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*Full Length Research Paper*

# Antioxidant condensed tannins from *Machilus pauhoi* leaves

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**The acetone-water (1:1, v/v) was more effective solvent for extracting total phenolics and extractable condensed tannins from *Machilus pauhoi* leaves than methanol, ethanol, acetone, water, methanol-water (1:1, v/v), and ethanol-water (1:1, v/v). The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis revealed that catechin/epicatechin was the basic units occurring in *M. pauhoi* leaf condensed tannins, and A-type and B-type linkages were most common among the structural units of polymers. Thiol degradation with cysteamine indicated that the polymer was constituted of (+)-catechin and (-)-epicatechin as the terminal units and (-)-epicatechin as the extension units. The mean degree of polymerization (mDP) of the condensed tannins was 6.96. The condensed tannins from *M. pauhoi* leaves had more effective 2,2-diphenyl-1-picrylhydrazyl (DPPH) and diammonium salt (ABTS) free radicals scavenging abilities than the ascorbic acid and butylated hydroxyanisole (BHA), and may be considered as a new source of natural antioxidants for food and nutraceutical products.**

**Key words:** *Machilus pauhoi*, condensed tannins, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, reversed phase high performance liquid chromatography, antioxidant activity.

## INTRODUCTION

Tannins, secondary metabolites of higher plants, are oligomeric compounds with multiple structure units with free phenolic groups and molecular weight ranging from 500 to > 20000 (González et al., 2010). The main groups of tannins are hydrolyzable tannins and condensed tannins (Isaza, 2007). Condensed tannins or proanthocyanidins consist of coupled flavan-3-ol units that can appear as isolated dimers and compounds with high polymerization grade (Routaboul et al., 2006). The diversity of condensed tannins attribute to the hydroxylation pattern of the A- and B-rings, stereochemistry of C2, C3 and C4 of the central ring, and interflavan linkages (Aron and Kennedy, 2008; Porter, 1988).

Condensed tannins have attracted interest because of their antioxidant and other potentially health-promoting qualities (Alasalvar et al., 2009; Da Silva et al., 1991; Prior and Gu, 2005). However, the biological activity of plant condensed tannins depends on their chemical structure and concentration (Monagas et al., 2010). The structural elucidation of these compounds, especially the higher polymers, is difficult because of their heterogeneous character. Due to the complexity and diversity, the characterization of highly polymerized condensed tannins remains very challenging (Hümmer

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and Schreier, 2008), and condensed tannins thus were considered to be the final frontier of flavonoid research (Dixon et al., 2005).

Since its introduction by Karas and Hillenkamp in 1987 (Karas et al., 1987), matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) has greatly expanded the use of mass spectrometry towards large molecules and has revealed itself to be a powerful method for the characterization of synthetic and natural polymers (Bahr et al., 1992; Danis and Karr, 1993; Ehring et al., 1992; Pasch et al., 2001). It allows the determination of detailed information on the condensed tannins' profiles including the polymer chain length, chemical constitution of individual chains, and the sequential succession of monomer units in individual chains (Behrens et al., 2003).

MALDI-TOF MS produces only a singly charged molecular ion for each parent molecule and allows detection of high mass with precision (Fu et al., 2007; Montaudo et al., 2002).

*Machilus pauhoi* (Lauraceae) is a precious timber and economical tree species in the subtropical evergreen broadleaf forests of China. The leaf extracts have excellent antioxidant, anti-bacterial, anti-inflammatory and analgesic effects (Guangxi Institute of Botany, 1991). Therefore, this plant might be a good candidate for further development as a nutraceutical or for its antioxidant remedies. However, detailed information on the condensed tannins profiles from *M. pauhoi* leaves, especially with respect to polymer chain length, chemical constitution of individual chains, and the sequential succession of monomer units in individual chains present in *M. pauhoi* is currently lacking. In this study, concentrations of total phenolics and extractable condensed tannins of *M. pauhoi* leaves were determined, and the structures of the condensed tannins were characterized by MALDI-TOF MS and reversed phase high performance liquid chromatography (RP-HPLC) analyses. In addition, the potential antioxidant activity of the condensed tannins from *M. pauhoi* leaves was also discussed.

## MATERIALS AND METHODS

### Chemicals and plant materials

The solvents methanol, ethanol, acetone and hexane were of analytical reagent (AR) purity grade. The trifluoroacetic acid (TFA) and acetonitrile were of HPLC grade. Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), cysteamine hydrochloride, ascorbic acid, butylated hydroxyanisole (BHA), cesium chloride and gallic acid were purchased from Aldrich (USA). (-)-Epicatechin (EC) and (+)-catechin (Cat) were purchased from Sigma (USA). Sephadex LH-20 was purchased from Amersham (USA).

The leaves of *M. pauhoi* were collected from Yongan, Fujian province, China in April, 2010 and immediately freeze dried and ground.

### Solvent extraction and preparation of the purified condensed tannins

Freeze-dried leaf powder (0.05 g) was successively extracted with 5 ml of different solvents: methanol, ethanol, acetone, methanol-water (1:1, v/v), ethanol-water (1:1, v/v), acetone-water (1:1, v/v) and water separately. Extracts were centrifuged at 12000 g for 5 min and collected. Each extraction process was repeated three times. The collected extracts were combined and their total phenolics and extractable condensed tannins were determined.

The acetone-water (1:1, v/v), the most effective solvent for extracting total phenolics and extractable condensed tannins, was used further to obtain the purified condensed tannins. Briefly, the freeze-dried leaf powders were extracted thrice with the acetone-water (1:1, v/v) solvent at room temperature. The ratio of sample to extraction medium was 1:100. Each extract was filtered and pooled, and the solvent was removed under reduced pressure by use a rotary evaporator at 38°C. The remaining aqueous fraction was extracted thrice with hexane in order to remove chlorophyll and lipophilic compounds. The remaining crude tannin fraction was chromatographed on an LH-20 column (Pharmacia Biotech, Uppsala, Sweden) which was first eluted with methanol-water (1:1, v/v) and then with acetone-water (7:3, v/v). The last fraction of purified condensed tannins was freezed-dried and stored at -20°C before analysis by MALDI-TOF mass spectrometry and thiolysis.

### Determination of the amount of total phenolics and extractable condensed tannins

The established procedures (Lin et al., 2006) were used. The amount of total phenolics was determined using the Folin-Ciocalteu method (Makkar et al., 1993). Briefly, 0.2 ml aliquot of extract was added to a test tube containing 0.3 ml of distilled $H_2O$. 0.5 ml of Folin-Ciocalteu reagent and 2.5 ml 20% $Na_2CO_3$ solution 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 phenolics concentrations of extracts were expressed as mg gallic acid equivalents/g dry weight (DW).

The extractable condensed tannins concentration was assayed by the butanol-HCl method (Terrill et al., 1992), using the 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, USA), 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 ($[M+Cs]^+$) (Xiang et al., 2006).

### Thiolysis reaction

Thiolysis was carried out according to the method of Torres and Lozano (2001). Briefly, the purified condensed tannins from the

leaves of *M. pauhoi* (4 mg/ml in methanol, 50 µl) were placed in a vial hydrochloric acid in methanol (3.3%, v/v; 50 µl) and cysteamine hydrochloride in methanol (50 mg/ml, 100 µl) was then added. 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 RP-HPLC.

The high performance liquid chromatograph was an Agilent 1200 system (USA) equipped with a diode array detector and a quaternary pump. The thiolysis medium was further analyzed using LC/MS (QTRAP 3200, USA) with a Hypersil ODS column (4.6 x 250 mm, 2.5 µm) (China). Two solvents, namely A = 0.1% (v/v) TFA in aqueous and B = $CH_3CN$, were used. The gradient condition was: 0 to 5 min, 3% B (isocratic); 5 to 15 min, 3 to 9% B (linear gradient); 15 to 45 min, 9 to 16% B (linear gradient). The column temperature was ambient and the flow-rate was set at 1 ml/min. Detection was at a wavelength of 280 nm and the ultraviolent (UV) spectra were acquired between 200 to 600 nm. Degradation products were identified on chromatograms according to their relative retention times and LC/MS.

#### DPPH radical scavenging activity

The free radical scavenging activity of purified condensed tannins from the leaves of *M. pauhoi* on the DPPH radical was determined according to the method described by Brand-Williams et al. (1995). A 0.1 ml of various concentrations of each freeze-dried sample at different concentrations (12.5, 25, 50, 100 and 200 µg/ml) was added to 3.9 ml of DPPH solution (25 mg/l in methanolic solution). 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 using 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.

#### ABTS radical scavenging activity

This antioxidant capacity assay was carried out using a Unico UV-2000 spectrophotometer (USA) by the improved $ABTS^{\cdot+}$ method (Re et al., 1999). $ABTS^{\cdot+}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 h until reaching a stable oxidative state. On the day of analysis, the $ABTS^{\cdot+}$ solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.050 at a wavelength of 734 nm. 0.1 ml of the purified condensed tannins (12.5, 25, 50, 100 and 200 µg/ml dissolved in 80% ethanol) was added to $ABTS^{\cdot+}$ solution (3.9 ml; absorbance of 0.700 ± 0.050) and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. The results were expressed the same to DPPH assay described above, with ABTS% inhibition and $IC_{50}$ value.

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

## RESULTS AND DISCUSSION

### Effect of the extract solvents on the total phenolics and extractable condensed tannins concentrations

The choice of the appropriate solvent is one of the most important factors for obtaining extracts with a high concentration of bioactive compounds. In general, the highly hydroxylated aglycones forms of phenolic compounds are soluble in alcohols such as ethanol and methanol (Arts and Hollman, 1998). Less polar solvents such as ethyl acetate, acetone and chloroform are used for the less polar and the highly methoxylated aglycones forms (Lafka et al., 2007). The most polar phytochemical compounds can be extracted using water (González-Montelongo et al., 2010).

The total phenolics and extractable condensed tannins concentrations in different solvent extracts of *M. pauhoi* leaves ranged from 125.27 ± 2.19 to 213.77 ± 2.11 mg/g DW and 22.03 ± 1.87 to 135.80 ± 2.68 mg/g DW, with the highest in acetone-water (1:1, v/v) extract and the lowest in acetone extract, respectively (Table 1). The results indicated that the acetone-water (1:1, v/v) was more effective solvent for extracting total phenolics and extractable condensed tannins from *M. pauhoi* leaves than methanol, ethanol, acetone, water, methanol-water (1:1, v/v), and ethanol-water (1:1, v/v) (Table 1).

### MALDI-TOF MS analysis

MALDI-TOF MS is very sensitive to molecular weight, and nowadays is considered a method of choice for analysis of tannins exhibiting large structural heterogeneity (Reed et al., 2005). It has the power to reveal the nature of the interflavan bonds (A-type, Δ 2 Da) and has been used to confirm those linkages in cranberry tannins (Foo et al., 2000a), sorghum tannins (Krueger et al., 2003), mangosteen pericarp tannins (Fu et al., 2007), and persimmon tannins (Li et al., 2010).

Figure 1 shows the MALDI-TOF mass spectrum of the condensed tannins isolated from *M. pauhoi* leaves, recorded as $CS^+$ adducts in the positive ion reflectron mode. The included magnification demonstrated the good resolution of the spectrum. Leaf condensed tannins were characterized by mass spectrum with a series of peaks with distance of 288 Da, corresponding to a mass difference of one catechin/epicatechin between each polymer. Therefore, prolongation of condensed tannins was due to the addition of catechin/epicatechin monomers (Table 2).

In addition, a series of compounds that are Δ 2

**Table 1.** Total phenolics and extractable condensed tannins concentrations of different solvent extracts of *M. pauhoi* leaves.

| Solvents used for extraction | Total phenolics (mg/g DW) [a] | Extractable condensed tannins (mg/g DW) [b] |
|---|---|---|
| Methanol | 203.65 ± 3.04 b | 124.42 ± 6.04 b |
| Ethanol | 185.09 ± 2.34 c | 103.42 ± 5.15 c |
| Acetone | 125.27 ± 2.19 e | 22.03 ± 1.87 g |
| Water | 166.80 ± 1.76 d | 44.08 ± 2.04 f |
| Methanol-water (1:1, v/v) | 200.43 ± 4.35 b | 69.88 ± 5.36 e |
| Ethanol-water (1:1, v/v) | 199.56 ± 2.36 b | 87.27 ± 8.30 d |
| Acetone-water (1:1, v/v) | 213.77 ± 2.11 a | 135.80 ± 2.68 a |

[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.

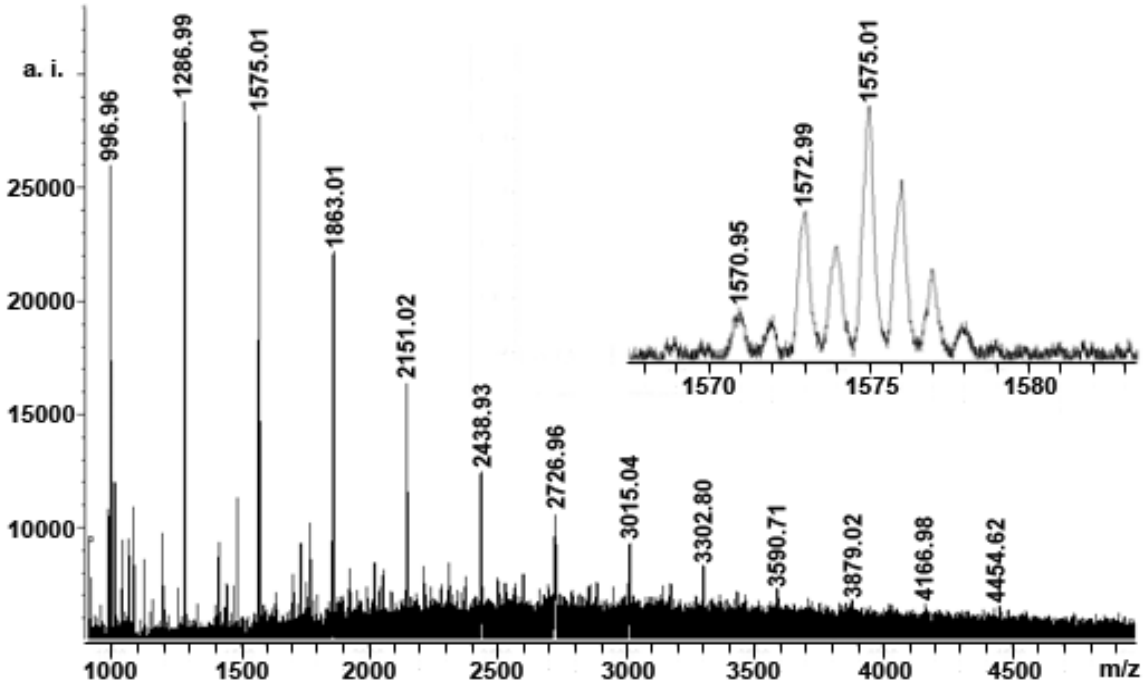


**Figure 1.** MALDI-TOF positive reflectron mode mass spectrum of the condensed tannins from *M. pauhoi* leaves.

**Table 2.** MALDI-TOF MS of condensed tannins from the leaves of *M. pauhoi*.

| Polymer | Number of catechin/epicatechin units | Number of A-type bonds | Number of B-type bonds | Calculated $[M + Cs]^+$ | Observed $[M + Cs]^+$ |
|---|---|---|---|---|---|
| Trimer | 3 | 0 | 2 | 999 | 998.97 |
| | 3 | 1 | 1 | 997 | 996.96 |
| Tetramer | 4 | 0 | 3 | 1287 | 1286.99 |
| | 4 | 1 | 2 | 1285 | 1284.97 |
| Pentamer | 5 | 0 | 4 | 1575 | 1575.01 |
| | 5 | 1 | 3 | 1573 | 1572.99 |
| | 5 | 2 | 2 | 1571 | 1570.95 |

**Table 2.** Continued.

| | | | | | |
|---|---|---|---|---|---|
| Hexamer | 6 | 0 | 5 | 1863 | 1863.01 |
| | 6 | 1 | 4 | 1861 | 1860.96 |
| Heptamer | 7 | 0 | 6 | 2151 | 2151.02 |
| | 7 | 1 | 5 | 2149 | 2149.36 |
| Octamer | 8 | 0 | 7 | 2439 | 2438.93 |
| | 8 | 1 | 6 | 2437 | 2437.04 |
| Nonamer | 9 | 0 | 8 | 2727 | 2726.96 |
| | 9 | 1 | 7 | 2725 | 2725.01 |
| Decamer | 10 | 0 | 9 | 3015 | 3015.04 |
| | 10 | 1 | 8 | 3013 | 3012.98 |
| Undecamer | 11 | 0 | 10 | 3303 | 3302.80 |
| Dodecamer | 12 | 0 | 11 | 3591 | 3590.71 |
| Tridecamer | 13 | 0 | 12 | 3879 | 3879.02 |
| Tetradecamer | 14 | 0 | 13 | 4167 | 4166.98 |
| Pentadecamer | 15 | 0 | 14 | 4455 | 4454.62 |

Da multiples lower than those described peaks for homopolyflavan-3-ols were also detected (Figure 1, Table 2).

Takahata et al. (2001) ascertained that these peaks did not originate from fragmentation or dehydration in the MALDI-MS detection process. These masses might represent a series of compounds in which the A-type interflavan ether linkage occurs between adjacent flavan-3-ol subunits because two hydrogen atoms are lost in the formation of this interflavan bond. The similar mass distribution has been reported in cranberries (Porter et al., 2001), a fruit that contains A-type linkage (Foo et al., 2000b).

On the basis of the above analysis, an equation was formulated to describe the structures of the condensed tannins from *M. pauhoi* leaves. The equation is $M = 290 + 288a - 2b + 133$, where M is calculated mass, 290 is the molecular weight of the terminal catechin/epicatechin unit, *a* is the degree of polymerization contributed by the catechin/epicatechin extending unit, *b* is the number of A-type linkage, and 133 is the weight of cesium. 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. Condensed tannins from *M. pauhoi* leaves ranged from the trimer (*m/z* 998.97) to the pentadecamer (*m/z* 4454.62).

Catechin/epicatechin was the basic units occurring in the condensed tannins, and A-type and B-type linkages were most common among the structural units of polymers (Table 2).

**Thiolysis with cysteamine followed by reversed phase HPLC**

A complementary technique to further investigate whether the condensed tannins from *M. pauhoi* leaves were composed of epicatechin and catechin, depolymerization through thiolysis reaction was carried out in the presence of cysteamine as the thiol reagents (Torres and Selga, 2003), which was preferred to toluene-α-thiol for being more user-friendly and much less toxic (Jerez et al., 2007). In thiolysis reactions, all the extension subunits of condensed tannins were attacked by cysteamine to form the 4-(2-aminoethylthio) flavan-3-ols. Only the terminal unit was released as the free flavan-3-ol (Gu et al., 2002; Torres and Lozano, 2001). Both the extension units and terminal units could be distinguished by reversed phase HPLC analysis (Figure 2). The major product was the 4β-(2-aminoethylthio) epicatechin (Cya-EC) along with a small amount of (+)-catechin (Cat) and (-)-epicatechin (EC). This result suggested that the condensed tannins from *M. pauhoi* leaves mainly constituted with epicatechin units. The mDP of leaf condensed tannins was calculated to be 6.96 by comparing the peak areas based on the following equation:

mDP = (area of cysteamine derivative of epicatechin)/(total area of catechin and epicatechin) + 1

**Determination of antioxidant activity**

The potential antioxidant activity of the condensed

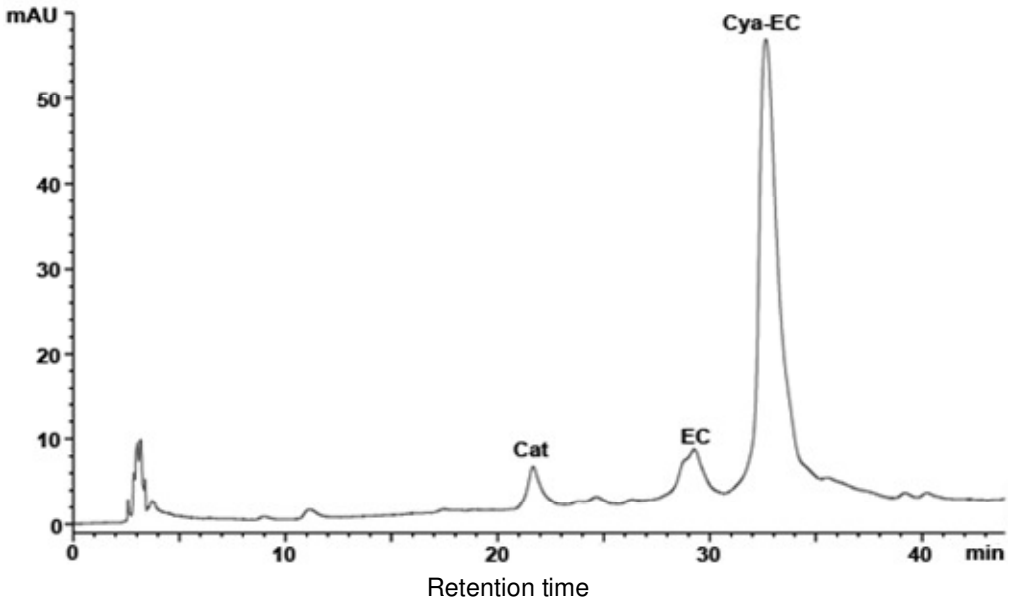


**Figure 2.** Reversed phase HPLC chromatogram of the condensed tannins of *M. pauhoi* leaves degraded in the presence of cysteamine; Cat, (+)-catechin; EC, (-)-epicatechin; Cya-EC, 4β-(2-aminoethylthio) epicatechin.

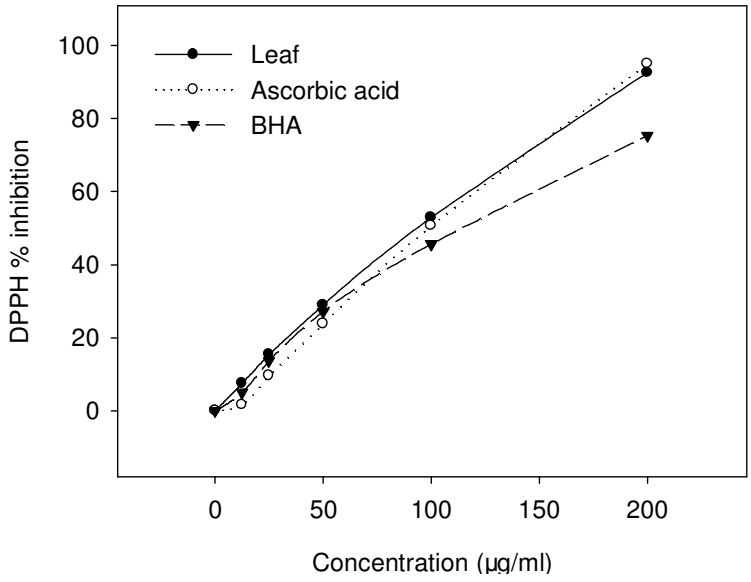


**Figure 3.** DPPH free radical scavenging activities of ascorbic acid, BHA and the condensed tannins of *M. pauhoi* leaves at different concentrations.

tannins from *M. pauhoi* leaves was evaluated by measuring the scavenging effect on DPPH and ABTS radicals. DPPH is a stable radical and has been widely used for screening the radical scavenging activity of antioxidant compounds (Amarowicz et al., 2004; Ruan et al., 2008; Wei et al., 2010; Zhang and Lin, 2008; Zhang et al., 2010). This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H (Dinis et al., 1994; Singleton et al., 1999). Figure 3 shows the DPPH radical scavenging activity of the condensed tannins from *M. pauhoi* leaves and the reference antioxidant compounds, ascorbic acid and BHA. The condensed tannins significantly inhibited

**Table 3.** Antioxidant activity of the condensed tannins from *M. pauhoi* leaves using the DPPH and ABTS radical scavenging assay.

| Samples | Antioxidant activity | |
|---|---|---|
| | $IC_{50/DPPH}$ (µg/ml) [a] | $IC_{50/ABTS}$ (µg/ml) [b] |
| Leaf | 93.22 ± 1.52 c | 81.09 ± 0.67 c |
| Ascorbic acid | 105.38 ± 1.25 b | 104.99 ± 2.08 a |
| BHA | 122.34 ± 1.46 a | 97.43 ± 0.50 b |

[a] The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance of DPPH at 517 nm by 50% in comparison to the control; [b] The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance of ABTS at 734 nm by 50% in comparison to the control; 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.

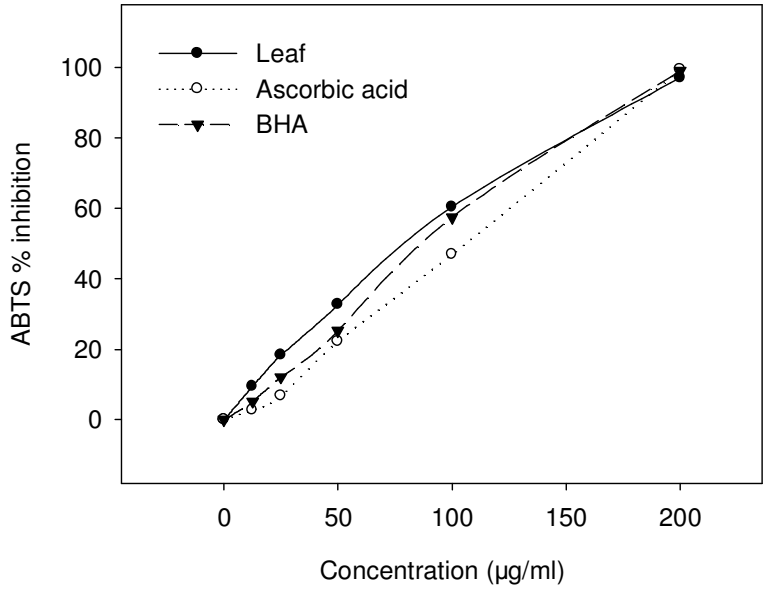


**Figure 4.** ABTS free radical scavenging activities of ascorbic acid, BHA and the condensed tannins of *M. pauhoi* leaves at different concentrations.

the activity of DPPH radicals in a dose-dependent manner. At 100 µg/ml, the scavenging activity of *M. pauhoi* leaves (52.86%) was higher than that of ascorbic acid (50.65%) and BHA (45.63%).

The $IC_{50}$ values of the leaves, ascorbic and BHA are shown in Table 3. Lower $IC_{50}$ value reflects better DPPH radical scavenging activity (Gülçin et al., 2007). Leaf condensed tannins was more effective against DPPH radical than ascorbic acid and BHA (Table 3).

ABTS radical cation decolorization assay is a rapid and reliable method for the measurement of the total radical scavenging of pure substances and aqueous mixtures (Cai et al., 2004; Re et al., 1999). The reaction between ABTS and potassium persulfate directly generates the blue/green ABTS chromophore, which can be reduced by an antioxidant, thereby resulting in a loss of absorbance at 734 nm (Gao et al., 2007). Scavenging activity increased as the condensed tannins concentration increased. At 100 µg/ml, the order of scavenging activity of *M. pauhoi* leaves and standards was: *M. pauhoi* leaves > BHA > ascorbic acid (Figure 4). The $IC_{50}$ value of the leaves (81.09 ± 0.67 µg/ml) was significantly lower than that of standards (Table 3), indicating the condensed tannins from *M. pauhoi* leaves exhibited the higher ABTS radical scavenging effect than ascorbic acid and BHA.

## Conclusions

The acetone-water (1:1, v/v) was more effective solvent for extracting total phenolics and extractable condensed tannins from *M. pauhoi* leaves than methanol, ethanol,

acetone, water, methanol-water (1:1, v/v), and ethanol-water (1:1, v/v). The MALDI-TOF MS and RP-HPLC analyses revealed that catechin/epicatechin was the basic units occurring in *M. pauhoi* leaf condensed tannins, and A-type and B-type linkages were most common among the structural units of polymers. Constitutive units were mainly epicatechin, and the mDP of the condensed tannins was 6.96. The condensed tannins from *M. pauhoi* leaves had more effective DPPH and ABTS free radicals scavenging abilities than the ascorbic acid and BHA, and may be considered as a new source of natural antioxidants for food and nutraceutical products.

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### REFERENCES

Alasalvar C, Karamać M, Kosińska A, Rybarczyk A, Shahidi F, Amarowicz R (2009). Antioxidant activity of hazelnut skin phenolics. J. Agric. Food Chem., 57: 4645-4650.

Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem., 84: 551-562.

Aron PM, Kennedy JA (2008). Flavan-3-ols: nature, occurrence and biological activity. Mol. Nutr. Food Res., 52: 79-104.

Arts ICW, Hollman PCH (1998). Optimization of a quantitative method for the determination of catechins in fruits and legumes. J. Agric. Food Chem., 46: 5156-5162.

Bahr U, Deppe A, Karas M, Hillenkamp F, Giessmann U (1992). Mass spectrometry of synthetic polymers by UV-matrix-assisted laser desorption/ionization. Anal. Chem., 64: 2866-2869.

Behrens A, Maie N, Knicker H, Kögel-Knabner I (2003). MALDI-TOF mass spectrometry and PSD fragmentation as means for the analysis of condensed tannins in plant leaves and needles. Phytochem., 62: 1159-1170.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Cai YZ, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Da Silva JMR, Darmon N, Fernandez Y, Mitjavila S (1991). Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. J. Agric. Food Chem., 39: 1549-1552.

Danis PO, Karr DE (1993). A facile sample preparation for the analysis of synthetic organic polymers by matrix-assisted laser desorption/ionization. Org. Mass Spec., 28: 923-925.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophy., 315: 161-169.

Dixon R, Xie D, Sharma S (2005). Proanthocyanidins–a final frontier in flavonoid research?. New Phytol., 165: 9-28.

Ehring H, Karas M, Hillenkamp F (1992). Role of photoionization and photochemistry in ionization processes of organic molecules and relevance for matrix-assisted laser desorption Ionization mass spectrometry. Org. Mass Spec., 27: 472-480.

Foo LY, Lu Y, Howell AB, Vorsa N (2000a). The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli in vitro*. Phytochem., 54: 173-181.

Foo LY, Lu Y, Howell AB, Vorsa N (2000b). A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. J. Nat. Prod., 63: 1225-1228.

Fu C, Loo AEK, Chia FPP, Huang D (2007). Oligomeric proanthocyanidins from mangosteen pericarps. J. Agric. Food Chem., 55: 7689-7694.

Gao H, Shupe TF, Eberhardt TL, Hse CY (2007). Antioxidant activity of extracts from the wood and bark of Port Orford cedar. J. Wood Sci., 53: 147-152.

González-Montelongo R, Gloria Lobo M, González M (2010). Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. Food Chem., 119: 1030-1039.

González MJ, Torres JL, Medina I (2010). Impact of thermal processing on the activity of gallotannins and condensed tannins from *Hamamelis virginiana* used as functional ingredients in seafood. J. Agric. Food Chem., 58: 4274-4283.

Guangxi Institute of Botany, Chinese Academy of Sciences (1991). Flora of Guangxi. Science and Techology Press of Guangxi, Guangxi.

Gülçin I, Elias R, Gepdiremen A, Boyer L, Köksal E (2007). A comparative study on the antioxidant activity of fringe tree (*Chionanthus virginicus* L.) extracts. Afr. J. Biotechnol., 6: 410-418.

Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, Prior RL (2002). Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. J. Agric. Food Chem., 50: 4852-4860.

Hümmer W, Schreier P (2008). Analysis of proanthocyanidins. Mol. Nutr. Food Res., 52: 1381-1398.

Isaza JH (2007). Taninos o polifenoles vegetales. Scientia et Technica. (Univ. Tecnol. Pereira)., 33: 13–18.

Jerez M, Touriño S, Sineiro J, Torres JL, Núñez MJ (2007). Procyanidins from pine bark: Relationships between structure, composition and antiradical activity. Food Chem., 104: 518-527.

Karas M, Bachmann D, Bahr U, Hillenkamp F (1987). Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int. J. Mass Spectrom. Ion Proc., 78: 53-68.

Krueger CG, Vestling MM, Reed JD (2003). Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry of heteropolyflavan-3-ols and glucosylated heteropolyflavans in sorghum [*Sorghum bicolor* (L.) Moench]. J. Agric. Food Chem., 51: 538-543.

Lafka TI, Sinanoglou V, Lazos ES (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem., 104: 1206-1214.

Li CM, Leverence R, Trombley JD, Xu S, Yang J, Tian Y, Reed JD, Hagerman AE (2010). High molecular weight persimmon (*Diospyros kaki* L.) proanthocyanidin: a highly galloylated, A-linked tannin with an unusual flavonol terminal unit, myricetin. J. Agric. Food Chem., 58: 9033-9042.

Lin YM, Liu JW, Xiang P, Lin P, Ye GF, Sternberg LDSL (2006). Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China. Biogeochem. 78: 343-359.

Makkar HPS, Blümmel M, Borowy NK, Becker K (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric., 61: 161-165.

Monagas M, Quintanilla-López JE, Gómez-Cordovés C, Bartolomé B, Lebrón-Aguilar R (2010). MALDI-TOF MS analysis of plant proanthocyanidins. J. Pharm. Biomed., Anal., 51: 358-372.

Montaudo G, Montaudo MS, Samperi F (2002). Martix-assisted laser desorption/ionization mass spectrometry of polymers (MALDI-MS). In Mass Spectrometry of Polymers; Montaudo G, Lattimer, RP, Eds.; CRC Press: Boca Raton, FL, Pp. 419-521. .

Pasch H, Pizzi A, Rode K (2001). MALDI-TOF mass spectrometry of

polyflavonoid tannins. Polymer, 42: 7531-7539.
Porter LJ (1988). The Flavonoids, Chapman and Hall, New York, Pp. 21-62.
Porter ML, Krueger CG, Wiebe DA, Cunningham DG, Reed JD (2001). Cranberry proanthocyanidins associate with low-density lipoprotein and inhibit in vitro $Cu^{2+}$-induced oxidation. J. Sci. Food Agric, 81: 1306-1313.
Prior RL, Gu L (2005). Occurrence and biological significance of proanthocyanidins in the American diet. Phytochem., 66: 2264-2280.
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.
Reed JD, Krueger CG, Vestling MM (2005). MALDI-TOF mass spectrometry of oligomeric food polyphenols. Phytochem., 66: 2248-2263.
Routaboul JM, Kerhoas L, Debeaujon I, Pourcel L, Caboche M, Einhorn J, Lepiniec L (2006). Flavonoid diversity and biosynthesis in seed of *Arabidopsis thaliana*. Planta Med., 224: 96-107.
Ruan ZP, Zhang LL, Lin YM (2008). Evaluation of the antioxidant activity of *Syzygium cumini* leaves. Mole., 13: 2545-2556.
Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.
Takahata Y, Ohnishi-Kameyama M, Furuta S, Takahashi M, Suda I (2001). Highly polymerized procyanidins in brown soybean seed coat with a high radical-scavenging activity. J. Agric. Food Chem., 49: 5843-5847.
Terrill TH, Rowan AM, Douglas GB, Barry TN (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric., 58: 321-329.
Torres JL, Lozano C (2001). Chromatographic characterization of proanthocyanidins after thiolysis with cysteamine. Chromatographia, 54: 523-526.
Torres JL, Selga A (2003). Procyanidin size and composition by thiolysis with cysteamine hydrochloride and chromatography. Chromatographia, 57: 441-445.
Wei SD, Zhou HC, Lin YM (2010). Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. Int. J. Mol. Sci., 11: 4080-4093.
Xiang P, Lin YM, Lin P, Xiang C (2006). Effects of adduct ions on matrix-assisted laser desorption/ionization time of flight mass spectrometry of condensed tannins: a prerequisite knowledge. Chin. J. Anal. Chem., 34: 1019-1022.
Zhang LL, Lin YM (2008). HPLC, NMR and MALDI-TOF MS analysis of condensed tannins from *Lithocarpus glaber* leaves with potent free radical scavenging activity. Molecules, 13: 2986-2997.
Zhang LL, Lin YM, Zhou HC, Wei SD, Chen JH (2010). Condensed tannins from mangrove species *Kandelia candel* and *Rhizophora mangle* and their antioxidant activity. Molecules, 15: 420-431.