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Improving the antioxidant and antibacterial activities of fermented *Bletilla striata* with *Fusarium avenaceum* and *Fusarium oxysporum*

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ABSTRACT

The tubers of *Bletilla striata* were fermented with *Fusarium avenaceum* and *Fusarium oxysporum*. The total phenolic contents (TPCs) and antioxidant and antibacterial activities of the extracts from the fermented products were measured. Additionally, the extracts of both *F. avenaceum* and *F. oxysporum* fermented *B. striata* (FBS) were characterized by UV–vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. The results showed that the *F. avenaceum* and *F. oxysporum* FBSs possessed higher TPCs and exhibited stronger antioxidant and antibacterial activities (*Bacillus subtilis*) in comparison with original material not co-fermented with microorganisms. Chemical characterization of the two extracts with UV–vis, FT-IR, and ^{1}H NMR implied that some of the phenolic glycosides may be deglycosylated and that bibenzyl compounds and saccharides may be metabolized in both FBS systems. Microbial fermentation enhances the TPC as well as the antioxidant and antibacterial activities of *B. striata*. The present study suggests that *F. avenaceum* and *F. oxysporum* fermentations were effective in *B. striata* processing.

1. Introduction

Bletilla striata (Orchidaceae) is a perennial herb found mainly in China, Japan, and Northern Burma at altitudes between 100 and 3200 m. The tubers of *B. striata* are widely used in East Asian countries to treat alimentary canal mucosal damage, ulcers, bleeding, bruises, and burns [1]. Phytochemical research revealed that *B. striata* contains bibenzyl [2,3], phenanthrene [4,5], dihydrophenanthrene [6,7], and diphenanthrene [8–10] compounds (Fig. 1), which are known to have various biological activities including antimicrobial [11,12], antioxidant [13,14], and anti-inflammatory activities [15]. In the Yunnan Province of China, naturally fermented *B. striata* tubers combined with honey are used as a health food to alleviate coughing. Our previous study revealed that *Helminthosporium maydis* fermentation could improve the TPC and antioxidant activities of *Bletilla formosana*, which also belongs to the genus of *Bletilla*. Notably, *Bletilla formosana* is an edible medicinal material used to treat coughs in Yunnan [16].

Herbal fermentation processing, which began approximately 4000 years ago in China, is used to produce secondary metabolites from domestic plants in bulk by utilizing the metabolic mechanisms of microorganisms. In ancient China, solid-state natural fermentation was very popular in the processing of herbal medicines, such as *Semen Sojae Praeparatum* (Dandouchi), *Massa Medicata Fermentata* (Liushenqu), *Rhizoma Pinelliae Fermentata* (Banxiaqu) and *Mass Galla chinesis et camelliae Fermentata* (Baiyaojian). In modern times, pure strain fermentation of herbal medicines has become increasingly acceptable and reliable. In recent years, fermented traditional medicine (FTM) has increasingly garnered attention in East Asia, especially in Taiwan and Korea. Some FTMs exhibit stronger biological activities or higher bioavailability in the human body compared with the original medicines. For example, Kim et al. [17] reported that pretreating fermented *Curcuma longa* L. with *Aspergillus oryzaeis* effectively prevents CCl_4-induced hepatic damage in rats. Lin et al. [18] reported that the fermentation of *Panax notoginseng* with lactic acid bacteria enhances its anti-hepatoma activity. Moreover, the major anticancer compounds formed during fermentation are protopanaxatriol and ginsenosides Rg3 and Rh1. Hsu et al. [19] reported that fermenting *Radix astragali* with *Bacillus subtilis* significantly stimulates the biosynthesis of type I procollagen in a dose-dependent manner in both aged and young human dermal fibroblast cells.

In the present study, the tubers of *B. striata* were fermented with four plant pathogenic fungi, including *Fusarium avenaceum*, *Fusa-*

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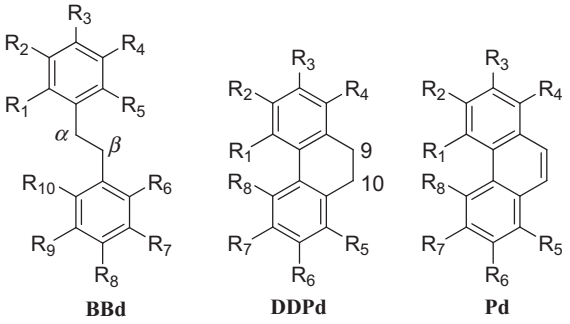

Fig. 1. The main classes of chemicals found in *B. striata*.

rium oxysporum, *Aspergillus niger*, and *Botrytis cinerea*. The TPCs and antioxidant and antibacterial activities of the acetone extracts from the four FBSs were measured. The results showed that the *F. avenaceum* and *F. oxysporum* FBSs possessed higher TPCs and exhibited stronger antioxidant activities and antibacterial activities (*Bacillus subtilis*) than the other two FBSs and the original materials. To understand the chemical changes that occur during fermentation, the *F. avenaceum* and *F. oxysporum* FBS extracts were characterized by UV–vis spectrophotometry, FT-IR spectroscopy, and NMR spectroscopy. To the best of our knowledge, this paper is the first to report the extraction of *B. striata* phenolics using a fermentation method.

2. Materials and methods

2.1. Chemicals

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ); and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from J&K Scientific Ltd. (Beijing, China). Rutin and gallic acid were purchased from Aladdin-Reagent (Shanghai, China). Dimethyl sulfoxide-d_6 (99.9 atom% D, contains 0.03% TMS) was obtained from Sigma–Aldrich (Shanghai, China). The water (resistivity $\geq 18.25\,M\Omega\,cm^{-1}$) used was purified with a purity water system (Chengdu, China). All other chemicals used were of analytical grade.

2.2. Plant material

B. Striata was collected in Shuangshao County, Kunming, Yunnan Province, China in November 2012 and authenticated by Prof. Shugang Lu of the School of Life Sciences at Yunnan University. A voucher specimen (No. YNU-BS01) has been deposited in the Key Laboratory of Medicinal Chemistry for Natural Resources at Yunnan University.

2.3. Microorganisms and fermentation

F. avenaceum (YM 3065), *F. oxysporum* (YM 3068), *A. niger* (YM 3029), and *B. cinerea* (YM 3061) were obtained from Stains Collection of Yunnan Institute of Microbiology, Yunnan Province, China.

PDA (1 L water, 200 g potato, 20 g dextrose, and 15 g agar) slant culture mediums were inoculated with the four plant pathogen fungi above and incubated in a constant temperature incubator at 28 °C for 5 days. Five grams of *B. striata* were added to a 50-mL Erlenmeyer flask to function as the fermentation culture medium. After being infiltrated with 8 mL water and sterilized at 121 °C for 30 min, the mature slants were added and incubated at 28 °C for 45 days.

2.4. Extraction

B. striata (5 g) and each of the four individual FBSs were immersed in 50 mL acetone for 24 h and ultrasonicated three times for 30 min each time. The extracting solution was decanted, filtered under vacuum and concentrated in a rotary evaporator to afford five extracts: E1 (*B. striata*, 0.4306 g), E2 (*F. avenaceum* FBS, 0.4327 g), E3 (*F. oxysporum* FBS, 0.4164 g), E4 (*A. niger* FBS, 0.8809 g), and E5 (*B. cinerea* FBS, 0.6665 g).

2.5. Antioxidant activities

2.5.1. DPPH radical-scavenging activity

The DPPH free radical-scavenging activity was estimated by the method described previously [20]. DPPH radical-scavenging activity of the sample was calculated as follows:

$$\text{Inhibition}\,(\%) = 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{sample} is the absorbance of sample solution mixed with DPPH and A_{control} is the absorbance of the blank solution (ethanol). Rutin was used as a positive control. All tests were performed in triplicate. The IC_{50} value was defined as the effective concentration at which the DPPH radical was scavenged by 50%.

2.5.2. ABTS assay

ABTS antioxidant capacity was measured using a previous ABTS method [21] that was slightly modified. The ABTS radical cation (ABTS+) solution was prepared by reacting 7 mM ABTS with 2.5 mM $K_2S_2O_8$. The mixture was incubated at room temperature in the dark for 12–16 h. The ABTS+ solution was then diluted with PBS (200 mM, pH = 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. A one-hundred microliter sample was added to 3.9 mL of ABTS+ solution and mixed vigorously. After the reaction mixture was incubated at room temperature for 6 min, the absorbance at 734 nm was recorded immediately. A standard curve was obtained using a standard solution of trolox at various concentrations in PBS. ABTS radical-scavenging activity was calculated as follows:

$$\text{Inhibition}\,(\%) = 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{sample} is the absorbance of sample solution mixed with ABTS and A_{control} is the absorbance of the blank solution (PBS). The antioxidant capacity was expressed as the trolox equivalent antioxidant capacity (TEAC), which was calculated according to the standard curve.

2.5.3. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay measures the reduction of ferric iron to the ferrous form in the presence of antioxidants. The assay protocol followed a previously reported procedure [22] that was modified slightly. The working FRAP reagent was prepared freshly by mixing 5.0 mL of TPTZ (10 mM in 40 mM HCl), 5.0 mL of $FeCl_3$ (20 mM) and 50 mL of sodium acetate buffer (300 mM, pH = 3.6). One hundred microliters of each sample was mixed with 300 μL of water and 3.0 mL of working FRAP reagent. The mixture was shaken vigorously and allowed to stand at 37 °C for 30 min. Then, the absorbance at 595 nm was recorded immediately. The antioxidant capacity of the sample was expressed as μmol ferrous sulfate per g extract (μmol FS/g Ex), which was calculated from the standard graph obtained with the ferrous sulfate solution.