Optimisation of pressurised liquid extraction of antioxidants from black bamboo leaves

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**A B S T R A C T**

To develop an efficient green extraction approach for recovering bioactive compounds from natural plants, the potential of using pressurised liquid extraction (PLE) was examined on black bamboo (Phyllostachys nigra) leaves, with ethanol/water as solvents. The superheated PLE process showed a higher recovery of most constituents and antioxidative activity, compared to reflux extraction, with a significantly improved recovery of the total phenolic (TP) and flavonoid (TF) content and DPPH radical scavenging ability. For a broad range of ethanol aqueous solutions and temperatures, 50\% EtOH and 200 \degree C (static time: 25 min) gave the best performance, in terms of the TP and TF (75\% EtOH) content yield and DPPH scavenging ability (25\% EtOH). Under the optimised extraction conditions, eight main antioxidative compounds were isolated and identified with HPLC-ABTS\textsuperscript{+} assay guidance and assessed for radical scavenging activity. The superheated extraction process for black bamboo leaves enhanced the antioxidant properties by increasing the extraction of the phenolic compounds.

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1. Introduction

A high level of fruit and vegetable consumption has shown the association of phenolic compounds with health benefits (Parr & Bolwell, 2000). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim, Tagliaferro, & Bobilya, 2002). Phenolic compounds could be a major determinant of the antioxidant potential of foods (Parr & Bolwell, 2000), and therefore could be a natural source of antioxidants.

It has become increasingly important to obtain antioxidants from common edible plant materials. In Korea, local inhabitants enjoy black bamboo teas made from roasting black bamboo leaves. The roasting process enhances the antioxidative activity as well as the flavours and taste (Kim, Jeon, Kang, Kim, Lee, & Um, 2012). Bamboo leaves have been used as a Chinese medicinal material for reducing the energy of “fire” (an element usually related to inflammation) and to treat cardiovascular disease, arteriosclerosis and hypertension (Yuan, 1983). In a previous study, we reported that black bamboo (Phyllostachys nigra) leaf extracts and the isolated phenol compounds were effective at inhibiting aldose reductase and advanced glycation endproducts in vitro (Jung et al., 2007) and protecting against oxidative stress-induced retinal ganglion cells death (Lee et al., 2010).

The search for plant-derived biomaterials has stimulated research interest in using more efficient methods for extracting polyphenolic compounds. The pressurised liquid extraction (PLE) method is an important sample preparation technique for extracting bioactive compounds. This technique is a green extract method due to its decreased solvent use, short operating time and light- and oxygen-free environment (Acar, Gokmen, Pellegrini, & Fogliano, 2009). This technique causes desirable and/or undesirable changes in the physical, chemical and nutritional properties of the extracted materials due to the control experiment conditions (Rostagno, Palma & Barroso, 2004; José, Pedro, & María, 2006). One of the main desired outcomes of the PLE process is the increase in antioxidant activity, which occurs mainly due to the formation of Maillard reaction products (MRPs). The PLE process has been used to increase the extraction efficiency of various bioactive compounds (Shang et al., 2010; José et al., 2006).

In this study, the optimised conditions for extracting black bamboo leaves under a superheated PLE process were investigated for total polyphenol and flavonoid content as well as the DPPH radical scavenging ability. The newly produced and increased antioxidative compounds at the optimised extraction condition were identified and assessed for antioxidative activity.
2. Materials and methods

2.1. Chemicals and plant materials

All HPLC-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA). The organic solvents used for extraction were purchased from Daejung (Gyonggi, Korea). 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH•), ascorbic acid, Folin–Ciocalteu’s phenol reagent, catechin and potassium persulfate were purchased from Sigma-Aldrich Chemicals (Saint Louis, MO, USA). Deuterium solvent methanol-d4 was purchased from Cambridge Isotope Laboratories (USA). Fresh leaves (500 g) of *P. nigra* Munro were harvested on September 12, 2010, in Gangnueng City (Gangwon-do, Korea), and a voucher specimen (No. BBL-0003) was deposited at the Herbarium of KIST, Korea. The leaves were air-dried in the shade and kept at room temperature until use. Column chromatography was performed over silica gel 60 (Merck, particle size 230–400 mesh).

2.2. PLE procedure

PLE was carried out using a fully automated ASE 200 system (Dionex, Sunnyvale, CA, USA). Samples were loaded into the stainless steel cell (11 mL) with sea sand (particle size 30–50 mesh, Fisher Chemicals) above the sample to avoid any void spaces. Various temperature ranges, ethanol:water concentrations, sample sizes, and extraction times were used to identify the optimal conditions for extracting polyphenols and investigating the antioxidative activity. A standard stepwise PLE extraction protocol was used for all extractions. (1) The extraction cell was placed in the carrousel and heated up to the temperature specified by the univariate design (A °C). (2) The cell was filled with the specified ethanol and water concentration (B%) until a pressure of 1500 psi was reached. (3) Static extraction (C min) when the pressure and temperature were reached was then maintained. (4) The cell was rinsed with an additional 40% of the volume of the solvent mixture that had already crossed the cell. Extracts were collected in 60 mL glass vials. The volume was made up to 25 mL with extraction solvent and filtered through a 0.45 µm membrane filter (Agilent Technologies, Santa Clara, CA, USA) before the total phenolic and flavonoid and DPPH radical scavenging analysis and injection into the HPLC system.

2.3. Polyphenol assays

2.3.1. Determination of total phenolic content

The total phenolic content of the extracts was determined with the Folin–Ciocalteu colorimetric method, with some modification. Briefly, black bamboo leaf extract supernatant was diluted twice. Each sample solution (2 µL) was mixed with 78 µL water and Folin–Ciocalteu’s reagent 20 µL for 5 min. Then sodium carbonate solution (100 µL 20%, v/v) was added. The mixture stood for 30 min in the dark at room temperature and was measured at 730 nm with the Synergy HT-Multi-microplate reader (Bio-Tek Instruments). The final result was expressed as milligrams of catechin equivalents (CE) per 100 g dry sample of black bamboo leaves.

2.3.2. Determination of total flavonoid content

The total flavonoid content was measured using a colorimetric method, with modification. For this, 2% AlCl3/H2O 100 mL was prepared, and a 10 µL sample was taken from the extraction solution, which had been diluted twice and mixed with 90 µL ethanol and 100 µL AlCl3/H2O for 5 min. The absorbance was read at 430 nm with a Synergy HT-Multi-microplate reader in triplicate. The final result was expressed as the milligram of quercetin equivalents per 100 g dry sample of black bamboo leaves.

2.4. Measurement of in vitro antioxidative activity

2.4.1. DPPH assay

A modified version of the diphenyl picrylhydrazyl (DPPH) assay was used to measure the in vitro antioxidant activity, using ascorbic acid as the standard (Goupy, Hugues, Boivin, & Amiot, 1999). Standard samples were prepared by diluting an ethanolic ascorbic acid stock solution (0.1 mg/mL). The ascorbic acid standard and blanks were used for the calibration curve. The results are expressed in mg ascorbic acid/100 g dry weight (mg AA/100 g DW). A working DPPH solution (0.15 mg/mL) was prepared by dissolving 15 mg DPPH in 100 mL methanol. Before analysis, all extracts were diluted twice from the 25 mL original extracts. A serial dilution of the extracts (100 µL) was prepared and added to 100 µL of the DPPH working solution in a 96 well-plate. The 96 well-plates were left in the dark for 30 min at room temperature. The absorbance was then measured against ethanol at 515 nm with a Synergy HT Multimicroplate reader (Bio-Tek Instruments). The decrease in the absorbance of a sample was calculated compared to a blank sample and corrected for the absorbance of the sample extract itself. The relative decrease in absorbance (PI) was then calculated as follows: PI (%) = 1 – (As/Ab), where As is the absorbance of sample extract and Ab the absorbance of blank. The antioxidative activity was defined as the concentration of the sample extract necessary to scavenge 50% of the DPPH radicals (SC50). For the samples, the SC50 unit was the µL of extracts. In all experiments, the SC50 of ascorbic acid (µg/mL) was determined as well. The final results for the antioxidative activity were determined using the following equation: antioxidant activity = (SC50/SCsample)×4. The antioxidative activity was expressed in g ascorbic acid equivalent (AAE) per 100 g dry weight sample (g AAE/100 g DW).

2.4.2. Antioxidant activity determination with on-line HPLC-ABTS+ screening system

To investigate the antioxidant activity profile of the black bamboo extracts, the on-line HPLC-ABTS+ screening system was applied. The Agilent 1200 analysis HPLC system (Agilent Technologies, Santa Clara, CA, USA) was fitted with an additional pump to supply the ABTS radical solution. The ABTS solution was prepared with the same method as that used for off-line. An aliquot of 10 µL black bamboo extract methanol solution (10 mg/mL) (40 °C and 200 °C with PLE) was injected into the on-line HPLC-ABTS+ system. The individual compound was separated with an YMC-PACK C18 analytical column (150 × 4.6 mm i.d., 3 µm particle size, YMC, American, Inc.). The HPLC condition was expressed as follows: The mobile phase was acetonitrile (solvent A) and water (solvent B), a gradient system of solvent A from 10% to 64% in 40 min with flow rate 1 mL/min was used. The ABTS solution was supplied with a flow rate of 0.5 ml/min. The chromatogram was recorded at 210 nm and 280 nm as the positive peaks, and the visible detector was set at 734 nm to measure the decrease in the ABTS radicals as the negative peak (Fig. 1).

2.5. Experimental design for optimisation

The univariate method was used to optimise polyphenol and antioxidative activity extraction from black bamboo leaves. The sample surface area affects the interaction between the sample and solvent; therefore, various sample sizes (A: 425 µm, 850 µm, 2 mm, 4.75 mm, 6 mm) were tested. Since an increase in temperature can improve the extraction of natural compounds, a series of experiments at different temperatures (B: 40, 80, 120, 160, 200 °C) was performed to determine the best extraction tempera-
ture. The most widely used solvents for extracting phenolic substances are methanol, ethanol, acetone and their water mixtures, acidified or not. Here, an environmentally friendly solvent mixture of ethanol and water was selected (ethanol concentration, C: 0%, 25%, 50%, 75%, 100%, and extraction time, D: 5, 10, 15, 20, 25 min). All experiments were performed in triplicate.

2.6. Isolation and identification of antioxidative compounds

The compounds extracted at 200 °C with PLE were investigated by obtaining pure compounds from extracts. Dry black bamboo leaves (78 g) were extracted at 200 °C with PLE with the conditions described in the superheated processing section. The residue was evaporated in vacuo to yield the total ethanol extract (21.8 g). This extract was then suspended in distilled water and partitioned sequentially with n-hexane, methylene chloride (MC), ethyl acetate (EA) and n-butanol. Then each fraction yield was obtained after evaporation in vacuo. All fractions were analysed by HPLC analysis. The condition and column were the same as those of the on-line HPLC-ABTS+ conditions. The n-hexane fraction (3.5 g) was separated by preparative HPLC JASCO, with a YMC-PACK C18 column (250 × 4.6 mm i.d., 5 μm particle size, YMC, American, Inc.), the mobile phase acetonitrile was increased from 10% to 90% in 60 min, and compounds 1 (1 mg) and 2 (1 mg) were obtained. The EA fraction (3.5 g), which was subjected to silica gel column chromatography (5 × 60 cm) using an ethyl acetate-n-hexane (2:8) isocratic system, provided 4 fractions (A–D). From fraction C (200 mg), compounds 3 (2 mg), 4 (2 mg), 5 (3 mg), 7 (1.5 mg), and 8 (0.7 mg) (Fig. 1) were obtained by using the same preparative HPLC conditions as with the n-hexane fraction.

The structure of the isolated compounds was elucidated by spectroscopy, including NMR (Varian, USA, 500 MHz equipped with carbon enhanced cold probe) and MS consisting of an LCQ FLEET™ ion trap mass spectrometer equipped with high-temperature electrospray ionisation (HESI) (Thermo, San Jose, CA, USA). The mass spectrometer conditions were as follows: positive ion mode; mass range, m/z 100–1500; the capillary voltage, 500 V; nebulising gas pressure (N2), 30 psi; drying gas (N2) flow rate, 13.0 L/min; drying temperature, 350 °C. A HPLC-DAD-ESI/MS was obtained using the Agilent 1100 Series HPLC-MSD System equipped with an auto sampler, a column oven, a quaternary pump, a DAD detector and a degasser (Hewlett-Packard, Waldbronn, Germany).

3. Results and discussion

3.1. PLE

PLE is an extraction technique that uses elevated temperatures and pressures to extract target components from a sample matrix. By using a liquid solvent at temperatures and pressures above its boiling point, the extraction process becomes more efficient due to faster diffusion rates, higher thermal energy and solvent strength, as well as lower viscosity and surface tension, compared to using the same solvent at room temperature. This implies that the extraction times and solvent volume for extraction can be significantly reduced. Furthermore, the solvent strength for extraction can easily be changed by varying the temperature.

The major factors contributing to the efficiency of extraction for pressurised liquid extraction are the solvent, material state, temperature and static time (Ju & Howard, 2003). For healthy extracts in the food industry and other applications, water and ethanol were used as the extract solvent.

The variables with the largest effect on the TP, TF, and DPPH radical scavenging ability were temperature (B) and the ethanol concentration (C), followed by the sample size (A) and extraction time (D) (Fig. 2). For TP, the best extraction condition was a 425 μm size material, 200 °C and 50% EtOH for 25 min. For TF, the best extraction condition was a 4.75 mm size material, 200 °C and 75% EtOH for 25 min. For DPPH radical scavenging ability, the best extraction condition was a 425 μm size material, 200 °C and 25% EtOH for 25 min.

Cellular deformations take place during the high-temperature process, which facilitates the extraction ability of the phytochemicals and causes the release or chemical alteration of phenolic compounds from bound structures, such as the degradation of lignin in black bamboo leaves (Durmaz & Gökmen, 2011) during the extraction process. In addition, when the temperature of the water and ethanol is increased under constant pressure, their respective dielectric constants decrease, thus reducing their polarity as extraction solvents. These polarity changes are more pronounced for water than for ethanol, which shows only minor changes. This results in water exhibiting a dielectric constant similar to that of
methanol and ethanol at room temperature (TP at 200 °C, 100% water is much higher than 40 °C (data not shown)).

The properties of the solvents significantly affected the measured TP content (±64% variation), TF content (±84% variation) and DPPH radical scavenging ability. Fig. 2 shows that the best TP extraction was at 50% and 75% EtOH (200 °C) for TF, and 25% EtOH for DPPH radical scavenging ability (Fig. 2(c)). A certain amount of water (30–50%) in the extracting solvent is helpful to improve the phenolic extraction (Fig. 2). Since polyphenols with several hydroxyl groups, such as glucosides, are hydrophilic and generally present higher solubilities in hydroalcoholic mixtures than in a pure alcoholic solvent (Rostagno, Palma & Barroso, 2004), hydroalcoholic mixtures showed the best extraction result for TP and DPPH (Fig. 2). However, 100% EtOH gave the best extraction efficiency for TF at 120 °C. To verify the interaction effects of the solvent and temperature, we conducted another series of experiments. The ethanol concentrations have different effects at different temperatures. It was found that only TF was different from the first group experiment. The highest extraction efficiency was found at 75% EtOH with 200 °C (data not shown). This may contribute to the temperature effects on the solvent and then the extraction efficiency for the TF.

Fig. 2 also shows that the TP and DPPH radical scavenging ability gave the highest values when the sample size was around 425 μm, which may contribute to the higher sample surface area giving shorter diffusion paths. The static time did not show a large difference in the results, which showed that PLE can have a high extraction efficiency in a short time.

3.2. On-line HPLC-ABTS+ radical scavenging analysis of the extracts

The extracts were analysed with the on-line HPLC-ABTS+ method. Two extracts (40 and 200 °C) by PLE were made into 10 mg/mL solution and then injected in the HPLC. The compounds were separated by a column. The ABTS+ solution had a deep green colour with maximum absorbance at 734 nm, and any quenching of the radical resulted in a loss of colour was indicated by a negative peak on the HPLC profile. The HPLC profiles (Fig. 1) showed that the main compounds in the extracts changed dramatically during thermal processing. The 40 °C HPLC profile was similar to the reference (Kim et al., 2009), in which six flavonoids were identified as the main compounds from black bamboo leaves in the hot methanol (50 °C) extract. The 200 °C extract had more peaks than the room temperature extract over 20–35 min; most of these peaks had antioxidant activity. Using the negative peak area, it was calculated that these new peaks accounted for 56.3% of the extract’s total antioxidant capacity. These peaks appeared as the temperature surpassed 160 °C in the 10 mg/mL solutions. (To verify when the new compounds were produced, we extracted samples at 130, 140, and 150 °C, respectively. The HPLC spectrums (data not shown) showed these peaks appeared when the temperature higher than 160 °C. The results indicated that some antioxidant
compounds were difficult to extract with normal methods in black bamboo leaves or some newly produced compounds formed at high temperature. The huge difference between the two temperatures extracts guided us to investigate the main antioxidative compounds produced at high temperature with PLE.

3.3. Isolation and identification of antioxidative compounds

The EA and n-hexane fractions of black bamboo leaf extracts at 200 °C were used to isolate compounds. The compounds were identified with NMR and MS. The compounds: trans-coniferyl alcohol (1), p-coumaric acid (2), n-feruloyl serotonin (3), caffeic acid ethyl ether (4), tricin (5), coumaryl alcohol (6), coumaric acid ethyl ether (7) and ferulic acid ethyl ether (8) were identified with NMR and MS and direct comparison with authentic compounds. The structures are shown in Fig. 3. Compounds 2 and 5 were reported in black bamboo from a previous study (Jung, Lee, Lee, Kim, Lee & Um, 2007), compounds 1, 3, 4, 6, 7 and 8 are the first reported from black bamboo leaf.

Compound (6) coumaryl alcohol showed the best antioxidative activity in the on-line HPLC-ABTS+ system. This compound fraction was obtained from the EA fraction and verified from the HPLC retention time and LC–MS data. First, it was speculated that this compound was phenolic from its UV spectrum. From the ESI–MS data peak with \( m/z \) 149 was found. In the MS–MS analysis, the \( m/z \) was 130 and 102 as the predominant fragment ions, derived from \( m/z \) 149 in the negative mode. We speculated it was coumaryl alcohol (Whitaker, Schmidt, Kirk, & Barnes, 2001). From the literature (Ly, Shimoyamada, Kato, & Yamauchi, 2004), the compound has strong antioxidant activity, which was consistent with this result. However, further research is needed to collect more information about the NMR data.

These small phenols may derive from the degradation of bamboo lignin at high temperatures, as bamboo lignin is a typical Gramineae lignin composed of a mixed dehydrogenation polymer of coniferyl, sinapyl and p-coumaryls alcohols (Higuchi, 1987). 5–10% of p-coumaric acid esterified to the \( \alpha- \) or \( \gamma- \) hydroxyl groups of the lignin side chain were conformed well (Higuchi, 1987). Coniferyl alcohol (1), p-coumaric acid (2) and coumaryl alcohol (6) were found as the main peaks in extract, which were in agreement with the previous report that more phenolic hydroxyl groups were detected in bamboo lignin after the heating process (Shao, 2007).
Tricin (of human liver microsome CYP1A2 activity (Churdsak et al., 2011). The migration of monocytes on human aortic endothelial cells (Piga, & Tsuda, 2003); it has strong antioxidant activity and was verified as eased bamboo in a previous study (Tanaka, Tanaka, Mori, Kuwahara, 1983). Coumaric acid ethyl ether (Zhou & Ibrahim, 2010). 

Wen, & Jin, 2008). In addition, during the superheated process, the corresponding ethyl esters in 50% aqueous ethanol were reported, and coumaric acid ethyl ether (7) and ferulic acid ethyl ether (8) were found as the main peaks during the high temperature process (José et al., 2006).

The compounds identified from the superheated process are bioactive, and are natural chemopreventive agents for human health. Trans-coniferyl alcohol (1), like many natural phenols, is an antioxidant in vitro in the sense that it is reactive toward free radicals such as reactive oxygen species (ROS) (Lee, Yoon, Kim, & Lim, 2004). Animal studies and in vitro studies suggest that ferulic acid may have direct antitumour activity against breast cancer and liver cancer (Valentão et al., 2001). Ferulic acid also has potent suppressive effects of SOS-inducing activity by chemical and physical mutagens and potent inhibition of the mutagenicity against chemical mutagens (Miyazawa & Hisama, 2003). P-Coumaric acid (2) can be found in a wide variety of edible plants such as peanuts, tomatoes, carrots and garlic, which has antioxidant properties and is believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines (Kikugawa, Hakamada, Hasunuma, & Kurechi, 1983). N-feruloyl serotonin (3) was reported in withes’ broom diseased bamboo in a previous study (Tanaka, Tanaka, Mori, Kuwahara, & Tsuda, 2003); it has strong antioxidant activity and was verified as having an inhibitory effect on high glucose-induced adhesion and the migration of monocytes on human aortic endothelial cells (Piga, Naito, Kokura, Handa, & Yoshikawa, 2009). Caffeic acid ethyl ether (4) is a good antioxidant on free radicals, such as the nitric radical, superoxide anion and DPPH radicals and showed good inhibition of human liver microsome CYP1A2 activity (Churdsak et al., 2011). Tricin (5), a biologically active flavone, was first isolated from rust-infected wheat leaves, which was highlighted as a potential multifunctional nutraceutical (Zhou & Ibrahim, 2010). Beneficial health effects were also described, such as antioxidant effects, inhibition of lipid peroxidation, antiviral, antibacterial, immunomodulatory, antimutagenic, antiulcerogenic, mildly estrogenic, anti-inflammatory, and potent cancer chemopreventive agent (Zhou & Ibrahim, 2010). Coumaric acid ethyl ether (7) also has antioxidant activity and showed a high antisepic effect in cosmetics, with no disadvantage in preservability and safety (Tanjiguchi, Indu, & Shibayama, 2003). Ferulic acid ethyl ether (8) has anti-inflammatory and antioxidant properties and protects against amyloid beta-peptide-induced oxidative stress, the loss of phospholipid asymmetry and neurodegenerative disorders (Rukhsana, 2012).

The radical scavenging activity of the ABTS and DPPH radicals of the isolated compounds were examined according to the method by Kim et al. (2009). Table 1 shows that compounds 1, 2, 3, and 4 had high radical scavenging activity compared to Trolox in the ABTS assay. Compound 3 and 4 exhibited high DPPH radical scavenging ability compared to gallic acid.

### 3.4. Comparison of extraction methods

The PLE (40 °C and the best extraction condition) and reflux extraction methods were compared. Table 2 shows that the extract weight from 136 mg/g dry black bamboo leaves (40 °C) increased to 500 mg/g dry black bamboo leaves (200 °C), which increased almost fourfold. Increasing the temperature facilitates analyte diffusion and reduces interactions between the analyte and the matrix by disrupting intramolecular forces, such as van der Waal’s, hydrogen bonding and dipole attractions, as well as decreasing the viscosity of a liquid solvent, thus enabling better penetration of matrix particles (Palma, Piheiro, & Barroso, 2001). Compared to the reflux extraction method (~90 °C, 1 L solvent, 60 min), the PLE method showed more than twice the extraction efficiency on the extraction yields; TP, TF and the antioxidative activity of the crude extract, using seventy-six times less extraction solvent (13 mL instead of 1000 mL) in 2.4 times less time (25 min instead of 60 min) (Table 2).

The PLE also showed its environmentally friendly feature, as it reduced solvent waste and the time for additional clean-up and concentration steps before chromatographic analysis. The high efficiency of antioxidant extraction with less solvent usage could compensate the drawback of PLE expensive equipment, as the antioxidants can be used for diet components as its substantial beneficial health effects for human are very important. Taken together, PLE not only has high extraction efficiency in a short time with less solvent addition, but also changes the extract components, especially to produce health beneficial components.

### 4. Conclusions

The results revealed that the superheated process may significantly modify the composition of extracts from black bamboo leaves, with a 300% increase in the total phenolic and flavonoid recovered, compared to low temperature extraction. High temperatures reduced the solvent viscosity and surface tension and disrupted the black bamboo leaves matrix to allow for greater solvent connected with phenolics and flavonoids. At the same time, the modified solvent properties under high temperatures are

### Table 1

Antioxidant activity of isolated compounds from black bamboo leaves by PLE under the optimised conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SC50(μM)a for ABTSb</th>
<th>SC50(mM)c for DPPHd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.9</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>28.5</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>0.0077</td>
</tr>
<tr>
<td>4</td>
<td>8.4</td>
<td>0.087</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>95.9</td>
<td>–</td>
</tr>
</tbody>
</table>

a SC50: radical scavenging activity (concentration in μM necessary for 50% reduction of corresponding radical).
b Trolox was used as a positive control, SC50 is 57 μM. – Not detected under experiment concentration.
c Gallic acid was used as a positive control, SC50 is 0.037 mM.
d Ascorbic acid equivalent for scavenging 50% DPPH radical/ 100 g dry weight sample.

### Table 2

Total phenolics, flavonoids contents and anti-oxidative activity of black bamboo extracts under different extraction methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Time (min)</th>
<th>Solvent</th>
<th>Extract weight (mg/1 g DLa)</th>
<th>TPb (mg/100 g DL)</th>
<th>TFc (mg/100 g DL)</th>
<th>SC50(μg/mL)d for ABTS</th>
<th>AAEe (g AA/100 g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 °C PLE</td>
<td>25</td>
<td>13 mL</td>
<td>136</td>
<td>775 ± 1.2</td>
<td>252 ± 2.4</td>
<td>140 ± 4.5</td>
<td>1.23 ± 7.0</td>
</tr>
<tr>
<td>200 °C PLE</td>
<td>25</td>
<td>13 mL</td>
<td>500</td>
<td>2682 ± 0.9</td>
<td>657 ± 1.7</td>
<td>123 ± 3.6</td>
<td>3.05 ± 3.9</td>
</tr>
<tr>
<td>Reflux</td>
<td>60</td>
<td>1 L</td>
<td>240</td>
<td>1510 ± 3.2</td>
<td>182 ± 2.7</td>
<td>135 ± 3.8</td>
<td>1.68 ± 11.0</td>
</tr>
</tbody>
</table>

a DL: Dried leaves.
b TP: Total phenolics, expressed as quercetin equivalent, values represent means ± SD (n = 3).
c TF: Total flavonoids, expressed as gallic acid equivalent, values represent means ± SD (n = 3).
d SC50: radical scavenging activity (concentration in μg/mL necessary for 50% reduction of ABTS radical).
e Ascorbic acid equivalent for scavenging 50% DPPH radical/ 100 g dry weight sample.
helpful for extracting more non-polar phenolics and producing ethyl ester components in extracts. Results demonstrated clearly that PLE is a powerful method for increasing extraction efficiency and the content of biological compounds. Black bamboo leaves extracts from PLE with a high antioxidant content could serve as valuable sources in the nutraceutical and cosmetic industries as a natural antioxidants.

Acknowledgments

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