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## Original Article

# Chemical quality evaluation of *Antrodia cinnamomea* fruiting bodies using phytomics similarity index analysis



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## ABSTRACT

The fruiting body of *Antrodia cinnamomea* is used as a medicinal mushroom in Taiwan and is found on the inner cavity of the endemic species *Cinnamomum kanehirai*. In this study, phytomics similarity index (PSI) analysis was employed for the chemical quality evaluation of the *A. cinnamomea* fruiting bodies from different strains, and grown on various substrates. The results indicated that the different types of *A. cinnamomea* fruiting bodies contain eight index compounds, and that it was difficult to discriminate between them solely on the basis of those index compounds. In our research, we used PSI scores to assess the metabolite similarity of the fruiting bodies of *A. cinnamomea*. It was revealed that fruiting bodies from various *A. cinnamomea* strains grown on different culture substrates produce distinct PSI scores. We concluded that PSI analysis had good selectivity on the different types of *A. cinnamomea* fruiting bodies.

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## 1. Introduction

*Antrodia cinnamomea* is used as a medicinal mushroom in Taiwan and is found on the inner cavity of the endemic species *Cinnamomum kanehirai* [1]. The fruiting body of *A. cinnamomea* from wood is expensive and rarely found in nature because of its slow growth rate and the limited availability of

*C. kanehirai* trees. The selling price of *A. cinnamomea* fruiting bodies is > US\$10,000 per kg in Taiwan [2]. The fruiting bodies of *A. cinnamomea* have been used as a folk remedy for the prevention and treatment of various diseases, including liver disease, food intoxication, drug intoxication, hypertension, and cancer [1]. Recent studies indicated that there are multiple methods that can be used to culture the fruiting bodies of *A. cinnamomea*, including cultivation of *A. cinnamomea* on tree

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species other than *C. kanehirai* [1] and dish culture on artificial agar media [3,4]. These types of fruiting bodies have been promoted in the market of Asian countries.

The hundreds of characteristic phytochemicals found in *A. cinnamomea* extracts pose a challenge to the development of robust quality control metrics. While the current standards for the quality control of fruiting bodies and mycelium of *A. cinnamomea* involve quantization of a few chemical marker compounds using high-performance liquid chromatography (HPLC) coupled with diode array detection and mass spectrometry (MS) [5–9], other approaches use complete fingerprint patterns to characterize the multi-chemical Chinese medicine more thoroughly [10–12]. According to one study [13], > 80 compounds have been identified in the fruiting body of *A. cinnamomea*. However, it was difficult to analyze every potential phytochemical class of molecules. A recent study indicated that the fruiting body extract of *A. cinnamomea* exhibited similar HPLC profiles from different wood-cultured and dish-cultured samples [13]. This demonstrated that it was difficult to evaluate the *A. cinnamomea* samples from only a small number of index compounds. Thus, a similarity analysis would be a useful tool for the evaluation of the metabolite profiles of *A. cinnamomea*. Phytomics similarity index (PSI) analysis is a methodology of chemical analysis used to evaluate the similarity of metabolites, and can be used to evaluate the chemical fingerprints of Chinese medicine [14–16]. Several strains (3 red strains and 1 white strain) of *A. cinnamomea* have been successfully cultured, and these strains of *A. cinnamomea* which were grown on different substrates have been collected in our laboratory. Distinguishing between the white and red *A. cinnamomea* strains solely on the basis of their index compounds was challenging, and there were also no significant differences between the index compounds of bodies from the various wood substrates. In this study, we use a PSI to evaluate the metabolite similarity of *A. cinnamomea* produced from different strains and grown on a variety of substrates.

## 2. Materials and methods

### 2.1. *A. cinnamomea* fruiting bodies

The *A. cinnamomea* fruiting bodies used in this study are shown in Fig. 1. The strains BCRC 35398, AC-R02, AC-R06, and AC-W01 were grown on *Cinnamomum kanehirai* wood for 18 months and cultured by Joben Bio-Medical Co. Ltd., Ping-Tung, Taiwan. The strain BCRC 35398 was obtained from the Bioresource Collection and Research Center (BCRC) at the Food Industry Research and Development Institute (Hsin-chu, Taiwan). The samples AC-CK, AC-CC, and AC-CL were the fruiting bodies of *A. cinnamomea* grown for 18 months on *Cinnamomum camphora* (CC), *Cunninghamia konishii* (CK), and *Cunninghamia lanceolata* (CL), respectively. The *A. cinnamomea* dish culture (AC-DC) was grown on malt extract agar for 3 months. These four samples were obtained commercially from the market. The strains of *A. cinnamomea* were confirmed by the polymerase chain reaction fragments of the internal transcribed spacers (ITS) of their ribosomal RNA genes.

### 2.2. Preparation of *A. cinnamomea* fruiting body ethanol extract

Fruiting bodies of *A. cinnamomea* (approximately 2 g) were soaked in 20 mL of ethanol for 3 days. The sample was filtered using filter paper (Advantec No. 1, Tokyo, Japan) and the residue was further extracted under the same conditions twice. The filtrates collected from the three extractions were combined and evaporated to dryness under vacuum. Five milligrams of ethanol extract was dissolved in 1 mL of methanol for analysis.

### 2.3. HPLC and electrospray ionization-mass spectrometry analysis

The *A. cinnamomea* ethanol extract was further separated by HPLC using a Thermo HPLC system equipped with a UV detector. The analytical column was an Eclipse XDB-C18 (4.6 × 150 mm, 5 μm particle size, Agilent Tech.). Gradient elution was performed with a mobile phase of 0.1% formic acid aqueous solution (A) and acetonitrile (B). The gradient elution profile was as follows: 0–3 minutes, A:B = 70:30 to A:B = 60:40 (linear gradient); 3–15 minutes, A:B = 60:40 to A:B = 42:58 (linear gradient); 15–21 minutes, A:B = 42:58 (isocratic); 21–26 minutes, A:B = 42:58 to A:B = 35:65 (linear gradient); 26–35 minutes, A:B = 35:65 to A:B = 0:100 (linear gradient); and 35–50 minutes, A:B = 0:100 (isocratic). The flow rate was 0.8 mL/min and the photo diode array detector wavelength was set at 254 nm [6]. The electrospray ionization-mass spectrometry (ESI-MS) analysis was carried out using a Thermo LCQ FLEET (Thermo Fisher Scientific, Waltham, MA, USA) in the negative ion mode and a scan range of 200–2000 m/z.

### 2.4. PSI method

The PSI is a statistical method that compares the fingerprint patterns by computing a correlation value from the ratio of the N peaks of data computed for each of the N data points with each of the other (N-1) data points. The ratio information is incorporated into the analysis as it provides relative information between various peak intensities reflecting the importance of the balance of the compound amounts. The integrated ion counts for each of the N peaks (retention time) are extracted from the overall spectra of two different samples. A total of N(N-1)/2 unique nondiagonal elements describe the full set of intensity ratio information between all of the peaks with each peak contributing (N-1) ratios. The PSI score would range from -1.0 (perfect anticorrelation) to 0.0 (complete dissimilarity) to 1.0 (complete identity). According to one study [14], highly similar batches tend to have PSI values > 0.85, and batches with low similarity tend to have PSI values < 0.75. The PSI method was employed in conjunction with tools for filtering and sorting the peaks in the HPLC data, acquired at 254 nm. Similarity analysis was performed using the professional software Similarity Evaluation System (SES) for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was recommended by the State Food and Drug Administration (SFDA) of China. Each chromatogram was exported from Chem Quest 4.1 software (Thermo Electron Corporation, Altrincham, UK) as \*AIA

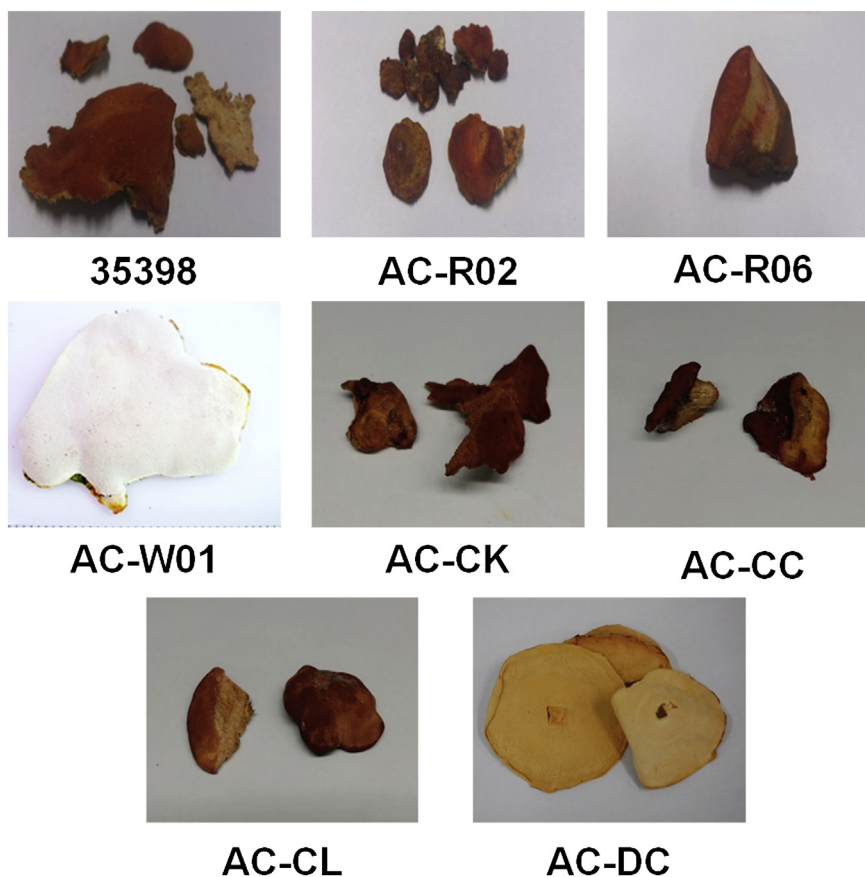


Fig. 1 – Morphological observations of *Antrodia cinnamomea* fruiting bodies in this study.

format, which was then imported into SES. The mean chromatogram was generated, and the similarity values were calculated after multipoint correction was performed.

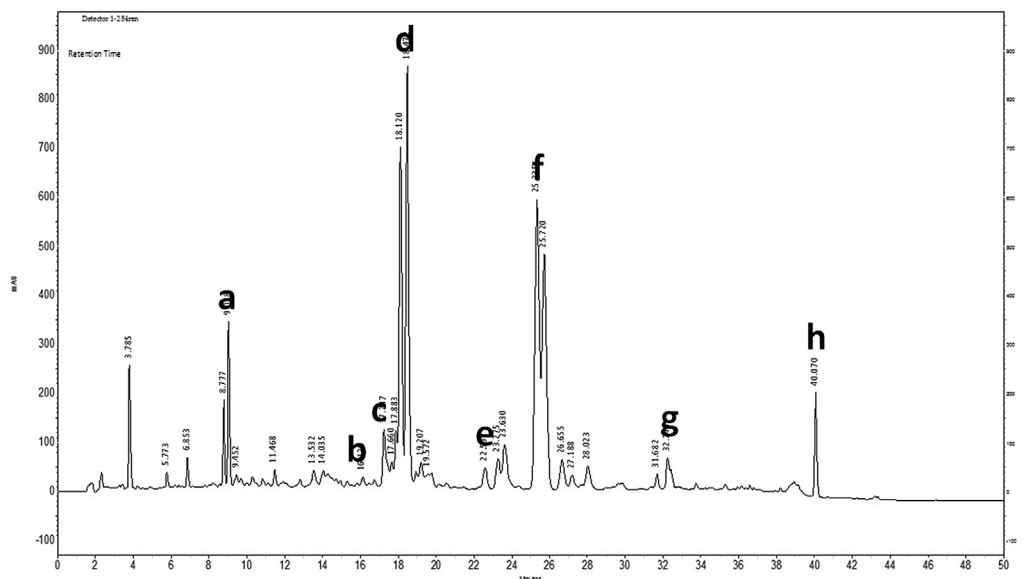
### 3. Results and discussions

#### 3.1. HPLC-MS chromatography of index compounds in *A. cinnamomea* BCRC 35398 fruiting bodies

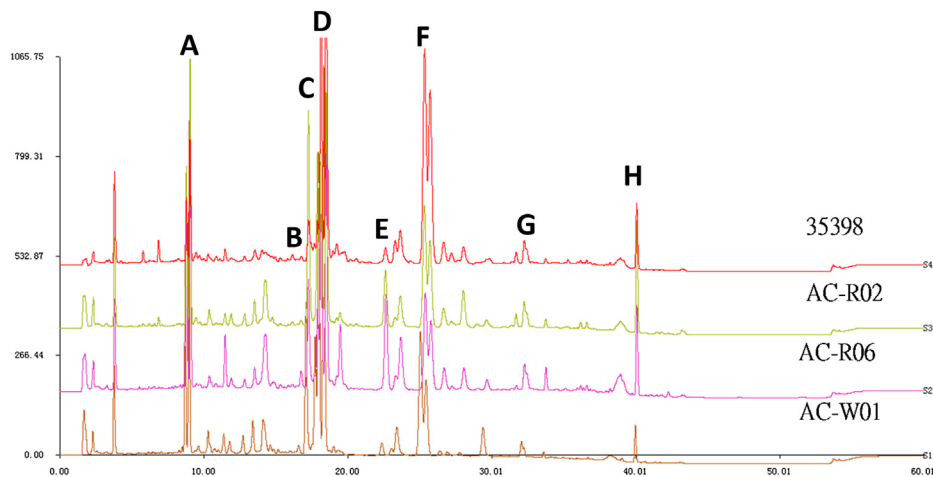
Eight samples of *A. cinnamomea* fruiting bodies were analyzed in this study. We used the strain BCRC 35398 as the standard fruiting body, and we used eight index compounds to profile metabolite composition in *A. cinnamomea* fruiting body extracts from strain BCRC 35398 (Fig. 2). The compounds are represented by the eight main chromatographic peaks (peaks A–H) and were confirmed by MS analysis. The index compounds [6,17] were: (A) (R,S)-antcin K, (B) 1,4-dimethoxy-2,3-methylenedioxy-5-methylbenzene, (C) (R,S)-antcin C, (D) (R,S)-antcin H, (E) dehydrosulfurenic acid, (F) (R,S)-antcin B, (G) (R,S)-antcin A, and (H) dehydroeburicoic acid. To investigate the metabolite profiles of the various strains and the samples obtained from different growth substrates, the HPLC fingerprint of the fruiting body ethanol extract of *A. cinnamomea* BCRC 35398 was used as the standard to compare the fingerprints of different fruiting bodies by phytochemical similarity analysis in the following experiments.

#### 3.2. Phytochemical similarity index analysis of ethanol extracts of the fruiting bodies of different *A. cinnamomea* strains

Four different strains of *A. cinnamomea* fruiting bodies were analyzed by PSI analysis: three of them were red strains (35398, AC-R02, AC-R06) and one was a white strain (AC-W01). Metabolite profiles for the four *A. cinnamomea* strains grown on *Cinnamomum kanehirai* (the original host) and harvested at a culture age of 18 months are displayed in Fig. 3. The eight index compounds were detected in these four strains. We then compared the metabolite fingerprint by PSI analysis, and the PSI values are presented in Table 1. Comparative analysis of the different *A. cinnamomea* strains revealed PSI values between 0.55 and 0.94. The PSI values between the three *A. cinnamomea* red strains (35398, AC-R02, and AC-R06) range from 0.81 to 0.94, and the PSI values between the *A. cinnamomea* white strain (AC-W01) and the three *A. cinnamomea* red strains range from 0.55 to 0.74. The data sets yielded high PSI values (> 0.8) between the three different red *A. cinnamomea* strains and lower PSI values between the three *A. cinnamomea* red strains and the one white strain. The *A. cinnamomea* red strains showed highly similar fingerprints, while the fingerprint of the white strain displayed low similarity to the fingerprints of the three red strains. Although all four *A. cinnamomea* strains contained the eight index compounds, the results indicated that PSI analysis can indeed be used to distinguish between



**Fig. 2** – HPLC chemical fingerprint of ethanol extract of *A. cinnamomea* fruiting body strain BCRC 35398. The index compounds are A: (R, S)-Antcin K. B: 1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene. C:(R,S) -Antcin C. D:(R,S)- Antcin H. E: Dehydrosulfurenic acid. F: (R,S)-Antcin B. G: (R,S)-Antcin A. H: Dehydroeburicoic acid.



**Fig. 3** – HPLC chemical fingerprint of ethanol extract of fruiting bodies of different *A. cinnamomea* strains. The index compounds are A: (R, S)-Antcin K. B: 1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene. C:(R,S) -Antcin C. D:(R,S)- Antcin H. E: Dehydrosulfurenic acid. F: (R,S)-Antcin B. G: (R,S)-Antcin A. H: Dehydroeburicoic acid.

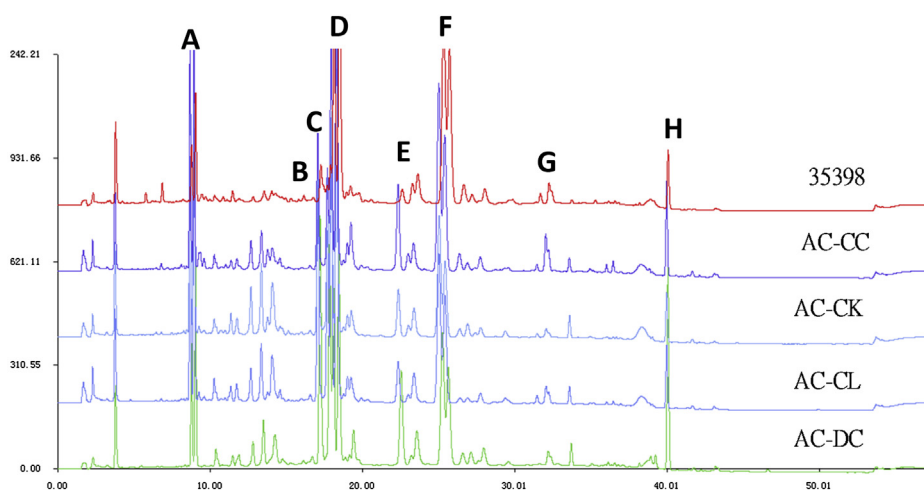
the different *A. cinnamomea* strains based on their PSI values. The results also demonstrated that the PSI values can be used to differentiate between the red and white strains of *A. cinnamomea* despite the similarity in the metabolite fingerprints.

**Table 1** – Phytonomics similarity index (PSI) values for the chemical fingerprints of ethanol extracts of fruiting bodies of different *Antrodia cinnamomea* strains.

	35398	AC-R02	AC-R06	AC-W01
35398	1	0.82	0.81	0.55
AC-R02		1	0.94	0.74
AC-R06			1	0.68
AC-W01				1

### 3.3. Phytonomics similarity index analysis of ethanol extracts of *A. cinnamomea* fruiting bodies grown on different substrates

According to one study [6], culture substrates also influence the metabolites produced. The fruiting bodies of the same *A. cinnamomea* strain grown for 18 months on *Cinnamomum camphora* (CC) wood, *Cunninghamia konishii* (CK) wood, and *Cunninghamia lanceolata* (CL) wood were analyzed in this study. We also cultured a different strain on malt extract agar dish culture (DC) for 3 months to compare the metabolite fingerprints. The metabolite fingerprints of the four *A. cinnamomea* fruiting body samples (AC-CC, AC-CK, AC-CL, and AC-DC) are displayed in Fig. 4, and the PSI analysis is shown in Table 2.



**Fig. 4** – HPLC chemical fingerprint of ethanol extract of *A. cinnamomea* fruiting bodies grown on *Cinnamomum kanehirai* wood (35398), *Cinnamomum camphora* (CC) wood, *Cunninghamia konishii* wood (CK), *Cunninghamia lanceolata* (CL) wood, and dish culture (DC). The index compounds are A: (R, S)-Antcin K. B: 1,4-Dimethoxy-2,3-methylenedioxy-5-methylbenzene. C:(R,S) -Antcin C. D:(R,S)- Antcin H. E: Dehydrosulfurenic acid. F: (R,S)-Antcin B. G: (R,S)-Antcin A. H: Dehydroeburicoic acid.

The eight index compounds were detected, and comparative analysis of *A. cinnamomea* grown on different substrates yielded values of PSI between 0.48 and 0.99. The PSI values between BCRC 35398 and *A. cinnamomea* grown on three different wood substrates (AC-CC, AC-CK, and AC-CL) range from 0.48 to 0.54, indicating that *A. cinnamomea* grown on the original host wood produced metabolites of low similarity to *A. cinnamomea* cultured on the other wood species. The PSI value between BCRC 35398 and AC-DC was 0.79. These results indicated that *A. cinnamomea* grown on different substrates produce distinct chemical fingerprints and can be distinguished by PSI analysis.

According to previous studies [1,13], > 80 compounds have been isolated from the fruiting body and mycelium of *A. cinnamomea*, and there are many techniques that can be used to culture the fruiting body of *A. cinnamomea*. Evaluation of the chemical fingerprints of *A. cinnamomea* fruiting bodies grown on different substrates is an important topic, and discrimination solely by index compounds was a challenging task. Thus, in our research, we used PSI analysis to assess the quality of the fruiting body of *A. cinnamomea*, and found that the PSI values can evaluate the chemical fingerprint similarity of the ethanol extracts of different *A. cinnamomea* fruiting

bodies. The present study suggests that fruiting bodies with chemical fingerprint PSI scores > 0.80 are likely to be highly similar chemically [14].

#### 4. Conclusion

According to previous studies, HPLC fingerprint analysis with PSI methods was used for the purposes of quality evaluation of *Polygoni Perfoliati Herba* [18], *Panax notoginseng* [16], and *Salvia yunnanensis* roots [15]. PSI analysis was used to evaluate the ethanol extracts of different *A. cinnamomea* fruiting bodies in this study. This method was successfully applied to evaluate the metabolite similarity of fruiting bodies from different *A. cinnamomea* strains grown on various culture substrates. The results of the study showed that PSI analysis had good selectivity on the different kinds of *A. cinnamomea* fruiting bodies. Moreover, PSI analysis is convenient for proper clinical use and pharmacological investigation of *A. cinnamomea*. Future study on PSI analysis will include analyzing the data of multiplexed chemical fingerprints and biological response to identify the bioactivity subset of the metabolites, and to use PSI values to combine chemical and biological information.

#### Conflicts of interest

All contributing authors declare no conflicts of interest.

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**Table 2** – Phytomics similarity index (PSI) values for the chemical fingerprints of ethanol extract of *Antrpedia cinnamomea* fruiting body grown on *Cinnamomum kanehirai* wood (35398), *Cinnamomum camphora* (CC) wood, *Cunninghamia konishii* (CK) wood, *Cunninghamia lanceolata* (CL) wood, and dish culture (DC).

	35398	AC-CC	AC-CK	AC-CL	AC-DC
35398	1	0.54	0.48	0.52	0.79
AC-CC		1	0.94	0.97	0.60
AC-CK			1	0.99	0.57
AC-CL				1	0.59
AC-DC					1

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