POLYKETIDES FROM ENDOPHYTIC FUNGI: A REVIEW

Abstract

Endophytic fungi are a significant resource of bioactive metabolites and natural products. Here, the biological properties of endophytes that produce polyketides are presented and reviewed. Fungal polyketides play an important role in drug discovery. This review highlights the molecular approaches done in the past years for the identification of PKS genes and also the polyketides from endophytic fungi that possess inhibitory activity against pathogenic microbes and cancer cell lines.

Keywords: Polyketide synthase, KS domain, Secondary metabolites, IC_{50} value, spectroscopic methods

Authors

Swetha P.R
Dr. Resmi M.S
Dr. Vivek P.J

I.   WHAT ARE FUNGAL ENDOPHYTES?

The popular definition of endophytes was given by Bacon and White. [**1**]: 'Microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effect'. Almost all of the host plants that have been explored have yet have be isolated. A symbiotic relationship exists between plants and endophytes. Plants provide nutrition and shelter to the endophyte and in exchange, they give the host protection from biotic and abiotic stress. [**2**]. Even though they were discovered in 1904, they got attention only after recognition of their ecological and pharmaceutical significance [**3**]. Endophytic fungi can thrive without any symptoms in healthy tissues of living plants above and/or below the ground. It is assumed that above one million endophytic fungal species are present in nature [**4**]. Endophytes are carried horizontally by airborne spores to their host plants. Some endophytes, however, may vertically transfer seeds to their subsequent generation.[**5**]. Endophytic fungi are a storehouse of known and new potential bioactive compounds. They can produce compounds that are specific to the host plants, it helps both the host and plant to thrive in biotic abiotic stresses. [**6**, **2**]. Thus, endophytes have generated significant interest due to their bioactive production capacity. In contrast to epiphytes or soil-associated microbes, it has been hypothesized that the association between a host and an endophyte produces a wider range and number of biological molecules [**7**].

A great breakthrough in derived natural products happened when the multibillion-dollar anticancer drug taxol-producing endophyte was isolated. At first, the However, yew tree, *Taxus brevifolia* was the main source of the compound. However the plants are slow-growing and result in low-yield taxol per gram. Endophytic fungi *Taxomyces andreanae,* found from the host plant produced this compound [**8, 9**]. Hence, an alternative method for taxol production has been explored. Co-produced bioactive substances by the endophytic fungus and its host include the anticancer drugs camptothecin [**10**] and podophyllotoxin [**11**] and the natural insecticide azadirachtin [**12**].

II.   POLYKETIDES- AN INTRODUCTION

Polyketides are a different group of secondary metabolites that have a range of biological properties and structural changes. They have wide applications in various industries such as agriculture, chemical industry, and human and animal medicine. [**13, 14**, **15**]. Because of these properties, they are known as the richest "drug gold mines" [**16**]. Polyketides are present in bacteria, fungi, and plants [**17**] and are synthesized by the enzyme Polyketide synthases (PKSs). They catalyze the condensation of extender units onto either acyl starter substrate or a developing polyketide chain by their ketosynthase (KS) activity. PKSs retain their substrates and reaction intermediate products as thioester conjugates to an acyl carrier protein (ACP) or a small molecule, Coenzyme A (CoA). Acyl transferase (AT) enzyme is responsible for recognising a particular acyl starter or extender unit and catalyzes the transfer reaction onto ACP's phosphopantetheine arm. PKSs catalyze the intramolecular cyclization of the resulting polyketide chain to produce monocyclic or polycyclic polyketides by performing a specific reduction and dehydration reaction on each resulting β-keto carbon [**18, 14**]. PKSs fall under three categories based on the catalytic domain and enzymatic mechanisms [**19, 20**]. Proteins of type I PKSs are multimodular and include unique catalytic domains. Three catalytic domains are essential for PKS I: β – ketosynthase (KS), acyl transferase (AT), and an acyl carrier protein (ACP). Type II PKSs consist of individual

protein complexes which act iteratively and most commonly produce aromatic polyketides. Type III PKSs are the simplest PKS with homodimers of KS which catalyze the reaction without ACP [18, 21, 19].

Production of most fungal polyketides are carried out by type I modular PKSs, which function repeatedly and are analogous to fungal PKSs. Enoyl reductase (ER), dehydratase (DH), and keto reductase (KR) may catalyze optional β-keto processing reactions in addition to the basic catalytic domains of type I PKS [22, 14]. Only a small amount of Type III PKSs are found in fungal genomes [23, 24].

Fungal PKSs fall into 3 categories, non-reducing or aromatic (NR-PKS), partially reducing (PR-PKS) and highly reducing PKSs according to their architecture and the presence or absence of additional β-keto processing domains. [25, 26].

III. POLYKETIDE SYNTHASE (PKS) FROM ENDOPHYTIC FUNGI

PCR with degenerated primers or genomic DNA and cDNA libraries screened by heterologous or homologous probes are used for targeting a specific PKS gene from a strain. In 1995, degenerated β- ketoacyl synthase domain primers (KS1 and KS2) were designed [27]. These were designed based on the conserved amino acid regions in the β- ketoacyl synthase domains of type I fungal and bacterial PKS. For the amplification of KS domain fragments from fungal PKS included in one of the two subclasses NR and PR-PKS, WA-type and MSAS- type, two sets of degenerate primers (LC1 and LC2c, LC3 and LC5c) were created. It was done by Bingle et al [25]. Additional sets of degenerate primers were created to target conserved areas of various fungal PKS domains, such as β-ketoacylsynthase, ketoreductase, and C-methyltransferase, enabling for the selective and quick cloning of specific fungal PKS genes [26].

The KS domain of 24 endophytic fungi isolated from Oryza rufipogon was tested for using the KAF1/KAR1 or KAF1/KAR2 assays. [28]. Nine strains were KS positive. KS positive strains are *Aspergillus*, *Penicillium*, *Phoma*, *Dendryphiella*, *Sarocladium*, *Fusarium*, *Leptosphaerulina*, *Trichoderma, Chaetomium, Sordariales*, *Eurotiales*, *Hypocreales,* and *Pleosporales.* Most of the fungal PKSs were Type I identified by phylogenetic analysis. PCR was used to check for the presence of the PKS gene in a group of 17 ascomycetes that were isolated from sugarcane. [29]. LC1/2c and KS3/4c were used for the KS domain amplification and Cmet1/3c for CMT domain amplification. The genomes encoded 36 putative PKS sequences, of which 26 shared sequence homology with KS domain and the remaining 10 shared CMT homology. The PKS gene was tested in 63 endophytic fungi isolated from Annona squamosa using the degenerate primer pairs LC1-LC2c, LC3-LC5c, and KS3-KS4c. [30]. All the three domains were present in 11 of the 63 isolates.

Manoharan et al [23] attempt to pick out Type III PKS in fungal endophytes isolated from ethnomedicinal plants including *Arbus precatorius, Bacopa monnieri, Citrus aurantifolia,* and *Datura metel.* Using rDNA-ITS phylogenetic analyses, seventeen endophytic fungal strains were identified. Analysis of eight endophytes revealed partial PKS gene presence. Amplification of the full-length gene was done by the partial sequencing of FiPKS from *Fusarium incarnatum*. FiPKScDNA was expressed in E. coli after being cloned.

IV. POLYKETIDES ROM ENDOPHYTIC FUNGI

The search for bioactive compounds from endophytic fungi resulted in the discovery of many novel and existing polyketides. The most often used approach for identifying polyketides is by HPLC of the crude extract of endophytic fungi. After proper fermentation, crude extracts were obtained, which showed several bioactivities. In majority of cases, individually purified new natural compounds showed promising activities like cytotoxic, antimicrobial, or anti-inflammatory.

The endophytic fungus *Aplosporella javeedii* was isolated from the plant *Orychophragmus violaceus*, and produced six new polyketides called aplojaveediins A–F. **[31]**. NMR and MS data analysis elucidated the structure of these metabolites. Antibacterial activities were done for all the compounds. Only compounds 1 and 6 displayed antibacterial activities against ATCC 29213 and ATCC 70699 strains of *Staphylococcus aureus.* Among these compounds, only compound 1 showed antifungal activity against *Candida albicans* strain ATCC 2433 in both agar plate diffusion assay and micro broth dilution assay. Time kill assay of compound 1 (Fig 1.1) against *Candida albicans* exhibited a faster killing rate than the positive control hygromycin B.

Penicillium commune QQF , which was isolated from the mangrove plant *Kandelia candel,* produced ten novel isocoumarins known as peniisocoumarins A–J, along with three known analogs **[32]**. Spectroscopy was used to determine the structure of the coumarins, including single-crystal x-ray diffraction, Mosher's method, and electronic circular dichroism data. The inhibitory actions of these compounds against α-glucosidase were investigated. Compounds 3 (Fig 1.3), 7 (Fig 1.2), 9 (Fig 1.5) and 11 (Fig 1.4) were more potent than acarbose, which served as a positive control. The IC_{50} values were in the range of 38.1 to 78.1 μM. With an IC_{50} value of 20.7μM, compound 7 showed significant MptpB inhibitory action.

Four α-pyrone derivatives, xylariaopyrones A-D got from the ethyl acetate extract of *Xylariales* sp. (HM1), an endophytic fungus isolated from *Distylium chinense*. **[33]**. Antimicrobial inhibitory activity was observed in xylariopyrones A-D, with MIC values in the range from 20.5 to 50.6 μg/mL. Cytotoxicity was absent against the tested four cancer cell lines. Additionally, the results of the metabolites' inhibitory action on brine shrimp showed inhibition percentages varying from 42 to 82%.

The endophyte, *Penicillium ochrochloronthe* isolated from *Taxus media* yielded three new 3,4,6- trisubstituted α-pyrone derivatives, namely 6-(2′*R*hydroxy-3′*E*,5′*E*-diene-1′-heptyl)-4-hydroxy3methyl-2H-pyran-2-one, 6-(2′*S*-hydroxy-5′*E*-ene-1′-heptyl)-4-hydroxy-3-methyl-2H-pyran-2-one, and 6-(2′*S*-hydroxy-1′-heptyl)-4-hydroxy-3-methyl-2Hpyran-2-one **[34]**. With a MIC value of 12.5 μg/ml, Significant activity was demonstrated by the compounds against the tested fungal strains and MIC of 25 μg/ml implies that it has moderate antibacterial activity against the tested bacterial strains.

Aspergillus fumigatiaffnis, an endophytic fungus isolated from the medicinal plant *Tribulus terestris* yielded a new polyketide palitantin **[35]**. The 1D and 2D NMR along with mass spectroscopy revealed the structure of this metabolite (Fig 1.6). The broth microdilution method was used to study the antibacterial activity of the compound against the strain panel *S. aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, *E. coli* ATCC 25922, (multi) drug-resistant *S. aureus* 25697, and *Enterococcus faecalis* UW 2689. MIC 64 μg/ml

suggested that the compound exhibited antibacterial activity against *Enterococcus faecalis* and *Streptococcus pneumoniae.*

A rare compound class of polyketides, curvularides A–E were isolated from the endophytic fungus *Curvularia geniculata* CTOM11 associated with the limbs of *Catunaregam tomentosa* **[36]**. Even though all the compounds are cytotoxically inactive, compound B showed inhibitory activity against *Candida albicans* ATCC 90028. It also demonstrated a synergistic impact when used in conjunction with fluconazole medication. *Aspergillus alliaceus,* from a marine algae, was co-cultured in two distinct developmental stages to yield new chlorinated bianthrones, including allianthrone A and its two diastereomers [37]. With IC50 values of 9.0 and 11.0 μM, respectively, Allianthrone A was found to exhibit mild cytotoxic activity in the HCT-116 colon cancer and SK-Mel-5 melanoma cell lines **[37]**.

Curtachalasins A and B, a novel family of cytochalasans with a 5/6/66- fused tetracyclic structure, were produced by the endophytic fungus *Xylaria curta* 10, which was isolated from the healthy stem tissues of potato (*Solanum torvum*). **[38]**. The cytotoxic potential of these compounds was examined against five novel human cancer cell lines but no cytotoxic activities are found. The antibacterial properties of the metabolites were evaluated against four strains of bacteria and four strains of fungi. At a concentration of 200 μM, both compounds showed weak antifungal activity against the *Microsporum gypseum.* Again, three new novel metabolites, curtachalasins C-E were isolated from the endophytic fungus *Xylaria* cf. *curta* from the same plant **[39]**. Curtachalasins C together with 10 μg/mL fluconazole showed dose-dependent resistance. When compared to fluconazole alone, the inhibitory ratio against the strain reported a substantial improvement.

Five new polyketides, paralactonic acids A-E were isolated from an endophytic fungus *Paraconiothyrium* sp. SW-B-1 inhabiting the seaweed *Chondrus ocellatus* **[40]**. Among the all isolates only paralactonic acid E (Fig 1.7) shown to have moderate antibacterial activity with a MIC value of 3.2 μg/mL at a concentration of 100μg/disk against *S. aureus* NBRC 13276. Using a hypersensitive drug screening approach based on the YNS17 [*Saccharomyces cerevisiae*] strain, all metabolites were evaluated for their ability to block Ca^{2+} signal transduction. In the mutant yeast strain YNS17, only paralactonic acid E exhibited dose-dependent growth restoring activity around the inhibitory zone.

Penicillium purpurogenum IMM003, an endophytic fungus isolated from the solid-substrate cultures, yielded three new polyketides including two benzophenone derivatives, penibenzones A and B, and a novel phthalide derivative, penibenzone C **[41]**. By spectroscopic studies, the structures of the compounds were interpreted. None of the compounds displayed inhibitory activity against pancreatic lipase.

Penicillium sp. ZJ-SY2, isolated from *Sonneratia apetala* leaves, fermented to yield nine polyketides, which include two new benzophenone derivatives, peniphenone and methyl peniphenone, and seven known xanthones. **[42]**. The structure of the compounds was determined spectroscopically using MS, 1D, and 2D NMR data. All of the metabolites were examined for their ability to suppress the immune system in response to LPS-induced B cell proliferation and Con A-induced T cell proliferation in mouse splenic lymphocytes.

Immunosuppressive activity was demonstrated by compounds peniphenone and three xanthones, with IC50 values ranging from 5.9 to 9.3 µg/mL.

Daldinia eschscholtzii, fungal strain inhabiting the terrestrial orchid *Paphiopedi lumexul* (Ridl.) Rolfe, was found to produce aromatic polyketides which include new naphthalene derivatives daldionin (Fig 1.8), nodulones B and C, and daldinones F and G along with eight known compounds **[43]**. MS and NMR spectroscopic studies revealed the structure of these compounds. Dadionin showed mild antiproliferative activity towards HUVEC and K-562 cell lines. The medicinal plant, *Globularia alypum* associated fungal strain *Preussia similis* produced six novel bicyclic polyketides, *Preussilides A−F* **[44]**. The biological activity of all the metabolites was tested towards bacteria, yeast, and filamentous fungi. With a MIC value of 150 and 37.5 µg/mL, compounds A and C showed mild antifungal activity respectively. All the compounds showed the weakest to modest cytotoxic activity against tested cell lines.

Phoma bellidis, endophytic fungi obtained from the medicinal plant *Tricyrtis maculate*, produced four novel polyketides, bellidisins A-D and also produced three familiar compounds, pinolidoxin 5, 6-epoxypinolidoxin, and 2-epiherbarumin II **[45]**. The structure of these compounds was identified by 1D, 2D and NMR, HRESIMS, and ECD calculation. All of the metabolites' cytotoxic potential was evaluated using a human leukemia cell lines HL-60, adenocarcinomic human alveolar basal epithelial cells A549, human breast cancer cell line MCF-7, human colorectal adenocarcinoma cell lines SW480, and human hepatocarcinoma cell line SMMC-7221. With an IC$_{50}$ value of $3.40 \pm 0.11 \mu M$, compound D (Fig 1.9) exhibited cytotoxic activity against the human leukemia cell line.

The fungus, *Phomopsis* sp. CFS42 was isolated from a medicinally important plant, *Cephalotaxus fortunei* Hook **[46]**. The fungus yielded a polyketide having an unprecedented carbon-carbon skeleton with a rare C_6 unit connected to a C_{12} unit via CAC bond, Phomotide A. Spectroscopic data analyses, and single X-ray diffraction revealed the structure of the metabolite. The study also suggested a plausible biogenetic pathway of the compound.

Penicillium chermisinum, isolated from the root of a mangrove tree, *Hertiera littoralis,* yielded a new polyketide derivative, 2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl-6H-benzo[c]chromen-6-one **[47]**. The structure of the compounds was identified by spectroscopic methods including UV, IR, HR-ESI MS, and 1D and 2D NMR experiments. With an IC$_{50}$ value of 14.94 µM, the metabolite was shown to have selective cytotoxic activity towards the MOLT-3 cell line.

The endophytic fungus *Pestalotiopsis clavispora,* from the mangrove plant *Rhizophora harrisonii,* yielded six new polyketide derivatives, which include pestalpolyol I, pestapyrones A and B, (R)-periplanetin D, pestaxanthone, norpestaphthalide A, and an isolation artifact pestapyrone C **[48.]**. 1D and 2D NMR spectroscopy was used to identify the structure of these compounds. All the compounds were inactive towards the MTT assay with the murine lymphoma cell line L5178Y, except pestapolyol I which showed an IC$_{50}$ value of 4.1 µM.

Three polyoxy-generated polyketides, namely epicolactone and epicocolides A and B yielded from ethyl acetate extract of the fungus *Epicoccum* sp., an endophyte isolated from *Theobroma cacao* **[49]**. All the isolates were evaluated for their antibacterial activity against

Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Epicolactone showed maximum activity against the tested strains. All the metabolites showed significant antifungal activity against phytopathogens, *Pythium ultimum* and *Aphanomyces cochlioides,* and the basidiomycetous fungi *Rhizoctonia solani.*

The endophytic fungus *Fusarium* sp. LN-10 isolated from *Melia azedarach* yielded a new isocoumarin derivative named fusariumin (Fig 1.11), along with two familiar related resorcylic acid lactones aigialomycin D and ponchonin N **[50]**. 1D and 2D NMR spectroscopic studies revealed the structure of the compounds. All three compounds were evaluated for growth inhibitory activity against brine shrimp. All the metabolites were shown to have significant toxicity towards brine shrimp larvae at a concentration of 10µg/mL.

Three endophytic strains of *Talaromyces* from the Amazonian rainforest yielded 6 polyketide compounds **[51]**. These compounds were tested for their antibacterial activity against a set of pathogenic microorganisms, wild hospital strains of gram-positive *Bacillus cereus*, *Staphylococcus aureus*, gram-negative *Escherichia coli*, and a wild environmental strain of the plant pathogen *R. solanacearum*. All the metabolites showed moderate antibacterial activity.

From the cultures of *Phoma* sp., an endophytic fungus isolated from the medicinal plant *Cinnamomum mollissimum*, a polyketide chemical known as 4-hydroxymellein (Fig. 1.10) was discovered and characterised. **[52]**. The crude extracts were assessed for cytotoxicity against P388 murine leukemic cells and antimicrobial activity towards the fungi *Aspergillus niger* and *A. fumigatus* and the bacteria *Bacillus subtilis*. The compound exhibited high inhibitory activity against P388 murine leukemic cells (94.6%) and *B. subtilis (97.3%). Aspergillus* sp., isolated from *Tripterygium wilfordii,* yielded four new butenolides, terrusnolides A-D **[53]**. LPS-stimulated RAW264.7 macrophages were used to evaluate the *in vitro* anti-inflammatory effects of these fungal isolates. All the compounds showed admirable inhibitory effects on the production of interleukin- 1β (IL-1β), tumour necrosis factor-α (TNF-α), and Nitric oxide (NO) in LPS-induced RAW264.7, equivalent to the positive control indomethacin.

The endophytic fungus *Diaporthe* sp. from *Datura inoxia,* produced three new compounds xylarolide A, diportharine A, and xylarolide B, and a known compound xylarolide **[54]**. All the compounds were screened for their antioxidant, antibacterial, and cytotoxicity activities. None of these compounds showed any antibacterial activity. Xylarolide A exhibited growth inhibition in MIA-PaCa-2 and PC-3 cells with IC_{50} values of 20 and 14 µM respectively and for xylarolide, 32 and 18 µM respectively.

Two new polyketides, fusariumins C and D, yielded from the endophytic fungus *Fusarium oxysporum* ZZP-R1, isolated from the traditional Chinese medicinal herb *Rumex madaio* Makino **[55]**. With a MIC value of 25.0 µM, fusariumins D exhibited moderate inhibitory effect against *S. aureus,* and fusariumins C (Fig 1.12) showed strong activity against *S. aureus* with a MIC value of 6.25 µM. The endophytic fungus *Dothiorella* sp., obtained from the mangrove *Xylocarpus granatum* Koenig, yielded three new cytosporone derivatives, dothiorelones K-M **[56]**. The compounds were tested for their inhibitory activities against α-glycosidase, antibacterial activity against five pathogenic bacteria, and cytotoxicity activity against human A549, Hela, and HepG2 cell lines. With IC_{50} values of

22.0 and 77.9 μg/mL, compound K (Fig 1.13) and M (Fig 1.14) showed α-glucosidase inhibitory activity respectively. These compounds also showed moderate antibacterial activities against *S. aureus.* None of the metabolites showed cytotoxic activity

The endophytic fungus *Diaporthe* sp. JC-17 isolated from the stems of *Dendrobium nobile* was subjected to chemical analysis.[57]. In steatoic L-02 cells, Diaporthsin E (Fig 1.16) had a 26% inhibitory ratio on triglycerides, with a concentration of 5 μg/mL. All the compounds exhibited an inhibition ratio of less than 10%. The endophytic fungus *Ascomycota* sp., obtained from the mangrove plant *Kandelia candel* produced two prenylated polyketides, ascomfurans C and ascomarugosin A [58]. With an IC_{50} value of 72.3 μM, ascomarugosin A showed weak anti-inflammatory activity.

Byssochlamys spectabilis, an endophytic fungus that lives in the leaf tissues of *Edgeworthia chrysantha*, a traditional Chinese medicinal plant, produces an organic extract that results in the production of bysspectins A, an octaketide dimer derived from polyketides with a unique carbon skeleton, and two new precursor derivatives, bysspectins B and C. [59]. Only bysspectins C exhibited a weak inhibition with MIC values of 32 and 64 μg/mL against *E. coli* and *S. aureus* respectively. Bysspectin A showed inhibition against human carboxylesterase hCE2 with an IC_{50} value of 2.01 μM.

Four novel polyketides, emericelactones A-D, were obtained from the endophytic fungal isolate *Emericella* sp. XL029, isolated from the leaves of *Panax otoginseng* [60]. For this strain, the one-strain many compounds (OSMAC) method was used. Against the agricultural pathogenic fungi *Verticillium dahlia* kleb, *R. solani*, and *Gibberella saubinetii*, as well as the human pathogenic bacteria *M. lysodeikticus* and *S. typhi*, all of the compounds showed moderate antifungal and antibacterial activity.

Two novel cryptic 3,4-dihydronaphthalen-(2H)-1-one (1-tetralone) derivatives, aspvanicin A and its epimer aspavanicin B, were identified from the ethyl acetate extract of a co-culture of the bacterium *B. subtilis* 168 trpC2 and the endophytic fungus *Aspergillus versicolor* KU258497, isolated from the leaves of *Eichhornia crassipes*. [61]. Antiproliferative activity of these metabolites was measured in the mouse lymphoma cell lines L5178Y. With an IC_{50} value of 22.8 μM, aspavanicin B (Fig 1.15) showed cytotoxic potential. The endophytic fungus *Colletotrichum* sp. JS-0367, inhabiting the leaves of mulberry tree *Morus alba,* yielded a new anthroquinone and three known anthroquinones [62]. One anthroquinone demonstrated potent neuroprotection in the immortalized mouse HT22 hippocampus neuronal cell line toward excessive glutamate-induced cell death.

The endophytic fungus *Neofusicoccum austral* SYSU-SKS024, obtained from a mangrove plant *Kandelia candel* yielded nine new metabolites, including three new ethylnaphthoquinone derivatives [63]. Among the compounds, six exhibited indoleamine 2,3-dioxygenase (IDO) inhibitory activity. Chemical investigation of the endophytic fungus *Talaromyces wortmannii* LGT-4 isolated from *Tripterygium wilfordii* resulted in two new pyrones, Wortmannine F and G [64]. Both metabolites exhibited potent phosphoionositide 3-kinase α (PI3Kα) inhibition with IC_{50} values of 25 and 5 μM, respectively. *Nigrospora* sp. BCC47789, an endophytic fungus isolated from the leaf of *Choerospondias axillaris,* produced novel hydroanthraquinone, nigrosporone A, and a new naturally present nigrosporone B along with eleven familiar compounds [65]. To evaluate each compound's

antiplasmodial, antimycobacterial, antibacterial, and cytotoxic potential in cancer cell lines, assays were performed on them all.Nigrosporone B (Fig 1.17) showed antiplasmodial activity against *P. falciparum,* antimycobacterial activity against *M. tuberculosis,* antibacterial activity against *B. cereus* and *E. faecium.* With an IC$_{50}$ value of 0.25 µM, it showed cytotoxicity in NCI-H187. Nigrosporone B exhibited only mild cytotoxicity in MCF-7 and NCI-H187

Table 1: Polyketides Producing Endophytes and Their Hosts.

Source	Host	Polyketide	Reference
Aplosporella javeedii	*Orychophragmus violaceus*	Aplojaveediins A-F	[31]
Penicilium commune QQF3	*Kandelia candel*	Peiisocoumarins A-J	[32]
Xylaria sp. HM1	*Distylium chinense*	Xylariopyrones A-D	[33]
Penicilium ochrochloron	*Taxus media*	3,4,6- trisubstituted α-pyrone derivatives	[34]
Aspergillus fumigatiaffnis	*Tribulus terrestris*	Palitantin	[35]
Curvularia geniculata CTM011	*Catunaregram tomentosa*	Curvularides A-E	[36]
Aspergillus alliaceus	Marine alga	Allianthrone A	[37]
Xylaria curta E10	*solanum torvum*	Curtachalasins A&B	[38]
		Curtachalasins C-E	[39]
Paraconiothyrium sp. SW-B-1	*Chondrus ocellatus*	Paralactonic acid A-E	[40]
Penicilium purpogenum IMM3	Solid substrate cultures	Penibenzone A-C	[41]
Penicilium sp. ZJ-SY2	*Sonneratia apetala*	Peniphenone,Methyl peniphenone and xanthones	[42]
Daldinia escholtzii	*Paphiopedilum exul.*(Ridl) Rolfe	Nodulones B&C, Daldinones F&G	[43]
Preussia similis	*Globularia alypum*	Preussilides A-F	[44]
Phoma bellidis	*Tricyrtis maculatae*	Bellidisins A-D	[45]
Phomopsis sp. CFS42	*Cephalotaxus fortuni* Hook	Phomotide A	[46]

| *Penicilium chemisinum*	*Hertiera littoralis*	2-chloro-3,4,7-trihydroxy-9-methoxy-1-methyl 6H-benzo[c]chromen-6-one	[47]
Pestalotiopsis clavispora	*Rhizophora harrisonii*	Pestalpolyol I, Pestapyrones A&B, Pestaxanthone	[48]
Epicoccum sp.	*Theobroma cacao*	Epicolactone, Epicocolides A&B	[49]
Fusarium sp. LN-10	*Melia azedarach*	Fusariumin, Algialomycin D, Ponchonin N	[50]
Phoma sp.	*Cinnamomum mollissimum*	4-hydroxymellein	[52]
Aspergillus sp.	*Tripterygium wilfordii*	Butenolides, Terrusnolides A-D	[53]
Diaporthe sp.	*Datura inoxia*	Xylarolide A&B, Diportharine A	[54]
Fusarium oxysporum ZZP-R1	*Rumex madaio Makino*	Fusariumin C&D	[55]
Dothiorella sp.	*Xylocarpus granatum. Koeing*	Dothiorelones K-M	[56]
Ascomycota sp.	*Kandelia candel*	Ascomfurans C and ascomarugosin A	[58]
Byssochlaemys spectabilis	*Edgeworthia chrysantha*	Bysspectins A,B & C	[59]
Emericella sp. XL029	*Panax otoginseng*	Emericelactones A-D	[60]
Aspergillus versicolor KO258497	*Eichhornia crassipes*	3,4-dihydronaphthalen-(2H)-1-one, Aspavanicin A&B	[61]
Colletotrichum sp. JS-0367	*Morus alba*	Anthroquinone	[62]
Neofusicoccum austral SYSU-SKS024	*Kandelia candel*	Ethylnaphthoquinone derivatives	[64]
Nigrospora *sp.* BCC47789	*Choerospondias axillaris*	Hydroanthraquinone, Nigrosporone A&B	[65]
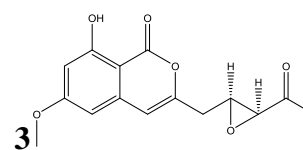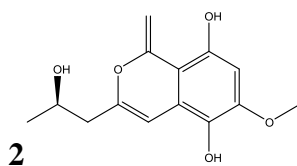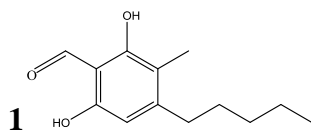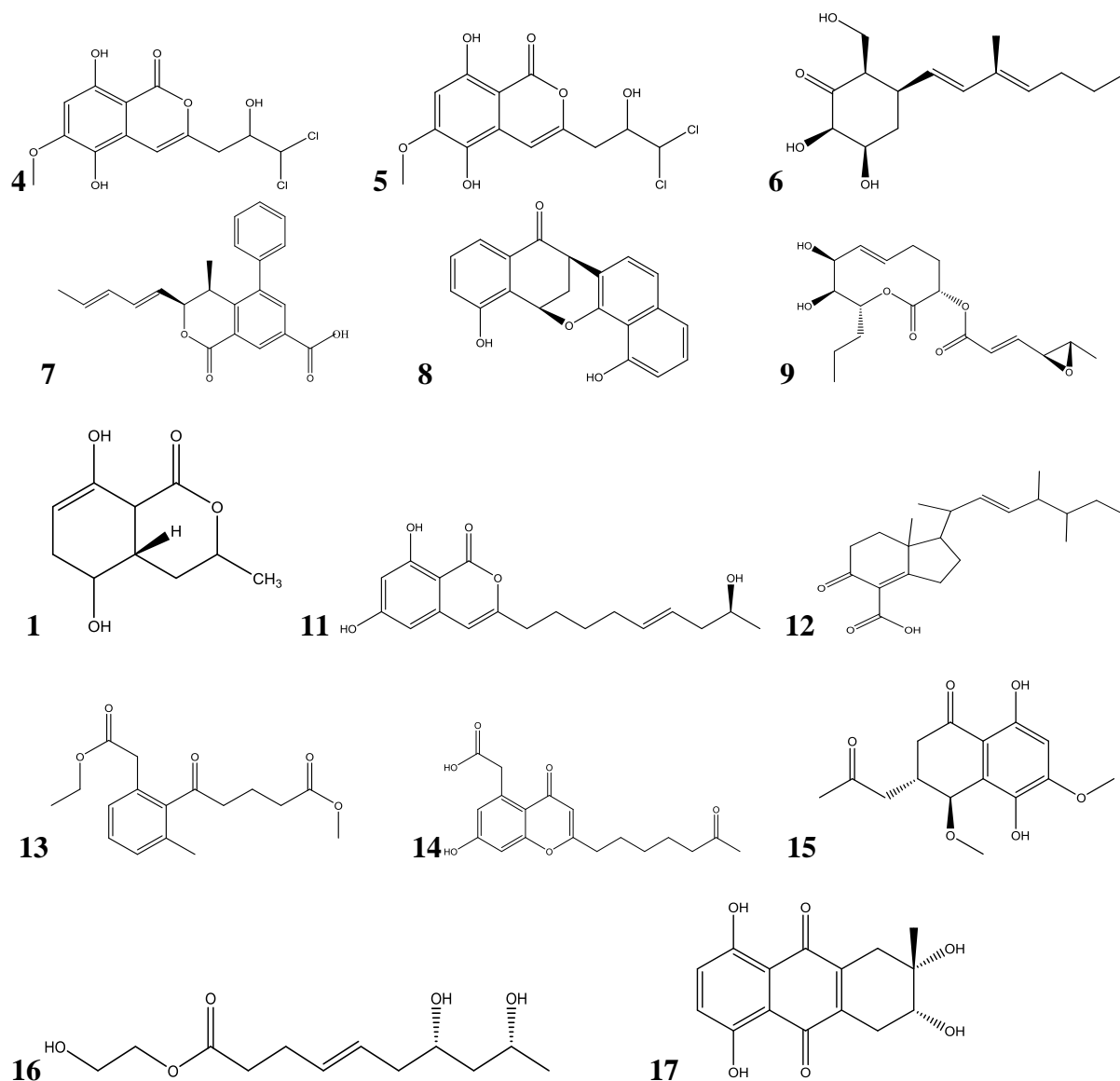
Figure 1: The structures of polyketides (1-17) isolated from endophytic fungi

V. CONCLUSION

This review demonstrated the polyketides and the enzyme polyketide synthases (PKS) yielded by various endophytic fungi. Most of the endophytic crude extracts yielded after fermentation exhibited various biological activities including antimicrobial, anti-inflammatory, and cytotoxic activities. Some isolated polyketides had a low level of activity. However, their distinct structures may provide fertile ground for additional research. Thus, these polyketides are valuable and can be used for drug development. Plant endophytes, on the other hand, are still poorly studied in terms of their medicinal qualities. For the structure elucidation of these polyketides, spectroscopic methods such as 1D, and 2D NMR studies are often used. In order to identify if endophytic fungus have the PKS gene, degenerate primers were utilized. Heterologous expression facilitates the production of native polyketides. There

are still a lot of biosynthetic potentials to be discovered. This is a fruitful study area that will continue to advance in the coming years.

REFERENCE

[1]   Bacon, Charles W, James White (2000) Microbial endophytes. CRC press, New York.

[2]   Rodriguez RJ, White JF.Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182: 314–330. doi: 10.1111/j.1469-8137.2009.02773.x

[3]   Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implication of their occurrence. J Nat Prod 69:509–526.

[4]   Faeth SH, Fagan WF (2002) Fungal endophytes: common hostplant symbionts but uncommonmutualists. Integr Comp Biol 42:360–368. doi: 10.1093/icb/42.2.360

[5]   Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol 54:323–342

[6]   Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat.Prod.Rep 23:753–771. doi:10.1039/b609472b

[7]   Strobel GA (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544.

[8]   Roopa G, Madhusudhan MC, Sunil KCR, Lisa N, Calvin R, Poornima R, Zeinab N, Kini KR, Prakash HS, Geetha N (2015) Identification of Taxol-producing endophytic fungi isolated from Salacia oblonga through genomic mining approach. J Genet Eng Biotechnol 13:119-127.

[9]   Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of pacific yew. Sci. 260:214-216.

[10]   Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from Nothapodytes foetida that produces camptothecin. J Nat Prod 68:1717–1719.

[11]   Puri SC, Nazir A, Chawla R, Arora R, Riyaz-ul-Hasan S, Amna T, et al (2006) The endophytic fungus Trametes hirsuta as a novel alternative source of podophyl-lotoxin and related aryl tetralin lignans. J Biotechnol 122:494–510.

[12]   Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012) An endophytic fungus from Azadirachta indica A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28:1287–94.

[13]   O'Hagan D (1991) The Polyketide Metabolites. Ellis Horwood, New York

[14]   Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chem Int Ed 48:4688-4716.

[15]   Cox RJ (2007) Polyketides, proteins and genes in fungi: programmed nano-machines begin to reveal their secrets. Org Biomol Chem 5:2010–2026.

[16]   Borchardt JK (1999) Combinatorial biosynthesis: panning for pharmaceutical gold. Mod Drug Discov 2:22–29.

[17]   Hopwood DA, Khosla C (1992) Genes for polyketide secondarymetabolic pathways in microorganisms and plants. Ciba Found Symp 171:88–106.

[18]   Miyanaga A (2017) Structure and function of polyketide biosynthetic enzymes: various strategies for production of structurally diverse polyketides. Biosci Biotechnolo Biochem 81:2227-2236.

[19]   Shen B (2003) Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. Curr Opin Chem Biol 7**:**285-295.

[20]   Weissman KJ (2009) Introduction to polyketide biosynthesis. Methods Enzymol 459:3–16.

[21]   Yu D, Xu F, Zeng J, Zhan J (2012) Type III polyketide synthases in natural product biosynthesis. IUBMB life 64**:**285-295.

[22]   Herbst DA, Townsend CA, Maier T (2018) The architectures of iterative type I PKS and FAS. Nat Prod Rep 35:1046–1069. doi: 10.1039/c8np00039e

[23]   Manoharan G, Sairam T, Thangamani R., Ramakrishnan D, Tiwari MK, Lee JK et al (2019). Identification and characterization of type III polyketide synthase genes from culturable endophytes of ethnomedicinal plants. Enzyme Microb Technol 131:109396. doi: 10.1016/j.enzmictec.2019.109396

[24]   Sun L, Wang S, Zhang S, Yu D, Qin Y, Huang H et al (2016) Identification of a type III polyketide synthase involved in the biosynthesis of spirolaxine. Appl Microbiol Biotechnol 100:7103–7113. doi: 10.1007/s00253-016-7444-5

[25]   Bingle LE, Simpson TJ, Lazarus CM (1999) Ketosynthase domain probes identify two subclasses of fungal polyketide synthase genes. Fungal Genet Biol 26:209–223

[26]   Nicholson TP, Rudd BA, Dawson M, Lazarus CM, Simpson TJ, Cox RJ (2001) Design and utility of oligonucleotide gene probes for fungal polyketide synthases. Chem Biol 8:157–178.

[27] Keller NP, Brown D, et al (1995) A conserved polyketide mycotoxin gene cluster in Aspergillus nidulans. Molecular approaches to food safety issues involving toxic microorganisms. Alaken Inc, Fort Collins

[28] Wang Y, Gao BL, Li XX, Zhang ZB, Yan RM, Yang HL, Zhu D (2015) Phylogenetic diversity of culturable endophytic fungi in Dongxiang wild rice (Oryza rufipogon Griff), detection of polyketide synthase gene and their antagonistic activity analysis. Fungal Biol 119:1032-1045.

[29] Rojas JD, Sette LD, de Araujo WL, Lopes MSG, da Silva LF, Furlan RL, Padilla G (2012) The diversity of polyketide synthase genes from sugarcane-derived fungi. Microb Ecol 63:565-577.

[30] Lin X, Huang YJ, Zheng ZH, Su WJ, Qian XM, Shen YM (2010) Endophytes from the pharmaceutical plant, Annona squamosa: isolation, bioactivity, identification and diversity of its polyketide synthase gene. Fungal Divers 41:41-51.

[31] Gao Y, Wang L, Kalscheuer R, Liu Z, Proksch P (2020) Antifungal polyketide derivatives from the endophytic fungus Aplosporella javeedii. Bioorg Med chem 28:115456.

[32] Cai R, Wu Y, Chen S, Cui H, Liu Z, Li C, She Z (2018) Peniisocoumarins A–J: isocoumarins from Penicillium commune QQF-3, an endophytic fungus of the mangrove plant Kandelia candel. J Nat Pro 81:1376-1383.

[33] Guo ZY, Lu LW, Bao SS et al (2018) Xylariaopyrones A–D,four new antimicrobial a-pyrone derivatives from endophytic fungus Xylariales sp. Phytochem Lett 28:98–103.

[34] Zhao T, Xu LL, Zhang Y et al (2018) Three new α-pyrone derivatives from the plant endophytic fungus Penicillium ochrochloronthe and their antibacterial, antifungal and cytotoxic activities. J Asian Nat Prod Res 21:851-858.

[35] Ola AR, Tawo BD, Belli HLL, Proksch P, Tommy D, Hakim EH (2018) A new antibacterial polyketide from the endophytic fungi Aspergillus fumigatiaffinis. Nat Prod Commun 13:1934578X1801301202.

[36] Chomcheon P, Wiyakrutta S, Aree T, Sriubolmas N, Ngamrojanavanich N, Mahidol C, Ruchirawat S, Kittakoop P (2010) Curvularides A–E: antifungal hybrid peptide–polyketides from the endophytic fungus Curvularia geniculate. Chem Eur J 16:11178-11185.

[37] Mandelare PE, Adpressa DA, Kaweesa EN, Zakharov LN Loesgen S (2018) Coculture of two developmental stages of a marine-derived Aspergillus alliaceus results in the production of the cytotoxic bianthrone allianthrone. J Nat Prod 81:1014-1022.

[38] Wang WX, Li ZH, Feng T, Li J, Sun H, Huang R, Yuan QX, Ai HL Liu JK (2018) Curtachalasins A and B, two cytochalasans with a tetracyclic skeleton from the endophytic fungus Xylaria curta E10. Org Lett 20:7758-7761.

[39] Wang WX, Lei X, Ai HL, Bai X, Li J, He J, Li ZH, Zheng YS, Feng T, Liu JK (2019) Cytochalasans from the endophytic fungus Xylaria cf. curta with resistance reversal activity against fluconazole-resistant. Candida albicans. Org Lett 21:1108-1111.

[40] Suzuki T, Arifta NR, Koseki T, Furuno H, Kwon E, Momma H, Harneti D, Maharani R, Supratman U, Kimura K, Shiono Y (2019) New polyketides, paralactonic acids A–E produced by Paraconiothyrium sp. SW-B-1, an endophytic fungus associated with a seaweed, Chondrus ocellatus Holmes. Fitoterapia 132:75-81.

[41] Xia GY, Wang LY, Xia H, Wu YZ, Wang YN, Lin PC, Lin S (2019) Three new polyketides from the endophytic fungus Penicillium purpurogenum. J Asian Nat Prod Res 1-8.

[42] Liu H, Chen S, Liu W, Liu Y, Huang X, She Z (2016) Polyketides with immunosuppressive activities from mangrove endophytic fungus Penicillium sp. ZJ-SY2. Mar Drug 14:217.

[43] Barnes EC, Jumpathong J, Lumyong S, Voigt K, Hertweck C (2016) Daldionin, an unprecedented binaphthyl derivative, and diverse polyketide congeners from a fungal orchid endophyte. Chem Eur J 22:4551-4555.

[44] Noumeur SR, Helaly SE, Jansen R, Gereke M, Stradal TE, Harzallah D Stadler M (2017) Preussilides A–F, bicyclic polyketides from the endophytic fungus Preussia similis with antiproliferative activity. J Nat Prod 80:1531-1540.

[45] Wang WX, Zheng MJ, Li J, Feng T, Li ZH, Huang R, Zheng YS, Sun H, Ai HL, Liu JK (2019) Cytotoxic polyketides from endophytic fungus Phoma bellidis harbored in Ttricyrtis maculate. Phytochem Lett 29:41-46.

[46] Ma KL, Wei WJ, Li HY, Wang LD, Dong SH, Gao, K (2019) Phomotide A, a Novel Polyketide, from the Endophytic Fungus Phomopsis sp. CFS42. Tetrahedron Lett 151468.

[47] Darsih C, Prachyawarakorn V, Mahidol C, Ruchirawat S, Kittakoop P (2017) A New Polyketide from the Endophytic Fungus Penicillium chermesinum. Indones J Chem 17:360-364.

[48] Hemphill CFP, Daletos G, Liu Z, Lin W, Proksch P (2016) Polyketides from the mangrove-derived fungal endophyte Pestalotiopsis clavispora. Tetrahedron Lett 57:2078-2083.

[49] Talontsi FM, Dittrich B, Schüffler A, Sun H, Laatsch H (2013) Epicoccolides: Antimicrobial and antifungal polyketides from an endophytic fungus Epicoccum sp associated with Theobroma cacao. Eur J Org Chem 15:3174-3180.

[50] Yang SX, Gao JM, Zhang Q, Laatsch H (2011) Toxic polyketides produced by Fusarium sp., an endophytic fungus isolated from Melia azedarach. Bioorg Med Chem Lett 21: 1887-1889.

[51] da Silva PH, Souza MPD, Bianco EA, da Silva SR, Soares LN, Costa EV, Silva F, Barison A, Forim MR, Cass QB, de Souza AD (2018) Antifungal polyketides and other compounds from Amazonian endophytic Talaromyces fungi. J Braz Chem Soc 29:622-630.

[52] Santiago C, Sun L, Munro MHG, Santhanam J (2014) Polyketide and benzopyran compounds of an endophytic fungus isolated from C innamomum mollissimum: biological activity and structure. Asian Pac J Trop Biomed 4:627-632.

[53] Qi C, Gao W, Wang J, Liu M, Zhang J, Chen C, Hu Z, Xue Y, Li D, Zhang Q Lai, Y (2018) Terrusnolides AD, new butenolides with anti-inflammatory activities from an endophytic Aspergillus from Tripterygium wilfordii. Fitoterapia 130:134-139.

[54] Sharma V, Singamaneni V, Sharma N, Kumar A, Arora D, Kushwaha M, Bhushan S, Jaglan S, Gupta P (2018) Valproic acid induces three novel cytotoxic secondary metabolites in Diaporthe sp., an endophytic fungus from Datura inoxia Mill. Bioorg Med Chem Lett 28:2217-2221.

[55] Chen J, Bai X, Hua Y, Zhang H, Wang H (2019) Fusariumins C and D, two novel antimicrobial agents from Fusarium oxysporum ZZP-R1 symbiotic on Rumex madaio Makino. Fitoterapia 134:1-4.

[56] Zheng CJ, Huang GL, Liao HX, Mei RQ, Luo YP, Chen GY, Zhang QY (2019) Bioactive cytosporone derivatives isolated from the mangrove-derived fungus Dothiorella sp. ML002. Bioorg Chem 85:382-385.

[57] Hu M, Yang XQ, Wan CP et al (2018) Potential antihyperlipidemic polyketones from endophytic Diaporthe sp. JCJ7 in Dendrobium nobile. RSC Adv 8:41810–41817.

[58] Liu z, Qiu P, Li J, et al (2018) Anti-inflammatory polyketides from the mangrove derived fungus Ascomycota sp. SK2YWS-L. Tetrahedron 74: 746-751.

[59] Wu YZ, Zhang HW, Sun ZH, Dai JG, Hu YC, Li R, Lin PC, Xia GY, Wang LY, Qiu BL, Zhang JF (2018) Bysspectin A, an unusual octaketide dimer and the precursor derivatives from the endophytic fungus Byssochlamys spectabilis IMM0002 and their biological activities. Eur J Med Chem 145:717-725.

[60] Pang XJ, Zhang SB, Chen HL, Zhao WT, Yang DF, Xian PJ, Xu LL, Tao YD, Fu HY, Yang XL (2018) Emericelactones AD: four novel polyketides produced by Emericella sp. XL 029, a fungus associated the leaves of Panax notoginseng. Tetrahedron Lett 59:4566-4570.

[61] Abdel-Wahab MF, Kurtan T, Mandi A et al (2018) Induced secondary metabolites from the endophytic fungus Aspergillus versicolor through bacterial co-culture and OSMAC approaches. Tetrahedron Lett 59:2647-2652.

[62] Song JH, Lee C, Lee D, Kim S, Bang S, Shin MS, Lee J, Kang KS, Shim SH (2018) Neuroprotective compound from an endophytic fungus, Colletotrichum sp. JS-0367. J Nat Prod 81:1411-1416.

[63] Cui H, Zhang H, Liu Y, Gu Q, Xu J, Huang X, She, Z (2018) Ethylnaphthoquinone derivatives as inhibitors of indoleamine-2, 3-dioxygenase from the mangrove endophytic fungus Neofusicoccum austral SYSU-SKS024. Fitoterapia 125:281-285.

[64] Zhao JW, Yang ZD, Zhou SY, Yang LJ, Sun JH, Yao XJ, Shu ZM, Li S (2019) Wortmannine F and G, two new pyranones from Talaromyces wortmannii LGT-4, the endophytic fungus of Tripterygium wilfordii. Phytochem Lett 29:115-118.

[65] Kornsakulkarn J, Choowong W, Rachtawee P, Boonyuen N, Kongthong S, Isaka M Thongpanchang C (2018) Bioactive hydroanthraquinones from endophytic fungus Nigrospora sp. BCC 47789. Phytochem Lett 24:46-50.