Metabolite Profiling and Comparison of Bioactivity in *Antrodia cinnamomea* and *Antrodia salmonea* Fruiting Bodies

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**Abstract**

*Antrodia cinnamomea* is a precious edible mushroom endemic to Taiwan that has been claimed to have significant health promotion activities. *Antrodia salmonea* is a new species of the genus *Antrodia*. In this study, we compared the metabolites and bioactivity of *A. cinnamomea* and *A. salmonea* fruiting bodies. The volatiles of *A. cinnamomea* and *A. salmonea* were characterized and 3,4,5-trimethoxybenzaldehyde was found to be the most abundant compound in *A. cinnamomea*; the other abundant compounds were δ-guaiane, isolongifolene, 1-octen-3-ol, 4-terpinenol, α-guaiane, and p-cymene. In *A. salmonea*, the main volatiles were α-cedrene, 1-octen-3-ol, D-limonene, cadinadiene, germacrene D, isolongifolene, and α-muurolene. Furthermore, five ergostane-type triterpenoids and two lanostane-type triterpenoids were selected as index compounds characterizing *A. cinnamomea* and *A. salmonea* extracts. The content of each compound varied between the two species. (R,S)-antcin B was the most abundant compound in *A. cinnamomea* fruiting bodies (75.18 ± 0.11 µg/mg). However, (R,S)-antcin C (184.85 ± 0.96 µg/mg) was the major triterpenoid in the *A. salmonea* fruiting body. Furthermore, two compounds, antcin M and methyl antcin K, were only present in the *A. salmonea* fingerprint; therefore, antcin M and methyl antcin K may be important for distinguishing between *A. cinnamomea* and *A. salmonea* fruiting bodies. Finally, examination of anti-inflammation activity and cytotoxicity showed that *A. salmonea* had more anti-inflammatory activity than *A. cinnamomea*; however, *A. salmonea* was more cytotoxic than *A. cinnamomea*. In conclusion, the composition and bioactivity of the fruiting bodies of *A. cinnamomea* and *A. salmonea* varies. Therefore, it is recommended that further toxicological evaluation and investigation of the biological activity of *A. salmonea* is carried out to ensure its safe and efficacious use as an alternative to *A. cinnamomea*.

**Abbreviations**

- DMEM: Dulbecco’s modified Eagle medium
- FBS: fetal bovine serum
- GC/MS: gas chromatography/mass spectrometry
- HPLC: high-performance liquid chromatography
- LPS: lipopolysaccharide
- MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
- NMR: nuclear magnetic resonance
- NO: nitric oxide
- SPME: solid-phase microextraction
- UPLC-MS: ultra-performance liquid chromatography-mass spectrometry

**Introduction**

*Antrodia cinnamomea* (syn. *Antrodia camphorata* and *Taiwanofungus camphoratus*) is a precious edible mushroom that has long been the most highly valued medicinal fungus in Taiwan. Traditionally, *A. cinnamomea* has been used as a folk remedy for various diseases including cancer, hypertension, abdominal pains, and diarrhea [1]. The extract of *A. cinnamomea* has also been used as a food intoxicant since antiquity [2]. Owing to its perceived efficacy, *A. cinnamomea* dry fruiting bodies are sold at prices exceeding US$15,000 per kilogram in the local market in Taiwan, and the total market value of *A. cinnamomea* products, including raw fruiting bodies and health foods, is estimated to be over US$100 million per year [3]. One of the most important reasons for the high price of...
A. cinnamomea is a new species of the genus Antrodia, which was originally described in the indigenous coniferous tree Cunninghamia konishii Hayata (Cupressaceae) [4]. This mushroom is similar to A. cinnamomea. Both A. salmonoea and A. cinnamomea have a strong bitter taste; however, the color of A. cinnamomea is cardinal red whereas A. salmonoea is salmon-pink, and it is often easy to confuse the two species. Because of the difficulty in producing A. cinnamomea, as well as its high market price, A. salmonoea is often used as a substitute for A. cinnamomea in the marketplace. So far, more than 250 scientific papers have been published on A. cinnamomea [1,5–7], but literature on A. salmonoea is rare. To our knowledge, around ten research articles have been published on A. salmonoea to date. For the development of functional foods or phytomedicines, quality control, efficacy approbation, and safety are the three most important requirements. Since A. salmonoea now is commonly used as a substitute for A. cinnamomea, verification of the chemical ingredients and bioactivity of A. cinnamomea and A. salmonoea is an urgent issue. In the present study, the chemical compositions of A. salmonoea and A. cinnamomea were analyzed by using SPME-GC/MS and HPLC profiling, and the cytotoxicity and anti-inflammatory activity of these two mushrooms were also studied.

Results and Discussion

Odor is a significant distinguishing characteristic of the fruiting bodies of A. cinnamomea and A. salmonoea. The volatile compounds of A. cinnamomea and A. salmonoea were collected by using SPME to obtain the volatiles, and analysis was done by GC/MS. Table 1 shows the compositions of volatile compounds emitted from A. cinnamomea and A. salmonoea fruiting bodies. In the A. salmonoea fruiting body, 3,4,5-trimethoxybenzaldehyde (30.04%) was the most abundant compound, followed by δ-guaiane (9.88%), isolongifolene (8.00%), 1-octen-3-ol (7.08%), 4-terpineol (5.99%), α-guaiane (5.99%), and p-cymene (4.96%). In contrast, the main compositions of volatiles of A. salmonoea were α-cedrene (14.68%), 1-octen-3-ol (9.31%), D-limonene (9.21%), cadinadiene (7.65%), germacrene D (7.22%), isolongifolene (6.72%), and α-muurolene (5.31%). To date, more than 80 compounds have been identified from A. cinnamomea, including triterpenoids, benzolics, and polyacetylenes [5]. In our previous study, we selected 13 index compounds to establish a comprehensive profile of the ethanol extract of A. cinnamomea fruiting body [8]. Triterpenoids in both the mycelium and fruiting body are now considered to be the most biologically active components of A. cinnamomea [9]. Thus, five ergostane-type triterpenoids, i.e., (R,S)-antcin K, (R,S)-antcin C, (R,S)-antcin H, (R,S)-antcin B, and (R,S)-antcin A, as well as two lanostane-type triterpenoids, dehydrodiphenurenic acid and dehydroeburicoic acid, were used as the index compounds to characterize the A. cinnamomea and A. salmonoea extracts in this study. The structure and MS analysis data are shown in Fig. 2 and Table 2. Compounds a to g in Fig. 2 were then used as index compounds to profile metabolites in A. cinnamomea and A. salmonoea extracts. Fig. 3 shows the HPLC metabolite profiles for A. cinnamomea (Fig. 3A) and A. salmonoea extracts (Fig. 3B).
Some of the compounds, including (R,S)-antcin K, (R,S)-antcin C, (R,S)-antcin H, and (R,S)-antcin B, showed two peaks, for R and S configurations, as these compounds contain a chiral center at the C25 position. We were not able to characterize the absolute configuration for this group of compounds directly by NMR; so further R/S derivatization will be needed to determine their absolute configuration. Thus, both R-form and S-form triterpenoids were treated as the same compound in this study. To measure the content of the index compounds in A. cinnamomea and A. salmonea samples, calibration curves of the index compounds were established using five dilution standards from 10 to 1000 µg/mL. The contents of eight index compounds were determined by the peak area in the HPLC profile and calculated by using the calibration curves of index compounds (purity > 99.5%; Table 3). The ergostane-type triterpenoid (R,S)-antcin B was the most abundant compound in the A. cinnamomea fruiting body (75.18 ± 0.11 µg/mg) followed by (R,S)-antcin H (48.77 ± 0.31 µg/mg), (R,S)-antcin C (43.36 ± 0.76 µg/mg), and (R,S)-antcin A (19.86 ± 0.12 µg/mg). However, (R,S)-antcin C was the dominant triterpenoid in the A. salmonea fruiting body with the content of (R,S)-antcin C in the extract being up to 184.85 ± 0.96 µg/mg. (R,S)-antcin A was the second most abundant triterpenoid in A. salmonea with the content being 57.85 ± 0.11 µg/mg, followed by (R,S)-antcin H (19.86 ± 0.28 µg/mg) and (R,S)-antcin K (18.61 ± 0.33 µg/mg). A comparison of the quantity of the lanostane-type triterpenoids with ergostane-type-triterpenoids (dehydrosulphurenic acid and dehydroeburicoic acid) showed that the amounts of lanostane-type triterpenoids in A. cinnamomea and A. salmonea were lower than ergostane-type triterpenoids.

The results of the triterpenoid analysis for A. cinnamomea and A. salmonea showed that all the index compounds selected in this study could be detected in both A. cinnamomea and A. salmonea fruiting bodies, but the content of each triterpenoid varied in the two mushrooms. A comparison of the metabolite profiles of A. cinnamomea and A. salmonea (Fig. 3) showed that there were two more peaks (X and Y) present in A. salmonea at the retention time of 60 to 62 min (Fig. 3B). The MS, 1HNMR, and 13CNMR spectral data were in good agreement with antcin M (X) and methyl antcinate K (Y), which were reported previously [10]. Antcin M and methyl antcinate K (Fig. 4) might be important constituents for distinguishing A. cinnamomea and A. salmonea fruiting bodies.

To evaluate the anti-inflammation activity of the extracts from A. cinnamomea and A. salmonea fruiting bodies, an LPS-stimulated murine macrophage assay system was used. Table 4 shows the inhibitory effects of the extracts of A. cinnamomea and A. salmonea fruiting bodies. IC50 values (50% inhibitory concentration) of A. cinnamomea and A. salmonea were 73.89 µg/mg and 66.3 µg/mg, respectively. Test cells were healthy and viable at doses ranging from 10 to 80 µg/mL, as determined by the MTT colorimetric assay (data not shown). In addition to anti-inflammation activity, the cytotoxicity was evaluated for A. cinnamomea and A. salmonea extracts. As shown in the results presented in Table 4, the A. cinnamomea extract possessed stronger cyto-

**Table 2** Molecular ions [M + H+] product ions (35 eV), and percentage of relative intensities (RI%) of triterpenoids for characterization of the fruiting bodies of A. cinnamomea and A. salmonea.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(M ± H)</th>
<th>Product ions m/z at 35 eV (RI%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,S)-Antcin K</td>
<td>489 [M + H]+</td>
<td>435 (100), 453 (67), 443 (38), 407 (33), 417 (31)</td>
</tr>
<tr>
<td>(R,S)-Antcin H</td>
<td>485 [M – H]</td>
<td>441 (100), 413 (69), 457 (41), 442 (32), 414 (23)</td>
</tr>
<tr>
<td>Dehydrosulphurenic acid</td>
<td>485 [M + H]+</td>
<td>425 (100), 426 (31), 427 (5), 341 (4), 407 (3)</td>
</tr>
<tr>
<td>(R,S)-Antcin B</td>
<td>467 [M – H]</td>
<td>423 (100), 424 (32), 425 (6), 408 (2)</td>
</tr>
<tr>
<td>(R,S)-Antcin A</td>
<td>483 [M – H]</td>
<td>409 (100), 410 (30), 411 (3)</td>
</tr>
<tr>
<td>Dehydroeburicoic acid</td>
<td>467 [M – H]</td>
<td>337 (100), 371 (94), 339 (87), 373 (47), 373 (47), 338 (28)</td>
</tr>
</tbody>
</table>

**Table 3** Index compounds of A. cinnamomea and A. salmonea fruiting bodies. a (R,S)-Antcin K, b (R,S)-Antcin C, c (R,S)-Antcin H, d Dehydrosulphurenic acid, e (R,S)-Antcin B, f (R,S)-Antcin A, and g Dehydroeburicoic acid.
toxicity than \textit{A. salmonea}. The IC\textsubscript{50} value against MCF-7 cells was 59.18 µg/mL for \textit{A. cinnamomea} and 91.45 µg/mL for \textit{A. salmonea}. \textit{A. cinnamomea} grown on its original host, \textit{C. kanehirai}, has the highest market value [4]. However, excessive felling of \textit{C. kanehirai} is prohibited by the government of Taiwan. \textit{A. salmonea} is a similar mushroom to \textit{A. cinnamomea}, and because the cultivation of wood for \textit{A. salmonea} is easy to obtain, \textit{A. salmonea} has become a common substitute for \textit{A. cinnamomea} in the marketplace. Numerous studies have discussed the metabolites of \textit{A. cinnamomea} and its bioactivities; however, to date, investigations of \textit{A. salmonea} are rare.

Although some studies have analyzed the volatile compounds emitted from the mycelium of \textit{A. cinnamomea}, no volatiles have been characterized for \textit{A. cinnamomea} and \textit{A. salmonea} fruiting bodies until now [11]. In the current study, first we characterized the volatiles of \textit{A. cinnamomea} and \textit{A. salmonea} fruiting bodies. According to our analysis, 3,4,5-trimethoxybenzaldehyde was the most abundant compound in the \textit{A. cinnamomea} fruiting body. The other abundant compounds were \(\delta\)-guaiene, isolongifolene, 1-octen-3-ol, 4-terpinenol, \(\alpha\)-guaiene, and p-cymene. In the case of \textit{A. salmonea}, the most abundant volatiles were \(\alpha\)-cedrene, 1-octen-3-ol, D-limonene, cadinadiene, germacrene D, isolongifolene, and \(\alpha\)-muurolene. The odor of the \textit{A. cinnamomea} and \textit{A. salmonea} fruiting bodies is quite different. \textit{A. cinnamomea} has a relatively rich woody odor, while \textit{A. salmonea} has a lighter odor. \(\delta\)-guaiene, isolongifolene, 4-terpinenol, and \(\alpha\)-guaiene have distinguished woody odors and, therefore, the volatile compound composition might determine the different odors of \textit{A. cinnamomea} and \textit{A. salmonea}.

In addition to a comparison of the chemicals of \textit{A. cinnamomea} and \textit{A. salmonea}, the metabolite fingerprints of \textit{A. cinnamomea} and \textit{A. salmonea} were established by using HPLC. We purified the major triterpenoids, including five ergostane-type triterpenoids and two lanostane-type triterpenoids, the compound

![Fig. 3](image-url)
The established previously [8]. All of the index compounds shown in however, the content of each compound was different in the two mea
Interestingly, (R,S)-antcin C was the dominant compound in 75.18 ± 0.11, 48.77 ± 0.31, and 43.36 ± 0.76 µg/mg, respectively. (R,S)-antcin H, and (R,S)-antcin C were abundant compounds at
fruiting body with an IC50 = 5.48 µg/mL [8]. However, the strongest anti-inflammatory triterpenoid in the fingerprints of A. cinnamomea and A. salmonoe. The A. cinnamomea fingerprint was similar to the metabolite profiling, which we established previously [8]. All of the index compounds shown in the A. salmonoe fingerprint were observed in A. cinnamomea; however, the content of each compound was different in the two mushrooms. For the A. cinnamomea fruiting body, (R,S)-antcin B, (R,S)-antcin H, and (R,S)-antcin C were abundant compounds at 75.18 ± 0.11, 48.77 ± 0.31, and 43.36 ± 0.76 µg/mg, respectively. Interestingly, (R,S)-antcin C was the dominant compound in A. salmonoe with levels of up to 184.85 ± 0.96 µg/mg. The second most abundant triterpenoid in A. salmonoe was (R,S)-antcin A (57.85 ± 0.11 µg/mg) followed by (R,S)-antcin H (19.86 ± 0.28 µg/mg) and (R,S)-antcin K (18.61 ± 0.33 µg/mg). In addition, we found a further two compounds in A. salmonoe that were absent in A. cinnamomea. According to the spectral analysis, the compounds were antcin M and methyl antcinate K. These two compounds might be important for distinguishing A. cinnamomea and A. salmonoe fruiting bodies. Obviously, the compositions of A. cinnamomea and A. salmonoe were dissimilar. We speculate that these different compositions might affect the bioactivity of A. cinnamomea and A. salmonoe. In our previous study, the ethanolic extracts of A. cinnamomea exhibited potent anti-inflammatory activity in vitro and in vivo [2]. To evaluate the anti-inflammatory activity of A. cinnamomea and A. salmonoe extracts, an LPS-stimulated murine macrophage assay system was used. The anti-inflammatory activity of A. salmonoe extract (IC50 = 66.3 µg/mg) was slightly higher than that of A. cinnamomea (IC50 = 73.89 µg/mg). (R,S)-antcin C is the strongest anti-inflammatory triterpenoid in the A. salmonoe fruiting body with an IC50 = 5.48 µg/mL [8]. However, A. salmonoe contained a higher amount of (R,S)-antcin C than A. cinnamomea. This might be the reason why A. salmonoe possessed higher anti-inflammatory activity than A. cinnamomea. In addition to evaluating the anti-inflammatory activity, we examined the cytotoxicity of A. cinnamomea and A. salmonoe against MCF-7 cell lines. The results showed that A. cinnamomea (IC50 = 59.18 µg/mg) was more cytotoxic than A. salmonoe (IC50 = 91.45 µg/mg). According to the results of a cytotoxicity assay by Du and his coworkers, (R,S)-antcin H and (R,S)-antcin B exhibited stronger cytotoxicity against human leukemia cell lines in comparison with other ergostane-type triterpenoids in the A. cinnamomea fruiting body [9]. (R,S)-antcin H and (R,S)-antcin B were abundant in A. cinnamomea. This might be why A. cinnamomea possessed stronger cytotoxicity than A. salmonoe.

According to this study, we can distinguish between A. cinnamomea and A. salmonoe by fingerprints, especially since two compounds, antcin M and methyl antcinate K, were only present in the A. salmonoe fingerprint. A. salmonoe had more anti-inflammatory activity than A. cinnamomea, however. A. salmonoe was more cytotoxic than A. cinnamomea. Nowadays, A. salmonoe is often used as a substitute for A. cinnamomea. However, the composition and levels of bioactivity are different in these two mushrooms. Therefore, for safety reasons, we recommend a toxicological evaluation and biological activity investigation of A. salmonoe for use as an alternative to A. cinnamomea.

### Materials and Methods

**Antrodia cinnamomea and Antrodia salmonea fruiting bodies**

The A. cinnamomea and A. salmonea fruiting bodies were collected by Mr. Wen-Wei Hsiao who is an assistant research fellow in Experimental Forest, National Taiwan University. A. cinnamomea was collected from the remained wood of C. kanehirai distributed at the secondary growth of forest, which is located at Suili countryside, Nantou County, Taiwan. A. salmonea was also collected from its host wood, C. konishii. The remained wood of C. konishii was located at Sun-Link-Sea region in Nantou County, Taiwan. The samples were identified by Dr. Sheng-Yang Wang (Professor of Department of Forestry, Nation Chung-Hsing University). The voucher specimens (TCFAC0001 and TCFAS0001) were deposited in the herbarium of the same university. Fig. 1 shows the morphology of the fruiting bodies analyzed in this study.

**Volatile metabolite analysis of Antrodia salmonoea and Antrodia cinnamomea fruiting bodies by using solid-phase microextraction combined with gas chromatography coupled to mass spectrometry**

An SPME holder and carboxen-polydimethylsiloxane-coated fibers (75 mm) were purchased from Supelco (Bellefonte). The sample bottle was placed in a water bath (40 ± 2 °C) and conditioned (15 min, without fiber). Before use, SPME fibers were conditioned by heating in a hot injection port of a GC at 200 °C for 15 min to remove contaminants. After the equilibration time, the fiber was introduced into the sample bottle and exposed to the gases in the headspace of the fruiting bodies for 15 min to remove contaminants. After the equilibration time, the fiber was introduced into the sample bottle and exposed to the gases in the headspace of the fruiting bodies for 15 min to remove contaminants. After the equilibration time, the fiber was introduced into the sample bottle and exposed to the gases in the headspace of the fruiting bodies for 15 min to remove contaminants.

<table>
<thead>
<tr>
<th>IC50 (µg/mg)</th>
<th>A. cinnamomea</th>
<th>A. salmonea</th>
<th>Plumagin</th>
<th>Curcumín</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO inhibition activity</td>
<td>73.89</td>
<td>66.30</td>
<td>–</td>
<td>4.50</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>59.18</td>
<td>91.45</td>
<td>4.54</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 4 Anti-inflammation activity and cytotoxicity against MCF-7 cells of A. cinnamomea and A. salmonea.**
Metabolite profiling and quantification of ethanol extract of *A. cinnamomea* and *A. salmonea* fruiting bodies

The protocol for the preparation of the ethanol (EtOH) extract of *A. salmonea* and *A. cinnamomea* fruiting bodies was according to our previous method [8]. Briefly, fresh material was lyophilized for 72 h and *A. cinnamomea* and *A. salmonea* were ground to a powder (particle diameter < 0.7 mm), accurately weighed (around 5 g), placed in an Erlenmeyer flask (250 mL) with 100 mL EtOH, and sonicated in an ultrasonicator (Branson 5510, Branson Ultrasonic) for 60 min. The extracts were then decanted, filtered under vacuum, concentrated in a rotary evaporator, and lyophilized. The metabolite profile of the ethanol extract prepared from the *A. cinnamomea* and *A. salmonea* were established by using seven index compounds, namely (R,S)-antcin K, (R,S)-antcin C, (R,S)-antcin H, dehydroesulphenic acid, (R,S)-antcin B, (R,S)-antcin A, and dehydroeburicoic acid, which were identified by us previously [8] and the structure was reconfirmed by using UPLC-MS analysis. An amaZon speed ion trap (Bruker) was set to an ion source temperature 250 °C with N2 as dry gas at 9.0 L min-1 with the capillary voltage at 4500 V, the end plate offset at 500 V, and a scan range of 70 to 600 m/z in the positive and negative ionization modes. The unknown peaks presented in the metabolite profiling of *A. salmonea* were collected and identified by spectra analysis. UV spectra were recorded on a Jasco V-550 spectrophotometer and IR spectra were recorded on a Bio-Rad FTS-40 spectrometer. Electrospray ionization-mass spectrometric spectrometry data were collected with a Finnigan MAT-95 S mass spectrometer, and NMR spectra were recorded with Bruker Avance 400 MHz FT-NMR spectrometers at 400 MHz (1 H) and 100 MHz (13C). d-Chloroform (CDCl3) was used for NMR analysis. All spectrometry data were in good agreement with the literature. The standard calibration curves (peak area vs. concentration) of each index compound were determined at the range of compound concentrations of 10, 25, 50, 100, 250, 500, and 1000 µg/mL. Quantification of the content of each index compound in fruiting bodies from *A. cinnamonomea* and *A. salmonea* was then performed by HPLC analysis. The peak areas of the index compounds in the chromatogram of the EtOH extracts (with known loading concentration) were then defined, and their contents in the extracts were calculated on the basis of the quantity calibrated from the standard calibration curves. The analyses were performed in triplicate and the results are presented as mean ± SE.

**Statistical analysis**

Data are expressed as means ± SE. The significance of the differences between group means was determined by analysis of variance (ANOVA) using Dunnett’s test. Mean values within each column with different labels (a, b, c, d) are significantly different (p < 0.05).

**Conflict of Interest**

The authors declare that they have no competing interests.

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