1. Introduction

Fungi are potentially known as a promising source of bioactive compounds for drug discovery [1]. Mushrooms and other Basidiomycota, in particular, are widely used in traditional Chinese medicines and have been shown to provide beneficial activities against cancer and other ailments [2,3], but even the microfungi have various other potential benefits [4]. Dothideomycetes (Ascomycota) is a large and diverse class comprising of mostly microfungi. New species are constantly being discovered from this group and could be promising sources of novel bioactive compounds [5–7]. A few contemporary studies in Thailand have been focusing on saprobic fungi in...
Dothideomycetes as a source for finding novel bioactive compounds. For example, a novel Thai Dothideomycete, *Pseudobambusicola thailandica*, has yielded six new compounds with nematicidal and antimicrobial activity [8]. A new abscisic acid derivative with anti-biofilm activity against *Staphylococcus aureus* was isolated from cultures of a *Roussella* sp. inhabiting *Clematis subumbellata* in northern Thailand [9], while *Sparticola junici*, another new Thai dothideomycete, yielded seven new spirodioxynaphthalenes with antimicrobial and cytotoxic activities [10]. Recently some phenalenones from another new Thai *Pseudolophiostoma* species were found to selectively inhibit α-glucosidase and lipase [11]. In spite of these recent discoveries, the study of bioactive compounds from Thai and other tropical Dothideomycetes is still in the initial stages of research.

In this study, we provide morphological descriptions and illustrations of a new Dothideomycetes fungus *Pseudopalawania siamensis*, collected from *Caryota* sp. (Areaceae) in northern Thailand, based on multi-gene analyses and morphological comparison to confirm the current taxonomic placement of the fungus. In addition, we studied the new fungus for the production of bioactive compounds because its extracts showed significant antimicrobial activities in a preliminary screening. Thus, we here report the first secondary metabolites from this species, including their isolation, structure elucidation, and biological activity.

2. Materials and Methods

2.1. Sample Collection, Specimen Examination and Isolation of Fungi

Fresh material was collected from Nan Province, Thailand, in 2016. Fungal micromorphology was examined using a Motic, (Hongkong, China) SMZ 168 Series microscope. The appearance of ascomata on substrate was captured using a (stereo microscope fitted with an AxioCam camera (Carl Zeiss GmbH, Jena, Germany). Sections of ascomata were made by hand. Fungal material was mounted in water and photographed with a Nikon (Bangkok, Thailand) ECLIPSE Ni compound microscope fitted with a Canon (Singapore) EOS 600D digital camera. Fungal photoplate was processed with Adobe Photoshop CS6 version 13.1.2 (Adobe Systems, CA, USA). All microscopic characters were measured using Tarosoft Image Frame Work program (IFW) version 0.97 (Nonthaburi, Thailand). Single spore isolations were obtained using the methods of Chomnunti et al. [12]. Germinating ascospores were transferred to a new malt extract agar (MEA) media and incubated at room temperature (25 °C) in the dark. Fungal cultures were used for molecular study and secondary metabolite production. The specimens and living cultures are deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Culture collection Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand. Nomenclature and taxonomic information were deposited in MycoBank [13].

2.2. DNA Extraction, PCR Amplification and Sequencing

The genomic DNA from the fungal mycelium was extracted by using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Irvine, CA, USA) following the manufacturer’s instructions. DNA amplifications were performed by polymerase chain reaction (PCR). The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5 [14]. The internal transcribed spacer (ITS) was amplified by using primer pairs ITS5 and ITS4 [15]. The partial small subunit nuclear rDNA (SSU) was amplified with primer pairs NS1 and NS4 [15]. The translation elongation factor 1-alpha gene (TEF1) was amplified by using primers EF1-983F and EF1-2218R [16]. The partial gene encoding for the second largest RNA polymerase subunit (RPB2) was amplified by using primers fRPB2-5F and fRPB2-7cR [17]. Methods for PCR amplification and sequencing were carried out according to previously described procedures [18,19].

2.3. Phylogenetic Analysis

The closest matched taxa were determined through nucleotide BLAST searches online in GenBank (http://www.ncbi.nlm.nih.gov/). Combined LSU: 28S large subunit of the nrRNA gene; ITS: internal transcribed spacer regions 1 and 2 including 5.8S nrRNA gene; SSU: 18S small subunit of the
nrRNA gene; TEF1: partial translation elongation factor 1-α gene; and RPB2: partial RNA polymerase II second largest subunit gene sequence data from representative closest relatives to our strains were selected following Hongsanan et al. [20], Crous et al. [21], Hernández-Restrepo et al. [22], and Mapook et al. [23,24], to confirm the phylogenetic placement of our new strains. The phylogenetic analysis based on maximum likelihood (ML) and Bayesian inference (BI) were following the methodology as described in Mapook et al. [23,24]. The sequences used for analyses with accession numbers are given in Table 1. Phylogram generated from ML analysis was drawn using FigTree v. 1.4.2 [25] and edited by Microsoft Office PowerPoint 2013. The new nucleotide sequence data are deposited in GenBank.

Table 1. Taxa used in this study and their GenBank accession numbers. New sequences generated in the present study are in bold.

| Taxa                     | Strain No. | GenBank Accession Numbers | References                  |
|--------------------------|------------|---------------------------|-----------------------------|
| Acrospermum adaeum       | M133       | EU94/104, EU94/0031, EU94/0320, EU94/0180 - | Stenoos et al. [26]          |
| Acrospermum compressum   | M151       | EU94/084, EU94/0012, EU94/0301, EU94/0161 - | Stenoos et al. [26]          |
| Acrospermum gramineum    | M152       | EU94/085, EU94/0013, EU94/0302, EU94/0162 - | Stenoos et al. [26]          |
| Alternaria alternata     | KFRD-18    | XX60/781, XX60/9769, XX34/6897, KY09/4931 - | Li et al. [27]               |
| Alternariaster bidentis  | CBS 130421 | KC60/341, KC60/9347, KC60/9333 - | Alves et al. [28]            |
| Antennariella placitae   | CBS 12478  | GQ303/299, - , MH8/63403 - | Cheewangko et al. [29]       |
| Arxiella dolichandrae    | CBS 138853 | KP004/477, - , KP10/4449 - | Crous et al. [30]            |
| Arxiella terrestris      | CBS 268.65 | MH8/0201, - , MH8/58565 - | Vu et al. [31]               |
| Asterina fuchsae         | TH590      | GU58/216, GU58/6210, - , - - | Hofmann et al. [32]          |
| Asterina phenacis        | TH589      | GU58/217, GU58/6211, - , - , - | Hofmann et al. [32]          |
| Bambusicola massarinia   | MFUCC 11-0389 | JX442/37, JX442/041, EU94/0169, JX442/033 - | Dai et al. [33]              |
| Bambusicola splendida    | MFUCC 11-0439 | JX442/38, JX442/042, - , JX442/034 - | Dai et al. [33]              |
| Botryosphaeria agaraves   | MFUCC 11-0125 | JX646/08, JX646/825, - , JX646/791, JX646/856 - | Liu et al. [34]              |
| Botryosphaeria tsugae    | AFTOL-ID 1586 | DQ76/655, - , DQ76/7644, DQ76/7914 - | Schoch et al. [35]           |
| Calicium salicinum       | CBS 100898 | KF157/982, KF15/7970, KF15/7998 - | Beimorode et al. [36]        |
| Calicium viride          | 10-VIII-1997 (DUKE) | AF356/670, AF35/6669, AF35/1031, - | Lutzoni et al. [37]          |
| Camarosporium quaternatum | CBS 483.95 | GU301/806, GU29/6141, GU35/7761, KY92/9149, GU34/9044 | Schoch et al. [38]          |
| Capnodioides salicinum   | AFTOL-ID 937 | DQ678/050, DQ67/7997, - , DQ67/7889 | Schoch et al. [37]          |
| Cargospora minima        | -          | EU19/550, EU19/6551, - , - , - | Cai and Hyde [39]            |
| Chaetothyriothecium elegans | CPC 21375 | KF268/420, - , - , - | Hongsanan et al. [40]       |
| Corynespora cassicola     | CBS 100822 | GU301/808, GU29/6144, GU37/1742, GU34/9052 | Schoch et al. [38]          |
| Corynespora smithii      | CABI 5649b | GU323/201, - , GU37/1783, GU34/9018 | Schoch et al. [38]          |
| Cucurbitaria berberidis   | MFUCC 11-0387 | KC506/796, KC50/6800, - , - , - | Hyde et al. [41]            |
| Taxa                              | Strain No. 1 | GenBank Accession Numbers 2 | References                  |
|----------------------------------|--------------|----------------------------|-----------------------------|
| *Cyphelium inquinans*            | Tibell 22253 (UPS) | AY453 639 U866 95 - - | Tibell [42]                |
| *Cyphelium tigillare*            | Tibell 22343 (UPS) | AY453 AF24 641 1545 - - | Tibell [42]                |
| *Cystoleucus ebeneus*            | L161         | EU048 578 EU304 8571 - - | Muggia et al. [43]         |
| *Didymella exigua*               | CBS 183.35   | JX6810 89 EU75 4056 1764 MH8 57436 KR18 4187 | Verkley et al. [44]       |
| *Didymosphaeria rubi-umifolii*   | MFLUCC 14-0023 | KJ4365 86 KJ436 588 - - | Ariyawansa et al. [45]     |
| *Dothiara cannabinae*            | AFTOL ID 1359 | DQ470 984 DQ47 9933 DQ47 0936 - DQ47 1107 | Spatafora et al. [46]     |
| *Dyfrolomyces phetchaburienis*   | MFLUCC 15-0951 | MF615 402 MF61 5403 - - | Hyde et al. [47]           |
| *Dyfrolomyces rhizophorae*       | BCC15481     | - - KF16 0009 - - - | Pang et al. [48]           |
| *Elsinoe fawcettii*              | JN940 382 JN940 559 - KX88 7207 KX88 6853 | Schoch et al. [52]        |
| *Elsinoe verbenae*               | JN940 391 JN940 562 - KX88 7298 KX88 6942 | Schoch et al. [53]        |
| *Extremus antarcticus*           | CCFEE 5312   | KF310 020 - KF31 0086 KF30 9979 - | Egidi et al. [54]         |
| *Gonatophragmium triuniae*       | CBS 138901   | KF004 479 - - KF00 4451 - | Crous et al. [30]         |
| *Helicascus nypae*               | BCC 36751    | GU479 788 GU47 9754 GU47 9826 - GU47 9854 | Sueterong et al. [49]     |
| *Julella avicenniae*             | BCC 20173    | GU371 822 GU37 1830 GU37 1786 - GU37 1815 | Schoch et al. [38]        |
| *Karschia cezannei*              | Cezanne-Eichler B26 | KP456 152 - - - - | Ertz and Diederich [55]   |
| *Katamota bambusicola*           | KT 1517a     | AB524 595 AB52 4454 AB53 9095 NR_1 54103 AB53 9108 | Tanaka et al. [56]       |
| *Labrocarpon canariense*         | Ertz 16907 (BR) | KP456 157 - - - - | Ertz and Diederich [55]   |
| *Lentithecium flaviatilense*     | CBS 123090   | FJ795 50 FJ795 492 FJ795 467 - - | Zhang et al. [57]         |
| *Leptodiscella africana*         | CBS 400.65   | MH8 0275 - - MH8 58635 - | Vu et al. [31]            |
| *Leptodiscella brevicatenata*    | FMR 10885    | FR821 311 - - FR82 1312 - | Madrid et al. [58]        |
| *Leptodiscella chlamydospora*    | MUCL 28859   | FN869 567 - - FR74 5398 - | Madrid et al. [58]        |
| *Leptodiscella rintelii*         | CBS 14427    | LR025 181 - - LR02 5180 - | Papendorf [52]            |
| *Leptosphaeria doliolum*         | MFLUCC 15-1875 | KT45 719 KT45 4734 - KT45 4727 - | Ariyawansa et al. [59]    |
| *Leptosphaerulina australis*     | CBS 317.83   | EU754 166 GU29 6160 GU37 1790 MH8 61604 GU34 9070 | de Gruyter et al. [60]   |
| *Leptoxyphium cacuminum*         | MFLUCC 1-0049 | JN832 602 JN832 587 - - - | Chomuntu et al. [61]      |
| Taxa                      | Strain No. | GenBank Accession Numbers | References                        |
|---------------------------|------------|---------------------------|-----------------------------------|
| Lophiotrema nucula        | CBS 627-86 | GU301 GU29 GU37 LC19 GU34 | Schoch et al. [38]                |
| Lophium mytilinum         | AFOTL-JD 1669 | DQ678 DQ67 DQ67 DQ67 | Schoch et al. [35]                |
| Massarina bambusina       | H 4321     | AB807 AB79 LC01 AB80     | Tanaka et al. [56]                |
| Massarina eburnea         | CBS 473-64 | GU301 GU29 GU37 GU34     | Schoch et al. [38]                |
| Melanomma pulvis-pyrius   | CBS 371-75 | GU301 FJ201 GU37         | Schoch et al. [38]                |
| Melaspileopsis cf.        | Ertz 16247 | KP456 164 - - -          | Ertz and Diederich [55]           |
| diplasiospora             |            |                          |                                   |
| Melomastia maolanensis    | GZCC 16-0102 | KY111 KY11 1906         | Zhang et al. [51]                 |
| Microsphaeropsis oliveacea | CBS 233-77 | GU237 988 - KT38 9643 MH8 | Aveskamp et al. [62]              |
| Microthyrium buxicola     | MFLUCC 15-0213 | KT306 KT30 552 6550 - | Ariyawansa et al. [63]            |
| Microthyrium microsCopicum | CBS 115976 | GU301 GU29 GU37 1734 - GU34 | Schoch et al. [44]                |
| Multiseptospora thailandica | MFLUCC 11-0183 | KP744 KP75 490 3955 - KIP74 4447 | Liu et al. [64]                  |
| Marispora rubicunda       | IFRD 2017  | FJ955 07 GU45 6308 - GU45 | Zhang et al. [57]                 |
| Mugocopron alcornii       | BRIP 43897  | MK48 7708 - MK49 2712 MK48 | Hernández-Restrepo et al. [22]   |
| Mugocopron atromaculans   | MUCL 34983  | MK48 7709 - MK49 2713 MK48 | Hernández-Restrepo et al. [22]   |
| Mugocopron castanopsis    | MFLUCC 10-0042 | - - - - - - - - | Mapook et al. [23]               |
| Mugocopron castanopsis    | MFLUCC 14-1108 | KU726 KU72 6956 6968 - KU22 KY22 MT13 | Mapook et al. [23]               |
| Mugocopron chromolaenae   | MFLUCC 17-1513 | MT13 MT13 876 781 - 6761 MT13 | Mapook et al. [24]               |
| Mugocopron chromolaenicola | MFLUCC 17-1470 | MT13 MT13 877 7882 - MT13 | Mapook et al. [24]               |
| Mugocopron coloratum       | CBS 720-95  | MK48 7710 - MK49 2714 NR1 60197 | Hernández-Restrepo et al. [22]   |
| Mugocopron dipterocarpi    | MFLUCC 14-1103 | Ku726 Ku72 966 6969 - KY22 KY22 MT13 | Mapook et al. [23]               |
| Mugocopron dipterocarpi    | MFLUCC 17-0075 | MH98 MH9 6833 86829 - MH9 | Senwanna et al. [65]              |
| Mugocopron dipterocarpi    | MFLUCC 17-0354 | MH98 MH9 6834 86830 - MH9 | Senwanna et al. [65]              |
| Mugocopron dipterocarpi    | MFLUCC 17-0356 | MH98 MH9 6835 86831 - MH9 | Senwanna et al. [66]              |
| Mugocopron dipterocarpi    | MFLUCC 18-0470 | MK34 MK34 8001 7890 - MK34 | Jayasiri et al. [67]              |
| Mugocopron garethjonesii   | MFLUCC 16-2664 | KY070 KY07 274 0275 - KY07 274 | Tibpromma et al. [68]            |
| Mugocopron genicalatum     | CBS 721-95  | MK48 7711 - MK49 2715 MK49 | Hernández-Restrepo et al. [22]   |
| Mugocopron heveae          | MFLUCC 17-0066 | MH98 MH9 6832 86828 - MH9 | Senwanna et al. [66]              |
| Mugocopron laterale        | CBS 141029  | MK48 7712 - MK49 2716 MK49 | Hernández-Restrepo et al. [22]   |
| Taxa                  | Strain No.  | GenBank Accession Numbers | References                  |
|----------------------|-------------|---------------------------|-----------------------------|
|                      |             | LSU | SSU | RPB2 | ITS | TEF |                          |
| *Muyocopron laterale*| IMI 324533  | MK48 | MK49 | MK48 | MK49 | 7713 | MK49 7739 5961            |
|                      | CBS 719.95  | MK48 | MK49 | MK48 | MK49 | 7714 | MK49 7740 5962            |
|                      | CBS 141033  | MK48 | MK49 | MK48 | MK49 | 7715 | MK49 7741 5963            |
|                      | URM 7802    | MK48 | MK49 | MK48 | MK49 | 7716 | MK49 7742 5964            |
|                      | URM 7801    | MK48 | MK49 | MK48 | MK49 | 7717 | MK49 7743 -               |
|                      | CBS 127677  | MK48 | MK49 | MK48 | MK49 | 7718 | MK49 7744 5965            |
|                      | CBS 145310  | MK48 | MK49 | MK48 | MK49 | 7719 | MK49 7745 5966            |
|                      | CBS 145315  | MK48 | MK49 | MK48 | MK49 | 7720 | MK49 7746 5967            |
|                      | CBS 145313  | MK48 | MK49 | MK48 | MK49 | 7721 | MK49 7747 5968            |
|                      | CBS 145309  | MK48 | MK49 | MK48 | MK49 | 7722 | MK49 7748 5969            |
|                      | CBS 145314  | MK48 | MK49 | MK48 | MK49 | 7723 | MK49 7749 5970            |
|                      | CBS 145311  | MK48 | MK49 | MK48 | MK49 | 7724 | MK49 7750 -               |
|                      | CBS 145312  | MK48 | MK49 | MK48 | MK49 | 7725 | MK49 7751 5971            |
|                      | CBS 145316  | MK48 | MK49 | MK48 | MK49 | 7726 | MK49 7752 5972            |
|                      | FMR13797    | MK87 | MK87 | MK87 | MK87 | 4616 | MK87 4615 5803            |
| *Muyocopron lithocarpi* | MFLUCC 10-0041 | JQ036 | JQ036 | JQ036 | JQ036 | 230  | JQ036 226  -             |
|                      | MFLUCC 14-1106 | KU726 | KU72  | KU72 | KY22 | 6970  | KU72 6970 5780            |
|                      | MFLUCC 18-2087 | KU34  | KU34 | 7821 | KU34 | 7930  | KU34 7821 5776            |
|                      | MFLUCC 18-2088 | KU34  | KU34 | 7822 | KU34 | 7931  | KU34 7822 5777            |
|                      | MFLUCC 16-0962 | KU34  | KU34 | 7923 | KU34 | 8034  | KU34 7923 5780            |
|                      | MFLUCC 17-1465 | MT13 | MT13 | MT13 | MT13 | 878  | MT13 7883 7779            |
|                      | MFLUCC 17-1466 | MT13 | MT13 | MT13 | MT13 | 879  | MT13 7884 7780            |

References:
- Mapook et al. [23]
- Jayasiri et al. [66]
- Hernández-Restrepo et al. [22]
| Taxa                                                | Strain No. | GenBank Accession Numbers 2 | References                      |
|-----------------------------------------------------|------------|-----------------------------|---------------------------------|
| *Myococron lithocarpi*                             | MFLUCC 17-1500 | MT137 880 MT13 7885 MT13 6762 MT13 7781 MT13 6760 | Mapook et al. [24]              |
| *Myococron zamiae*                                 | CBS 203.71 | MK48 7727 - MK49 2731 - MK49 5973 | Hernández-Restrepo et al. [22]  |
| *Mycoleptodiscus endophytica*                      | MFLUCC 17-0545 | MG64 6946 MG6 46978 - MG6 46961 MG6 46985 | Tibpromma et al. [69]           |
| *Mycoleptodiscus suttonii*                         | CBS 276.72 | MK48 7728 - MK49 2732 - MK49 7753 MK49 5974 | Hernández-Restrepo et al. [22]  |
| *Mycoleptodiscus suttonii*                         | CBS 141030 | MK48 7729 - MK49 2733 - MK49 7754 MK49 5975 | Hernández-Restrepo et al. [22]  |
| *Mycoleptodiscus terrestris*                       | CBS 231.53 | MK48 7730 - MK49 2734 - MK49 7755 MK49 5977 | Hernández-Restrepo et al. [22]  |
| *Mycoleptodiscus terrestris*                       | IMI 159038 | MK48 7731 - MK49 2735 - MK49 7756 MK49 5978 | Hernández-Restrepo et al. [22]  |
| *Myriangium duriae*                                | CBS 260.36 | NG0 27579 AF24 2266 KT21 6528 MH8 55793 - | Schoch et al. [35]              |
| *Myriangium hispanicum*                            | CBS 247.33 | GU301 854 GU29 6180 GU37 1744 MH8 55426 GU34 9655 | Schoch et al. [38]              |
| *Mytilinidion rhenanum*                            | CBS 135.34 | FJ161 75 FJ161 136 FJ161 115 FJ161 092 | Boehm et al. [70]               |
| *Natipusilla decorospora*                           | AF236.1a  | HM19 6369 HM1 96376 - - - | Ferrer et al. [71]              |
| *Natipusilla naponensis*                            | AF217.1a  | HM19 6371 HM1 96378 - - - | Ferrer et al. [71]              |
| *Neocochlearomyces chronolaenae*                   | BCC 68250 | MK04 7514 MK04 7552 - MK04 7464 MK04 7573 | Crous et al. [21]               |
| *Neocochlearomyces chronolaenae*                   | BCC 68251 | MK04 7515 MK04 7553 - MK04 7465 MK04 7574 | Crous et al. [21]               |
| *Neocochlearomyces chronolaenae*                   | BCC 68252 | MK04 7516 MK04 7554 - MK04 7466 MK04 7575 | Crous et al. [21]               |
| *Neocylindroseptoria pistacae*                     | CBS 471.69 | KF251 656 - KF25 2161 KF25 1152 KF25 3112 | Quaedvlieg et al. [65]          |
| *Neomycoleptodiscus venezuelense*                  | CBS 100519 | MK48 7732 - MK49 2736 MK49 7756 MK49 5978 | Hernández-Restrepo et al. [22]  |
| *Palawania thailandensis*                          | MFLUCC 14-1121 | KY086 493 KY08 6495 KY08 6496 MT13 7787 | Mapook et al. [24]              |
| *Palawania thailandensis*                          | MFLUCC 14-1871 | KY086 494 - - MT13 7788 - | Mapook et al. [24]              |
| *Paramycoleptodiscus albizziae*                    | CPC 27552 | MH87 8220 - - - - | Vu et al. [31]                 |
| *Paramycoleptodiscus albizziae*                    | CBS 141320 | KX228 330 - MK49 2737 KX22 8279 MK49 5979 | Crous et al. [72]               |
| *Phaeodimeriella cissampeli*                       | MFLUCC 16-0558 | KU746 806 KU74 6808 KU74 6810 - KU74 6812 | Mapook et al. [73]              |
| *Phaeodimeriella dilleniæ*                         | MFLUCC 14-0013 | KU74 805 KU74 6807 KU74 6809 - KU74 6811 | Mapook et al. [73]              |
| *Phacotrichum benjamiini*                          | CBS 541.72 | AY004 340 AY01 6348 GU35 7788 MH8 60561 DQ67 7892 | Lumbsch et al. [74]            |
| *Physcia aipolia*                                  | AF470-ID 84 | DQ782 904.1 DQ78 2876 DQ78 2862 DQ78 2836 DQ78 2892 | James et al. [75]              |
| *Piedraia hortae*                                  | CBS 480.64  | GU214 466 - KF90 2289 GU21 4647 - | Crous et al. [76]              |
| *Platystomum crataegi*                             | MFLUCC 14-0925 | KTO26 109 KTO2 6113 - NG0 63580 KTO2 6121 | Thambuduga et al. [77]          |
| Taxa                                           | Strain No. | GenBank Accession Numbers | References                  |
|------------------------------------------------|------------|---------------------------|-----------------------------|
| Pleomassaria siparia                           | AFTOL-ID   | DQ678 DQ67 078 DQ67 078  | Schoch et al. [35]          |
| Pleospora herbarum                             | IT 956     | KP734 KF33 KP73 709      | Aiyawansa et al. [78]       |
| Preussia funiculata                            | CBS 659.74 | GU301 GU29 864 GU37 6187 | Schoch et al. [38]          |
| Pseudomassariosphaeria bromicola               | IT-1333    | KT305 KT30 KT30 KT30    | Aiyawansa et al. [63]       |
| Pseudopalawania siamensis                      | MFLUCC     | MT13 MT13 MT13 MT13     | This study                  |
| Pseudopalawania siamensis                      | MFLUCC     | MT13 MT13 MT13 MT13     | This study                  |
| Pseudostrickeria muriformis                    | MFLUCC     | KT934 KT93 254 KT93 4268 | Tian et al. [79]            |
| Pseudovirgaria grisea                         | CPC 19134  | JF957 JF957 14 JF957 609 | Braun et al. [80]           |
| Pseudovirgaria hyperparasitica                 | CPC 10753  | EU041 EU04 824 EU04 1767 | Arzanlou et al. [81]        |
| Ramularia endophylla                           | CBS 113265 | KP251 KP25 833 KP89 4673 | Verkley et al. [82]         |
| Rasutoria pseudotsugae                         | rapsd      | EF114 EF114 704 EF114 729 | Winton et al. [83]          |
| Rasutoria tsugae                               | ratsk      | EF114 EF114 705 EF114 730 | Winton et al. [83]          |
| Salsuginea ramicola                            | KT 2597.1  | GU479 GU47 800 GU47 9768 | Suertong et al. [40]        |
| Schizothyrium poni                             | CBS 406.61 | EF134 EF134 949 EF134 2384 | Batzer et al. [84]          |
| Setoapispora thailandica                       | MFLUCC     | MN63 MN6 8847 MN6 38851 | Hyde et al. [85]            |
| Stictographa lentiginosa                       | Erz 17570  | KP456 KP45 170 KP45 170  | Erzt and Diederich [55]     |
| Sympoventuria capensis                         | CBS        | KF156 KF15 104 KF15 6094 | Samerpiatk et al. [86]      |
| Teratosphaeria fibrillosa                      | CBS        | GU323 GU29 213 GU35 6199 | Schoch et al. [38]          |
| Trichodelitschia munkii                        | DQ384      | DQ384 096 DQ384 096     | Krays et al. [87]           |
| Tumidispora shoreae                            | MFLUCC     | KT314 KT31 074 KT31 4076 | Aiyawansa et al. [63]       |
| Uwebraunia commune                             | NC132C1d   | - - KT21 KT21 6546     | Ismail et al. [88]          |
| Venturia inaequalis                            | CBS 594.70 | GU301 GU29 879 GU35 6205 | Schoch et al. [38]          |
| Xenolophium applanatum                         | CBS        | GU45 GU45 330 GU45 6313 | Zhang et al. [89]           |
| Zeloasperisporium hypophodioiides              | CBS 218.95 | EU035 EU035 442 EU035 442 | Crous et al. [90]           |
| Zeloasperisporium siamense                     | IFRDCC     | JQ036 JQ036 228 JQ036 223 | Mapook et al. [73]          |
| Zeloasperisporium weightiae                    | MFLUCC     | KT387 KT38 737 KT38 737  | Hongsanan et al. [91]       |

1 AFTOL-ID: Assembling the Fungal Tree of Life; BCC: BIOTEC Culture Collection; BRIP: Biosecurity Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCFEE: Culture Collection of Fungi from Extreme Environments, The University of Tuscia; CPC: Culture collection of Pedro Crous, the Netherlands; FMR: Facultad de Medicina, Reus, Tarragona, Spain; GZCC: Guizhou Culture Collection; IFRDCC = International Fungal Research and Development Centre Culture Collection, China; IMI: The International Mycological Institute Culture Collections; JK: J. Kohlmeyer; MFLU: the Herbarium of Mae Fah Luang University; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL:
Isolation of its active ingredients.

Compounds 1–5

The supernatant crude extract was dissolved in methanol and initially fractionated on preparative HPLC manufactured by Gilson (Middleton, Wi, USA), comprised of a GX-271 Liquid Handler, a 172 DAD, a 305 and 306 pump, with 50SC Piston Pump Head. A Phenomenex (Torrance, California) 241 polarimeter in a 100 × 2 mm cell at 22 °C. ECD spectra were recorded on a J-815 spectropolarimeter (JASCO, Pfungstadt, Germany). UV spectra were obtained on a Shimadzu (Duisburg, Germany) UV-Vis spectrophotometer UV-2450 with 1 cm quartz cells. IR spectra were measured with a Nicolet Spectrum 100 FT-IR spectrometer (Perkin-Elmer, Waltham, MA, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 700 MHz Avance III spectrometer with a 5 mm TXI cryoprobe (1H 700 MHz, 13C 175 MHz) and a Bruker 500 MHz Avance III spectrometer with a BBFO (plus) SmartProbe (1H 500 MHz, 13C 125 MHz). In all cases, spectra were acquired at 25 °C (unless otherwise specified) in solvents as specified in the text, with referencing to residual 1H or 13C signals in the deuterated solvents (CDCl3 or MeOH-d4). HPLC-DAD/MS analysis was conducted using an amaZon Speed ETD ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). HR-ESI mass spectra was measured using an Agilent 1200 series HPLC-UV system (column 2.1 × 50 mm, 1.7 μm, C18 Waters Acquity UPLC BEH) combined with an maXis (Bruker) ESI-TOF-MS instrument. The mobile phase was composed of H2O + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B), with the following gradient: 5% solvent B for 0.5 min with a flow rate of 0.6 mL/min, increasing to 100% solvent B in 19.5 min and then maintaining 100% solvent B for 5 min. UV/Vis detection at 200–600 nm. Chemicals and solvents were obtained from AppliChem GmbH, Avantor Performance Materials, Carl Roth GmbH & Co. KG (Karlsruhe, Germany) and Merck KGaA (Darmstadt, Germany) in analytical and HPLC grade.
Ca., USA) Gemini 10u C₁₈ 110Å column (250 × 21.20 mm, 10 μm) was used as a stationary phase. The mobile phase was composed of deionised water (Milli-Q, Millipore, Schwalbach, Germany) with 0.05% of trifluoroacetic acid (TFA) as a solvent A and acetonitrile (ACN) HPLC grade with 0.05% TFA as a solvent B. The fractionation proceeded with the following gradient: linear gradient of 10% solvent B for 5 min with a flow rate of 35 mL/min, followed by 10% to 100% solvent B for 30 min, and 100% solvent B for 10 min. The UV detection was carried out at 210, 254 and 350 nm. Final five compounds were purified from initially 16 fractions (Figure 1). Compound 1 (pseudopalawanone; 5.51 mg) eluted at \( t_R = 7.8 \) min from fraction 12, compound 2 (4,4′-secalonic acid D; 5.48 mg) eluted at \( t_R = 10.5 \) min from fraction 15, compound 4 (paecilin B; 1.08 mg) eluted at \( t_R = 6.9 \) min from fraction 4, and compound 5 (cephalanone F; 1.52 mg) eluted at \( t_R = 3.0 \) min from fraction 3, while compound 3 (penicillixanthone A; 0.86 mg) eluted at \( t_R = 11.3 \) min was resulted from the purification of fraction 16 (4.12 mg) on a VarioPrep Nucleodur 100-10 C₁₈ ec column (150 × 40 mm, 7 μm; Macherey-Nagel, Düren, Germany) using the following gradients: linear gradient of 30% solvent B for 5 min with a flow rate of 15 mL/min, followed by 30% to 100% solvent B for 20 min, and 100% solvent B for 10 min.

![HPLC-(DAD)-UV chromatogram of the crude ethyl acetate extract of the culture filtrate of Pseudopalawania siamensis (MFLUCC 17-1476).](image)

2.7. Spectral Data

2.7.1. Pseudopalawanone (1)

Pale yellowish gum. \([\alpha]^{25}_D = +30.0 \text{ (c 1.0, MeOH)}\). \(^1\)H NMR (500 MHz, CDCl₃): see Table 2; \(^{13}\)C NMR (125 MHz, CDCl₃): see Table 2. HR-ESIMS \( m/z \) 641.1492 \([\text{M} + \text{H}]^+\), calcd for C₃₁H₂₉O₁₅, 641.1501).
Table 2. NMR spectroscopic data for pseudopalawanone (1).

| No. | δ_H, m | J (Hz) | δ_C, m | No. | δ_H, m | J (Hz) | δ_C, m |
|-----|--------|--------|--------|-----|--------|--------|--------|
| 1   | -      |        |        | 1’  | -      |        |        |
| 2   | 160.1, C |        |        | 110.4, CH |        |        |
| 3   | 7.82, d (8.6) | 143.8, CH | 3’ | 5.74, d (8.7) | 141.2, CH |        |
| 4   | 6.77, d (8.6) | 108.3, CH | 4’ | -        | 114.0, C |        |
| 4a  | -      |        |        | 158.3, C | 4a’  | -      | 155.6, C |
| 5   | 4.44, d (4.0) | 74.1, CH | 5’ | 4.38, d (2.5) | 88.1, CH |        |
| 6   | 2.13, m | 30.4, CH | 6’ | 2.65, m | 29.9, CH |        |
| 7a  | 2.36, dd (15.9, 13.6) | 33.8, CH | 7a  | 2.18, m | 35.8, CH |        |
| b   | 2.12, m |         |        | b   | 1.99, dd (18.3, 3.1) |        |
| 8   | -      | 108.9, C | 8’ | -      | 176.5, C |        |
| 8a  | -      | 73.6, C | 8a’a | 3.14, d (16.9) | 39.6, CH |        |
| 9   | -      | 194.9, C | 9’ | -      | 193.6, C |        |
| 9a  | -      | 106.8, C | 9a’ | -      | 107.6, C |        |
| 10a | -      | 84.7, C | 10a’ | -      | 84.8, C |        |
| 11  | 1.20, d (6.5) | 14.9, CH | 11’ | 1.16, d (7.2) | 20.9, CH |        |
| 12  | -      | 176.6, C | 12’ | -      | 168.5, C |        |
| 13  | -      | 13’, s  | 3.80, s | 53.7, CH |        |
| 1-OH | 11.35, s |        |        | 1’-OH | 11.51, s |        |
Figure 2. Phylogram generated from maximum likelihood analysis based on combined dataset of LSU, SSU, RPB2, ITS and TEF sequence data. Bootstrap support values for maximum likelihood (ML) equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.90 are given above the nodes. Newly generated sequences are in dark red bold. The tree is rooted with Lecanoromycetes. Small red arrows point towards the bootstrap values of the clades representing genera of the order Muyocopronales, while some other monophyletic clades that represent monophyletic clades have been collapses (indicated by red triangles).
3.2. Taxonomy

3.2.1. *Pseudopalawania* Mapook and K.D. Hyde, gen. nov.

*Mycobank number*: MB834934.

*Etymology*: The generic epithet refers to the similarity to *Palawania*.

*Saprobic* on dead rachis of Arecales. **Sexual morph**: Ascomata superficial, solitary or scattered, sub-carbonaceous to carbonaceous, appearing as circular, flattened, dark brown to black spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. **Ostioles** central. **Peridium** comprising dark brown or black to reddish-brown cells of *textura epidermoidea* to *textura angularis*. **Hamathecium** cylindrical to filiform, septe, hyaline, branching pseudoparaphyses. **Asci** eight-spored, bitunicate, fissitunicate, cylindric-clavate, straight or slightly curved, with an ocular chamber observed clearly when immature. **Ascospores** overlapping, 2–3-seriate, broadly fusiform to inquilinate, pointed ends, hyaline, 1-septate, constricted at the septum, guttulate when immature, surrounded by hyaline and thin layers of gelatinous sheath, observed clearly when mounted in Indian ink. **Asexual morph**: Undetermined.

*Type species*: *Pseudopalawania siamensis* Mapook and K.D. Hyde

3.2.2. *Pseudopalawania siamensis* Mapook and K.D. Hyde, sp. nov.

*Mycobank number*: MB834935; Figure 3

*Etymology*: Named after the country from where the fungus was collected, using the former name of Siam.

*Saprobic* on dead rachis of *Caryota* sp. **Sexual morph**: Ascomata 29–40 μm high × 270–290(–315) μm diam. ( x = 32.5 × 292 μm, n = 5), superficial, solitary or scattered, sub-carbonaceous to carbonaceous, appearing as circular, flattened, dark brown to black spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. **Ostioles** central. **Peridium** comprising dark brown or black to reddish-brown cells of *textura epidermoidea* to *textura angularis*. **Hamathecium** comprising 1–2.5 μm wide, cylindrical to filiform, septe, hyaline, branching pseudoparaphyses. **Asci** 65–85 × 15–21 μm ( x = 75 × 18 μm, n = 10), eight-spored, bitunicate, fissitunicate, cylindric-clavate, straight or slightly curved, with an ocular chamber observed clearly when immature. **Ascospores** 25–37 × 5–11 μm ( x = 29 × 7 μm, n = 20), overlapping, 2–3-seriate, broadly fusiform to inquilinate, pointed ends, hyaline, 1-septate, constricted at the septum, guttulate when immature, surrounded by hyaline and thin layers of gelatinous sheath, observed clearly when mounted in Indian ink. **Asexual morph**: Undetermined.

**Culture characteristics**: Ascospores germinating on MEA within 24 hrs. at room temperature and germ tubes produced from the apex. Colonies on MEA circular, slightly raised, filamentous, mycelium white at the surface and initially creamy-white to pale brown in reverse, becoming dark brown from the centre of the colony with creamy-white at the margin.

**Pre-screening for antimicrobial activity**: *Pseudopalawania siamensis* (MFLUCC 17-1476) showed antimicrobial activity against *B. subtilis* with a 16 mm inhibition zone and against *M. plumbeus* with a 17 mm inhibition zone, observable as full inhibition, when compared to the positive control (26 mm and 17 mm, respectively), but no inhibition of *E. coli*.

**Material examined**: THAILAND, Nan Province, on dead rachis of *Caryota* sp. (Arecales), 23 September 2016, A. Mapook (MFLU 20-0353, holotype); ex-type culture MFLUCC 17-1476.

**Notes**: *Pseudopalawania* is similar to *Palawania* in its superficial and flattened ascomata, with hyaline, 1-septate ascospores, but differs in its peridium wall patterns, shape of asci (cylindric-clavate vs. inquilinate to ovoid) with an ocular chamber and shape of ascospores (broadly fusiform to inquilinate vs. oblong to broadly fusiform) with a thin layer of gelatinous sheath. The gelatinous sheath in *Palawania* is thicker [24]. *Pseudopalawania* is also similar to *Muyocopron* in its superficial, flattened ascomata with similar peridium wall patterns, and asci with an ocular chamber; but differs in its sub-carbonaceous to carbonaceous ascomata, shape of asci and ascospores with surrounded by hyaline gelatinous sheath, 1-septate, while *Muyocopron* have coriaceous ascomata, aseptate ascospores with granular appearance and without gelatinous sheath [23]. In addition, the genus was
compared with genera in Microthyriaceae of which no DNA sequence data are available, but the holotype specimens were re-examined in previous studies with morphological descriptions and illustrations [94–99], and neither of them matched our new fungus. Therefore, we introduce *Pseudopalawania* as a new genus with a new species *P. siamensis* from Thailand. The fungus is placed in Muyocopronaceae (Muyocronales) with evidence from morphology and phylogeny.

Figure 3. *Pseudopalawania siamensis* (holotype) (a,b) Appearance of ascomata on substrate. (c) Squash mounts showing ascomata. (d) Section of ascoma. (e) Peridium. (f) Pseudoparaphyses. (g–j) asci. (K–p) Ascospores. (q) Ascospores in Indian ink. Scale bars: a, b = 500 μm, c, d = 100 μm, g–j = 50 μm, e, k–q = 10 μm, f = 5 μm.

3.3. Structure Elucidation of the New Compound

HPLC chromatographic fractionation of the crude ethyl acetate extract from the yeast malt (YM 6.3) broth of *Pseudopalawania siamensis* resulted in the isolation of a new heterodimeric
bistetrahydroxanthone, pseudopalawanone (1) together with three known tetrahydroxanthones, 4,4'-secalonic acid D (2) [100], penicillixanthone A (3) [101], paecilin B (4) [102] and the benzophenone, cephalanone F (5) [103] (Figure 4).

![4. Secondary metabolites from Pseudopalawania siamensis.](image)

Pseudopalawanone (1) was obtained as optically active, pale yellow gum. The IR spectrum showed the presence of hydroxyl groups (3387 cm⁻¹), carbonyl functionalities (1787, 1741 cm⁻¹) and aromatic residues (1648, 1622 cm⁻¹) while the UV spectrum was indicative of absorptions due to chromanone units [102,104]. The molecular formula C₃₁H₃₈O₁₅, indicating eighteen double bond equivalents, was established by HR-ESIMS based on its protonated pseudomolecular ion peak ([M + H]+) at m/z 641.1492. Observation of two sets of signals in the NMR spectra (Figure S1 and S2) and careful comparison of the ¹H and ¹³C NMR spectroscopic data of 1 (Table 2) with those of 2-4 immediately revealed 1 to be an asymmetric dimer of an unfamiliar highly oxygenated tetrahydroxanthone subunit and 7-deoxyblennolide D [102]. Thus, the gross structure of the latter fragment along with its connection to 7-deoxyblennolide D was established through analysis of 1D and 2D NMR spectroscopic data and will be the subject of the following discussions. The ¹³C and HSQC-DEPT edited spectra (Figure S3) showed the presence of fifteen resonances comprised of a ketone (δc 194.9), a carboxyl group of an ester functionality (δc 176.6), a hemiacetal carbon (δc 108.9), four quaternary aromatic carbons (δc 106.8, 117.6, 158.3, 160.1), two aromatic methine carbons (δc 108.3, 143.8), two aliphatic quaternary carbons (δc 73.6, 84.7), two methine carbons (δc 30.4, 74.1), a methylene carbon (δc 33.8) and a methyl group (δc 14.9). The ¹H and COSY NMR spectrum (Figure S4) revealed two ortho-coupled aromatic protons (J = 8.6 Hz) for H-3 (δH 7.82) and H-4 (δH 6.77), and a seven-proton spin system comprised of H-5 (δH 4.44) – H-6 (δH 2.23) (H-11) (δH 1.20) – H-7 (δH 2.12, 2.36). A C-2 substituted 1-hydroxychromanone unit was elucidated on the basis of HMBC correlations of chelated 1-OH (δH 11.35) with C-1 (δc 160.1), C-2 (δc 117.6) and C-9a (δc 106.8) and of H-4 (δH 6.77) with C-2 and C-4a (δc 158.3). The remaining portion of the molecule was constructed through HMBC correlations of H-6 (δH 2.13) and H-11 (δH 1.20) with C-8 (δc 108.9), of H-5 (δH 4.44) with C-8a (δc 73.6), C-10a (δc 84.7) and C-12 (δc 176.6), and of H-7 (δH 2.12, 2.36) with C-8 and C-8a. The chemical shifts assigned for C-8 and C-12 were ascribed to hemiacetal and γ-lactone moieties, respectively, by using a combination of 2D NMR experiments (Figure 5). The lactone ester was plausibly attached to C-8 forming a γ-hydroxyxylactone subunit of a [3.2.1] bicyclic structure. The remaining 17 mass units was attributed to a hydroxyl group attached to the γ-carbon (C-8a) of the chromanone substructure. This unusual tetrahydroxanthone motif could putatively originate presumably from α-hydroxylation of the keto form of blennolide A, followed by nucleophilic attack of the hydrolyzed C-12 methyl ester (Figure 6). The relative configurations of C-5 and C-6 were readily established to be similar with blennolide A by the coupling constant (J₅,₆ = 4.0 Hz) and the
chemical shifts as 5S*, 6S* while that of C-10a was assigned R* based on the observed positive n-π* CD transition at around 331 nm [104]. The chirality of C-8a cannot be established using available methods due to its remoteness to most protons in the molecule.

The linkage between the chromanone subunit and the 8-lactone in the 7-deoxyblennolide D monomer was indicated by the HMBC correlation of H–5′ (δH 4.38) with C–10a′ (δC 84.8) and C–12′ (δC 168.5). The C–5′S* and C–6′S* relative configurations in the lactone moiety were established by coupling constant analysis (|J5′,6′| = 2.5 Hz) depicting a pseudodiadial orientation for H–5′/H–6′ and the NOE (Figure S6 and S7) noted between H–5′ and H–8′a (δH 3.14), H–8′a’b (δH 2.98) and H–6′ (δH 2.65), and that of H–6′ and H–13 (δH 3.80) [102]. The spatial arrangements in ring C were similar to 7-deoxyblennolide D corroborated by NOE correlations between H–5′, H–11′ (δH 1.16) and H–7′b (δH 1.99). Finally, the relative configuration of C-10a′ may be tentatively assigned as S* on the basis of negative π*-π* transitions below 330 nm and positive n-π* transitions at 346 nm in the ECD spectrum (Figure S9) of 1 [104]. The overall relative configuration of the blennolide-type tetrahydroxanthone substructure is 5S*, 6S*, and 10aS* thus, structurally similar to 7-deoxyblennolide D.

The planar structure of 1 was established by connecting the two monomers through the linkage of C–2 (δC = 117.6) of the oxidized secalonic acid subunit and C–4′ (δC 114.0) of 7-deoxyblennolide D evidenced by the diagnostic HMBC correlations of H–3 (δH 7.82) to C–4′ and H–3′ (δH 7.54) to C–2. The axial configuration of C-2/C-4′ was assigned as P based on the CD spectrum of 1 which showed a positive first Cotton effect (225 nm, De = −6.41) and a negative second cotton effect (250 nm, De = +3.15). Thus, compound 1 was given the trivial name pseudopalawanone. To establish unambiguously its relative and absolute configurations especially C–8a in the blennolide A substructure and C–10a′ in the 7-deoxyblennolide D substructure, we suggest additional experiments such as asymmetric total synthesis, derivatization with heavy atom/s followed by single crystal x-ray diffraction and/or further ECD-TDDFT measurements and calculations.

![Figure 5. COSY (bold bonds), HMBC (red arrows) (a), and ROESY (blue arrows) (b) correlations in pseudopalawanone (1).](image)

![Figure 6. Plausible biogenetic pathway towards pseudopalawanone (1).](image)

### 3.4. Biological Activity of Compounds 1–5

The polyketides 1–5 were evaluated for their antimicrobial activity against selected microorganisms (Table 3) and cytotoxicity against two mammalian cell lines, HeLa cells KB3.1 and mouse fibroblast cell line L929 (Table 4). The starting concentration for antimicrobial assay and cytotoxicity assay were 66.7 and 300 μg/mL, respectively and the substances were dissolved in MeOH (1 mg/mL). MeOH was used as the negative control and showed no activity against the tested organisms and mammalian cell lines. Results were expressed as MIC or minimum inhibitory concentration (μg/mL) and IC50 or half maximal inhibitory concentration (μM) (Tables 3 and 4). The
known compounds 4 and 5 showed neither antimicrobial nor cytotoxic activities. The dimeric tetrahydroxanthone 4,4′-secalonic acid D (2) showed inhibition against the pathogenic fungus Candida albicans while penicillixanthone A (3) inhibited Mucor hiemalis with activities comparable to the positive drug control nystatin. Prominent activities were observed for compounds 2 and 3 against Bacillus subtilis with MIC values of 1.0 and 4.2 μg/mL, respectively. Compound 2 also showed inhibitory activity against all Gram-positive bacteria (Bacillus subtilis, Micrococcus luteus, Mycobacterium smegmatis, and Staphylococcus aureus), while compounds 1 and 3 also showed inhibitory activity against the Gram-positive bacterium, Mycobacterium smegmatis. In general, only the dimeric tetrahydroxanthones 1–3 exhibited activity against fungi and bacteria with the secalonic acid-bearing derivatives 2 and 3 exhibiting better antimicrobial profile. However, the dimeric compounds 1–3 also showed moderate cytotoxic activities against two mammalian cell lines (Table 4). These inhibitory concentrations for cytotoxic activities are given traditionally in molar concentrations, but if they are calculated in μg/mL, the IC_{50} values would be equivalent to a range of 2-25 μg/mL (i.e., the same or only slightly higher activity range as compared to the MIC). This observation precludes the potential use of these metabolites as candidates for the development of antibiotics, because their selectivity indices are far too low. In addition, the fact that they are broadly active against both, prokaryotic and eukaryotic test organisms suggests that they may address multiple targets and are therefore less suitable for development of any drug.

Some information on these and chemically related compounds is even available from the literature. Compound 2 (4,4′-secalonic acid D; 4,4′-SAD) is a regioisomeric structure to SAD with 2,2′-biaryl connectivity, belonging to the secalonic acid family. This compound class has long been known to have non-selective antimicrobial and other biological activities [100–106]. The compound 4,4′-SAD (2) itself was recently reported to have low toxicity with “potent” antitumor activity against several cancer cell lines through cell proliferation inhibition and apoptosis induction [100]. However, when compared to the precursor for a marketed drug, epothilone, which we used as a positive control in our standard cytotoxicity assays (Table 4), the activities of all the metabolites from Pseudopalawania siamensis are much weaker. Promising candidate compounds for anticancer therapy should have at least activities in the 100 nM range such assays. Penicillixanthone A (3) was also already shown to possess moderate antibacterial activity against four tested bacterial strains (M. luteus, Pseudoalteromonas nigrifaciens, E. coli and B. subtilis) [100], and its moderate cytotoxic effects on MDA-MB-435 human melanoma cells and SW620 human colorectal adenocarcinoma cell lines had been previously reported [101]. Furthermore, compound 3 was previously isolated from the marine-derived fungus Aspergillus fumigatus, and was reported to exhibit anti-HIV-1 activities by inhibiting CCR5-tropic HIV-1 and CXCR4-tropic HIV-1 infection [103]. These data also point toward non-selective effects of this metabolite in biological systems.
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Table 3. Antimicrobial activity of compounds 1–5.

| Tested organisms                  | Strain No.          | Compounds | Positive control* |
|-----------------------------------|---------------------|-----------|-------------------|
| Fungi                             |                     |           |                   |
| *Candida albicans*                | DSM 1665            | - 66.7 - - | 66.7 (20 μL N)    |
| *Cryptococcus neoformans*         | DSM 15466           | - - - - | 66.7 (20 μL N)    |
| *Mucor hiemalis*                  | DSM 6766            | - 66.7 - | 66.7 (20 μL N)    |
| *Pichia anomala*                  | DSM 6766            | - - - - | 66.7 (20 μL N)    |
| *Rhodotula glutinis*              | DSM 10134           | - - - - | 16.7 (20 μL N)    |
| *Schizosaccharomyces pombe*       | DSM 70572           | - - - - | 33.3 (20 μL N)    |
| Bacteria                          |                     |           |                   |
| *Bacillus subtilis*               | DSM 10              | 66.7 1.0 | 8.3 (20 μL O)     |
| *Chromobacterium violaceum*       | DSM 30191           | - - - - | 1.7 (2 μL O)      |
| *Escherichia coli*                | DSM 1116            | - - - - | 3.3 (2 μL O)      |
| *Micrococcus luteus*              | DSM 1790            | 66.7 8.3 | 0.4 (2 μL O)      |
| *Mycobacterium smegmatis*         | ATCC 700084         | - 66.7 - | 3.3 (2 μL K)      |
| *Pseudomonas aeruginosa*          | PA14                | - - - - | 0.8 (2 μL G)      |
| *Staphylococcus aureus*           | DSM 346             | 66.7 4.2 | 0.2 (2 μL O)      |

* Positive drug controls: K = kanamycin, N = nystatin, O = oxytetracycline hydrochloride. (-): no inhibition. The starting concentration was 66.7 μg/mL.

Table 4. Cytotoxic activity of compounds 1–5.

| Cell Lines                       | Compounds | IC₅₀ (μM) | Epothilone B |
|----------------------------------|-----------|-----------|--------------|
|                                   | 1 2 3 4 5 |           |              |
| HeLa cells KB3.1                  | 29.7 3.9 17.2 - - | 8.9 × 10⁻⁵ |
| Mouse fibroblast L929             | 50.0 14.1 - - - | 1.8 × 10⁻³ |

The in vitro cytotoxicity test of polyketides 1–5 was conducted against two mammalian cell lines, with epothilone B as positive control. Starting concentration for cytotoxicity assay was 66 μg/mL, substances were dissolved in MeOH (1 mg/mL). MeOH was used as negative control and showed no activity against the tested mammalian cell lines. Results were expressed as IC₅₀: half maximal inhibitory concentration (μM). (-): no inhibition.

4. Conclusion

The current study showed that new genera and species of tropical fungi can still yield numerous new and interesting secondary metabolites. Even though the preliminary characterization of the metabolites 1–5 indicates that they act non-selectively in biological systems, their further evaluation could result in the discovery of additional, more specific biological effects. In any case, it is worthwhile to further explore tropical fungi whose cultures result from taxonomic and biodiversity studies for the production of secondary metabolites and other potentially beneficial properties [107].

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: S1.¹H NMR spectrum (CDCl₃, 700 MHz) of pseudopalawanone (1). Figure S2: ¹³C NMR spectrum (CDCl₃, 175 MHz) of pseudopalawanone (1). Figure S3: HSQC-DEPT spectrum of pseudopalawanone (1). Figure S4: COSY spectrum of pseudopalawanone (1). Figure S5: HMBC spectrum of pseudopalawanone (1). Figure S6: ROESY spectrum of pseudopalawanone (1). Figure S7: NOESY spectrum of pseudopalawanone (1). Figure S8: LC-HRESIMS spectrum of pseudopalawanone (1). Figure S9: ECD spectrum of pseudopalawanone (1).

Author Contributions: All the authors listed made substantial contributions to the manuscript. A.M.: contributed in fungal specimen collection and isolation, fungal identification, fermentation, isolation of the compounds, and manuscript writing; A.F.G.M.: contributed in the experimental guidance, isolation of compounds, structure elucidation, and manuscript writing; B.T.: contributed in determination of biological activities, analyses of the spectral data; K.D.H. and M.S.: contributed to project organization, materials, facilities, experiment guidance and contributed in the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.
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