Secondary Metabolites from Colletotrichum phyllanthi, an Endophytic Fungi from Stem of Physalis peruviana and Evaluation of Media Culture

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Abstract. Endophytic fungi is a microorganism that grows in plant tissue without giving any negative effect directly against its host. Phytochemistry studies from endophytic fungi have been widely reported and showed that the metabolites have excellent activities. The exploration of secondary metabolites from endophytic fungi of Physalis peruviana well known as a “cecendet” in Indonesia has never been reported before. Therefore, this research aims to isolate the secondary metabolites of the endophytic fungi from stem of P. peruviana. Five known compounds were isolated from MeOH and EtOAc extract of endophytic fungi Colletotrichum phyllanthi resulted two steroid compounds, ergosterol (1), and ergosterol peroxide (2), from MeOH extract of C. phyllanthi mycelia, and from EtOAc extract of C. phyllanthi. The metabolites production of C. phyllanthi was influenced with composition of media cultures. In this research endophytic fungi was cultivated using Potato Dextrose Broth (PDB) and Tryptic Soy Broth (TSB) media. Two metabolites including tyrosol (3), and uridine (4) were produced by C. phyllanthi through PDB, and another compound that is 1-chloronaphtalene-2-ol (5) was isolated from endophytic fungi while treated by fermentation in TSB. Structure elucidation was achieved using spectroscopic data including 1D, 2D NMR and comparison with reported data. Our results provide a scientific rationale for further explorations into metabolites from endophytic fungi of P. peruviana.

Keywords: Colletotrichum phyllanthi, endophytic fungi, media culture, Physalis peruviana

1. Introduction
Endophytic fungi is microorganism that live in plant tissues without giving a negative effect during the life cycle of host [1, 2]. The role of endophytic fungi, among others, can increase the tolerance of host plants to environmental stresses both biotic and abiotic, increase growth ability, decrease pathogen attack and predators to host plants [3]. One of the role of endophytic fungi, for its host, is production of various secondary metabolites with a variety of bioactivities. It is not surprisingly that endophytic fungi can also be a biological source in the search for bioactive compounds.

Some examples of successful endophytic fungi as a source of bioactive compounds can be seen in the following reports. One of them is the discovery of Paclitaxel (or taxol) from the endophytic fungi of medicinal plants Paraconiothyrium variabile [4]. Another case such as a polyphenol derivative, cholletotrate, which is a secondary metabolite of the Colletotrichum gloeosporioides, an endophytic fungi of medicinal plants and has antibacterial properties [5]. In addition, environmental factors of fungal fermentation of endophytes have a major effect on the production of secondary metabolites produced [6, 7]. Based on those reports indicate that the endophytic fungi is one source of secondary
metabolites that have the potential to be developed and one of them is derived from medicinal plants. Based on this background, research on secondary metabolite isolation, and evaluation of endophytic fungal culture media from *Physalis* genus plants need to be done. The exploration of secondary metabolites from endophytic fungi of *Physalis peruviana* well known as a “cecendet” in Indonesia has never been reported before. Therefore, this research aims to isolate the secondary metabolites of the endophytic fungi from stem of *P. peruviana*.

2. Experimental Setup

2.1. General Experimental Procedure

Optical rotations were carried out on an Autopol IV Rudolph Research Analytical polarimeter. All NMR experiments were performed on an Agilent, operating at 500 MHz (\(^1\)H) and 125 MHz (\(^13\)C) together with 2D NMR including DEPT135, HSQC, HMOC, HMBCC, \(\Delta\text{TOSY}1\)D, and \(^1\)H-\(^1\)H COSY with TMS as an internal standard. For the deuterium eluent using CDCl\(_3\), acetone-\(d_6\), and CD\(_3\)OD, all solvent used in chromatography were technical grade. Polyamide and Silica Gel Merck 60 GF\(_{254}\) used for column chromatography, Merck Silica Gel 7749 used for radial chromatography, Silica Gel 60 (35 – 70 mesh ASTM) used for chromatography and TLC analysis using Aluminium Silica Gel Merck 60 F254: 0.25 mm (20x20 cm). Spot were visualized using UV light and by spraying with Ce(SO\(_4\))\(_2\). Endophyte media culture using Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and Tryptic Soy Broth (TSB).

2.2. Plant Material

Samples of the stem of *P. peruviana* were taken from Bumi Herbal Dago in July 2017, Bandung, West Java Indonesia. The best stem tissue was chosen for further sterilization and inoculation process to isolate endophytic fungi (Figure 1).

![Figure 1. (a) *P. peruviana* plant; (b) Best stem tissue from *P. peruviana*.](image)

2.3. Fungi Material

*Colletorichum phyllanthi* was isolated from stem of *P. peruviana* through some steps of cultivation. First of all, selected stem tissue was sterilized in 70% EtOH, 0.5% NaOCl and 70% EtOH respectively for 30 seconds immersed in every step. After the stem tissue was sterilized, the tissue cut to the size of 1 x 1 cm using a sterile scalpel, then it was grown in PDA for seven days. Endophyte fungi with different morphology were repeated subculture until a single potential isolate was obtained. The single strain of endophyte fungi was identified through molecular identification based on Internal Transcribed Spacer (ITS) in Indonesian Culture Collection (InaCC), Biology Research Center-LIPI, Cibinong. Then the isolated endophytic fungi were known as a *Colletotrichum phyllanthi*. 
2.4. Extraction of Culture Media
C. phyllanthi was cultivated in two different broth media including PDB and TSB to determine the effect of environment for production of metabolites. The MeOH and EtOAc extract from each culture media were evaluated using TLC to obtain different spot of metabolites.

2.5. Extraction and Isolation
Mycelia was maceration in MeOH for 2x24 h, and obtain 6.54 g MeOH extract. The liquid medium was extracted using EtOAc through liquid-liquid extraction, gained 1.36 g and 1.65 g EtOAc extract from PDB and TSB medium respectively. Then all of the MeOH and EtOAc extract were fractionated using several chromatography methods.

2.5.1 Fractionation MeOH extract of C. phyllanthi cultivated with PDB
The methanol extract of C. phyllanthi (6.54 g) was sequentially fractionated through gravity column chromatography (polyamide as a stationary media, EtOAc, and MeOH in order of increasing polarity) to give five major fraction (Fr. A – E). The first major fraction (Fr. A 358.9 mg) was purified by recrystallization using MeOH, and precipitate 8.3 mg ergosterol peroxide (1) was obtained as a white needle crystalline compound. The filtrate from recrystallization of Fr. A was further subjected to radial chromatography (Silica Gel 7749, hexane, CHCl3-EtOAc, and MeOH in order of increasing polarity). Six major fractions were ultimately obtained on combining the eluates on the basis of TLC. The fifth major fraction was further subjected to gravity column chromatography and successfully isolated ergosterol peroxide (2) (3 mg) as a shaped white powder.

Ergosterol (a): Shaped white needle crystal. [α]D
25 = -132° (c 0.0012 g/ml, CHCl3). Spectrum 1H NMR (500 MHz, CDC13) δ (ppm): 1,30 (1H, m, H-1a), 1,89 (1H, m, H-1b), 1,90 (1H, m, H-2a), 1,49 (1H, m, H-2b), 3,64 (1H, m, H-3), 2,45 (1H, m, H-4a), 2,28 (1H, m, H-4b), 5,57 (1H, dd, J = 2,6 Hz, 5,6 Hz, H-6), 5,38 (1H, t, J = 2,9 Hz, H-7), 1,97 (1H, m, H-9), 1,73 (1H, m, H-11a), 1,60 (1H, m, H-11b), 2,07 (1H, m, H-12a), 1,26 (1H, m, H-12b), 1,88 (1H, m, H-14), 1,60 (1H, m, H-15a), 1,36 (1H, m, H-15b), 1,77 (1H, m, H-16a), 1,31 (1H, m, H-16b), 1,27 (1H, m, H-17), 0,63 (3H, s, H-18), 0,95 (3H, s, H-19), 2,05 (1H, m, H-20), 1,04 (3H, d, J = 6,6 Hz, H-21), 5,20 (1H, dd, J = 15,3 Hz, 7,1 Hz, H-22), 5,19 (1H, d, J = 15,3 Hz, 7,7 Hz, H-23), 1,85 (1H, m, H-24), 1,47 (1H, m, H-25), 0,82 (3H, d, J = 7,2 Hz, H-26), 0,84 (3H, d, J = 7,2 Hz, H-27), 0,91 (3H, d, J = 6,9 Hz, H-28). Spectrum 13C NMR (125 MHz, CDC13) δ (ppm): 38,3 (C-1), 31,8 (C-2), 70,4 (C-3), 40,7 (C-4), 139,7 (C-5), 119,4 (C-6), 116,2 (C-7), 141,3 (C-8), 46,2 (C-9), 46,9 (C-10), 21,1 (C-11), 38,9 (C-12), 42,7 (C-13), 54,5 (C-14), 22,8 (C-15), 28,1 (C-16), 55,6 (C-17), 11,9 (C-18), 16,2 (C-19), 40,3 (C-20), 21,0 (C-21), 131,8 (C-22), 135,4 (C-23), 42,7 (C-24), 32,9 (C-25), 19,5 (C-26), 19,9 (C-27), 17,5 (C-28).

Ergosterol peroxide (b): Shaped white powder. [α]D
25 = -28° (c 0.0008 g/ml, CHCl3). Spectrum 1H NMR (500 MHz, CDC13) δ (ppm): 1,70 (1H, m, H-1a), 1,94 (1H, m, H-1b), 1,83 (1H, m, H-2a), 1,53 (1H, m, H-2b), 3,97 (1H, m, H-3), 2,11 (1H, m, H-4a), 1,91 (1H, m, H-4b), 6,24 (1H, d, J = 8,6 Hz, H-6), 6,50 (1H, m, J = 8,5 Hz, H-7), 1,49 (1H, m, H-9), 1,51 (1H, m, H-11a), 1,23 (1H, m, H-11b), 1,95 (1H, m, H-12a), 1,24 (1H, m, H-12b), 1,56 (1H, m, H-14), 1,58 (1H, m, H-15a), 1,39 (1H, m, H-15b), 1,75 (1H, m, H-16a), 1,36 (1H, m, H-16b), 1,22 (1H, m, H-17), 0,81 (3H, s, H-18), 0,88 (3H, s, H-19), 2,01 (1H, m, H-20), 1,00 (3H, d, J = 6,7 Hz, H-21), 5,14 (1H, dd, J = 8,3 Hz, 15,3 Hz, H-22), 5,22 (1H, dd, J = 7,5 Hz, H-23), 1,85 (1H, m, H-24), 1,46 (1H, m, H-25), 0,84 (3H, d, J = 7,2 Hz, H-26), 0,81 (3H, d, J= 7,2 Hz, H-27), 0,91 (3H, d, J= 6,9 Hz, H-28). Spectrum 13C NMR (125 MHz, CDC13) δ (ppm): 34,5 (C-1), 30,0 (C-2), 66,4 (C-3), 36,8 (C-4), 82,0 (C-5), 135,3 (C-6), 130,6 (C-7), 79,3 (C-8), 51,0 (C-9), 36,9 (C-10), 23,3 (C-11), 39,2 (C-12), 44,5 (C-13), 51,6 (C-14), 20,5 (C-15), 28,6 (C-16), 56,1 (C-17), 12,8 (C-18), 18,1 (C-19), 39,6 (C-20), 20,8 (C-21), 135,1 (C-22), 132,2 (C-23), 42,7 (C-24), 33,0 (C-25), 19,9 (C-26), 19,6 (C-27), 17,5 (C-18).

2.5.2 Fractionation EtOAc extract of C. phyllanthi cultivated with PDB
The EtOAc extract of C. phyllanthi that cultivated in PDB (1.36 g) was sequentially fractionated using vacuum chromatography (Silica Gel, hexane, hexane-EtOAc, ETOAc-MeOH, MeOH in order of
increasing polarity) obtained nine major fractions (Fr. A-Fr.I) on combining the eluates on the basis of TLC. The fourth major fraction (Fr. D) (44.8 mg) was further fractionated by radial chromatography (Silica Gel 7749, CHCl₃, CHCl₃-EtOAc, EtOAc-MeOH, MeOH in order of increasing polarity) and gained tyrosol (c) (12 mg) was obtained as a shaped yellowish-coloured compound. While, from the last major fraction (208.6 mg) was further fractionated using radial chromatography (Silica Gel 7749, CHCl₃, CHCl₃-EtOAc, EtOAc-MeOH, MeOH in order for increasing polarity) and successfully isolated uridine (d) (4 mg) was obtained as a shaped white crystals compound.

**Tyrosol (c):** Shaped yellowish-coloured. Spectrum ¹H NMR (500 MHz, CDCl₃) δ (ppm): 6.66 (1H, d, J = 8.4 Hz, H-2, H-6), 6.97 (1H, d, J = 8.4 Hz, H-3, H-5), 3.62 (2H, t, J = 7.2 Hz, H-1′), 3.59 (2H, t, J = 7.2 Hz, H-2′), 8.03 (1H, s, -OH). Spectrum ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 156.6 (C-1), 115.9 (C-2), 130.7 (C-3), 131.1 (C-4), 130.7 (C-5), 115.9 (C-6), 39.5 (C-1′), 64.3 (C-2′).

**Uridine (d):** Dark yellow gum of. Spectrum ¹H NMR (500 MHz, aceton-d₆) δ (ppm): 5.70 (1H, d, J = 8.05 Hz, H-5), 8.01 (1H, d, J = 8.1 Hz, H-6), 5.90 (1H, d, J = 4.6 Hz, H-1′), 4.18 (1H, t, J = 4.85 Hz, H-2′), 4.15 (1H, t, J = 4.85 Hz, H-3′), 4.01 (1H, m, H-4′), 3.84 (1H, dd, J = 3.1 Hz, 12.25 Hz, H-5′), 3.73 (1H, dd, J = 3.1 Hz, 12.25 Hz, H-5′). Spectrum ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 152.5 (C-2), 166.2 (C-4), 102.6 (C-5), 142.7 (C-6), 90.7 (C-1′), 75.7 (C-2′), 71.3 (C-3′), 86.3 (C-4′), 62.3 (C-5′).

2.5.3 Fractionation EtOAc extract of C. phyllanthi cultivated with TSB

The EtOAc extract of C. phyllanthi that cultivated in PDB (1.65 g) was sequentially fractionated using gravity column chromatography (Silica Gel, hexane, EtOAc, EtOAc-MeOH, MeOH in order of increasing polarity) obtained four main fractions (Fr. A-Fr.D) on combining the eluates on the basis of TLC. The third major fraction (Fr. C) (22.7 mg) was further fractionated by gravity column chromatography (Silica Gel 7749, hexane, EtOAc:hexane, EtOAc in order of increasing polarity) gained tyrosol (c) (4 mg) was obtained as a shaped white crystals compound.

**1-chloronaphtalene-2-ol (e):** Shaped white crystals. Spectrum ¹H NMR 500 MHz, CDCl₃) δ (ppm): 7.39 (1H, d, J = 8.9 Hz) H-3, 7.98 (1H, d, J = 8.9 Hz) H-4, 7.16 (1H, d, J = 8.6 Hz) H-5, 7.31 (1H, td, J = 1.4 Hz, 8.3 Hz) H-6, 7.61 (1H, td, J = 1.3 Hz, 8.1 Hz) H-7, 7.89 (1H, d, J = 8.1 Hz) H-8, 5.07 (1H, s). Spectrum ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 110.7 (C-1), 152.7 (C-2), 117.6 (C-3), 131.3 (C-4), 129.4 (C-4a), 128.3 (C-5), 123.9 (C-6), 124.1 (C-7), 127.3 (C-8), 133.3 (C-8a).

Figure 2. Skeleton of (a) Ergosterol (b) Ergosterol peroxide (c) Tyrosol (d) Uridine (e) 1-chloronaphtalene-2-ol
3. Results and Discussion

3.1. Compound Elucidation

Compound (1) was obtained as a white needle crystals with mass 8.3 mg and was optically active $[\alpha]_{D}^{25} = -132^\circ$ (c 0.0012 g/ml, CHCl$_3$). Based on the $^{13}$C-NMR spectrum together with the spectrum of DEPT135 and HSQC, revealed 28 carbon signals consisting of six carbon peaks of olefin ($\delta_{C}$ 131.8, 135.4, 139.7, 119.4, 141.3, 116, 2 ppm) and 22 sp$^3$ carbon signals was comprising a methynoxy carbon signal ($\delta_{C}$ 70.4 ppm), six methyl carbon signals ($\delta_{C}$ 11.9, 16.2, 21.0, 19.5, 19.9, 17, 5 ppm), seven methylene carbon signals ($\delta_{C}$ 38.3, 31.8, 40.7, 21.1, 38.9, 22.8, 28.1 ppm), six methyl signals ($\delta_{C}$ 46.2, 54.5, 54.5, 55.6, 40.3, 42.7, 32.9 ppm) and two quaternary carbon signals ($\delta_{C}$ 36.9, 42.7 ppm). Considering the amount of sp$^3$ carbon (in total 22 carbon) and the three alkene groups (DBE 7), it was suspected that compound (1) was a tetracyclic steroid derivative with a C$_{28}$ ergostand (C$_{28}$H$_{44}$O) skeleton (Figure 3).

![Figure 3. Ergostand skeleton.](image)

The $^1$H-NMR spectra also indicated the presence of signals $\delta_{H}$ 5.20 and 5.19 ppm which were characteristic of proton olefins that having a multiplicity of dd. These two protons had a large coupling value ($J = 15.3$ Hz) which showed trans-position and have a small coupling value ($J = 7.2$ Hz) which informed both of the proton in a vicinal position. The certainty of the location of the alkene system was confirmed by the correlation of HMBC (Figure 4). The spectral data of zTOCSY1D which showed the proton-proton correlation on (H-21), (H-22), (H-23), (H-24), (H-25), (H-26), (H-27) and (H-28) exhibit the side chains of ergostand skeleton (Fig.2). The position of the hydroxy group on the skeleton of compound (1) lied on C-3, this corresponded to the biogenesis of the steroid group compounds [8]. These signals from the $^1$H, $^{13}$C NMR, HMBC, and zTOCSY1D suggested that compound (1) was ergosterol (1), similar to the NMR data of ergosterol that found in Chaetomium globosum 118, an endophytic fungi in the medical plant Curcuma wenyujin [9].

![Figure 4. HMBC and zTOCSY1D correlation of ergosterol (1).](image)

Compound (2) was obtained as a white amorphous solid and it was optically active $[\alpha]_{D}^{25} = -28^\circ$ (c 0.0008 g/ml, CHCl$_3$). Based on $^1$H and $^{13}$C-NMR data assisted with HSQC and HMBC data showed that compound (2) had similarities with ergosterol (1), so it was presumed to have the same basic skeleton with ergosterol (1). The differences between these compounds was the compound (2) only had two alkene groups at $\delta_{C}$ 135.3, 130.6, 135.1 and 132.2 ppm. There were also two C-quaternary peaks which bind the O atoms at carbon $\delta_{C}$ 82.0 and 79.3 ppm, this data suspected there were substituted peroxide groups in C-5 and C-8. This is confirmed by the DBE value of compound (2) was 7, which denotes 2 for two alkene units, 4 for ergostand cyclic and 1 for cyclic of peroxide bridge. In addition,
the $^1$H-NMR shift also indicated that the proton at $\delta_H$ 6.24 ppm and 6.25 ppm with value $J = 8.6$ Hz showing cis-position. This provided information that an alkene system presented in C-6 and C-7. The assurance of second position of proton methyn olefin could be seen from HMBC correlation. Proton $\delta_H$ 6.24 (H-6) correlated with three C-quaternary ($\delta_C$ 36.9 (C-10), 79.3 (C-8), 82.0 (C-5) ppm) and proton $\delta_H$ 6.50 (H-7) ppm correlated with two C-quaternary ($\delta_C$ 79.3 (C-8) and 82.0 (C-5) ppm) and one carbon methylene ($\delta_C$ 51.0 (C-9) ppm). Based on data $^1$H, $^1$C-NMR, HSQC and HMBC (Figure 5) it can be suggested that compound (2) is ergosterol peroxide (2). These data also compared with reported data of ergosterol peroxide isolated from Chaetomium globosum II18, an endophytic fungi in the medical plant Curcuma wenyu-jin [9].

![Figure 5. HMBC correlations of ergosterol peroxide (2).](image)

Compound (3) was obtained in the form of a yellowish solid. Based on HMQC measurement, DBE value of compound (3) was 4. Based on the $^{13}$C-NMR spectrum of compound (3) shows the presence of six carbon signal peaks consisting of two methylene carbon signals ($\delta_C$ 39.5 and 64.3 ppm) of two C-sp$^2$ methyl. Two carbon signals representing four symmetric aromatic methyn ($\delta_C$ 115, 9 and 130.7 ppm) and there are two quaternary C-sp$^2$ carbon signals ($\delta_C$ 131.0 and 156.6 ppm) suggesting that the compound (3) had a substituted aromatic ring structure in the para position. The presence of a substituted aromatic ring with the para position of compound (3) is amplified by the presence of two aromatic proton signals, each representing 2H ($\delta_H$ 6.66 and 6.97 ppm) with ortho-coupling multiplicity ($J = 8.4$ Hz). The presence of a highly downfield carbon signal ($\delta_C$ 156.6 ppm) indicates the presence of oxy-aryl carbon. Another substituent in the form of an alkyl chain group is characterized by the presence of one quaternary aromatic carbon with a carbon shift ($\delta_C$ 131.0 ppm). The spectrum analysis of zTOCSY1D shows the presence of $^1$H-$^1$H correlation in one unit. There are three correlated proton signals ($\delta_H$ 2.62, 3.59 and 8.03 ppm) with these multiplicities were $t$, $m$ and $s$ respectively. This data confirmed that the side groups for aromatic systems are hydroxyethyl groups. The proton and carbon position certainty was seen from the environmental influences on the structure of compound (3), in which proton carbon shifts in C-3 and C-5 will be more downfield compared to C-2 and C-6 carbon because of ortho positions with electron donating group (\(-\text{OH}, \text{C}-1\)). On the side chain, methylene-oxy carbon has a more downfield carbon and proton shift compared to the methylene group due to the electronegativity of the -OH group. Thus, based on the spectra $^1$H, $^{13}$C-NMR, HMQC and zTOCSY1D show that compound (3) was known to be a tyrosine amino acid derived that was tyrosol (3). These NMR data confirmed with previous research [10, 11].

Compound (4) was obtained as a dark yellow. Based on the $^{13}$C-NMR spectrum shown the presence of nine signals consisting of five C-methyn signals located in the C-sp$^3$ shift region ($\delta_C$ 62.3, 71.3, 75.7, 86.4 and 90.7 ppm) which were typical shift for ribofuranoside. In addition, the presence of a carbon signal at $\delta_C$ 90.7 ppm which was a typical shift for anomic carbon. Downfield carbon ($\delta_C$ 166.2 ppm) was carbonyl carbon with a system of $\alpha$, $\beta$ unsaturated carbonyl. In addition, the presence two proton signals ($\delta_H$ 5.70, 8.01 ppm) with multiplicity $d (J = 8.05$ and 8.01 Hz) that was indicated the coupling cis. Another carbonyl carbon has $\delta_C$ 152.5 ppm which was characteristic for the carbonyl group that was bonded by two N atoms in C-2. The four carbon signals indicate the carbon shift for the pyrimidine base group of nitrogen compounds such as uracil. Based on the 1D and 2D NMR (Figure 6) spectrum, it was found that compound (4) had a structure consisting of uracil and arabinose sugar which was to be a
nucleotides uridine. This was reinforced by the HMBC correlation between proton methyn olefin (δ_H 8.01 ppm (C-6)) correlated with C-anomeric (δ_C 90.7 ppm (C-1')), in the arabinose sugar system indicating a bond β-N1 glycosides which were characteristic of the nucleoside compound. So based on data spectrum ^1^H, ^13^C-NMR, HMBC and ^1^H-^1^H COSY it was known that compound (4) was uridine (4).

![Figure 6](image)

**Figure 6.** (a) HMBC correlations (b) ^1^H-^1^H COSY correlations of uridine (4).

Compound (5) was isolated as a white solid. Based on the ^13^C-NMR spectrum data, there were 10 carbon signals located in the C-sp^2^ shift region. The 10 carbon signals consist of four carbon quaternary signals (δ_C 110.7, 152.7, 129.4 and 133.3 ppm) and six carbon methyl olefins (δ_C 117.6, 131.3, 128.3, 123, 9, 124.1 and 127.3 ppm). Based on the value of the carbon shift it can be seen that compound (5) has a structure in the aromatic ring system where one of carbon quaternary was oxyaril carbon (δ_C 152.7 ppm). In addition, the proton shifts with a singlet-wide multiplicity at δ_H 5.07 ppm which was characteristic of -OH freely protons. One other carbon quaternary is thought to be a carbon olefin with a substituted chlorine atom. This is seen from the shifting of highly aromatic quaternary carbon shifts at δ_C 110.7 ppm and this quite similar with the data of 1-chloronaphthalene-2-ol from previous research that shown in Table 1 [12]. The ^1^H-NMR spectral data together with zTOCSY1D show that compound 5 has a naphthalene base framework. These data suggest that four signals in the aromatic proton region are an early feature of the substituted aromatic system (δ_H 7.16, 7.31, 7.38 and 7.89 ppm). Certainty of the substituent position is confirmed from the spectral data of zTOCSY1D showing the correlation of the four protons, so that both substituents are ortho positions. Based on ^1^H-NMR spectrum data shown that two other protons are δ_H 7.39 and 7.98 ppm with coupling J = 8.9 Hz indicating that both protons were in the ortho positions. This was also confirmed by the correlation of zTOCSY1D on both protons. Based on ^1^H, ^13^C-NMR spectrum data as well as zTOCSY1D and comparing with reported data compound (5) is proposed as 1-chloronaphthalene-2-ol (5) [12].

### 3.2. Evaluation of Media Culture

The results of qualitative evaluation on EtOAc extract TLC profile of PDB and TSB media and three pure isolated compounds (Figure 7) showed that composition of media culture could influence *C. phyllanthi* in producing secondary metabolite. The difference composition of PDB and TSB was the nitrogen sources, where nitrogen source in PDB medium was amino acid mean while nitrogen source in TSB medium were *Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal*. Interaction
between endophytic fungi and fermentation condition could affect the production of secondary metabolite [13].

Table 1. $^1$H dan $^{13}$C-NMR data of 1-kloronaftalen-2-ol (5).

| No. C | $^5$ δ$_H$ (mult., J in Hz) | $^5$ δ$_C$ | $^5$ δ$_H$ (mult., J in Hz) | $^5$ δ$_C$ |
|-------|-----------------------------|----------|-----------------------------|----------|
| 1     | -                           | 110,7    | -                           | 113,3    |
| 2     | -                           | 152,7    | -                           | 149,3    |
| 3     | 7,39 (d, 8,9)               | 117,6    | 7,59 (t, 8,8)               | 117,2    |
| 4     | 7,98 (d, 8,9)               | 131,3    | 8,07 (d, 8,6)               | 129,4    |
| 4a    | -                           | 129,4    | -                           | 128,4    |
| 5     | 7,16 (d, 8,6)               | 128,3    | 7,27 (s)                    | 128,1    |
| 6     | 7,31 (td, 1,4, 7,6)         | 123,9    | 7,42 (t, 7,9)               | 122,7    |
| 7     | 7,61 (td, 1, 3, 7,5)        | 124,1    | 7,73 (d, 8,9)               | 124,1    |
| 8     | 7,89 (d, 8,1)               | 127,3    | 7,81 (d, 8,1)               | 127,5    |
| 8a    | -                           | 133,3    | -                           | 131,0    |
| -OH   | 5,07 (s)                    | 5,90 (s) | 125 MHz ($^{13}$C)         |

*measured in CDCl$_3$, 500 MHz ($^1$H) and 125 MHz ($^{13}$C)

*measured in CDCl$_3$, 500 MHz ($^1$H) and 126 MHz ($^{13}$C)[12]

Ergosterol (1) and ergosterol peroxide (2) were found in C. phyllanthi MeOH extract from both media culture, because these compound were the major compound that consist in fungi cell membrane [14]. In other side, Tyrosol (3) was produced more by C. phyllanthi in EtOAc extract from cultivation medium with PDB, while production of tyrosol (3) decreased when C. phyllanthi was cultivated with TSB medium. This is demonstrated by the TLC profile under 254 nm UV light suspected of tyrosol (3) concentration in the EtOAc extract from TSB culture is decreased. The decline concentration of tyrosol (3) was also suspected because there were other secondary metabolites on TSB media which was not produced by C. phyllanthi when cultivated on PDB media such as 1-chloronaphthalene-2-ol (5). The presence of 1-chloronaphthalene-2-ol (5) could affect the concentrations of other compounds that found in PDB media. Based on the results of research on media culture evaluation proven that the state of fermentation environment could affect the production of secondary metabolites in endophytic fungi C. phyllanthi.

![Figure 7](image1.png)

**Figure 7.** TLC Profile comparison of EtOAc C. phyllanthi extract from cultivation medium with PDB and TSB and three pure isolated isolates ie tyrosol (3), uridine (4) and 1-chloronaphthalene-2-ol (5).
4. Conclusion

Based on the present research that has been done, it can be concluded that it has been successfully isolated single isolate of the endophytic fungi *Colletotrichum phyllanthi* from stem’s *Physalis peruviana*. Successfully isolated five known compounds, two steroid compound that is ergosterol (1) and ergosterol peroxide (2) from the MeOH extract of mycelia, a tyrosine amino acid derivative that is tyrosol (3) and uridine which is nucleoside compound (4) from EtOAc extract cultivated with PDB culture medium from endophytic fungi *C. phyllanthi*. Another compound that is 1-chloronaphthalene-2-ol (5) isolated from EtOAc extract from cultivation with TSB medium. Composition of cultural media can affect the growth capability of endophytic fungi as well as secondary metabolites produced by endophytic fungi *C phyllanthi*. *C. phyllanthi* can produce more tyrosol (3) when cultivated on PDB media compared with TSB medium while 1-chloronaphthalene-2-ol (5) is produced only when endophytic fungi *C. phyllanthi* is cultivated on TSB media.

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1: EtOAc extract of cultivation with PDB media, 2: EtOAc extract cultivated with TSB medium, 3: tyrosol (3), 4: uridine (4), 5: 1-chloronafatlen-2-ol (5), 6: extract media TSB