

Full Length Research Paper

Antioxidant tannins from *Syzygium cumini* fruit

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Hydrolysable and condensed tannins in the fruit of *Syzygium cumini* were identified using NMR, MALDI-TOF MS and HPLC analyses. Hydrolysable tannins were identified as ellagitannins, consisting of a glucose core surrounded by gallic acid and ellagic acid units. Condensed tannins were identified as B-type oligomers of epiafzelechin (propelargonidin) with a degree of polymerization up to eleven. The antioxidant activity were measured by two vitro models: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing/antioxidant power (FRAP). Tannins extracted from *S. cumini* fruit showed a very good DPPH radical scavenging activity and ferric reducing/antioxidant power. The results are promising thus indicating the utilization of the fruit of *S. cumini* as a significant source of natural antioxidants.

Key words: *Syzygium cumini*, tannins, antioxidant activity, MALDI-TOF MS, HPLC.

INTRODUCTION

Tannins are significant plant secondary metabolites subdivided into condensed and hydrolysable compounds in vascular plants. Condensed tannins are also known as proanthocyanidins (PAs), the oligomeric and polymeric flavan-3-ols, which are linked through C4 - C8 or C4 - C6 linkages. The diversity of condensed tannins is given by the structural variability of the monomer units (different hydroxylation patterns of the aromatic rings A and B, and different configurations at the chiral centers C2 and C3) (Figure 1). The size of PAs molecules can be described by their degree of polymerization (DP). The molecules are water-soluble and are able to form complexes with proteins and polysaccharides (Haslam, 1998). Hydrolysable tannins (HTs) represent a large group of polyphenolic compounds that are widely distributed in the plant kingdom. They are esters of a polyol (most often β -D-glucose) with either gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins). These ester forms vary from simple compounds such as β -D-glucogallin to compounds with M_r values in excess of 2,500 (Haslam, 1992). The structural elucidation of polymeric tannins is difficult because of their heterogeneous character. Due to this complexity and diversity, the cha-

racterization of highly polymerized condensed tannins thus remains very challenging, and less is known regarding structure-activity relationships. Various techniques including NMR and mass spectroscopy (MS) have been used to characterize hydrolysable and condensed tannins (Behrens et al., 2003; Chen and Hagerman, 2004; Vivas et al., 2004).

The fruits of *Syzygium cumini* (L.) Skeels are edible and are reported to contain gallic acid, tannins, anthocyanins and other components (Benherlal and Arumughan, 2007). The juice of unripe fruits is used for preparing vinegar that is considered to be a stomachic, carminative and diuretic. The ripe fruits are used for making preserves, squashes and jellies. The fruits are astringent. A wine is prepared from the ripe fruits in Goa (Wealth of India, 1976). Extract of seed, which is traditionally used in diabetes, has a hypoglycaemic action and antioxidant property in alloxan diabetic rats (Prince et al., 1998) possibly due to tannins (Bhatia et al., 1971).

Tannins are antioxidants often characterized by reducing power (Mi-Yea et al., 2003) and scavenging activities (Minussi et al., 2003). The antioxidant capabilities of tannins depend on: (1) the extent of their colloidal state; (2) the ease of interflavonoid bond cleavage or its stereochemical structure; (3) the ease of the pyran ring (C-ring) opening; and (4) the relative number of -OH groups on A and B rings (Noferi et al., 1997). Compounds with a trihydroxyl structure in the B-ring have the greatest

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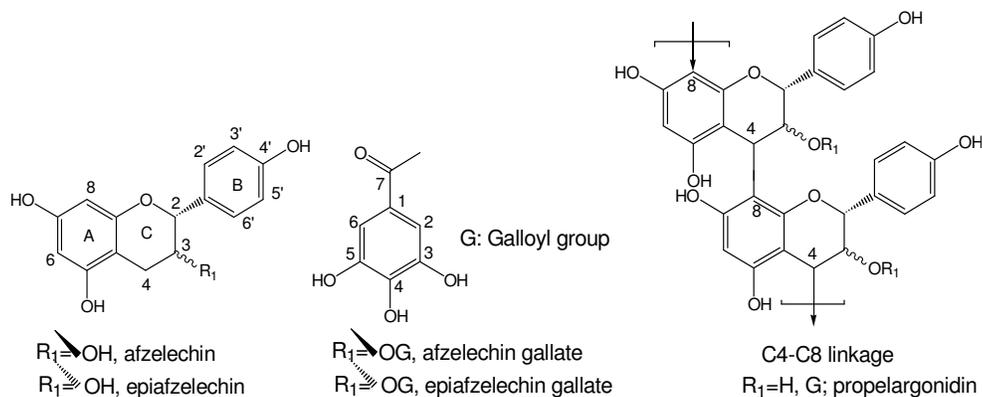


Figure 1. Chemical structures of flavan-3-ol monomers and polymers.

antioxidant activity (Rice-Evans et al., 1996).

The fruit of *S. cumini* has been shown to contain a large range of hydrolysable tannins (Benheral and Arumugan, 2007). However, neither hydrolysable nor condensed tannins have been characterized in *S. cumini*. In this study we undertook the structural characterization of tannins in *S. cumini* fruit using a combination of NMR, MALDI-TOF MS and HPLC analyses. This would contribute to a better understanding of the chemical composition of *S. cumini* and the applicability of NMR, MALDI-TOF MS and HPLC in the analyses of food tannins. We also report the evaluation of free-radical scavenging properties and ferric reducing/antioxidant power of tannins extracted from *S. cumini* fruit.

MATERIALS AND METHODS

Plant materials and chemicals

Mature fruits of *S. cumini* were collected at the campus of Xiamen University (Xiamen, China) and immediately freeze dried and ground. The resulting powder was extracted with acetone:water (7:3, v/v) and the organic solvent was eliminated by evaporation under vacuum. The remaining crude tannin fraction was chromatographed on an LH-20 column (Pharmacia Biotech, Uppsala, Sweden) which was first eluted with methanol:water (50:50, v/v) and then with acetone : water (7:3, v/v). The last fraction, containing the polymeric tannins was freeze-dried and stored at -20°C till further use.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), potassium ferricyanide, butylated hydroxyanisole (BHA), ascorbic acid, (+)-catechin, cesium chloride, gallic acid, ellagic acid and tannic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagents and solvents were of analytical or HPLC grade. Deionised water was used throughout.

NMR analysis

^{13}C NMR spectra were recorded in $\text{CD}_3\text{COCD}_3\text{-D}_2\text{O}$ mixture with a Varian Mercury-600 spectrometer (USA) at 150 MHz.

MALDI-TOF MS analysis

The tannin extract was mixed with a 1 M solution of dihydroxy-

benzoic acid (DHB) in 90% methanol in a 1:1 ratio, and 1 μl of the mixture was spotted onto a ground stainless steel MALDI target for MALDI analysis using the dry droplet method. Cesium chloride (1 mg/ml) was mixed with the analyte/matrix solution at the 1:3 volumetric ratio to promote the formation of a single type of ion adduct ($\text{M} + \text{Cs}^+$) (Xiang et al., 2006). A Bruker Reflex III MALDI-TOF MS (Germany) equipped with an N_2 laser (337 nm) was used in the MALDI analysis and all the data were obtained in a positive ion reflectron TOF mode.

Acid hydrolysis

Acid hydrolysis of the ellagitannins was performed as described by Oszmianski et al. (2007). The ellagitannins from *S. cumini* fruit stone (25 mg) were hydrolyzed with 2 ml of 2 mol/l hydrochloric acid in a boiling water bath for 1 h. After cooling, 2 ml of 2 mol/l NaOH and then 6 ml methanol were added to the vial. The slurry was sonicated for 20 min with occasional shaking. Further, the slurry was centrifuged at 10,000 g and the supernatant was used for HPLC analysis. The HPLC apparatus consisted of an Agilent 1100 diode array detector and a quaternary pump.

The samples were previously dissolved in a mobile phase and then filtrated through a membrane filter with an aperture size of 0.45 μm . 10 ml of the clear supernatant was injected. Separation was performed on a Hypersil ODS column (4.6 \times 250 mm, 5 μm) thermostatted at 30°C . The mobile phase was composed of solvent A (0.1% v/v) trifluoroacetic acid (TFA) in water) and solvent B (0.1% v/v) TFA in acetonitrile). The gradient condition was: 0~2nd min 100% A, 2nd~6th min 0~5% B, 6th~10th min 5% B, 10th~15th min 5~10% B, 15th~20th min 10% B, 20th~30th min 10~20% B, 30th~35th min 20% B and 35th~40th min 20~30% B. Other chromatographic conditions were as follows: flow rate at 1 ml/min, detection at 280 and 254 nm, and scanning performed between 200 and 600 nm.

The identification of chromatographic peaks was made by comparison of their relative retention times with those of external standards, as well as by their UV-visible spectra. Ellagic acid (detection 254 nm) and gallic acid (detection at 280 nm) was quantified using the calibration curve established with ellagic and gallic acid standards. Analysis was made in triplicate.

Free-radical scavenging activity

The free-radical scavenging activity was measured according to Braca et al. (2001). A 100 μl of the sample at different concentrations (15 – 500 $\mu\text{g}/\text{ml}$) was added to 3 ml of DPPH solution (0.1 M methanolic solution). 30 min later, the absorbance was measured

at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC₅₀ 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 using the following equation:

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

where A_1 is the absorbance of the control reaction and A_2 is the absorbance in the presence of the sample. BHA, (+)-catechin and ascorbic acid were used as controls.

Ferric reducing/antioxidant power (FRAP) assay

FRAP assay is a simple and reliable colorimetric method commonly used for measuring total antioxidant capacity (Benzie and Strain, 1996). Briefly, 3 ml of FRAP reagent, prepared freshly, was mixed with 100 μ l of the test sample, or methanol (for the reagent blank). The FRAP reagent was prepared from 300 mM, pH 3.6, acetate buffer, 20 mM ferric chloride and 10 mM 2,4,6-tripyridyl-S-triazine made up in 40 mM hydrochloric acid. All three solutions were mixed together in the ratio of 10:1:1 (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 mmol ascorbic acid equivalents (AAE)/g dried tannins, were derived from a standard curve.

Statistical analyses

All measurements were replicated three times and one-way analysis of variance (ANOVA) was used and the differences were considered to be significant at $P < 0.05$. All statistical analyses were performed with SPSS 11.0.

RESULTS AND DISCUSSION

NMR analysis

The signal assignment was made based on the publication of Czochanska et al. (1980). The spectrum shows distinct signals at 157 ppm, which are assignable to C4' in propelargonidin units (afzelechin/epiafzelechin). Indeed, procyanidin units (catechin/epicatechin) and prodelphinidin units (galocatechin/epigallocatechin) generally showed a typical resonance at 144 - 145 and 145 - 146 ppm respectively (Czochanska et al., 1980; Behrens et al., 2003). The absence of a clear signal with such chemical shift in the spectra of the condensed tannins from *S. cumini* fruit skin revealed that they are only composed of propelargonidin units.

The region between 70 and 90 ppm is sensitive to the stereochemistry of the C-ring. The determination of the ratio of the 2,3-*cis* to 2,3-*trans* stereochemistries could thus be achieved through distinct differences in their respective C2 chemical shifts (Czochanska et al., 1980). Whereas C3 of both *cis* and *trans* isomers occurs at 73 ppm, C2 gives a resonance at 76 ppm for the *cis* form and at 84 ppm for the *trans* form. The absence of the latter signal peak in the spectrum of the studied con-

densed tannin fraction indicated the presence of only epiafzelechin subunits. The presence of a signal at 35.9 ppm was consistent with a C4 being shifted upfield by the presence of a 3-O-gallate unit. This was further confirmed by the observation of signals for ester carbonyl carbons at 175.5 ppm (Gal-C7) and galloyl ring carbons at 114.0 ppm (Gal-C2, Gal-C6), 130.8 ppm (Gal-C1) and 143 - 145 ppm (Gal-C4). These results thus showed that the polymeric propelargonidin of the studied *S. cumini* fruit skin is predominantly constituted of propelargonidin with (-)-epiafzelechin as the main constitutive monomer, some with galloyl groups attached (Spencer et al., 2007).

As indicated above, ¹H and ¹³C NMR spectroscopy techniques were used to estimate the degree of polymerization and the number-average molecular weight. The C3 in terminal units generally have their chemical shift around 67 ppm. Theoretically, its intensity relative to that of the signal of the C3 in extension monomer units at 73 ppm could be used for elucidating the polymer chain length. However, in the case of the spectra presented here, the signal-to-noise ratio is too low to allow for such quantification.

MALDI-TOF MS analyses

To obtain more detailed information on the chemical structure of the condensed tannins and to overcome the problems with determination of polymer chain lengths by NMR spectroscopy, further characterization was continued by means of MALDI-TOF MS. MALDI-TOF MS has advantages over the other MS systems in terms of sensitivity and mass range. The single ionization event produced by MALDI-TOF MS allows the simultaneous determination of masses in complex mixtures of low and high molecular weight compounds. Several factors must be optimized to develop MALDI-TOF MS techniques. These factors include the selection of an appropriate matrix, optimal mixing and optimal selection of cationization reagent. In our study, the Cs⁺ was used as the cationization reagent. This resulted in the best conditions for their MALDI-TOF analysis and resulted in a relatively simple MALDI-TOF spectrum.

Figure 2 shows the MALDI-TOF mass spectrum of the studied polymeric mixture, recorded as Cs⁺ adducts in the positive-ion reflectron mode and showing a series of repeating propelargonidin polymers. The polymeric character is reflected by the periodic of peak series representing different chain lengths. The results indicated that *S. cumini* fruit skin tannins are characterized by mass spectra with a series of peaks with distances of 272 Da corresponding to a mass difference of one afzelechin/epiafzelechin between each polymer. Therefore, prolongation of condensed tannins is due to the addition of afzelechin/epiafzelechin monomers. The spectrum showed a series polyflavan-3-ols extending from the dimer (m/z 679) to the undecamer (m/z 3129) that did not contain ions with $\Delta 2$ amu lower than predicted in the

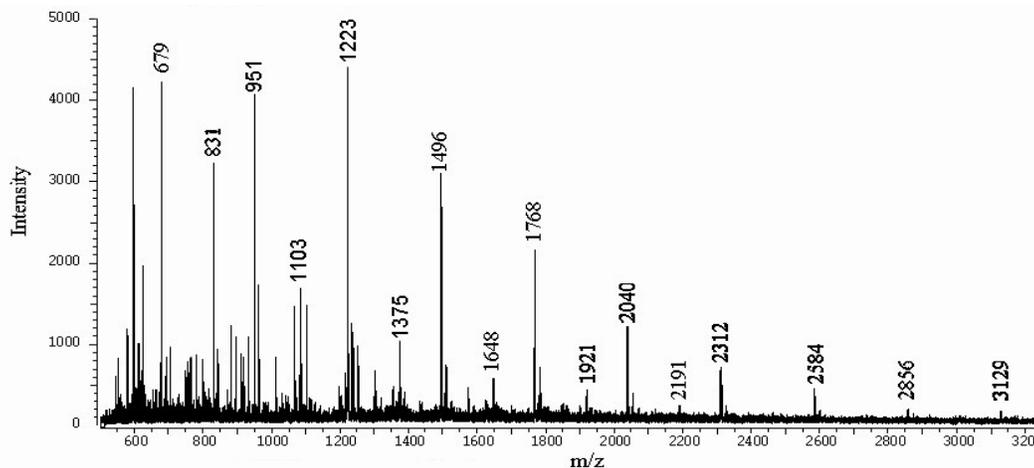


Figure 2. MALDI-TOF positive reflectron mode mass spectrum of tannins from *S. cumini* fruit skin. Masses represent the epiafzelechin homopolymer of the polyflavan-3-ol series $[M+Cs]^+$.

Table 1. Observed and calculated masses^a of heteropolyflavan-3-ols by MALDI-TOF MS.

Polymer	Number of galloylated esters	Calculated $[M+Cs]^+$ ^a	Observed $[M+Cs]^+$
Dimer	0	679	679
	1	831	831
Trimer	0	951	951
	1	1103	1103
Tetramer	0	1223	1224
	1	1375	1375
Pentamer	0	1495	1496
	1	1647	1648
Hexamer	0	1767	1768
	1	1919	1921
Heptamer	0	2039	2040
	1	2191	2192
Octamer	0	2311	2312
	1	2463	2463
Nonamer	0	2583	2584
	1	2735	2736
Decamer	0	2855	2856
	1	3007	3008
Undecamer	0	3127	3129
	1	3279	N

^aMass calculations were based on the equation $274 + 272a + 152b + 133$, where 274 is the molecular weight of the terminal epiafzelechin unit, a is the degree of polymerization (DP) contributed by the epiafzelechin extending unit, b is the number of galloyl esters and 133 is the atomic weight of cesium; N, no observed peaks corresponding to those calculated ones.

positive-ion reflectron mode (Table 1).

On the basis of the structures described by Krueger et

al. (2003), an equation was formulated to predict heteropolyflavan-3-ols of a higher DP. The equation is

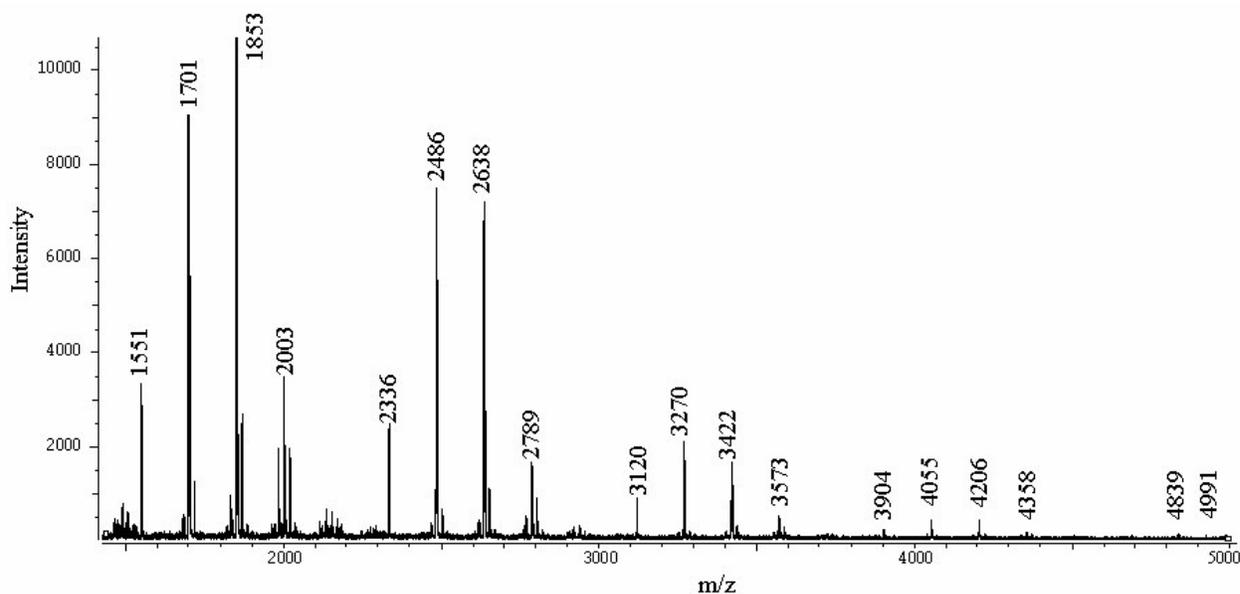


Figure 3. MALDI-TOF positive reflectron mode mass spectrum of oligomeric ellagitannins in *S. cumini* fruit stone. Labeled masses are the molecular ions minus 1 proton plus Cs^+ .

$274 + 272a + 152b + 133$; where 274 is the molecular weight of the terminal epiafzelechin unit, a is the degree of polymerization (DP) contributed by the epiafzelechin extending unit, b is the number of galloyl esters, 133 is the atomic weight of cesium. Application of this equation to the experimentally obtained data revealed the presence of a series of condensed tannins consisting of well-resolved oligomers. The broad peaks in these spectra indicate, however, that there is large structural heterogeneity within each DP.

For the condensed tannins indicated, each peak was always followed by mass signals at a distance of 152 Da corresponding to the addition of one galloyl group at the heterocyclic C-ring. Thus, peak signals corresponding to monogalloylated derivatives of various condensed tannin oligomers were easily attributed. No propelargonidin containing more than one galloyl group were detected. Therefore, MALDI-TOF MS indicates the simultaneous occurrence of a mixture of propelargonidin polymers, monogalloylated derivatives of propelargonidin polymers. This showed that there were a mixture of galloylated propelargonidin and propelargonidin in *S. cumini* fruit skin propelargonidin oligomers.

No series of compounds that are $\Delta 2$ amu multiples lower than those described in the predictive equation for heteropolyflavan-3-ols were detected. So there are no A-type interflavan ether linkages occurring between adjacent flavan-3-ol subunits. All compounds are linked by B-type.

In the case of *S. cumini* fruit stone tannins (Figure 3), a certain degree of regularity was observed in the MALDI-TOF mass spectrum and notably these tannins possess very similar mass distributions but different average

masses. In the spectra, four sets of peaks that are separated by 152 Da are evident and have been assigned to a unit of galloyl group using modeling correlations. Structural assignment of these tannins advocates that *S. cumini* fruit stone is composed of gallic acid units centred upon a core of glucose unit that is different from *S. cumini* fruit skin. So, our study confirms the general classification of the *S. cumini* fruit stone tannins as ellagitannins, that is consisting of a glucose core surrounded by gallic and ellagic acid units (Table 2). Almost superimposable mass spectra were obtained for all the *S. cumini* fruit stone ellagitannins analysed. Masses between 1500 and 5000 correspond to structures of oligomeric ellagitannins in which two or more core glucose units are cross-linked by dehydrodigalloyl or valoneoyl units. This is in agreement with previously reported data concerning the same genus plant *Syzygium aromaticum* (Tanaka et al., 1996) for which two new ellagitannins were reported. These different chemical groups are frequently composed of the same building blocks but in different combinations and numbers. For example, gallic acid occurs naturally but can dimerize to form ellagic acid. Ellagic acid can dimerize to form gallagic acid. Ellagic acid can combine with glucose to form the unique compounds punicalagin and punicalin. The different combinations and polymers of the aforementioned form the large, diverse group of compounds known as polyphenols, which show potent antioxidant capacity and possible protective effects on human health (Santos-Buelga and Scalbert, 2000). These oligomers have been detected in *S. cumini* for the first time in this study, although they are known to occur in other plants (Quideau and Feldman, 1996) and further study is required to elucidate their structure.

Table 2. Calculated and observed masses for oligomeric ellagitannins in *S. cumini* fruit stone and possible monomeric composition.

Mass + Cs	Observed mass	Monomeric composition				
		Glucosyl	Gallagic acid	Ellagic acid	Gallic acid	Dehydrodigallic acid
Dimers						
1551	1551	2	0	2	1	1
1553	1553	2	0	1	3	1
1701	1701	2	0	3	0	1
1703	1703	2	0	2	2	1
1853	1853	2	0	3	1	1
1855	1855	2	0	2	3	1
2003	2003	2	0	4	0	1
2005	2005	2	0	3	2	1
Trimers						
2335	2336	3	0	3	1	2
2486	2486	3	0	4	0	2
2488	2488	3	0	3	2	2
2638	2638	3	0	4	1	2
2788	2789	3	1	2	2	2
Tetramers						
3120	3120	4	0	4	1	3
3270	3270	4	0	5	0	3
3272	3272	4	0	4	2	3
3422	3422	4	0	4	3	3
3574	3573	4	0	5	2	3
Pentamers						
3905	3904	5	0	5	1	4
4055	4055	5	0	6	0	4
4057	4057	5	0	5	2	4
4207	4206	5	0	6	1	4
4209	4209	5	0	5	3	4
4359	4358	5	1	3	4	4
Hexamer						
4840	4839	6	0	7	0	5
4992	4991	6	0	7	1	5

Identification of hydrolytic products of ellagitannins

The ellagic acid in plants is present mainly in the form of ellagitannins and is bound to glucose. Acid hydrolysis transforms glucosylated and esterified ellagic acid into their aglycones, and liberates the parent compound ellagic acid and gallic acid (Daniel et al., 1989). Ellagic and gallic acids are major products, as shown by a typical HPLC chromatogram of the hydrolyzed products of ellagitannins from *S. cumini* fruit stone (Figure 4) with 35.46 ± 3.00 mg/g dry tannins for ellagic acid and 19.73 ± 0.81 mg/g dry tannins for gallic acid.

Most quantitative evaluation of ellagitannins in fruit has been on the hydrolyzed ellagitannins as ellagic acid equivalents (Wada and Ou, 2002; Siriwoharn and

Wrolstad, 2004). This has significant problems in terms of relating data to possible health effects, because there is significant evidence that larger molecular mass tannins (>1000 Da), including ellagitannins and procyanidins, are not absorbed to any appreciable extent in their native state (Cerdeira et al., 2004). Knowledge of ellagitannin molecular structure, composition and quantity is needed to understand their role in determining potential health effects.

Radical-scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

Figure 5 shows the dose-response curve of DPPH radical

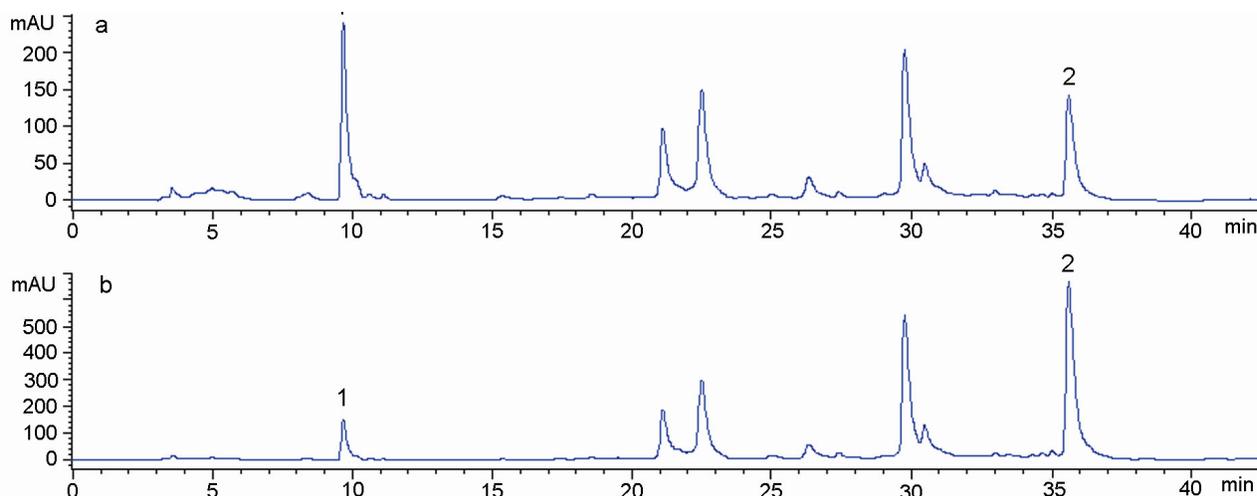


Figure 4. HPLC chromatograms of ellagitannins from *S. cumini* fruit stone after hydrolysis, detected by absorbance at 280 nm (a) and 254 nm (b). The peaks corresponding to gallic acid (1) and ellagic acid (2) are indicated on the chromatogram. Other peaks are unidentified phenolics.

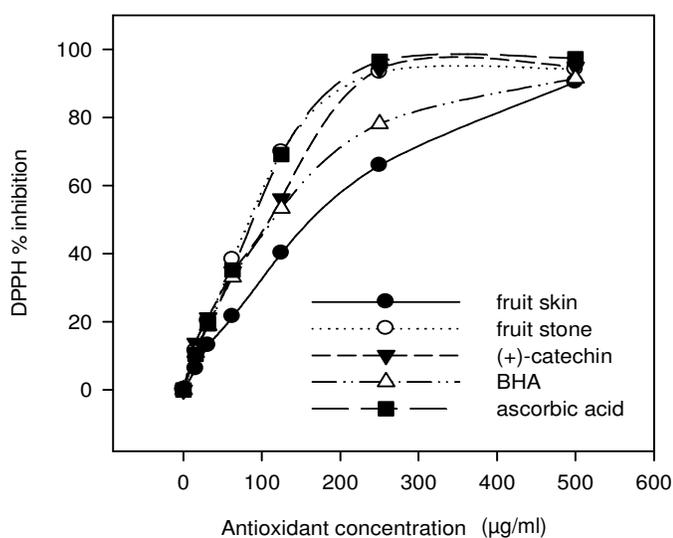


Figure 5. Free radical-scavenging activities of tannins, measured using ascorbic acid, BHA and (+)-catechin as DPPH assay reference compounds.

scavenging activity of the tannin fractions from the *S. cumini* fruits, compared with (+)-catechin, BHA and ascorbic acid. The tannins of the stone had a higher activity than that of the skin. At a concentration of 0.25 mg/ml, the scavenging activity of tannins of the skin reached 65.85%, while at the same concentration that of the stone was 93.31%. IC_{50} values were compared with those of ascorbic acid and BHA in the system to assess the antioxidant property of *S. cumini* fruit tannins (Table 3). A lower value of IC_{50} indicates greater antioxidant activity. IC_{50} values of tannins from stone were superior

Table 3. Antioxidant activities of tannins of *S. cumini* fruit using the (DPPH) free radical-scavenging assay and the (FRAP) ferric-reducing antioxidant power assay.

Sample	Antioxidant activity	
	$IC_{50}/DPPH$ ($\mu\text{g/ml}$) ^a	FRAP (mmol AAE/g) ^b
Fruit skin	165.05 \pm 3.90a	3.02 \pm 0.06d
Fruit stone	82.21 \pm 0.77c	6.21 \pm 0.19b
(+)-catechin	106.36 \pm 4.28b	4.34 \pm 0.07c
BHA	113.00 \pm 4.28b	7.43 \pm 0.14a
Ascorbic acid	85.68 \pm 0.46c	--

^aThe 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. ^bFRAP values are expressed in mmol ascorbic acid equivalent (AAE)/g sample in dry weight. Different letters on the same column show significant differences from each other at $P < 0.05$.

to those of the reference ascorbic acid, (+)-catechin and BHA. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann et al., 1979). Though the DPPH radical scavenging abilities of tannins from *S. cumini* fruit skin were less than that of the stone, the study showed that the tannins have proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Ferric reducing antioxidant power

The reducing ability of the tannin fractions from *S. cumini* fruit stone (6.21 mmol AAE/g) was higher than that of the skin (3.02 mmol AAE/g) (Table 3). The antioxidant potential of the tannins from *S. cumini* fruit were estimated from

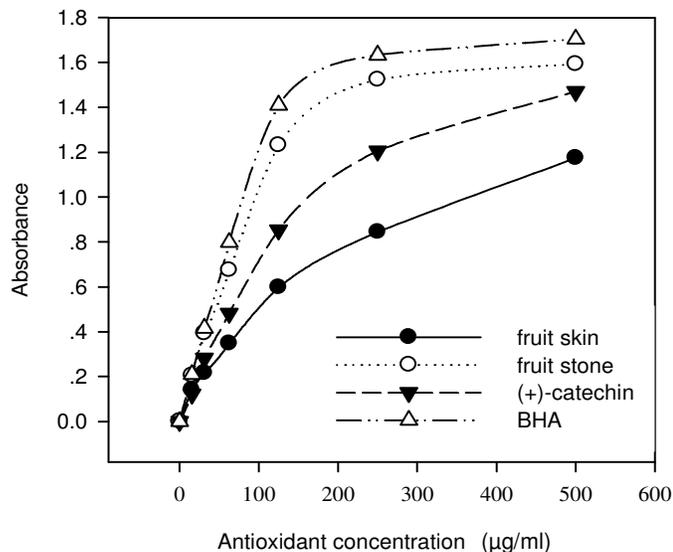


Figure 6. The reducing power of tannins as compared to (+)-catechin and BHA standards

their ability to reduce TPTZ-Fe³⁺ complex to TPTZ-Fe²⁺. The FRAP value of the skin tannins was significantly lower than those of BHA and (+)-catechin. The FRAP values for the stone tannins on the other hand were significantly lower than that of BHA but higher than that of (+)-catechin. Such potential reducing power activity might be attributed due to the presence of hydrolysable tannins present in the stone. Antioxidant activity increased proportionally with tannins content, and all tannins showed increased ferric reducing power with increasing concentration (Figure 6). According to Oktay et al. (2003), a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species.

Conclusion

The results obtained showed that the condensed tannins consisted of predominantly propelargonidin with 2,3-*cis* stereochemistry. The mean degree of polymerization determined through MALDI-TOF MS analysis was 5.0 and the number-average molecular weight was 1372.45 Da. Cationization by addition of Cs⁺ allowed us to eliminate the interference of the $\Delta 16$ mass differences between Na⁺ and K⁺ with $\Delta 16$ mass differences that results from pattern of hydroxylation. As a result of these techniques, we have observed larger structural heterogeneity of oligomers than is generally appreciated in the literature on plant tannins. The results from the application of MALDI-TOF MS clearly demonstrate its power as a tool to characterize the nature of tannins. Tannins extracted from *S. cumini* fruit showed a very good DPPH radical scavenging activity and ferric reducing/antioxidant power.

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