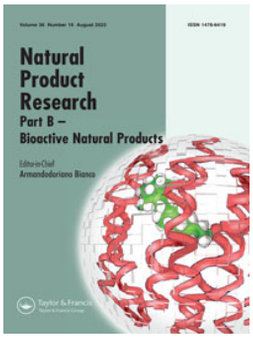

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# Characterisation of indole alkaloids and phenolic acids from wild mushroom *Tropicoporus linteus* and its chemical profiles compared with other Sanghuang mushrooms

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**ABSTRACT**

Two new indole alkaloids, 1-methylindole-7-methoxy-3-carboxaldehyde (**2**) and 7-methoxyindole-3-carboxaldehyde (**4**), together with 7-methoxyindole-3-carboxylic acid methyl ester (**1**) and 1-methylindole-3-carbaldehyde (**3**) were isolated from the fruiting bodies of wild Sanghuang mushroom *Tropicoporus linteus* (TL663). TLC, $^{1}$H-NMR and LC-MS chemical profiles of this mushroom compared with other three genera of wild Sanghuang mushroom extracts were investigated. The TLC, $^{1}$H-NMR and LC-MS profiles of TL663 and *Sanghuangporus sanghuang* (SS664) were similar and significantly different from other mushrooms. These two samples indicated the same TLC chromatograms by showing prominent bands of **1** – **4** when observed under UV 254 nm and having sharp aldehyde proton signals of 3-carboxaldehyde indole type in $^{1}$H-NMR spectra. From LC-MS analyses, peaks of isolated compounds **1** – **4** and indole-3-carboxaldehyde (**5**) in TL663 extract and peaks of protocatechuic acid (**6**), caffeic aldehyde (**7**), caffeic acid (**8**) and 3,4-dihydroxybenzalacetone (**9**) phenolic acids in TL663 fraction were identified.

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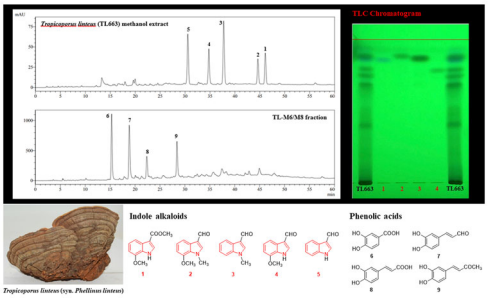


## 1. Introduction

*Phellinus linteus* (Sanghuang mushroom) is one of the most well-known valuable medicinal mushrooms in the genus *Phellinus*. It is mainly found in tropical America, Africa and East Asia. In East Asian countries this mushroom has long been used as traditional medicine for centuries (Wu et al. 2012). Medicinal properties of *P. linteus* have been studied extensively and well proven to be accepted for its anti-inflammatory (Kim et al. 2007), immunomodulatory (Wasser 2002; Suabjakyong et al. 2015a), antioxidative (Jung et al. 2008; Lee et al. 2010), antimicrobial and antiviral (Hur et al. 2004; Shirahata et al. 2017), anticancer (Sliva 2010; Konno et al. 2015; Lee et al. 2015a; Kim et al. 2016), antidiabetic (Kim et al. 2010; Feng et al. 2018), hepatoprotective (Huang et al. 2018) and neuroprotective (Choi et al. 2016) activities as well as increasing activity of immune cells (Suabjakyong et al. 2015b).

There are varieties of Sanghuang mushroom worldwide and many species of them remained unclear (Wu et al. 2012). According to taxonomic studies, numbers of *Phellinus* species were transferred to the genus *Inonotus*, *Sanghuangporus* and *Tropicoporus* (Wu et al. 2012; Tian et al. 2013; Zhou et al. 2016). From previous study, based on internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences and morphological features, *P. linteus* isolated from America and Africa was classified to *Tropicoporus linteus* (Zhou et al. 2016). Recently, some of *Phellinus* strains collected in Korea (Korea sanghwang) were cultivated on logs and investigated for their cultivation characteristics of the fruiting bodies production and bioactivities. Two of them expressed typical morphological characteristics of *T. linteus* (Min and Kang 2021). However, scientific names of *P. linteus* strains are still being used differently according to taxonomists based on the classical and molecular methods (Min and Kang 2021).

Chemical investigation and biological activity of *T. linteus* (syn. *P. linteus*) fruiting bodies are limited because of the difficulty to find and long cultivation of this mushroom to grow in nature. Most of the study of *T. linteus* nowadays are from cultivation

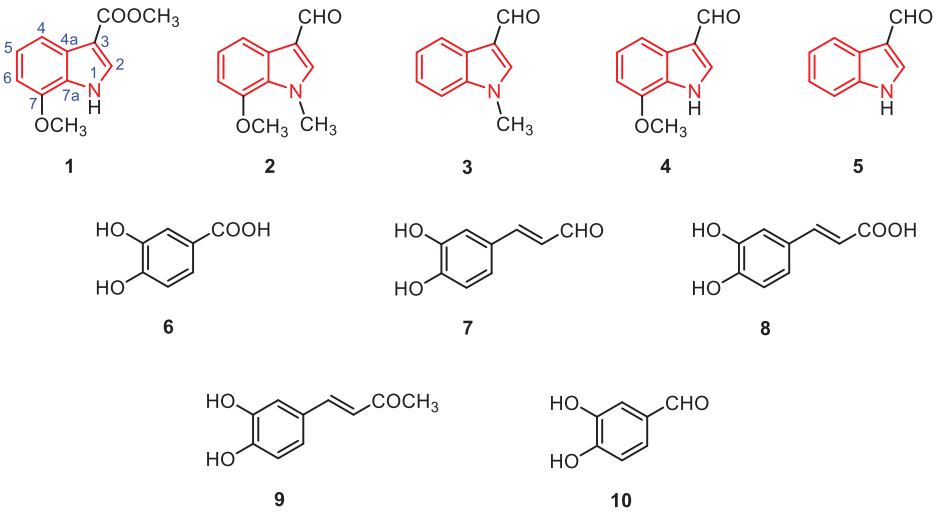


Figure 1. Structures of compounds 1-10.

mycelial materials which are convenient and affordable. Our continuing study on Sanghuang mushroom, chemical investigation of wild *T. linteus* (TL663) fruiting bodies and chemical profiles analysed by TLC, $^{1}$H-NMR and LC-MS compared with other three genera of wild Sanghuang mushrooms *Phellinus igniarius* (PI446, PI447, PI532, PI660, PI661, PI662), *Formitiporia robusta* (FR445) and *Sanghuangporus sanghuang* (SS664) were conducted. The aims of this study were to provide useful information of remarkable chemical constituents of wild *T. linteus* and methods to identify Sanghuang mushroom from forest by using their chemical profiles. Two new indole alkaloids, 1-methylindole-7-methoxy-3-carboxaldehyde (**2**) and 7-methoxyindole-3-carboxaldehyde (**4**), were isolated together with two known indole alkaloids, 7-methoxyindole-3-carboxylic acid methyl ester (**1**) and 1-methylindole-3-carbaldehyde (**3**), from the fruiting bodies of wild *T. linteus* (TL663). Based on LC-MS analysis, peaks belong to indole alkaloids **1** − **4** and a known indole-3-carboxaldehyde (**5**) (Figure 1) in TL663 extract including peaks of four phenolic acids, protocatechuic acid (**6**), caffeic aldehyde (**7**), caffeic acid (**8**) and 3,4-dihydroxybenzalacetone (**9**) (Figure 1), from a mixed TL-M6/M8 fraction of TL663 were identified.

## 2. Results and discussion

### 2.1. Structure elucidation of isolated compounds from TL663

Compound **2** was isolated as a brownish-black amorphous powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 310 (3.77), sh. 240 (3.97), 216 (4.34) nm; ATR-FTIR $\upsilon_{max}$ 3109, 3041, 1654, 1539, 1499, 1458, 1260, 1105, 779 cm$^{-1}$. The IR absorption bands at 3109 and 3041 cm$^{-1}$ confirmed characteristic of C-H stretching of formyl group when bands at 1654, 1539 and 1499 cm$^{-1}$ indicated carbonyl and carbon-carbon double bonds in the structure of **2**. From HRESIMS analysis, the formula of the ion $[M+H]^+$ of **2** is as $C_{11}H_{12}NO_2^+$ showing the molecular ion at *m/z* 190.0862 $[M+H]^+$ and its calculated monoisotopic mass is 190.0863. $^{1}$H and $^{13}$C NMR spectra of **2** (Table S1) were similar to those

reported for **3** which was isolated previously from *P. linteus* (Samchai et al. 2011) by our group except for an addition of methoxy proton $\delta_H$ 3.91 (s, 7-$OCH_3$) and methoxy carbon $\delta_C$ 55.5 (7-$OCH_3$). The presence of an aldehyde proton $\delta_H$ 9.92 (s, 3-CHO) and aldehyde carbon $\delta_C$ 184.4 (3-CHO), and *N*-methyl proton $\delta_H$ 4.09 (s, 1-$CH_3$) and carbon $\delta_C$ 37.8 (1-$CH_3$) signals in $^1$H and $^{13}$C spectra of **2** confirmed a formyl group and *N*-methyl substitution on the indole ring. These assignment were also based on coupling constants (*J*), COSY and HMBC correlations (Figure S1) of four aromatic methine protons $\delta_H$ 7.52 (s, H-2), 7.84 (d, $J = 7.9$ Hz, H-4), 7.17 (t, $J = 7.9$ Hz, H-5) and 6.72 (d, $J = 7.9$, H-6) protons. The HMBC correlations of H-2 to aldehyde carbon and correlations of *N*-methyl protons to $\delta_C$ 139.9 (C-2), 127.4 (C-4a) and 127.7 (C-7a) confirmed the positions of the formyl group at C-3 and *N*-methyl group (Figure S1).

Compound **4** was isolated as a brownish-red amorphous powder; UV (MeOH) $\lambda_{max}$ (log ε) 300 (3.62), 240 (3.86), 212 (4.16) nm; ATR-FTIR $\upsilon_{max}$ 3286, 3127, 2996, 1680, 1581, 1534, 1442, 1302, 1272, 1198, 1126, 1038, 787, 732 $cm^{-1}$. IR absorption bands at 3127 and 2996 $cm^{-1}$ of formyl group and 3286 $cm^{-1}$ of *N*-H stretching are consistent with the structure of **4**. From HRESIMS analysis, the formula of the ion $[M+H]^+$ of **4** is as $C_{10}H_{10}NO_2^+$ exhibiting the molecular ion at *m/z* 176.0707 $[M+H]^+$ and its calculated monoisotopic mass is 176.0706. $^1$H and $^{13}$C NMR spectra of **4** (Table S1) were very similar to **2** except for the absence of *N*-methyl signal and the presence of an amine proton signal $\delta_H$ 8.97 (brs., N*H*). These data suggested the *N*-methyl group of **2** was replaced by an exchangeable proton of amine for compound **4**. The connectivity of the structure of **4** was confirmed by COSY and HMBC correlation signals (Figure S1).

Indole alkaloids are a group of alkaloids with pharmacological interests (Taylor et al. 2014; Vitaku et al. 2014). Although they are mainly produced and widely distributed in plants (Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae) (Hamid et al. 2017) more than 140 indole alkaloids are well known to be mushroom-derived so far (Homer and Sperry 2017). Simple indole alkaloids such as 6-hydroxyindole-3-carbaldehyde and 6-hydroxyindole-3-acetamide isolated from *Agrocybe cylindracea* (also known as *Cyclocybe aegerita*) exhibited free radical scavenging and lipid peroxidation inhibitory activities (Kim et al. 1997; Kim et al. 2013).

### 2.2. TLC profiles

TLC analysis was performed to identify chromatographic fingerprint of the mushroom extracts. TL663 and SS664 displayed the same TLC chromatograms and they were significantly different from other PI446, PI447, PI532, PI660, PI661, PI662 and FR445 by showing high intensity of four upper prominent dark bands under UV 254 nm. These bands were identified to be indole alkaloids **1** − **4** by the comparison of TLC bands of the isolated **1** − **4** with TL663 extract.

There was a concern that the isolated compounds **1**-**4** may be artifacts due to the extraction in methanol. Therefore, small scale extraction in ethanol and TLC analyses of TL663 were carried out as the same procedure described in extraction for TLC and TLC analyses. Results indicated that TLC chromatograms of both TL663 methanol and ethanol extracts were identical.

### 2.3. $^1$H-NMR profiles

$^1$H-NMR spectra of all samples were recorded in MeOD-$d_4$ (Figure S2). TL663 and SS664 possessed similar $^1$H-NMR spectra with perceptible regions of *N*-methyl and methoxy groups ($\delta_H$ ~3.7 – 3.9), aromatic protons ($\delta_H$ ~6.7 – 8.3) and aldehyde proton ($\delta_H$ ~9.7 – 9.9) signals. Similarly, group of broad aromatic proton ($\delta_H$ ~6.6 – 7.6), tiny exchangeable *N*-H proton ($\delta_H$ ~8.4) and singlet aldehyde proton ($\delta_H$ ~9.9) signals were noticeable for PI446, PI447, PI532, PI660, PI661, PI662 and FR445.

### 2.4. HPLC and LC-MS profiles

TL663 extract solution was taken to HPLC analysis and its HPLC (UV 254 nm) chromatogram showed good separation under used condition (Figure S3). This HPLC condition was applied to LC-MS (UV 254 nm) analyses for TL663, its isolated fractions (a mixed TL-M6/M8, TL-M4 and TL-M7 fractions) and the other mushroom extract samples. The LC-MS chromatogram of TL663 exhibited good separation of four indole alkaloid peaks of **1** – **4** ($R_t$ ~46.10, 44.61, 37.75, 34.78 min, respectively) (Figure S4 (A) and Table S2) with their confirmed measured mass $[M+H]^+$ and/or $[M\text{-}H]^-$. The identification of an additional indole alkaloid **5** ($R_t$ ~30.55 min) was based on its identified mass $[M+H]^+$ at *m/z* 146.06 and $[M\text{-}H]^-$ at *m/z* 145.05 in LC-MS (Figure S4 (A) and Table S2). Phenolic acids **6** – **9** ($R_t$ ~15.30, 18.84, 22.36, 28.40 min, respectively) in a mixed TL-M6/M8 fraction were detected and identified on the basis of their detected molecular ion characteristics of both $[M+H]^+$ and $[M\text{-}H]^-$ (Figure S4 (B) and Table S2). Other two isolated fractions, TL-M4 showed peaks of **4**, **5**, **9** and protocatechuic aldehyde (**10**; $R_t$ ~16.76 min) (Figure S5 (A)), $[M\text{-}H]^-$ at *m/z* 137.02, while TL-M7 exhibited a peak of **10** together with numbers of broad peaks of unidentified mixture compounds (Figure S5 (B) and Table S3).

The LC-MS chromatograms of PI446, PI447, PI553, FR445 and SS664 were analysed compared with data of TL663 extract and TL-M6/M8 fraction (Figure S6). Result indicated that LC-MS chemical profile of SS664 is similar to TL663 but less intensity (Figure S7). The molecular ion masses of $[M+H]^+$ or $[M\text{-}H]^-$ of alkaloids **3** – **5** and phenolic acids **6** and **10** of SS664 were detected (Table S4). The phenolic acid peaks in PI446, PI447, PI553 and FR445 were shown the same patterns of TL663 but only few molecular ion masses of compounds were characterised (Figure S6 and Table S4). Although, peaks at $R_t$ ~44.0 – 44.6 min detected in PI446, PI447, PI553 and FR445 were likely to be a peak of indole alkaloid **2** its molecular ion mass of both $[M+H]^+$ and $[M\text{-}H]^-$ were not detected.

Indole alkaloid **5** and four phenolic acids **6** – **9** were identified from a mixed TL-M6/M8 fraction of TL663 based on their molecular ion peaks from LC-MS analyses compared with reported data (Nakajima et al. 2007; Lee et al. 2008b; Samchai et al. 2011; Lee and Yun 2011; Suabjakyong et al. 2015b; Lee et al. 2015b; Chen et al. 2019; Cao et al. 2021). These small phenolic molecules are common constituents of fungi including *Phellinus* and *Inonotus* (Fiasson 1982).

## 3. Experimental

See supplementary data.

## 4. Conclusion

Two new indole alkaloids **2** and **4** together with indole alkaloids **1** and **3** were isolated from the fruiting bodies of wild Sanghuang mushroom TL663 (*Tropicoporus linteus* or syn. *Phellinus linteus*). Peaks belong to the isolated compounds **1** – **4** and a peak of indole alkaloid **5** from TL663 extract including peaks of the phenolic acids **6**-**9** from the mixed TL-M6/M8 fraction of TL663 were identified by LC-MS. Among nine Sanghuang mushroom samples, TL663 and SS664 (*Sanghuangporus sanghuang*) exhibited similar TLC, $^{1}$H-NMR and LC-MS chemical profiles. Their TLC and $^{1}$H-NMR data were different from another group of PI446, PI447, PI532, PI660, PI661, PI662 (*Phellinus igniarius*) and FR445 (*Formitiporia robusta*). Comparison of the LC-MS profiles of TL663 and SS664 were also different from PI446, PI447, PI532 and FR445. From our studied, TLC and $^{1}$H NMR analyses of the extracts can be simply used to distinguish these two mushroom groups while LC-MS technique analysis provided peak information of identified compounds which is strongly confirm the chemical difference between these two groups of wild Sanghuang mushrooms.

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## Author contributions

P. Seephonkai and C. Kaewtong shared corresponding authorship.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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