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Effects of fermentation treatment on antioxidant and antimicrobial activities of four common Chinese herbal medicinal residues by *Aspergillus oryzae*

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ABSTRACT

The feasibility of using *Aspergillus oryzae* NCH 42, an extracellular tannase-producing fungus, for increasing the utilization of extraction residues of Chinese herbal medicines (CHMs) was evaluated in this study. Four types of CHMs, including *Trichosanthes kirilowii* Maxim, *Salvia miltiorrhiza* Bge, *Magnolia officinalis* and *Glycyrrhizae radix*, were used. Their aqueous extraction residues were inoculated with *A. oryzae* NCH 42, and solid-state fermentation was performed for 5 days at 30 °C with a moisture content of 86% (ratio of solid to water 1:6, w/v). The methanolic extracts of fermentation products (F&M) were examined for their functional components and properties, such as total phenolic content, antioxidant activity and tannin content. All experiments were performed using 0.1% (w/v) extracts, and the antibacterial activities of the 10% (w/v) extracts were tested against seven Gram-positive and Gram-negative bacteria, including *Bacillus cereus* BCRC 10603, *Listeria monocytogenes* BCRC 14848, *Staphylococcus aureus* BCRC 15211, *Staphylococcus aureus* BCRC 12154, *Escherichia coli* BCRC 10675, *Salmonella enterica* BCRC 10747, and *Salmonella enterica* BCRC 12948. Results showed that fermentation enhanced the release of functional ingredients from the extracts. The F&M products had higher total phenolic content and antioxidant activity than the unfermented ones, but the tannin content was lower. Moreover, the F&M products of each residue showed a wide spectrum of antibacterial activities against the seven pathogenic bacteria tested. These results revealed that solid-state fermentation using *A. oryzae* NCH 42 improves the utilization of extraction residues of CHMs.

1. Introduction

Research on Chinese herbal medicines (CHMs) is becoming increasingly popular since they are featured ingredients in many health and functional foods. Food and biotechnology industries have pursued some applications of CHMs and published research results. With the gradual establishment of regulation as in constituents with growing research developments, scientific studies on CHMs have become more popular [1].

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Microorganisms can break down and convert CHM ingredients, e.g., vitamins, into secondary metabolites [2]. Related research has shown that secondary metabolite products of CHMs could improve medical effectiveness and reduce medicinal toxicity [3]. Moreover, the bioconversion brought about by microorganisms has been shown to considerably reduce the disadvantages of traditional physical and chemical extraction methods [4].

The techniques used to manufacture fermented CHMs were usually developed as a result of a combination of microbiological studies and modern bioengineering, particularly the discovery of new treatment effects. The fermentation of CHMs has been traditionally performed in an environment similar to the natural habitat of the microorganism. However, the fermentation techniques currently adopted for the manufacture of CHM products use only one or a few types/strains of probiotics from a group of beneficial microorganisms as a starter culture. This culture is then inoculated with CHM extracts to produce fermented CHMs that contain the active ingredients, bacterial cells and other metabolites [5]. Since the 1980s, research reports on fermented CHMs have mostly been regarding single fermentations using fungal preparations such as *Ganoderma* mycelium, *Cordyceps* mycelium and *Trametes robiniophila* Murr [6]. The CHM extract was likely to be used as a substrate for mycelial fermentation. A substrate containing CHMs may affect the functionality of the original substance [6]. A study from the early 1990s showed that CHM ingredients such as sennoside could be converted into ingredients effective in the treatment of diarrhea through intestinal bacterial conversion [6]. Other studies have shown that many glycosides, flavones, flavanols, flavanones, and coumarins contained in CHMs are converted by intestinal bacteria into therapeutically effective compounds [7].

Tannins, which are the main components of drugs used in the past to treat burns, have been shown to be hepatotoxic. Furthermore, the toxicity of hydrolyzable tannins is greater than that of condensed tannins. Tannin content is therefore one of the main quality control indices for CHM products. The tannin content in tannin-containing herbal products also affects the stability and clarity of the products. The presence of tannins in CHM injections causes coagulation of blood proteins, resulting in hemorrhage. Tissue necrosis may occur at the site of multiple infections, causing aseptic inflammation, which in turn triggers a series of adverse physiological reactions. For example, some tannins are known to cause jaundice, hepatocellular necrosis and a series of related clinical manifestations while injected. Studies have shown that tannin accelerates cellular aggregation and can bind hemoglobin, resulting in sedimentation. Patients taking drugs containing tannins may show symptoms such as loss of appetite, nausea and headache [8,9].

CHMs have a widely distributed market and great future potential. However, extraction methods have been modified and improved since ancient times, and use of CHMs is still expanding, although their side effects and complicated composition are difficult to analyze. Although techniques in herbal fermentation have long been established, utilization of CHM residues (CHMRs) has been very little compared with the production per year. In the present study, the fungal strain *Aspergillus oryzae* NCH 42 and its bioconversion abilities were examined. Fermentation was carried out using the extracts of *Trichosanthes kirilowii* Maxim (*T. kirilowii*), *Salvia miltiorrhiza* Bge (*S. miltiorrhiza*), *Magnolia officinalis* (*M. officinalis*) and *Glycyrrhizae radix* (*G. radix*). The herbs are consumed at a high rate in Taiwan. Properties such as antioxidative activity, antibacterial activity and tannin content were investigated and compared before and after fermentation of the extracts. We have found that bioconversion by *A. oryzae* NCH 42 boosted the release of active ingredients and enabled greater utilization of these herbal extract residues.

2. Methods

2.1. Microorganisms and growth conditions

A. oryzae NCH 42 was previously isolated from material collected from the mountains of Nantou, Taiwan, by screening for its high tannase-producing ability in our laboratory. The working fungal strain was maintained on potato dextrose agar (Difco Laboratories Inc., Detroit, MI, USA) slants at 4 °C. A seed culture of the fungus was resuscitated by transferring one loop of spores from the potato dextrose agar slant onto a potato dextrose agar plate and incubated at 30 °C for 5 days before use. Seven food-borne pathogens were used in the antibacterial experiments. *Bacillus cereus* BCRC 10603, *Listeria monocytogenes* BCRC 14848, *Staphylococcus aureus* BCRC 15211, *S. aureus* BCRC 12154, *Escherichia coli* BCRC 10675, *Salmonella enterica* BCRC 10747 and *S. enterica* BCRC 12948. All microorganisms were purchased from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute in Hsinchu, Taiwan. They were inoculated on tryptic soy broth (Difco Laboratories Inc.,) and incubated for 12 hours at 37 °C. Stock cultures were maintained at −80 °C in tryptic soy broth containing 25% glycerine.

2.2. Preparation of CHMRs and solid-state fermentation (SSF)

Four CHMs containing *T. kirilowii*, *S. miltiorrhiza* Bge, *M. officinalis* and *G. radix* were purchased from a local Chinese medicine store in Taichung, Taiwan. CHMRs were prepared by aqueous extraction. In brief, distilled water (200 mL) was added to 5 g of each type of CHM and heated at 95 °C for 1 day. The residues were filtered using Whatman No. 1 filter paper and dried at 50 °C for 1 day. The extraction yields were about 30%. The dehydrated CHMRs were then pulverized into granular particles (40 mesh) and stored at 4 °C until further use. Erlenmeyer flasks (250 mL) containing 10 g CHMR and 60 mL water were sterilized at 121 °C for 1 hour. After sterilization, the flasks were allowed to cool to room temperature and inoculated with 1.2 mL of *A. oryzae* NCH 42 spore suspension (1×10^7 spores/mL). The spore suspension was prepared in sterilized distilled water containing 0.1% Tween 80 by scraping spores from the agar slant of *A. oryzae* NCH 42. After that, the inoculation flasks were statically incubated at 30 °C for 5 days in a humidity controlled incubator (Model-S302R; Firstek Scientific Co. Ltd., Taipei, Taiwan).

2.3. Preparation of sample extract by methanolic extraction

After fermentation, 100 mL of methanol was added to each flask and extraction was performed at room temperature with stirring for more than 24 hours. The extraction mixture was filtered through a Whatman No. 1 filter paper and concentrated in a vacuum evaporator (Model R210; Büchi Labortechnik AG, Flawil, Switzerland). The concentrated extracts (designated as F&M) were used for further analyses.

2.4. Determination of total phenolic content

Total phenolic content was determined by a previously described method [10] with modifications. In brief, a sample of each extract (0.1 g) was transferred to a test tube and mixed with 100 mL of ethanol. Aliquots of 0.4 mL of each sample extract (0.1%, w/v) and 0.4 mL of Folin–Ciocalteu reagent were mixed. After 3 minutes, 0.4 mL of 10% Na_2CO_3 was added and the reaction was allowed for 1 hour. Absorbance (ABS) was measured at 735 nm. ABS values were converted to total phenolic content and expressed as equivalents of gallic acid in micrograms per milliliter of the sample. Standard curves were established using various concentrations of gallic acid in 95% ethanol. Results were reported as averages of three experiments.

2.5. Determination of antioxidant activity

2.5.1. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) inhibition system

The effects of each sample extract at 0.1% (w/v) and the positive control (butylated hydroxytoluene, BHT) on DPPH radicals were evaluated as previously described [11]. To 0.1 mL of sample, 0.4 mL of Tris–HCl buffer (pH 7.4) and 0.5 mL of 250 mM DPPH in ethanol were added and mixed. The mixture was shaken vigorously and left to stand at room temperature for 20 minutes in the dark. The ABS of the reaction solution was measured spectrophotometrically at 517 nm. The percentage of DPPH decolorization was calculated according to the following equation:

$$\text{Scavenging activity } (\%) = \left[1 - \left(\text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}}\right)\right] \times 100$$

2.5.2. Measurement of reducing power

The reducing power of 0.1% (w/v) of each sample extract and BHT was measured as previously described [12]. Aliquots of 0.2 mL of sample and BHT were each mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes in a water bath. The resulting solution was cooled rapidly, spiked with 0.2 mL of 10% trichloroacetic acid and centrifuged at 4000 rpm for 10 minutes. The upper layer of the solution (0.6 mL) was mixed with an equal volume of distilled water and 0.1 mL of 0.1% $FeCl_3$, and ABS was measured at 700 nm. Increased ABS of the reaction was indicative of strong reducing power of the sample.

2.5.3. Measurement of ferrous ion-chelating ability

Ferrous ion-chelating ability was measured as previously described [13]. EDTA and 0.25 mL of 0.1% (w/v) of each sample extract were mixed with 0.8 mL of methanol and 0.025 mL of 2 mM $FeCl_2$. After reaction for 30 seconds, the mixture was added to 0.05 mL of 5 mM ferrozine solution and left to stand for 10 minutes in the dark. Fe^{2+}/ferrozine complex has a strong ABS at 562 nm; a high ferrous ion-chelating ability in the test sample results in a low ABS. The ferrous ion-chelating ability was calculated according to the following equation:

$$\text{Chelating ability } (\%) = \left[1 - \left(\text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}}\right)\right] \times 100$$

2.5.4. Measurement of Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity was determined by the $ABTS^+$ scavenging ability method [14,15]. Aliquots of 1.2 mL of deionized water were mixed with 0.2 mL of $ABTS^+$ (1000 μM), 0.2 mL of peroxidase (113 U/mL) and 0.2 mL of H_2O_2 (500 μM) for 1 hour at room temperature. Aliquots (0.2 mL) of each sample (0.1%, w/v) and vitamin C were then mixed in, and ABS was read at 734 nm after 10 minutes.

2.6. In vitro determination of antibacterial activity

Active cultures were prepared by transferring 0.1 mL of spore suspension from the stock culture to tryptic soy broth and incubated for 12 hours at 37 °C. Antibacterial activity was determined by the agar diffusion method [16]. In brief, 30 μL of each sample extract (0.1 g/mL in dimethyl sulfoxide) was added to a paper disc (7 mm in diameter) that was placed on Mueller–Hinton agar and inoculated with 0.1 mL of bacterial strain suspension (10^9 cfu/mL). The plates were refrigerated at 4 °C for 2 hours and then incubated at 37 °C for 24 hours. At the end of incubation, the diameters of the inhibition zones formed around the discs were measured with a caliper (mm).

2.7. Determination of residual condensed tannin content

The assay method, as described previously [17,18], was used with catechin as a standard. Each sample extract (0.2 mL) was mixed with 1.0 mL of vanillin reagent (4% vanillin/methanol:8% HCl/ methanol = 1:1), and the mixture was incubated in a water bath at 30 °C for 20 minutes. The amount of residual condensed tannins was analyzed by measuring ABS at 500 nm.

2.8. Statistical analyses

In this study, all experiments were performed in triplicate. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) for Windows was used for all statistical analyses. Differences were considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Changes in total phenolic content

The strain *A. oryzae* NCH 42 was previously isolated in our laboratory. It was identified by the sequence of the rDNA ITS1-5.8SITS2 region and morphological observations by growing on various types of media. The percentage yield for the F&M

products of *T. kirilowii*, *S. miltiorrhiza* Bge, *M. officinalis* and *G. radix* were about 11.1%, 20.6%, 18.4% and 21.4%, respectively.

S. miltiorrhiza, *M. officinalis* and *G. radix* contain natural antioxidants, which are mainly phenolic compounds. Total phenolic contents for the methanolic extracts of *S. miltiorrhiza*, *M. officinalis* bark and *T. kirilowii* peel have been reported to be 4.26, 3.15 and 0.50 g/100 g (DW), respectively [19]. Phenolic contents were quantified in other studies to be 9.68 ± 0.22 mg GAE/g for *M. officinalis* [20] and 2.13 ± 0.03 mg GAE/g for *T. kirilowii* [21].

The total phenolic contents of F&M products obtained by culturing *A. oryzae* NCH 42 with four CHMRs as substrate by SSF are shown in Fig. 1. The total phenolic contents for the F&M products of *T. kirilowii*, *S. miltiorrhiza* and *M. officinalis* were much higher than those for the unfermented extracts. After fermentation, *S. miltiorrhiza* showed the highest total phenolic content (698.97 g/100 g DW), while *M. officinalis* showed the lowest (9.18 g/100 g DW). The data demonstrated that fermentation using the fungus *Aspergillus* was necessary in order to release more total phenolic content from the extraction residues of CHMs.

3.2. Antioxidant activities

3.2.1. DPPH free radical scavenging activities

The antioxidant activities of CHM extracts, including reducing power, ferrous ion-chelating ability and total antioxidant capacity, were determined using different radical scavenging assays such as the DPPH inhibition system. A DPPH radical is scavenged by antioxidants through the donation of a proton to form reduced DPPH, and color changes can be measured at 517 nm. For example, data have revealed the presence of antioxidant compounds in fermented CHMs, and the main antioxidant component of *Monascus anka* has been found to be dimerumic acid, which has considerable DPPH free radical scavenging activity [22].

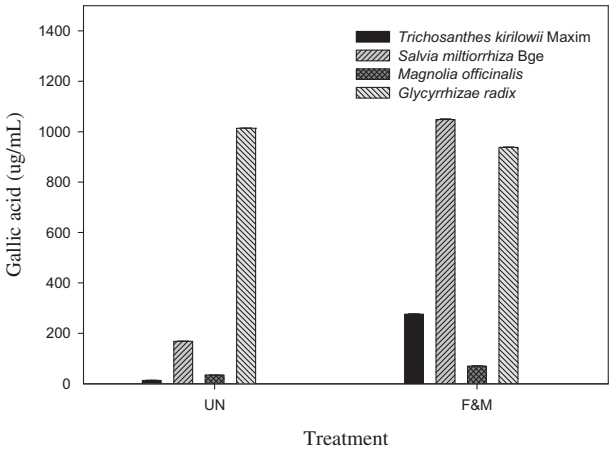

Fig. 1 – Effect of solid-state fermentation on the total phenolic content in the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs). CHMRs were produced after extracting 5 g of each Chinese herbal medicine (CHM) individually with hot water at 95 °C for 24 hours, followed by centrifugation and filtration. The residue from each CHM was dried and pulverized. Solid state fermentation was performed by inoculating *Aspergillus oryzae* NCH 42 spore suspension into a sterilized flask containing a CHMR (10 g) and 60 mL water, and incubated at 30 °C for 5 days. After fermentation, 100 mL methanol was added to the flask and the mixture was stirred for 24 hours. The mixture was filtered, and the filtrate was concentrated. UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

The DPPH free radical scavenging activities of the F&M products of the four CHMRs tested here are shown in Fig. 2. Except for *G. radix*, the scavenging activities of the F&M products of *T. kirilowii* (11.46%), *S. miltiorrhiza* (37.18%) and *M. officinalis* (85.13%) were greater than that obtained for the unfermented extracts. As a result, the F&M products of *M. officinalis* could be used as a natural antioxidant.

3.2.2. Reducing power

The F&M products from the four CHMRs showed a pattern of reducing power compared with the unfermented extracts, similar to that found for DPPH free radical scavenging activity (Fig. 3). Based on ABS at 700 nm, the F&M product of *G. radix* had reducing power (0.85) lower than that of the unfermented extract (0.92). However, the reducing powers of *T. kirilowii*, *S. miltiorrhiza* and *M. officinalis* were slightly higher after fermentation at 0.76, 0.72 and 1.82, respectively.

3.2.3. Ferrous ion-chelating ability

The ferrous ion-chelating abilities of the extracts of the four CHMRs are shown in Fig. 4. The results revealed sharp increases for the F&M products of both *S. miltiorrhiza* and *G. radix* after incubation with *A. oryzae* NCH 42. For *S. miltiorrhiza*, ferrous ion-chelating ability of the unfermented extract was 9.85% and that of the F&M product was 20.42%. For *G. radix*, ferrous ion-chelating ability was 14.0% higher for the F&M product than for the unfermented extract. After fermentation, the highest ferrous ion-chelating ability was found for *M. officinalis* (99.32%).

3.2.4. Total antioxidant capacity

Many studies have reported the total antioxidant capacities of methanolic herbal extracts. Values reported for *T. kirilowii* were 352 ± 8.1 μg/g DW [23], 1.05 ± 0.17 μmol Trolox/g [21] and 69.8 μmol Trolox/100 g DW [19]; that for *S. miltiorrhiza* root was 761.5 μmol Trolox/100 g DW [19]; and those for *M. officinalis* were 727.5 μmol Trolox/100 g DW [19] and 188.70 ± 12.01 μmol Trolox/g [20]. In this study, the total antioxidant capacities of the extracts of the four CHMRs were generally increased by SSF with *A. oryzae* NCH 42 (Fig. 5). Furthermore, for *T. kirilowii*, the total antioxidant capacities of the F&M products (0.11 Trolox, mM) were found to be nearly two-fold those of the unfermented extract (0.054 Trolox, mM). After fermentation, the highest total antioxidant capacity was found for *S. miltiorrhiza*, *M. officinalis* and *G. radix* (0.28 Trolox, mM). Previous studies have also indicated that *M. officinalis* has strong antioxidant activity [20,24].

3.3. Antibacterial activity

Several studies have shown that the biotransformation ability of microorganisms can enhance the antibacterial activity of

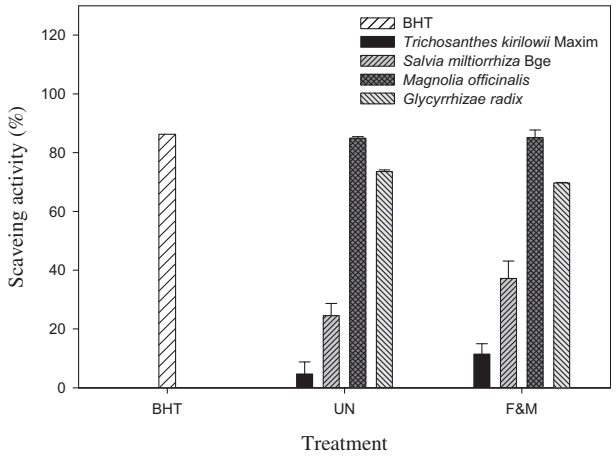

Fig. 2 – Effect of solid-state fermentation on the DPPH free radical scavenging activities of the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs) by growing *Aspergillus oryzae* NCH 42 at 30 °C for 5 days. BHT = butylated hydroxytoluene (0.1%, w/v); UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

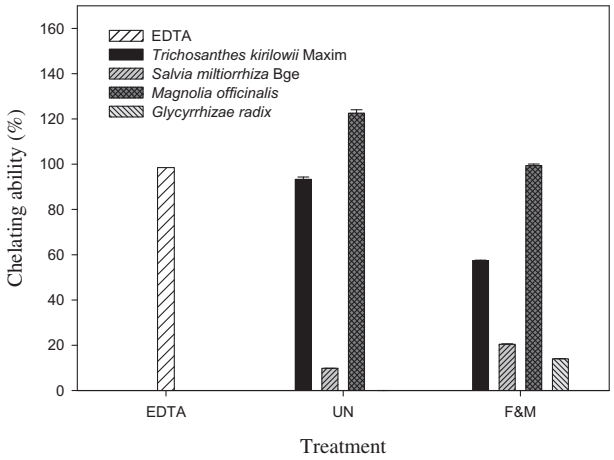

Fig. 4 – Effect of solid-state fermentation on the ferrous ion-chelating ability of the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs) by growing *Aspergillus oryzae* NCH 42 at 30 °C for 5 days. EDTA = EDTA 0.1% (w/v); UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

CHMs. The fermentation products of herbs such as lavender, lemon balm, mugwort and loquat with lactic acid bacteria (FHL) were tested for their antibacterial activity against tinea. These FHL could inhibit tinea growth at low pH values, while malonate and acetic acid produced in FHL had especially high antibacterial activity [25]. In another study, the SSF product of cranberry precipitate was used with the edible fungus *Lentinus edodes*, and its antioxidative activity and antibacterial activity against *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *E. coli* O157:H7 were studied. Gallic acid levels were increased after fermentation, accompanied by increases in antibacterial and antioxidative activities [26]. In addition, 50 types of fermented CHMs, including ginseng, American ginseng, red ginseng and *Codonopsis pilosula* with *Bacillus subtilis*, and the inhibitory effects of the fermented products on *Mycobacterium tuberculosis* and *Penicillium avellaneum* were investigated. The results showed that interaction between the microorganisms and CHMs resulted in enhanced antibacterial activity of the fermented products of certain herbs [27].

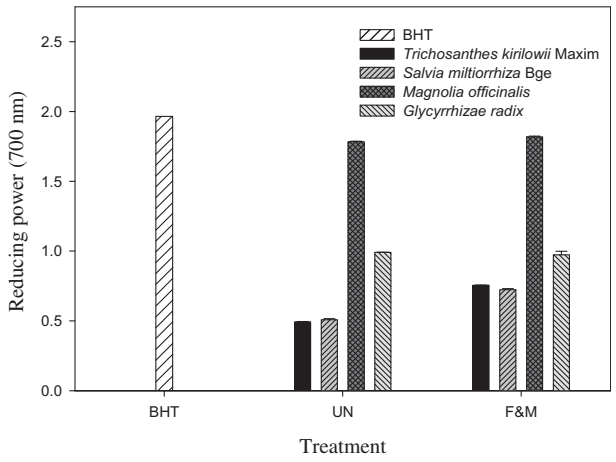

Fig. 3 – Effect of solid-state fermentation on the reducing power of the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs) by growing *Aspergillus oryzae* NCH 42 at 30 °C for 5 days. BHT = butylated hydroxytoluene (0.1%, w/v); UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

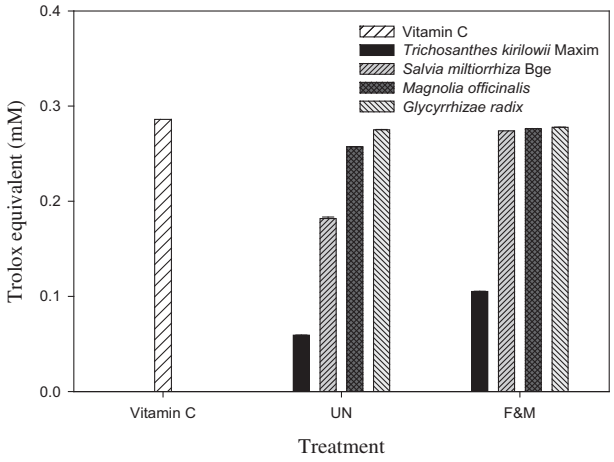

Fig. 5 – Effect of solid-state fermentation on the total antioxidant capacities of the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs) by growing *Aspergillus oryzae* NCH 42 at 30 °C for 5 days. Vitamin C = Vitamin C 0.1% (w/v); UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

Table 1 – Effect of solid-state fermentation of four Chinese herbal medicine residues by *Aspergillus oryzae* NCH 42 on the antimicrobial activities[a] of their methanolic extracts against pathogenic bacteria.

Species	Extracts	Diameter of the inhibition zone[b] (mm)						
		B. cereus BCRC 10603	*L. monocytogenes* BCRC 14848	*S. aureus* BCRC 12154	*S. aureus* BCRC 15211	*E. coli* BCRC 10675	*S. enterica* BCRC 10747	*S. enterica* BCRC 12948
Trichosanthes kirilowii Maxim	UN	9.5	8.0	7.5	7.5	—	—	—
	F&M	11.0 (H 15.0)	15.0	13.0	14.0	H 11.0	—	10.0
Salvia miltiorrhiza Bge	UN	8.0	—	13.0	10.0	—	—	15.0
	F&M	8.5	—	14.0	10.0	—	H 7.5	15.0
Magnolia officinalis	UN	15.5	16.0	14.0	16.0	—	—	13.0
	F&M	15.0	13.0	15.0	14.0	—	—	13.0
Glycyrrhizae radix	UN	13.0	8.5	13.5	12.0	—	H 7.5	12.0
	F&M	13.0	8.5	16.0	12.0	—	H 7.5	14.0

UN = methanolic extract of unfermented Chinese herbal medicine residue; F&M = methanolic extract of fermented Chinese herbal medicine residue; H = hazy zone; — = not detected.
a Antimicrobial activity was determined as described in Section 2.6. of this article.
b Inclusive of the 7-mm disc diameter.

In general, the results of the present study showed that antibacterial activities were not reduced in 10% (w/v) sample extracts of four CHMRs by SSF with *A. oryzae* NCH 42 compared with unfermented CHMRs (Table 1). *E. coli* BCRC 10675 and *S. enterica* BCRC 12948 were the most sensitive food-borne bacteria in the F&M product of *T. kirilowii* extracts. The F&M product of *S. miltiorrhiza* had greater inhibitory activity against *S. enterica* BCRC 10747 compared with the unfermented extract.

3.4. *Tannin content*

Tannins are present in a variety of plants [28]. CHM-containing liquid drinks, which have high concentrations of tannins, create a dry bitterness that hinders the consumer's acceptance. Conventional methods of removing tannins from herbal medicines include heat treatment/cold-set method, lime milk–H_2SO_4 precipitation, gelatin precipitation, polyamine

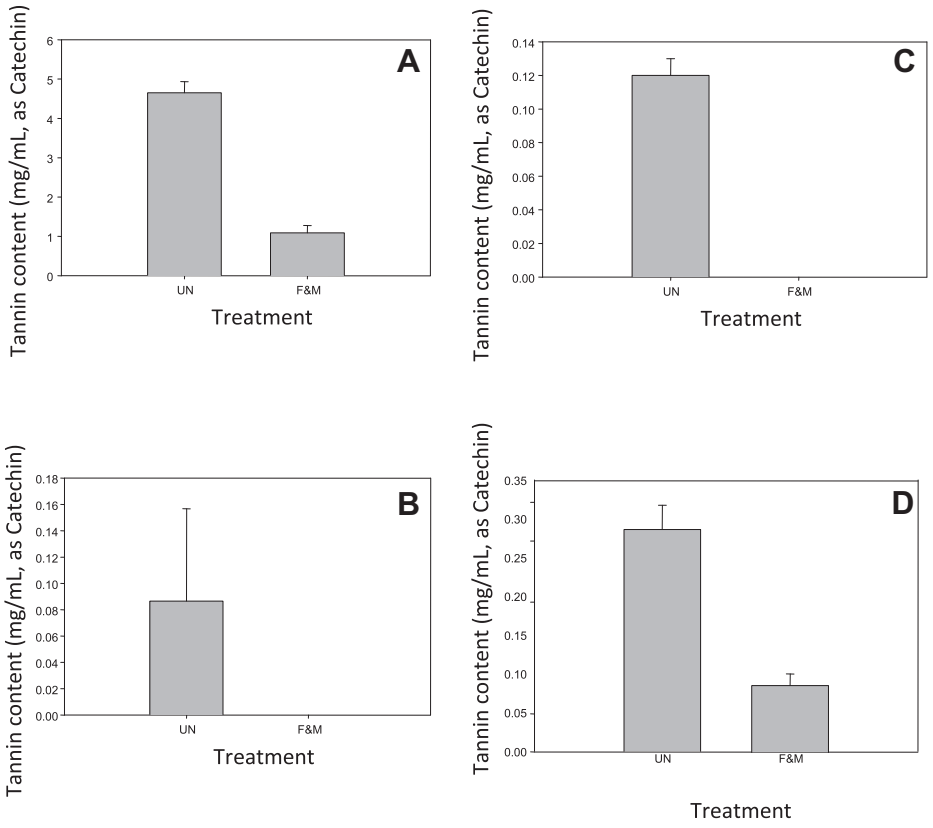

Fig. 6 – Effect of solid-state fermentation on the tannin contents in the methanolic extracts (0.1%, w/v) of four Chinese herbal medicine residues (CHMRs) by growing *Aspergillus oryzae* NCH 42 at 30 °C for 5 days. (A) *T. kirilowii* Maxim; (B) *S. miltiorrhiza* Bge; (C) *M. officinalis*; (D) *G. radix*. UN = methanolic extract of unfermented CHMR; F&M = methanolic extract of fermented CHMR.

adhesion, acidic or alkaline ethanol precipitation and lead salt precipitation. All of these methods have shortcomings because of poor selectivity and removal of large quantities of active ingredients from the herbs together with the tannins, while other methods may introduce impurities that impair the quality of the product [29].

The effects of SSF with *A. oryzae* NCH 42 on the tannin contents of the four CHMRs are shown in Fig. 6. The tannin contents of all four CHMRs were reduced after SSF with *A. oryzae* NCH 42. Reduction of tannins is beneficial because its significant protein binding ability is decreased, thus preventing the aggregation of proteins [30]. Although *A. oryzae* NCH 42 was originally screened for its high extracellular tannase-producing ability, tannase (tannin acyl hydrolase, EC 3.1.1.20) catalyzes the hydrolysis of ester and depside bonds present in hydrolyzable tannins, but not condensed tannins, accompanied by liberation of glucose [31]. Therefore, the reduction of tannins by *A. oryzae* NCH 42 here was probably due to enzymatic systems other than tannase. In fact, Lewis and Starkey [32] reported that condensed tannin such as wattle tannin could be degraded, with a slower rate as compared to hydrolyzable tannins, by the soil microorganism *Aspergillus fumigatus*.

4. Conclusion

Hot water or alcohols are commonly used as solvents in extraction methods. CHM extraction techniques have been developed in the past 10 years. Using various solvents and related media, extraction of functional ingredients from CHMs has been made possible. However, limitations have been encountered, such as: (1) low extraction rates of functional ingredients, (2) loss of nutrients, (3) difficulty in removing residual organic solvents, (4) problem of disposing of mass waste after extraction, and (5) problem of recycling organic solvents [33]. To improve the functionality and utility of CHM extracts, recent research has focused on the idea of bioconversion [33].

The results of this study showed that more functional ingredients were released from each residue after biotransformation by *A. oryzae* NCH 42. Compared with unfermented extracts, the total phenolic contents and antioxidant activities of F&M products increased while tannin contents were reduced. Moreover, the F&M products of each residue showed a wide spectrum of antibacterial activities against seven pathogenic bacteria. The results indicated that the F&M products of *T. kirilowii* and *S. miltiorrhiza* increased by 82.15% and 9.83%, respectively. Thus, fermentation of CHMRs with *A. oryzae* NCH 42 may improve the utilization of CHMs, enabling us to reduce the production cost of herbal drugs and to develop a new research sector of CHM fermentation in the medicinal and food industries.

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