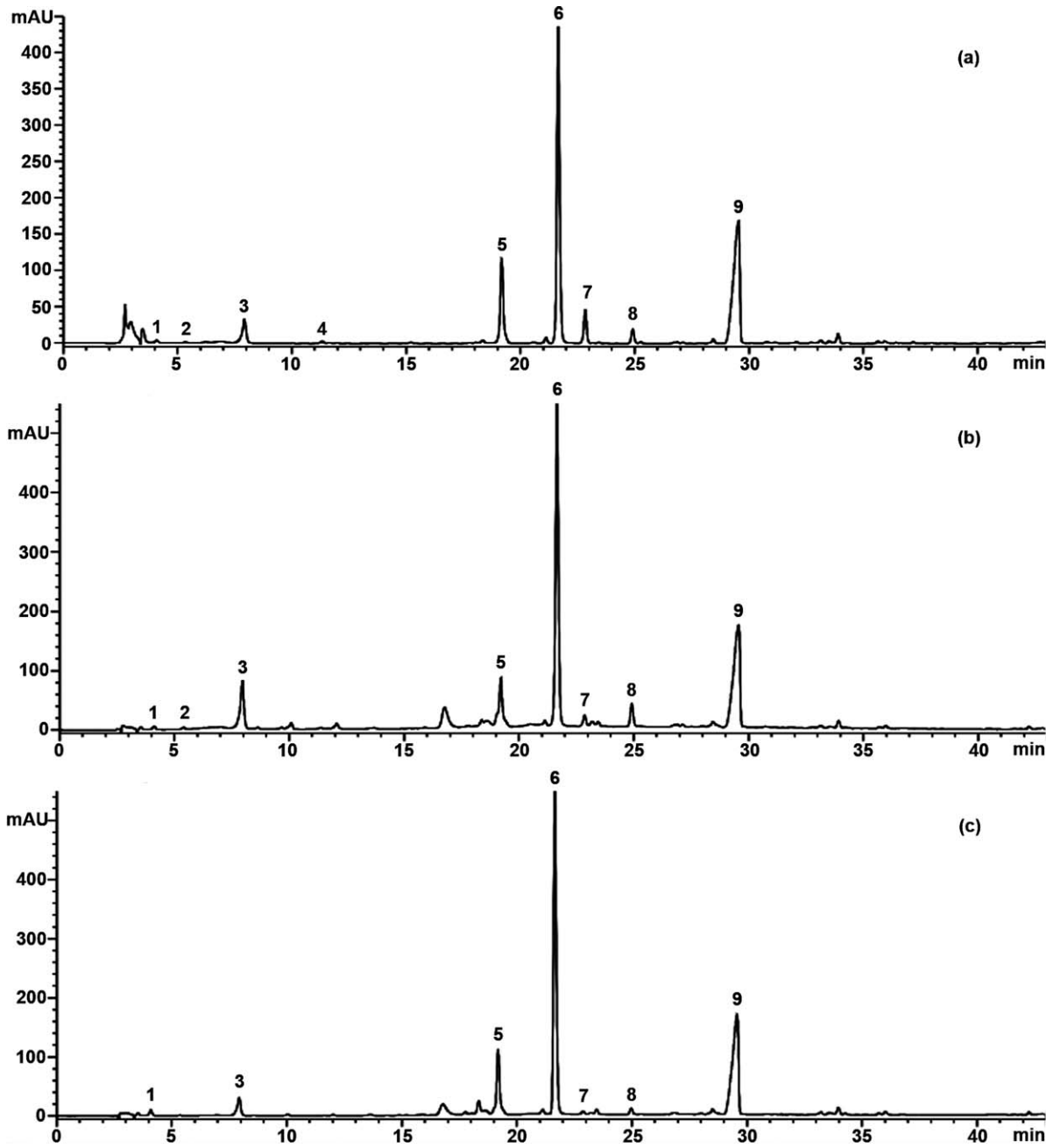

Figure 3 RP-HPLC chromatograms of condensed tannin from leaf (a), stem bark (b), and root bark (c) of *A. corniculatum* after thiolytic degradation. Key to peak labeling: 1, gallocatechin; 2, epigallocatechin; 3, epigallocatechin-3-*O*-gallate; 4, epicatechin-3-*O*-gallate; 5, (epi)gallocatechin benzylthioether; 6, (epi)gallocatechin-3-*O*-gallate benzylthioether; 7, (epi)catechin benzylthioether; 8, (epi)catechin-3-*O*-gallate benzylthioether; 9, benzyl mercaptan.

(epi)gallocatechin extending unit and (epi)catechin extending unit, respectively; *c* is the number of galloyl esters. Application of this equation to the experimental data obtained in the present study revealed the presence of a series of condensed tannins consisting of well-resolved polymers (Table II).

The series of compounds with 2 Da multiples lower than those described in the predictive equation for heteropolyflavan-3-ols were not detected, so A-type interflavan ether linkage did not occur between adjacent flavan-3-ol subunits for the leaf, stem bark, and root bark. All compounds were linked by B-type interflavan bonds. For the first time, the structures of condensed tannins from different parts of *A. corniculatum* were characterized by MALDI-TOF MS.

Estimation of the condensed tannin composition by thiolytic degradation

To further provide information on the nature of extension and terminal units and on the mean

TABLE III
Structural Composition and Mean Degrees of Polymerization (mDP) of Condensed Tannins from Leaf, Stem Bark, and Root Bark of *A. corniculatum*

	Terminal units (%)				Extension units (%)				
Samples	GC	EGC	EGCG	ECG	(Epi)GC	(Epi)GCG	(Epi)C	(Epi)CG	mDP
Leaf	0.5	0.2	6.2	0.5	18.7	64.2	6.8	2.9	13.5
Stem bark	1.6	0.3	11.7	—	13.8	65.7	2.4	4.5	7.4
Root bark	2.9	—	5.2	—	16.8	72.9	0.8	1.4	12.3

GC: gallocatechin; EGC: epigallocatechin; EGCG: epigallocatechin-3-*O*-gallate; ECG: epicatechin-3-*O*-gallate; (Epi)GC: (epi)gallocatechin; (Epi)GCG: (epi)gallocatechin-3-*O*-gallate; (Epi)C: (epi)catechin; (Epi)CG: (epi)catechin-3-*O*-gallate.

degree of polymerization (mDP) of the *A. corniculatum* condensed tannins, depolymerization through thiolysis reaction was carried out by following standard procedures using benzyl mercaptan. In the thiolysis reaction, the extension subunits are attacked by benzyl mercaptan to form the corresponding benzylthioether; only the terminal units are released as the free flavan-3-ols (Fig. 2). The reversed phase HPLC chromatograms of the thiolyzed condensed tannins recorded at 280 nm are shown in Figure 3.

Peaks 1, 2, 3, and 4 were identified to be gallocatechin, epigallocatechin, epigallocatechin-3-*O*-gallate, and epicatechin-3-*O*-gallate, respectively, as terminal units by comparison of retention times and mass spectra of those authentic standards. Peaks 5, 6, 7, and 8 gave rise to *m/z* 427, 579, 411, and 563 in negative ion mode, respectively. They were identified as (epi)gallocatechin benzylthioether, (epi)gallocatechin-3-*O*-gallate benzylthioether, (epi)catechin benzylthioether, and (epi)catechin-3-*O*-gallate benzylthioether derived from extension units, respectively. Due to lack of authentic standards, the stereochemistry of these compounds could not be confirmed based on mass spectra. The results after thiolytic degradation suggested that (epi)gallocatechin-3-*O*-gallate and (epi)gallocatechin were the mainly constitutive units of the *A. corniculatum* condensed tannins. In addition, the mean degrees of polymerization (mDP) of the condensed tannins for leaf, stem bark, and root bark were calculated to be 13.5, 7.4, and 12.3, respectively (Table III).

DPPH radical scavenging activity

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological system.[35] DPPH is a kind of stable-free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[36] Because these radicals are very sensitive to the presence of hydrogen donors, the whole system operates at a very low concentration, a large number of samples can be tested in a short time.[37,38] The DPPH assay has been used frequently for estimating free radical scavenging activities of various plants and some biological samples.[39,40] Figure 4(a) shows the percentages of scavenging DPPH in the presence of condensed tannins from different parts of *A. corniculatum* at different concentrations. The condensed tannins significantly inhibited the activity of DPPH radicals in a dose-dependent manner.

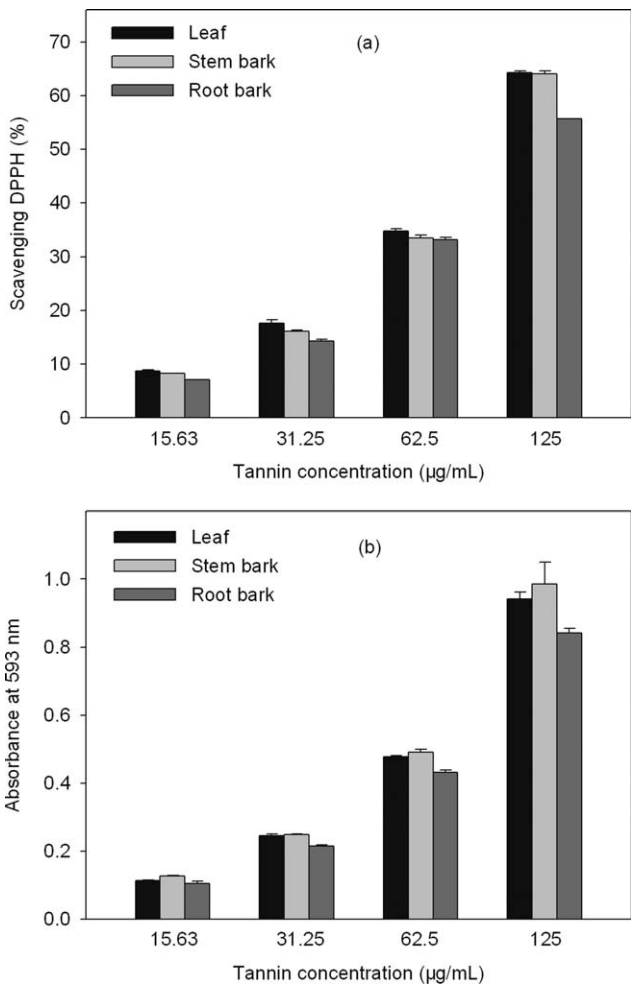

Figure 4 Antioxidant activities of condensed tannins from leaf, stem bark, and root bark of *A. corniculatum* were measured using DPPH (a) and FRAP (b) methods.

TABLE IV
Antioxidant Activities of Condensed Tannins from Leaf, Stem Bark, and Root Bark of *A. corniculatum* Using the DPPH Free Radical Scavenging Assay and the Ferric Reducing Antioxidant Power (FRAP) Assay

	Antioxidant activity	
Samples	$IC_{50/DPPH}$ (μg/mL)[a]	FRAP (mmol AAE/g)[b]
Leaf	95.11 ± 1.19d	5.10 ± 0.07b
Stem bark	96.68 ± 0.68d	5.67 ± 0.07a
Root bark	108.18 ± 0.51b	4.80 ± 0.12c
Ascorbic acid	101.96 ± 1.84c	—
BHA	123.91 ± 0.96a	4.85 ± 0.06c

[a] The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 517 nm by 50% in comparison to the control.
[b] FRAP values are expressed in mmol ascorbic acid equivalent/g sample in dry weight; BHA: butylated hydroxyanisole. Values are expressed as mean of duplicate determinations ± standard deviation.
Different letters in the same column show significant differences from each other at $P < 0.05$ level.

The quality of the antioxidants about the condensed tannins from different parts of *A. corniculatum* was determined by the IC_{50} values (the concentration with scavenging activity of 50%) (Table IV). The IC_{50} values of leaf and stem bark were significantly lower than those of root bark, ascorbic acid, and BHA, indicating the condensed tannins from leaf and stem bark exhibited a higher radical scavenging effect than the remainder. The scavenging effect on the DPPH radical followed the order: leaf ≈ stem bark > ascorbic acid > root bark > BHA.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant assay is based on the reduction of TPTZ-Fe (III) to the TPTZ-Fe (II) complex by a reductant at low pH.[41] The reduction capacity of a compound may serve as a significant indictor of its potential antioxidant activity.[42] A higher absorbance corresponds to a higher ferric reducing power. All condensed tannins showed increased ferric reducing power with increasing concentration [Fig. 4(b)]. At 125 μg/mL, the reducing powers of condensed tannins from leaf and stem bark were superior to that of root bark.

The antioxidant ability of different parts of *A. corniculatum* was estimated by FRAP values, which is expressed in ascorbic acid equivalent. The FRAP values for leaf, stem bark, and root bark ranged from 4.80 ± 0.12 to 5.67 ± 0.07 mmol AAE/g dried tannins, with the highest in stem bark and the lowest in root bark, respectively (Table IV). In brief, the reducing powers of different parts of *A. corniculatum* and standard were found in the following order: stem bark > leaf > root bark ≈ BHA.

CONCLUSIONS

It is better to use methanol than to use water and ethyl acetate for extracting phenolic compounds from different parts of *A. corniculatum*. The structures of condensed tannins from different parts of *A. corniculatum* were, for the first time, successfully characterized by MALDI-TOF MS and thiolytic degradation. (Epi)gallocatechin-3-*O*-gallate and (epi)gallocatechin were the main constitutional units of the purified condensed tannins from leaf, stem bark, and root bark of *A. corniculatum*, which had mean degrees of polymerization of 13.5, 7.4, and 12.3, respectively. Furthermore, condensed tannins from leaf and stem bark exhibited interesting DPPH radical scavenging activities and ferric reducing/antioxidant powers, suggesting that these tannins might mainly be responsible for the antioxidant properties of these medicinal plant materials from *A. corniculatum*, and may be considered as a new source of natural antioxidants for food products.

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