JOURNAL OF MEDICINAL FOOD
J Med Food 18 (4) 2015, 453–459

DOI: 10.1089/jmf.2014.3189

Functional Components of Bamboo Shavings and Bamboo Leaf Extracts and Their Antioxidant Activities *In Vitro*

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ABSTRACT This study was designed to detect characteristic compounds and evaluate the free radical scavenging capacity of the bamboo leaves extract and bamboo shavings extract (BSE). The antioxidant capacity of bamboo leaf n-butanol fraction (AOB) exhibited the highest total phenolic content (49.93%), total flavonoids content (24.11%), and characteristic flavonoids and phenolic acids, such as chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, orientin, homoorientin, vitexin, and isovitexin. Available data obtained with *in vitro* models suggested that AOB had higher free radical scavenging capacity with IC_{50} values of 1.04, 4.48, 5.37, and 1.12 μg/mL on DPPH$^{\bullet}$, $O_2^{\bullet -}$, $^{\bullet}OH$, and H_2O_2, respectively, than the other two extracts, bamboo leaf water extract and BSE. The results indicated that the extracts from different parts of the bamboo possess excellent antioxidant activity, which can be used potentially as a readily accessible and valuable bioactive source of natural antioxidants.

KEY WORDS: • *antioxidant effects* • *bamboo extract* • *flavonoids* • *functional components* • *polyphenols*

INTRODUCTION

FREE RADICALS AND OTHER reactive oxygen species (ROS), such as the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide radical (H_2O_2), are generated during normal aerobic metabolism. They can potentially damage the transient chemical species formed during aerobic life. Moreover, aerobic mammals use oxygen to maintain normal physiological functions and up to 2% of oxygen consumption may result in the formation of ROS.[1] ROS are considered to cause oxidative damage to biomolecules, such as DNA, lipids, and proteins.[2,3] Oxidative damage is believed to increase the risk of several human chronic diseases, including cancer, arteriosclerosis, neurodegenerative disorders, and aging processes.[4–6] Several scientific studies have demonstrated that polyphenols may protect cell constituents against oxidative damage and limit the risk of various degenerative diseases associated with oxidative stress.[7–12]

Bamboo, a giant and woody grass, is widely distributed in the tropics and subtropics. It represents a traditionally important commodity used as building materials, Chinese medicine, and as a food source. Bamboo leaves and bamboo shavings have been used in food and traditional Chinese medicine for over 1000 years.[13–15] Recently, several biologically active components from bamboo leaves and bamboo shavings were the subject of increasing research because of their potential health benefits. Bamboo leaf extract mainly contains flavone glycosides, phenolic acids, coumarin lactones, anthraquinones, amino acids, and polysaccharides.[16–19] The antioxidant of bamboo leaves (AOB), which is the trade name of n-butanol purified bamboo leaves water extract (BLWE), has been approved as a natural food additive for aquatic products, puffed foods, meat products, cereals, edible oils, bakery products, fruit and vegetable juices, tea, and fried foods by the Chinese Ministry of Health.[13,14] Bamboo shavings extract (BSE) contains abundant biologically active components, such as triterpenoids, phenolic acids, saponins, sterols, and lignans.[15,20–22] Previous studies on the safety of the AOB and BSE suggested that they have low toxicity.[13–15]

In 2000, Hu *et al.* prepared an ethanolic extract from bamboo leaf named BLE, which is different from AOB. They found that BLE has obvious antioxidant activity and also determined several functional components, such as chlorogenic acid, caffeic acid, and luteolin 7-glucoside.[23] Moreover, our previous studies have identified eight functional components from AOB.[13,14,24] However, there has been no systematic study on functional components of bamboo shavings as well as the free radical scavenging

Manuscript received 12 March 2014. Revision accepted 20 September 2014.

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capacity of its extract. According to the studies by our research team previously, different parts of the same edible food have similar components.[25–27] Thus, in this work, we not only detected eight functional components in BSE, BLWE, and AOB but also compared the free radical scavenging capacity of BSE with AOB and BLWE. It will provide the evidence for the exact utilization of bamboo shavings as a natural food additive or herbal drug, as well as the possibility of using bamboo shavings as a source of a low-cost natural antioxidant.

MATERIALS AND METHODS

Preparation of BLWE, AOB, and BSE

Bamboo leaf extract and BSE were prepared from bamboo leaves and shavings of *Phyllostachys nigra* var. *henonis* identified by the Research Institute of Subtropical Forestry of the Chinese Academy of Forestry (Hangzhou, China).

The BLWE was prepared from fresh bamboo leaves collected during the autumn and winter season in Anji County, Zhejiang Province, P.R. China. They were dried and ground into a powder. A total of 100 g of powder was extracted using a hot reflux method with 1 L water for 1.5 h, followed by filtration. The resulting filtrate was concentrated through vacuum, separated through centrifugation, and eventually dried into the final product by a spray dryer.

The AOB was prepared from BLWE, which was extracted with n-butanol thrice, and then the supernatant was collected, followed by vacuum concentration and centrifugation separation. The final product was obtained by drying using a spray dryer.

The BSE was prepared from the bamboo shavings of *Phyllostachys nigra* var. *henonis*. The fresh bamboo shavings were washed with water and air-dried. The coarse powder of the bamboo shavings was obtained through comminution and filtration. It was extracted for 1.5 h in 30% (v/v) ethanol solution using a hot reflux method after a carbon dioxide SFE extraction, followed by vacuum concentration and centrifugation separation, and dried into the final product using a spray dryer.[15]

The water-soluble tea extract (tea polyphenols [TP]) was a donation from Professor Xianqiang Yang at the Department of Tea Science (Zhejiang University, Hangzhou, China). The total content of four main flavanols (EC, ECG, EGC, and EGCG) was about 98%.

Determination of the contents of total flavonoids and total polyphenols of BLWE, AOB, and BSE

The contents of total flavonoids and total polyphenols were determined by a photocolorimetric method by comparison with a rutin standard and a para-hydroxybenzoic acid standard.[28]

Determination and quantification of characteristic compounds of BLWE, AOB, and BSE

Determinations of characteristic compounds from BLWE, AOB, and BSE were performed as previously described.[24] Homoorientin, orientin, isovitexin, vitexin, *p*-coumaric acid, chlorogenic acid, caffeic acid, and ferulic acid stock solutions were prepared in methanol at concentrations of 480, 500, 490, 510, 1070, 1150, 1160, and 1130 μg/mL, taking into account the purity of the standards. For quantitative analysis, matrix-matched calibration standards were prepared in triplicate at eight concentrations, ranging from 0.27 to 145 μg/mL for these four flavone *C*-glucosides and four phenolic acids. Working standards were analyzed on a Waters 2695 HPLC chromatograph at 330 nm with a Luna C_{18} column (250 × 4.6 mm id, 5 μm) from Phenomenex. A gradient program was used with the mobile phase, combining solvent A (acetonitrile) and solvent B (1% acetic acid, v/v) as follows: 15% A (15 min), 15–40% A (10 min), 40% A (9 min), and 40–15% A (6 min). The flow rate was 1.0 mL/min, the injection volume was 10 μL, and the column temperature was maintained at 40°C.

Scavenging activity of DPPH radical

The antioxidant activities of the extract were determined by measuring its capacity to bleach a purple-colored ethanol solution of $DPPH^{\bullet}$ as described by our research team.[25] The reaction mixture was prepared by mixing 2 mL of 0.2 mmol/L DPPH solution in ethanol and 2 mL of samples at various concentrations in a 30% (v/v) ethanol solution. After vortexing, the samples were kept at room temperature for 30 min, and then their optical density values were measured at 517 nm. The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging capacity } (\%) = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Water-soluble TP (≥98%) and 30% (v/v) ethanol solution were used as a positive control and a control, respectively.

Assay of scavenging activity against superoxide anion radical

The superoxide anion radical was generated from a pyrogallol autoxidation system.[29] Briefly, 50 μL of samples at various concentrations were incubated with 150 μL carbonic acid-buffered saline solution (pH 10.2) containing 0.1 mmol/L EDTA, 250 μL pyrogallol (3.125×10^{-5} mol/L), and 200 μL luminol (0.5 mmol/L). The luminescence was counted every 6 sec (total counts for 30 sec) using a Sirius luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany) equipped with a photon counter (370–630 nm) and an automatic injector. The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging activity } (\%) = [(A_{\text{control}} - A_0) - (A_{\text{sample}} - A_0)]/(A_{\text{control}} - A_0) \times 100$$

where A_{control} is the luminosity of the control, A_0 is the luminosity of the background, and A_{sample} is the luminosity of the test samples.

TP and phosphate-buffered saline (PBS, pH 7.8) were used as a positive control and a negative control, respectively.

Assay of scavenging activity against hydroxyl radical

The hydroxyl radical was generated by a Fenton-type reaction system containing 30 μL $FeSO_4$ (0.4 mmol/L), 30 μL H_2O_2 (1.5%), 450 μL 50 mmol/L PBS (pH7.8), and 100 μL of samples at various concentrations in PBS, and 50 μL luminol (0.1 mmol/L) in PBS.[25] Luminescence was counted every second on the Sirius luminometer. Scavenging activities (%) were calculated as described in the superoxide anion radical assay.

Assay of scavenging activity against hydrogen peroxide radical

The H_2O_2 scavenging assays were carried out according to the method described by Tian *et al.*[29] Briefly, 100 μL of

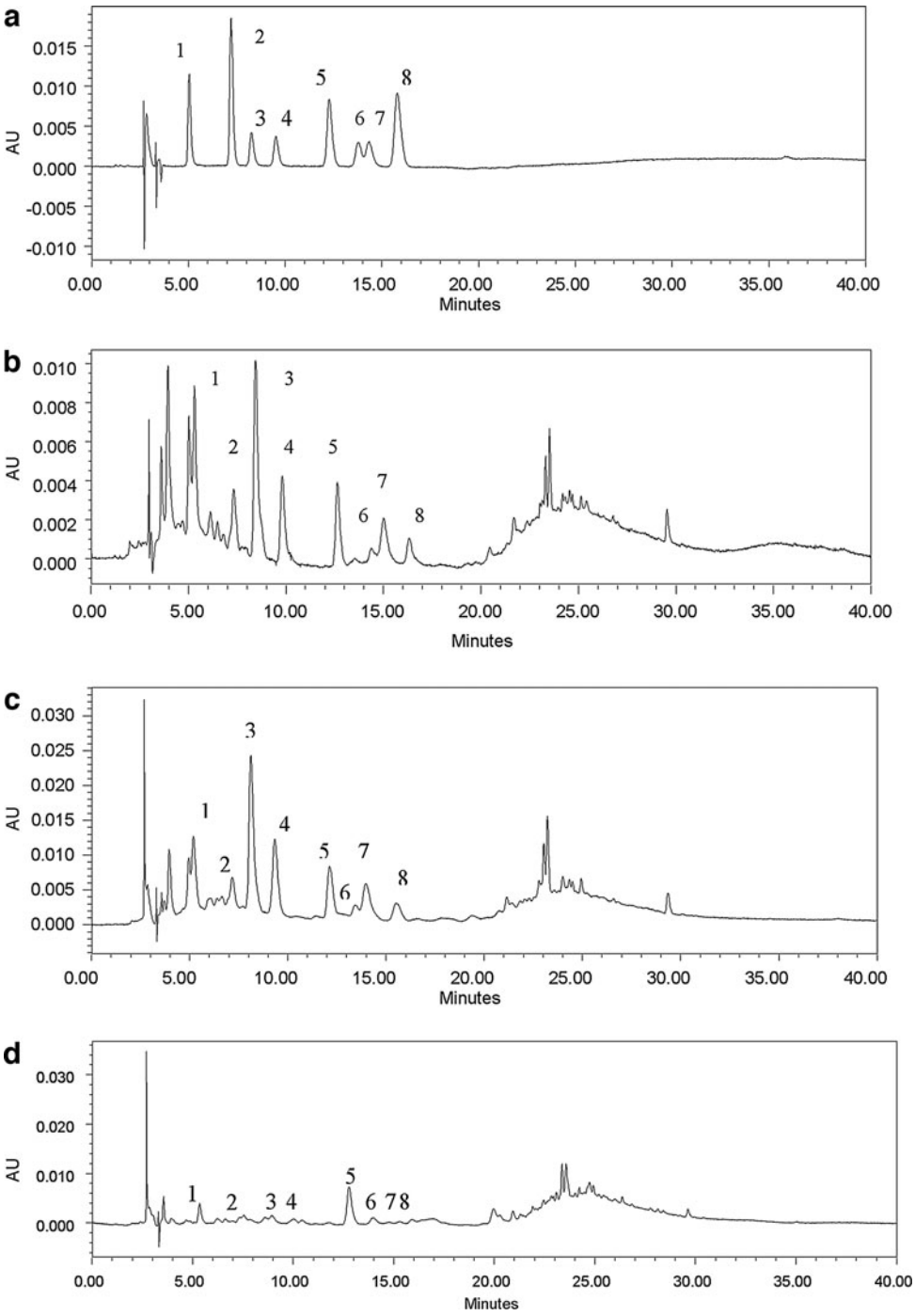

FIG. 1. The RP-HPLC chromatograms of the **(a)** four flavone *C*-glucosides and four phenolic acids in the standard mixture, **(b)** BLWE, **(c)** AOB, and **(d)** BSE, all detected at 330 nm. Peak No.: 1, chlorogenic acid; 2, caffeic acid; 3, homoorientin; 4, orientin; 5, *p*-coumaric acid; 6, vitexin; 7, isovitexin; 8, forulic acid. AOB, antioxidant of bamboo leaves; BLWE, bamboo leaf water extract; BSE, bamboo shavings extract.

Table 1. Calibration Data for the Standard Flavone *C*-Glucosides and Four Phenolic Acids

Compound	*Retention time (min)*	*Linearity range (μg/mL)*	*Calibration equation*	*Correlation factor (R^2)*	*LOD (ng/mL)*	*LOQ (ng/mL)*
Chlorogenic acid	5.4	0.29–143.8	y = 26345x – 4644.9	0.9995	20	60
Caffeic acid	7.4	0.29–145.0	y = 50534x + 4843.5	0.9995	15	45
Homoorientin	8.4	0.42–60.0	y = 20075x – 2957.5	0.9999	20	60
Orientin	9.8	0.40–62.5	y = 21009x – 1751.9	0.9999	30	90
p-Coumaric acid	12.6	0.27–133.8	y = 39890x – 3594.9	0.9995	15	45
Vitexin	14.4	0.41–61.3	y = 25695x – 3478	0.9999	30	90
Isovitexin	14.9	0.43–63.8	y = 22241x – 4319.3	0.9996	20	60
Ferulic acid	16.2	0.28–141.3	y = 50534x – 4843.5	0.9995	15	45

All data are expressed as mean ± SD ($n = 3$).

Working standards were analyzed on a Waters 2695 HPLC chromatograph at 330 nm with a Luna C_{18} column (250 × 4.6 mm id, 5 μm) from Phenomenex. A gradient program was used with the mobile phase, combining solvent A (acetonitrile) and solvent B (1% acetic acid, v/v) as follows: 15% A (15 min), 15–40% A (10 min), 40% A (9 min), and 40–15% A (6 min). The flow rate was 1.0 mL/min, the injection volume was 10 μL, and the column temperature was maintained at 40°C.

sample, 600 μL of 50 mmol/L PBS (pH 7.8), and 200 μL of 1% H_2O_2 (0.29 mol/L) were incubated for 20 min at 37°C. An aliquot of 150 μL of 1 mmol/L luminol was added to the mixture to start the chemiluminescence reaction. Luminescence was counted every 6 sec (total counts for 30 sec) using a Sirius luminometer. Scavenging activities (%) were calculated as described in the superoxide anion radical assay.

Statistical analysis

Data are expressed as mean ± standard deviation of three replicated determinations. One-way analysis of variance was used to determine significant difference at $P < .05$ using SPSS 11.

RESULTS

Characterization of BLWE, AOB, and BSE

The two bamboo leaf extracts (BLWE and AOB) and the BSE are complex mixtures containing many types of flavonoids and polyphenols. The contents of total flavonoids and total polyphenols were determined using two photocolorimetric methods. BLWE, AOB, and BSE contained 17.32%, 24.11%, and 15.90% total flavonoids and 30.28%, 49.93%, and 37.13% total polyphenols, respectively.

Table 2. Content of Four Flavone *C*-Glucosides and Four Phenolic Acids in BLWE, AOB, and BSE

Content (mg/g)	*BLWE*	*AOB*	*BSE*
Chlorogenic acid	1.33 ± 0.02	3.65 ± 0.32	0.67 ± 0.02
Caffeic acid	0.20 ± 0.01	1.69 ± 0.23	0.10 ± 0.01
Homoorientin	8.92 ± 0.09	23.16 ± 0.18	1.35 ± 0.05
Orientin	4.24 ± 0.05	15.34 ± 0.28	0.58 ± 0.06
p-Coumaric acid	2.32 ± 0.03	6.41 ± 0.27	6.80 ± 0.15
Vitexin	1.58 ± 0.01	2.32 ± 0.18	0.67 ± 0.04
Isovitexin	0.12 ± 0.07	5.73 ± 0.25	0.91 ± 0.05
Ferulic acid	0.61 ± 0.02	1.91 ± 0.12	0.18 ± 0.02

All data are expressed as mean ± SD ($n = 3$) on a dry basis.

Three extracts were analyzed on a Waters 2695 HPLC chromatograph at 330 nm with a Luna C_{18} column (250 × 4.6 mm id, 5 μm) from Phenomenex. A gradient program was used with the mobile phase, combining solvent A (acetonitrile) and solvent B (1% acetic acid, v/v) as follows: 15% A (15 min), 15–40% A (10 min), 40% A (9 min), and 40–15% A (6 min). The flow rate was 1.0 mL/min, the injection volume was 10 μL, and the column temperature was maintained at 40°C.

AOB, antioxidant of bamboo leaves; BLWE, bamboo leaf water extract; BSE, bamboo shavings extract.

Content of characteristic compounds

The main functional components of BLWE are flavonoids and phenolic acids. Four of the flavone C-glucosides, orientin, homoorientin, vitexin, and isovitexin, and four of the phenolic acids, chlorogenic acid, caffeic acid, ferulic acid, and *p*-coumaric acid, in BLWE have been previously reported,[24] but these functional components have not been reported in BSE. The three extracts were analyzed using reverse-phase HPLC, as shown in Figure 1, while the characteristic compounds are described in Table 1. Contents of eight functional components in the three extracts are shown in Table 2.

Scavenging capacity toward DPPH radical

The DPPH radical scavenging activity assay has been widely used to evaluate antioxidant capacities in a relatively short time compared with other methods.[30] This method is

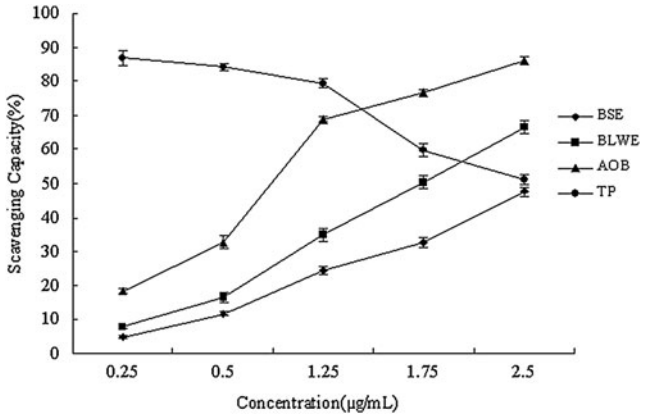

FIG. 2. Scavenging capacity of BLWE, AOB, BSE, and TP on the stable DPPH radical. TP, tea polyphenols.

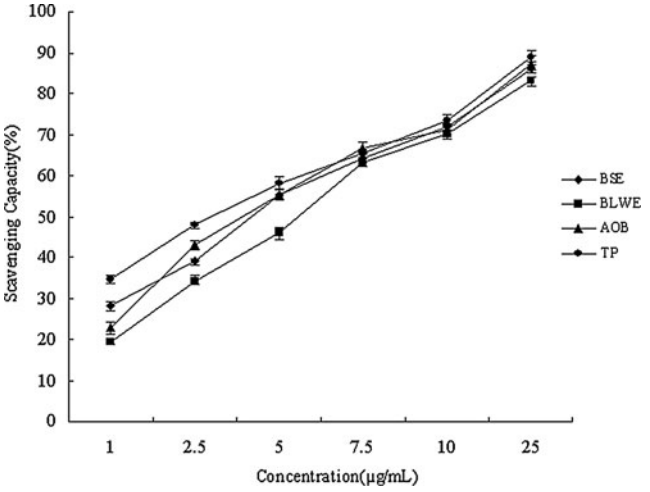

FIG. 3. Scavenging capacity of BLWE, AOB, BSE, and TP on the superoxide anion radical.

based on the reduction of DPPH• to DPPH-H in the presence of a hydrogen-donating antioxidant leading to the formation of the nonradical form DPPH-H.[31] The concentration-dependent scavenging activities of BLWE, AOB, and BSE against the stable DPPH radical are shown in Figure 2. AOB has the most scavenging activity of the three bamboo extracts, while the two bamboo leaf extracts have more scavenging activities than BSE. The IC_{50} values of AOB, BLWE, and BSE were 1.04, 1.81, and 2.65 μg/mL, respectively. The scavenging activity of the positive control (TP) decreased as its concentration increased (Fig. 2); the IC_{50} values of TP are below 0.25 μg/mL.

Scavenging capacity toward superoxide anion radical

In the present study, $O_2^{\bullet -}$ was generated using a pyrogallol autoxidation system. Figure 3 shows the percentage inhibition of superoxide anion radicals by BLWE, AOB, BSE, and TP at different concentrations ranging from 1 to 25 μg/mL. The inhibitory effect of all extracts toward the superoxide radical

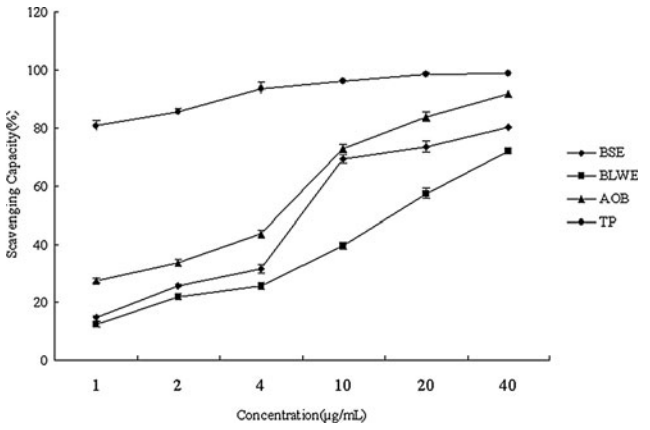

FIG. 4. Scavenging capacity of BLWE, AOB, BSE, and TP on the hydroxyl radical.

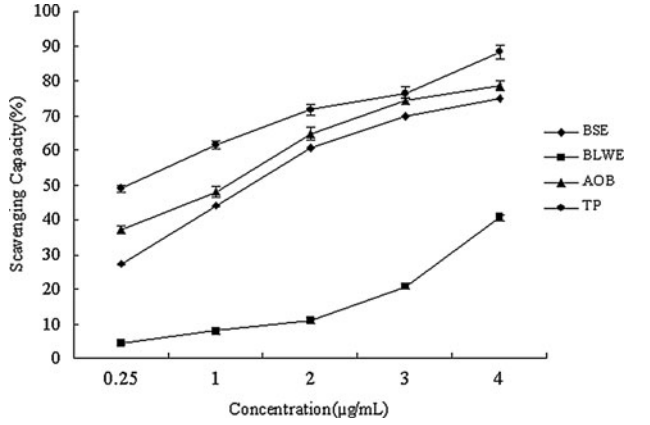

FIG. 5. Scavenging capacity of BLWE, AOB, BSE, and TP on the hydrogen peroxide radical.

correlated well with their concentrations, and the correlation coefficient is 0.991, 0.970, 0.989, and 0.987 (R^2), respectively. At low concentrations (< 1 μg/mL), the positive control (TP) was more efficient at scavenging $O_2^{\bullet -}$ than the three bamboo extracts. At high concentrations (> 7.5 μg/mL), BLWE, AOB, and BSE have comparable scavenging abilities as TP, as shown in Figure 3. The IC_{50} values of AOB (4.48 μg/mL), BLWE (5.81 μg/mL), and BSE (4.86 μg/mL) are close to that of TP (3.75 μg/mL). The radical scavenging activity is also consistent with the high level of phenolic compounds observed in the plant extract such as flavonoids, which are known to possess high $O_2^{\bullet -}$ anion scavenging abilities.[32]

Scavenging capacity toward hydroxyl radical

The hydroxyl radical is the most reactive type of oxygen radical and induces severe damage to adjacent biomolecules, including carbohydrates, proteins, lipids, and DNA in cells, causing tissue damage or cell death.[19]

The hydroxyl radical used in this study was generated from a Fe^{2+}-H_2O_2-luminol system. The scavenging ability of the samples against the hydrogen radical is shown in Figure 4. The inhibitory effects of all samples toward the hydrogen radical correlated well with their concentrations. BLWE, AOB, BSE, and TP exhibited 72.14%, 91.83%, 80.45%, and 98.89% of scavenging activity toward the hydrogen radical at 40 μg/mL, respectively. TP ($IC_{50} < 1$ μg/mL) had better scavenging capacity than the three bamboo extracts (IC_{50} was 15.87, 5.37, and 6.74 μg/mL for BLWE, AOB, and BSE, respectively).

Scavenging capacity toward hydrogen peroxide radical

Hydrogen peroxide (H_2O_2) is one of the many compounds that injure cells.[33] Figure 5 shows the dose-dependent (0.25–4 μg/mL) ability of BLWE, AOB, BSE, and TP to scavenge the hydrogen peroxide radical at different concentrations. The hydrogen peroxide radical scavenging capacities of three bamboo extracts were significant at all the tested concentrations. BLWE, AOB, BSE, and TP exhibited 40.55%, 78.65%, 74.98%, and 88.22% of scavenging

activity on the hydrogen peroxide radical at 4 μg/mL, respectively, as shown in Figure 5. TP (with an IC_{50} of 0.38 μg/mL) have better scavenging capacity than the three bamboo extracts (IC_{50} is > 4, 1.12, and 1.63 μg/mL for BLWE, AOB, and BSE, respectively).

DISCUSSION

The results of the present study showed that all of the three bamboo extracts contain chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, orientin, homoorientin, vitexin, and isovitexin. AOB was further extracted by an equal volume of *n*-butanol thrice, thus it is reasonable that it contains more total polyphenols content, total flavonoids content, chlorogenic acid, caffeic acid, ferulic acid, orientin, homoorientin, vitexin, and isovitexin than BLWE and BSE. BSE had the highest content of *p*-coumaric acid of the three extracts and more isovitexin than BLWE, but all of the other identified components were found in the lowest concentrations in BSE.

Results showed that the antioxidants of three bamboo extracts were not the same in different antioxidative test systems. Of the three samples, AOB had the strongest scavenging effect on the DPPH radical, superoxide anion radical, hydroxyl radical, and hydrogen peroxide radical, respectively. BSE had the middle scavenging effect on the superoxide anion radical, hydroxyl radical, and hydrogen peroxide radical. However, BLWE has more potent scavenging activity than BSE in the DPPH radical system. In general, there are two reasons to explain this observation. First, as the main antioxidants of BLWE and AOB, the characteristic flavonoids are flavone *C*-glucosides, but the main antioxidants of BSE are phenolic acids, such as *p*-Coumaric acid. Homoorientin, orientin, vitexin, and isovitexin are four representative compounds for flavone *C*-glucosides. Previous research work has shown that the antioxidant activities of flavones are in the sequence luteolin $\approx$ isoorientin > galuteolin >> tricin >> apigenin in DPPH analysis, and isoorientin > luteolin $\approx$ galuteolin > tricin > apigenin in FRAP analysis.[34] Therefore, the antioxidant activities of flavones in the three bamboo extracts are different when they are determined using different antioxidation systems. Second, there are many components in the three bamboo extracts that have the antioxidant activity, and the correlations between the antioxidant activity and these antioxidant components are different in different antioxidation systems. Moreover, some research studies have shown that flavones are not the exclusive antioxidant components, and phenolic acids, tannins, and amino acids also exhibit good antioxidant activities.[18] BSE had a higher concentration of *p*-coumaric acid than BLWE, which has the lowest molecular weight among the eight functional components, but was 2.93 times higher than BLWE.

Our results showed that TP act more as pro-oxidants than as antioxidants as the concentration increases (0.25–2.5 μg/mL) against the DPPH radical. Although the ability of polyphenols to protect the cell from oxidative stress has been demonstrated, there is increasing evidence for the existence of their pro-oxidant properties. The same polyphenol compounds could behave as both antioxidants and pro-oxidants, depending on their concentration and free radical source.[34–38] Some studies have reported that the green TP epicatechin, epigallocatechin-3-gallate, and catechins have pro-oxidant properties when they are employed at high concentrations.[36,37]

Three bamboo extracts containing a number of phenolic acids and flavonoids were shown to effectively scavenge the DPPH radical and various ROS. The significant antioxidant activity of BLWE, AOB, and BSE demonstrated that they might alleviate oxidative damage in cells, thereby being beneficial to human health and effectively employed as an ingredient in food applications. Therefore, bamboo extracts may be useful supplements in medicinal foods and pharmaceuticals; the identification of individual components in the BLWE, AOB, and BSE responsible for these different antioxidant properties is subject to further investigation.

ACKNOWLEDGMENTS

The work was financially supported by the Zhejiang Provincial Natural Science Foundation of China under (Nos. LQ13C200006 and LY14C200007), the National Natural Science Foundation of China (Nos. 81102861, 81374048, and 31470793), the Zhejiang Provincial Scientific Innovation of University Students (2013R415015), and the National Science & Technology Pillar Program during the 12th Five-year Plan Period of China (2011BAD23B02).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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