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Effects of drying methods on contents of bioactive compounds and antioxidant activities of *Angelica dahurica*

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Abstract Baizhi (*Angelica dahurica*) has been widely used as a traditional Chinese herbal medicine, functional food and cosmetic product ingredient, mostly because of the high furanocoumarin compounds in roots. Because the fresh root is perishable, drying techniques are needed to maintain a higher-quality product. Freeze-drying is the best method but energy-consuming and costly. The aim of this study was to analyze the quality (antioxidant and furanocoumarin content) of Baizhi roots after freeze-drying (the control) and in-the-shade, 40 and 70 °C drying. Antioxidant activity was revealed by 2,2-diphenyl-1-picrylhydrazyl and Fe^{2+} chelating assay, and the content of six furanocoumarin compounds, including xanthotoxin, bergapten, oxypeucedanin, imperatorin, phellopterin and isoimperatorin, was analyzed by liquid chromatography. Antioxidant activity was greater in roots with in-the-shade, 40 and 70 °C drying than freeze-drying. The furanocoumarin content pattern was similar with 70 °C drying and freeze-drying. *A. dahurica* roots dried at 70 °C may be an alternative method for maintaining high quality.

Keywords *Angelica dahurica* · Drying method · Furanocoumarin · Antioxidant

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Abbreviations

HPLC-DAD	High-performance liquid chromatography with diode-array detection
DPPH	2,2-Diphenyl-1-picrylhydrazyl

Introduction

Baizhi (*Angelica dahurica* BENTH. et HOOK.) is a perennial Apiaceae plant that originated in Taiwan [1] and is abundant in Korea, China, Japan and Russia [2, 3]. The root of Baizhi is a well-known traditional Chinese medicine that has been used for its antipyretic and analgesic properties for thousands of years in Asia. Today, researchers are analyzing the multiple biological functions of Baizhi and investigating its chemical constituents.

The multiple pharmacological effects of Baizhi root extracts include antioxidation [4], anti-inflammatory [5], antiproliferative [6], skin-whitening [7], anti-tumor [8], antimicrobial [9] and anti-Alzheimer disease effects [10]. These effects are believed to be attributed to the plant's rich furanocoumarin compounds such as imperatorin and isoimperatorin. Imperatorin has anti-inflammatory [11], anticonvulsant [12], hepatoprotective [13], myorelaxant [14], vasodilator [15] and anti-cancer effects [16]. Isoimperatorin has anti-inflammatory [17], antiallergic [18] and antimicrobial effects [19]. Besides the pharmacological effects of Baizhi furanocoumarins, root extracts contain a variety of phenolic compounds that are connected to its strong antioxidant activity [20]. Recently, foods with antioxidant effects have become valuable for their ability to remove reactive oxygen species, which oxidize lipids, DNA, membranes and proteins and are involved in atherosclerosis, cancers and other diseases [21].

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Fresh harvested Baizhi root can be easily infected by *Macrophomina phaseolina* (Tassi) Goid, a kind of fungal disease, causing root rot [22]. Moreover, with the harvesting of Baizhi concentrated in July and August [23], an efficient storage method is required to prevent root rot and maintain an annual supply. Drying is an efficient and easy method to store food, but it may cause the root to deteriorate. For example, Chinese farmers dry Baizhi with vulcanization fumigation to prevent pests and diseases while also decolorizing the root product, but one study showed that Baizhi loses about 58–67% coumarin after vulcanization fumigation [24]. Therefore, a good drying method should dry the product efficiently but also maintain its pharmacological effects.

The drying temperature may be the most influential factor of product quality. The stability of coumarin in Baizhi root extract increases then decreases with drying at increasingly high temperature [25]. Vacuum freeze-drying is the best method of water removal of food, with final products of the highest quality as compared with other drying methods [26, 27]. During the freeze-drying process, the water in the fresh sample is frozen and then sublimated. The low temperature and lack of water prevent sample deterioration and microbiological contamination. However, the cost for freeze-drying is 4–8 times higher than with hot-air drying, so it is little used in the food industry [27].

Generally, contents of imperatorin and isoimperatorin are used to assess the quality of Baizhi root [22, 28]. However, the multiple constituents in herbal drugs may work synergistically and cannot be easily separated into active parts. As well, the chemical constituents in herbs may vary by the drying process used [29].

In this study, to find an alternative drying process for Baizhi root, we used freeze-drying as the control and compared in-the-shade, 40 and 70 °C drying of root extracts. We also tested the content of six furanocoumarins—xanthotoxin, bergapten, oxypeucedanin, imperatorin, phellopterin and isoimperatorin—in roots with the four drying methods, as well as antioxidant activity by measuring the content of phenolic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and Fe^{2+} chelating capacity. Drying Baizhi root with 70 °C drying is better than in-the-shade and 40 °C drying.

Materials and methods

Reagents and chemicals

All solvents were purchased from Merck (Darmstadt, Germany) and were of analytical grade or high-performance liquid chromatography (HPLC) grade. Folin–Ciocalteu, Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 3-(2-pyridyl)-5,6, diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate were from Merck, and Fe^{2+} chloride tetrahydrate ferrozine, and furanocoumarin compound standards, including xanthotoxin, bergapten, oxypeucedanin, imperatorin, phellopterin and isoimperatorin were from Merck. The standards for HPLC with diode-array detection (HPLC–DAD) were dissolved in methanol to a concentration of 100 μg/mL and were stored at − 20 °C.

Plant materials

Baizhi was cultivated at the Hualien District Agricultural Research and Extension Station (121°33E, 23°58N), Council of Agriculture, Executive Yuan in November, 2014, and harvested in following May. Plantlets were harvested and the roots were then sliced and mixed. Dried roots were crushed to a powder by using a high-speed disintegrator (Solar Energy Co, Taiwan) and stored at − 20 °C.

Drying method

Four drying methods were applied in this study: freeze-drying and in-the-shade, 40 and 70 °C drying. A freeze-drying system (LYPH. LOCK 18, Labconco, USA) was used to dry root slices. For in-the-shade drying, the flattened root slices were placed under a shadowed and ventilated location at 30 °C for 4 h. Also, root slices were dried with a drying oven (Deng Yng, Taiwan) at 40 or 70 °C. All samples were treated by constant weighting till no weight change. For water-content corrections, a small portion of all samples were further treated by 70 °C drying to completely dry.

Root extracts and sample processing

Extraction for HPLC–DAD was performed as described [28] with some modifications. Methanol (20 mL) was added to 0.2 g ground powder, vortexed and let stand for 30 min. The powder with methanol was ultrasonic-shocked (DC600H, Delta, Taiwan) for 30 min, then centrifuged at 3320 rcf for 3 min. The supernatant was collected and the residue was re-extracted under the same conditions for complete extraction. Finally, the supernatant was mixed and filtered through a 0.2-μm Acrodisc Syringe Filter (Pall, USA) before being collected in the Automatic Sampler-approved certified vials (Agilent, USA). Antioxidant assay extraction was performed as described [30] with some modification. Methanol (20 mL) was added to 1.0 g ground powder, vortexed and shaken overnight (50 rpm), then centrifuged at 3320 rcf for 3 min. The supernatant was collected as the sample.

HPLC–DAD analysis

To identify the furanocoumarin compounds in root extracts, 25 μL samples were separated by using an analytical column (Syncrois C18, 250 × 4.6 mm, 5 μm particle; ThermoFisher, USA) with flow rate 1.0 mL/min at 30 °C and absorbance 310 nm monitored by the ThermoFisher LC system (ThermoFisher, USA) equipped with a ThermoFinnigan UV detector and ThermoFisher Automatic Sampler. The column was protected by a C18 Guard column (HI-5C18-10C5, Hichrom, UK). A binary gradient was used for H_2O (Solvent A) and methanol (Solvent B). Optimized pigment separation was achieved with a linear gradient of 40–20% Solvent A for 0–5 min, 20–10% Solvent A for 5–7 min, and 10–0% Solvent A for 7–9 min. After linear gradient was settled, the injections of the first sample were repeated until the displayed detector signal was stable, following samples were then injected.

Total polyphenol content

The total polyphenol content in Baizhi root was determined by using the Folin–Ciocalteu colorimetric method [31]. A mixture of 50 μL of aqueous extract (50 mg/mL) and 50 μL of 1 N Folin–Ciocalteu reagent was incubated for 3 min; 1.0 mL of 2% aqueous sodium carbonate was then added. The reaction mixture was incubated for 30 min in the dark and light absorbance was measured at 750 nm against distilled water as blank. Gallic acid was used as the standard to calculate the total polyphenol content of samples.

DPPH assay

DPPH radical scavenging activity was analyzed as described [30] with some modifications. The DPPH solution in methanol (0.4 mM) was prepared daily, and each antioxidant samples were diluted or concentrated to 83.3, 50, 35.7, 25, 17.86, 14.7, 10, 8.33, 6.25, 4.69, 3.13 mg/mL. An amount of 50 μL sample solutions was loaded on a 96-well plate. Each concentration was loaded in six wells. Three were mixed thoroughly with 150 μL fresh DPPH solution (A1) and the others were mixed thoroughly with 150 μL methanol (A2). Pure methanol was used as the control (C1, C2). The loaded 96-well plates were incubated for 30 min in the dark, then the decrease in light absorbance at 517 nm was measured. The DPPH radical scavenging effects were calculated as $[1 - (A1_{517} - A2_{517})/(C1_{517} - C2_{517})]$.

Fe^{2+} chelating assay

Fe^{2+} chelating activity was measured as described [32] with some modification. Antioxidant samples were diluted or concentrated to at least seven concentrations. The Fe^{2+} chloride tetrahydrate solution in methanol (12 mM) was prepared, and 10.5 μL of this solution was mixed thoroughly with 1400 μL sample solution. Ferrozine solution in methanol (5 mM) was prepared. An amount of 200 μL sample solution was loaded on 96-well plates, with each concentration loaded in six wells. Three wells were thoroughly mixed with 20 μL ferrozin solution (A1) and the others were thoroughly mixed with 150 μLmethanol (A2). Pure methanol was used as the control (C1, C2). The loaded 96-well plates were left to stand for 5 min, then the decrease in light absorbance at 562 nm was measured. The Fe^{2+} chelating effects were calculated as $[1 - (A1_{562} - A2_{562})/(C1_{562} - C2_{562})]$.

Statistical analysis

Data were analysed by using ANOVA. The source of variation was harvest stage. Significant differences by harvest stage were calculated by the Fisher least significant difference (LSD) test ($P < 0.05$). Data are expressed as mean ± SD. The IC50 value of DPPH radical scavenging activity and Fe^{2+} chelating activity was predicted by Loess regression. All analyses involved use of R 3.2.2. $P < 0.05$ was considered statistically significant.

Results and discussion

Characterization of furanocoumarin compounds in Baizhi root

In traditional Chinese medicine, crude Baizhi roots are used as medicine or as functional food for their antipyretic and analgesic activities because of abundant furanocoumarin compounds [3]. Here, we first determined the amount of furanocoumarin compounds in roots by the total coumarin content, then independent active compounds were determined to assess the medical functions [28, 33, 34]. For example, isoimperatorin, imperatorin and oxypeucedanin may be useful for treating Alzheimer disease [10]. Isoimperatorin, imperatorin and phellopterin have potential anti-inflammatory effects [35], whereas xanthotoxin, isoimperatorin, imperatorin and oxypeucedanin have anti-cancer effects [8]. In addition, the synergistic effect of the active compounds may affect the final quality of the dried root. Accordingly, we analyzed the absorbance at 310 nm for root extracts to represent the total coumarin content [36]. Absorbance was slightly greater for roots dried in-the-shade- and at 40 °C than freeze- and 70 °C drying (Fig. 1). Because the freeze-dried roots was used as the control, this result implied that additional coumarin compounds may result from in-the-shade and 40 °C drying.

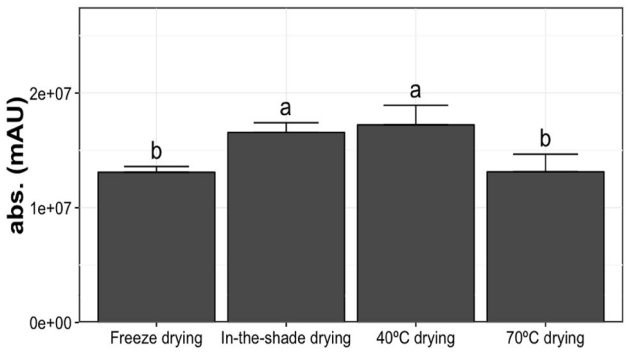

Fig. 1 Effect of drying methods on absorbance (310 nm) of dried root extracts, indicating total coumarin content. Different letters indicate difference ($P < 0.05$) from freeze-drying. Means followed by the same letter(s) are not significantly different at 5% level by Fisher's least significant difference (LSD) test

Coumarin in Baizhi is mainly composed of furanocoumarin compounds [3]. We extracted Baizhi root samples for HPLC–DAD chromatography and obtained seven clear peaks. By using reference compounds, we identified peaks 1, 2, 4, 5, 6 and 7 as xanthotoxin, bergapten, oxypeucedanin, imperatorin, phellopterin and isoimperatorin, respectively (Fig. 2). We then analyzed the peak area of six the furancoumarins by using HPLC–DAD at 310 nm to indicate the content of the compounds. We found different patterns of furancoumarin compounds from each dried root. The peak area of xanthotoxin in roots was about 3 and 5 times less with freeze-drying than 40 °C and in-the-shade drying, respectively, and the peak area of bergapten was about 1.3 and 1.6 times less (Fig. 3). The peak areas of the other furanocoumarins, including oxypeucedanin, imperatorin, phellopterin and isoimperatorin, were greater with freeze-drying than the other methods, but the variations were less than 30%.

The findings could be explained by the compound stability and the metabolisation of furanocoumarin. In terms of compound stability, xanthotoxin, bergapten, imperatorin and isoimperatorin are relatively stable [37]. Previously, drying under 40 °C did not affect the concentration of imperatorin and isoimperatorin [28]. In addition, the stability was poorer for phellopterin than imperatorin [38], with oxypeucedanin the most unstable compound among the six furanocoumarins. The epoxide ring of oxypeucedanin is easy to hydrolyze [39].

Xanthotoxin and bergapten result from complex furanocoumarin degradation during metabolisation, and imperatorin is demethylated to xanthotoxin [40]. As a result, xanthotoxin and bergapten content was increased with in-the-shade and 40 °C drying. The pattern of furanocoumarin contents changed with different drying processes, which will affect the pharmacological effect of dried root product. Therefore, we analyzed the proportion of peak area for each furanocoumarin compound. The primary difference in peak area proportions was observed for xanthotoxin with freeze-drying, in-the-shade-, 40 and 70 °C-dried root: 2.66 ± 0.21, 10.95 ± 0.21, 6.77 ± 0.78 and 2.82 ± 0.33, respectively (Table 1). The proportions for oxypeucedanin were 36.95 ± 1.45, 24.96 ± 0.75, 24.22 ± 1.75 and 32.13 ± 1.42, respectively. Thus, although the furanocoumarin compounds were degraded in 70 °C-dried root (Fig. 3), the pattern of peak area proportions was more similar with freeze-drying than other drying methods.

Total phenolic acid

Extracts of Baizhi root have anti-oxidant properties, mostly with high phenolic acid content [20]. We analyzed the total phenolic acid contents of roots by the four drying types. The contents were 1.24 ± 0.26, 2.04 ± 0.20, 2.68 ± 0.20 and 2.02 ± 0.26 mg/g for freeze-, in-the shade, 40 and 70 °C drying, respectively (Table 2). The total phenolic acid content was about 1.6–2.2 times lower with freeze-drying than with other drying methods. Therefore, during in-the-shade, 40 and 70 °C drying, phenolic acid content increased. This increased content may be due to some phenolic acids being produced or released during drying because the tissue structure of the sample was damaged and degrading enzymes, such as for oxidation, hydrolysis or glycolysis, were activated. In addition, damaged tissue structure and enzyme reaction occurs less with freeze-dried samples [41, 42].

DPPH radical scavenging activity

The variation in total phenolic acid content for roots by drying method should lead to different antioxidant effects. We determined the antioxidant activity of the four drying types by DPPH assay and observed a dose-dependent relation in DPPH scavenging activity of Baizhi root extracts [Fig. 4(A)]. The IC50 value for the extracts was 23.93 ± 9.39, 3.94 ± 17.15, 5.16 ± 14.69 and 8.35 ± 15.93 mg/mL for freeze-, in-the shade, 40 and 70 °C drying, respectively (Table 2). The activity was about 3–6 times less in freeze-drying than with other drying methods, which reflects the variation in phenolic acid content.

Fe^{2+} chelating activity

We also determined antioxidant activity and found a dose-dependent relation on Fe^{2+} chelating assay of Baizhi root extracts [Fig. 4(B)]. The activity in dried roots did not differ among the drying methods. The IC50 value for the extracts was 3.12 ± 1.05, 3.85 ± 0.58, 3.68 ± 0.69 and

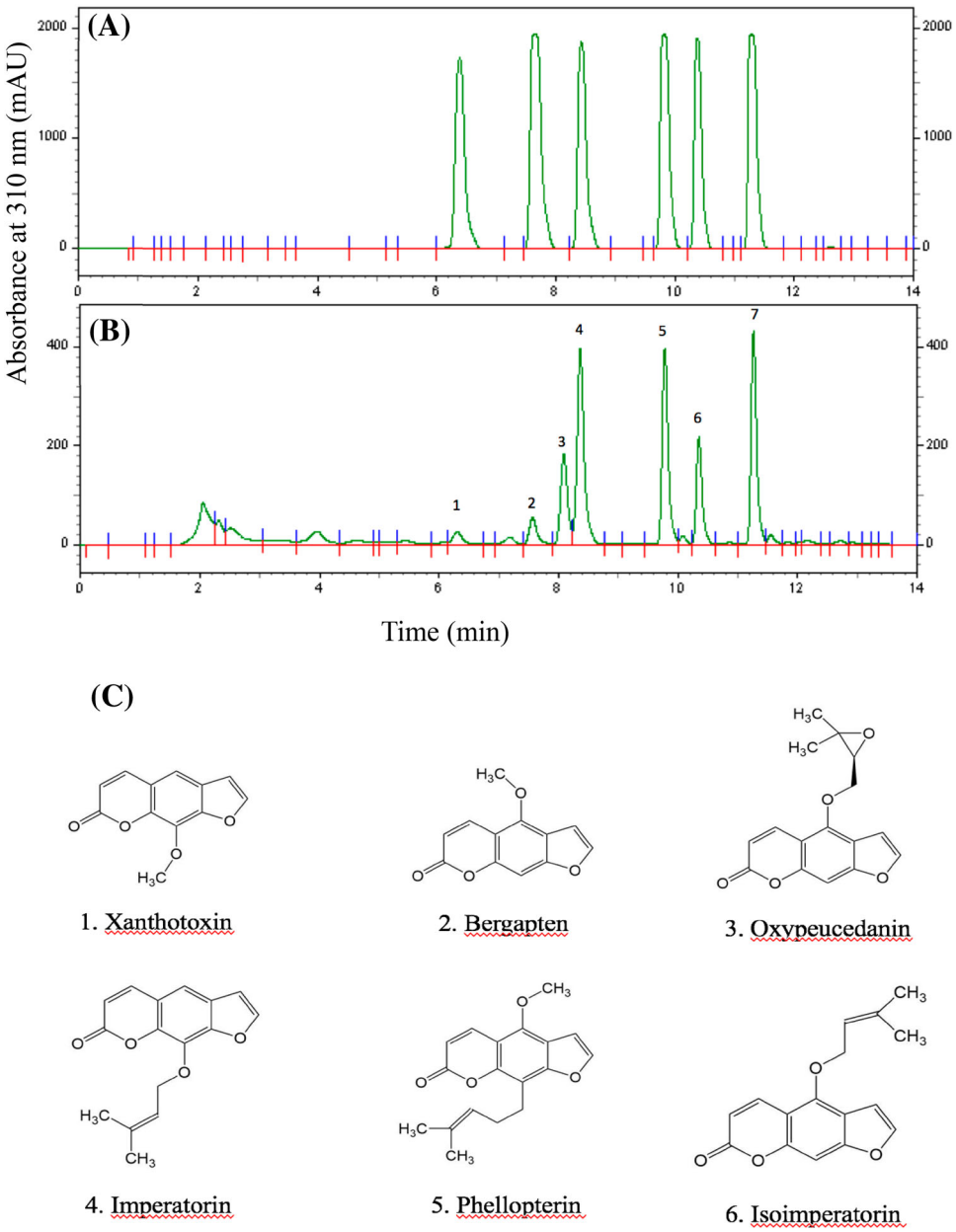

Fig. 2 HPLC–DAD chromatogram of standard (**A**) compared with Baizhi root tissue (**B**). Peaks 1, 2, 4, 5, 6 and 7 were identified as xanthotoxin, bergapten, oxypeucedanin, imperatorin, phellopterin and isoimperatorin, of which structural formula were shown respectively (**C**)

3.97 ± 0.40 mg/mL for freeze-, in-the shade, 40 and 70 °C drying, respectively (Table 2). Therefore, the active compounds for DPPH and Fe^{2+} chelating mechanisms differ, and the drying method mainly affected a DPPH radical scavenging mechanism instead of Fe^{2+} chelating.

Quality control for traditional herbs has always been a challenge because multiple factors such as harvest timing, storage condition and drying method affect the pattern of its active compound. Currently, the quality of Baizhi is evaluated by the content of imperatorin, isoimperatorin or total coumarin, which may be inaccurate according to our results. For example, Baizhi is a well-known natural-medicine cosmetic herb with skin-whitening activity with the active compounds imperatorin and isoimperatorin [7], whereas xanthotoxin has a melanogenesis-stimulation effect that may lead to skin pigmentation [43]. We found

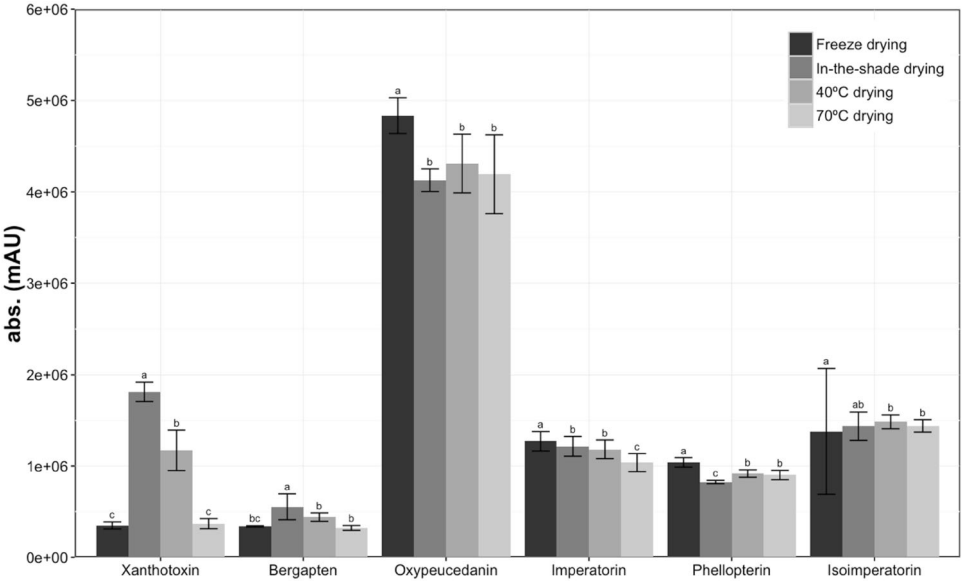

Fig. 3 Xanthotoxin, bergapten, oxypeucedanin, imperatorin, phelllopterin and isoimperatorin content in Baizhi roots dried by four methods. Roots were extracted with methanol and underwent HPLC–DAD analysis ($n = 5$). Data are mean ± SD absorbance (310 nm). Means within the same treatment followed by the same letter(s) are not significantly different at 5% level by Fisher's least significant difference (LSD) test

Table 1 Peak area proportion of furanocoumarins compounds in Baizhi roots with four drying methods

Drying methods	Xanthotoxin	Bergapten	Oxypeucedanin	Imperatorin	Phellopterin	Isoimperatorin
Freeze-dried	2.66 ± 0.21[1c]	2.59 ± 0.13[2b]	36.95 ± 1.45[a]	9.72 ± 0.53[a]	7.94 ± 0.31[a]	12.55 ± 0.64[a]
In-the-shade	10.95 ± 0.21[a]	3.31 ± 0.66[a]	24.96 ± 0.75[c]	7.33 ± 0.30[c]	4.98 ± 0.18[c]	8.67 ± 0.52[bc]
40 °C	6.77 ± 0.78[b]	2.55 ± 0.07[b]	24.22 ± 1.75[c]	6.99 ± 0.40[c]	5.34 ± 0.29[c]	8.32 ± 0.86[c]
70 °C	2.82 ± 0.33[c]	2.46 ± 0.14[b]	32.13 ± 1.42[b]	7.94 ± 0.21[b]	6.91 ± 0.52[b]	10.29 ± 2.31[b]

[1]Data are mean ± SD of 5 repeats

[2]Means within a column followed by the same letter(s) are not significantly different at 5% level by Fisher's least significant difference (LSD) test

Table 2 Antioxidant activity of Baizhi roots by four drying methods

Drying methods	Total phenolic acid content (mg/g)	DPPH radical scavenging capacity IC50 value (mg/mL)	Fe^{2+} chelating capacity IC50 value (mg/mL)
Freeze-dried	1.24 ± 0.26[1c]	23.93 ± 9.39[2b]	3.12 ± 1.05[a]
In-the-shade	2.04 ± 0.20[b]	3.94 ± 17.15[a]	3.85 ± 0.58[a]
40 °C	2.68 ± 0.20[a]	5.16 ± 14.69[a]	3.68 ± 0.69[a]
70 °C	2.02 ± 0.26[b]	8.35 ± 15.93[a]	3.97 ± 0.40[a]

Roots were extracted with methanol and underwent Folin–Ciocalteu colorimetric, DPPH and Fe^{2+} chelating assay

[1]Data are mean ± SD IC50 (mg/mL) predicted by Loess regression

[2]Means within a column followed by the same letter(s) are not significantly different at 5% level by Fisher's least significant difference (LSD) test

xanthotoxin content higher in Baizhi roots with 40 °C- and in-the-shade drying than with the other two drying methods. A similar result (high xanthotoxin) was observed by analyzing commercial Baizhi products (data not shown). Therefore, an inappropriate drying method may lead to higher xanthotoxin content and reduce the skin-whitening activity of Baizhi. Thus, in-the-shade and 40 °C drying are not good processes for drying Baizhi root. Also, the phenolic acid content and DPPH scavenging activity was

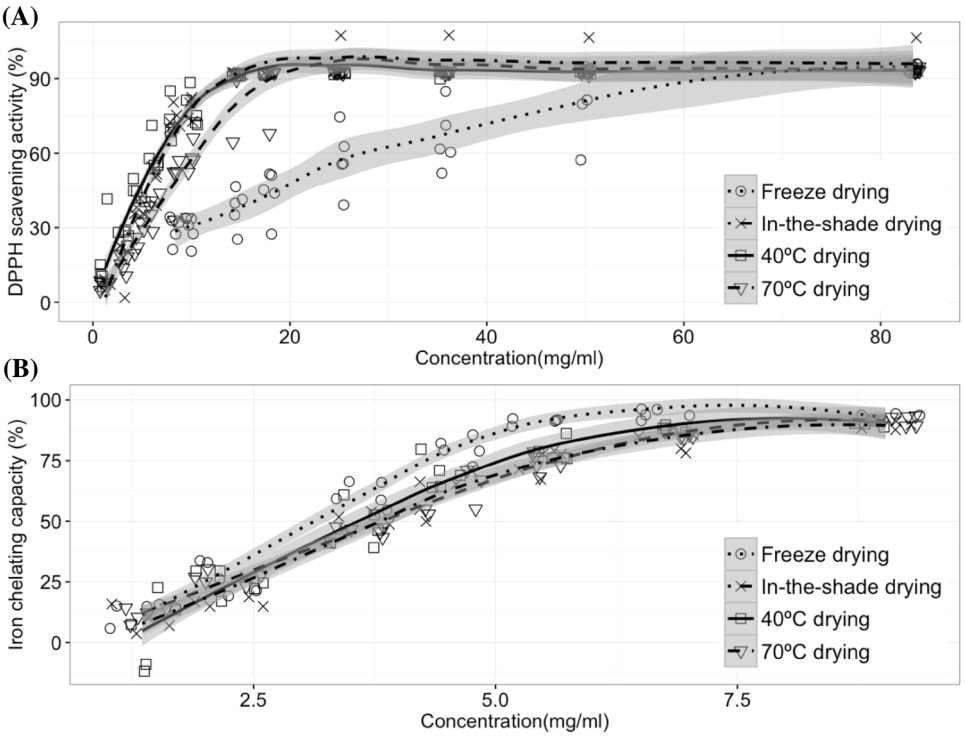

Fig. 4 Antioxidant activities of Baizhi root extracts from roots dried by four methods. Baizhi roots were extracted with methanol, and extracts were diluted or concentrated to at least seven concentrations, and underwent DPPH assay (**A**) and Fe^{2+} chelating assay (**B**)

increased in 70 °C-dried root. Hence, 70 °C drying is an appropriate method to reduce Baizhi drying costs.

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