



Research article

Chemical analysis and anticancer activity of sesterterpenoid from an endophytic fungus *Hypomontagnella monticulosa* Zg15SU and its host *Zingiber griffithii* BakerAnisa Lutfia^a, Erman Munir^{a,*}, Yurnaliza Yurnaliza^a, Mohammad Basyuni^b^a Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia^b Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, 20155, Indonesia

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ABSTRACT

Zingiber griffithii Baker is one of the native Zingiberaceae species in a tropical forest of North Sumatra, Indonesia. Zingiberaceae species have been intensively studied and reported as herbal ingredients in ethnomedicine and currently their endophytic fungal associates were studied for pharmacological importance. Fifteen endophytic fungi were isolated from *Zingiber griffithii* following morphological and molecular characterization. All isolates exhibited antibacterial properties to at least one of the tested pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, Methicilin-resistant *S. aureus* (MRSA), and Enteropathogenic *E. coli* (EPEC). The isolate, identified as *Hypomontagnella monticulosa* strain Zg15SU (syn. *Hypoxylon monticulosum* Mont.) based on its rDNA/ITS sequence, displayed antibacterial activities to all tested pathogens. The EtOAc extract of the *H. monticulosum* Zg15SU showed the highest activity for gram-negative bacteria, the *E. coli* and EPEC, while the extract of *Z. griffithii* rhizome displayed activity only for *E. coli*. The gas chromatography-mass spectrometry analysis (GC-MS) indicated a major portion of similar compounds found in both the endophytic fungus and plant extract, revealing the compounds of oleic acid, cyclononasiloxane, octadecamethyl, and eicosanoic acid. Furthermore, purification and structural elucidation on the EtOAc extract of both *Z. griffithii* rhizome and *H. monticulosa* Zg15SU yielded two bioactive compounds: a novel compound, griffithiène, a terpenoid-alkaloid bearing the skeleton of a scalarane (1) and scalaradiol (2) which were confirmed by ¹H- (500 MHz) and ¹³C-NMR (125 MHz) spectroscopy. Importantly, the elucidated compounds showed a cytotoxicity activity against cancer cell lines, the Panc-1, NBT-T2, and HCT116 based on in vitro MTT proliferation assay. This is the first report of *Z. griffithii* harboring an endophytic fungus, *H. monticulosa*, which produced potential antibacterial and anticancer metabolites along with its host to be utilized for future prospects.

1. Introduction

Plant materials have been considered the most promising source of biologically active compounds. Numerous plant species have been utilized in the local, regional and global scope as an isolation source of medicinal ingredients which have been studied thoroughly in pharmacological fields. A majority of medicinal plants have been reviewed to initiate and preserve symbiotic association with some endophytic fungi intensively involved in the co-synthesis of bioactive compounds (Strobel 2018). The fungi may have also been bestowed in the effectivity of phytochemical compounds produced by the plants for their defence against pathogen. The endophytic fungi are variably present in the plant parts by having ubiquitous symbiosis as slightly parasitic microorganisms

for all, or at least some, part of their life cycle (Strobel 2003; Strobel and Daisy 2003). The endophytic fungi are also considered to be a prominent producer of bioactive metabolites which can be developed in future drugs (Deshmukh et al., 2015).

Zingiberaceae is a medicinal plant family regularly used in traditional remedies and as herbal medicine, especially in Indonesia (Fernando and Nursyahra, 2008; Hartanto et al. 2014; Juwita et al. 2018). The associative endophytes have been reported from various sources leading to the progressive development of study and exploration of diverse endophytic fungi harbouring different habitats (Ginting et al. 2013; Anisha and Radhakrishnan, 2017; Hastuti et al., 2008). A vast number of endophytic fungi in Zingiberaceae species may also indicate an inter-relationship among the fungi within the plant tissue in the way of

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the co-synthesis of desired phytochemicals. However, studies of its phytochemical constituents produced by both the endophyte and its host are in progress to reveal the whole stable and mutual relationship (Aly et al. 2011).

Hypoxylon is a genus belonging to a family, Xylariaceae with identified 16 genera and 130 species (Sanchez-Ballesteros et al., 2007). The genus is the largest member within the family primarily distributed in diverse marine and terrestrial habitats, along with its potential as a producer of various bioactive compounds (Stadler et al. 2006, 2008). Recent investigations have been reported on natural products produced by *H. monticulosum*. The strain of *H. monticulosum* isolated from a sponge, *Sphaerocladina* in Tahiti, produced four novel phenolic acid derivatives, namely Sporochartines B-E to its culture broth. The purified compounds showed significant biological activities against human cancer cell lines in cytotoxic bioassays (Leman-Loubière et al., 2017a). In other reports, a similar strain also produced two sporothriolide-related compounds, later named Sporochartine A, Sporothriolide and Deoxysporothic acid (Leman-Loubière et al. 2017b). Both compounds were monocyclic acid precursors and reported as potential anti fungi without any inhibitory activity against bacteria. Based on recent taxonomical studies and polyphasic approaches, the species is now placed into its own clade as *Hypomontagnella monticulosa* (Mont.) Sir, L. Wendt & C. Lambert suggesting different family taxa or Hypoxylaceae (Lambert et al., 2019). Moreover, until now, the bioprospecting study of *H. monticulosa* metabolite is still limited and worth investigating.

In this study, an endophytic fungal isolate later identified as *Hypomontagnella monticulosa* was isolated from *Zingiber griffithii* Baker rhizome, in a tropical forest of Tongkoh Bukit Barisan, North Sumatra. The strain was identified based on its ITS-DNA region and exhibits potential antibacterial activities against pathogenic bacteria and multidrug-resistant strains. To our understanding, this is the first report on endophytic *H. monticulosum* from Zingiberaceous species, specifically from *Z. griffithii* Baker. In addition, the rhizome itself contained an unknown compound, proposed as a novel compound in the group of alkaloid-terpenoid with the potential as anticancer against cancer cell lines.

2. Materials and methods

2.1. Plant material

The fresh rhizome of *Zingiber griffithii* Baker was sampled from the forest area of Tahura Tongkoh Bukit Barisan, North Sumatra, Indonesia. The rhizome was collected and preserved in a sterile Ziploc bag, containing ice (4 °C) and transported or processed within 48 h. Voucher specimens were collected for authentication by the service from Herbarium Medanese, Department of Biology, Universitas Sumatera Utara, Medan, Indonesia.

2.2. Isolation of endophytic fungi from the rhizome interior

The procedure of fungal isolation followed method as described by Yurnaliza et al. (2014). Rhizomes (1–2 cm in length) were cleaned with tap water to remove dirt and soil particles. The rhizomes were sterilized by being immersed in sterilizing agents: 75% ethanol, 5.3% NaOCl, and 75% ethanol, for 2 min, 5 min, and 30 s, respectively. Thereafter, the sterilized fragments were washed in sterile distilled water to remove surface sterilization solutions. Each fragment was divided into smaller pieces (0.5 cm) and plated on Potato Dextrose Agar (Oxoid™) supplemented with 0.1% (w/v) chloramphenicol to suppress the bacterial growth. Cultures were incubated in an ambient condition for 3 days. Then, any visible fungal growth from each piece was sub-cultured into a new medium to maintain the strains. Each fungal strain was differentiated preliminarily based on morphological features.

2.3. Antagonism assay

PDA agar supplemented with 1% (w/v) yeast extracts (Merck, Germany) medium was used for the antagonism assay. *S. aureus* ATCC® 29213™, Methicillin-resistant *S. aureus* ATCC® 43300™, *E. coli* ATCC® 25922™, and Enteropathogenic *E. coli* K11 were of collections of the Hospital of Universitas Sumatera Utara, Medan, Indonesia. The antagonism assay of endophytic fungi was performed by dual culture assay (Balouiri et al. 2016). Direct bacterial suspensions were made by swabbing colonies and dipping them in a sterile saline solution (0.95% NaCl) to obtain OD₆₀₀: 0.5 ≈ 1.2 × 10⁸ CFU/mL. One mL of cell suspension was inoculated into 15 mL molten PDA (45 °C), and then plated to get microbial lawns. Three agar plugs of fungal mycelium were placed over the medium. The plates were incubated for 2 days in ambient temperature. Clear zones indicating inhibitory activities around mycelial plugs indicating antagonism were measured in millimetre unit (mm) using the standard caliper.

2.4. Molecular identification

The selected endophytic fungus, isolate Zg15SU, was identified based on its ITS-rDNA sequence region. The fungal DNA genome was extracted following the procedure as supplied by Wizard® Genomic DNA Purification Kit Protocol (United States). The ITS-rDNA sequence was amplified using the universal primer for fungi: ITS1 (5'-CTTGGTCATTAGAGGAAGTAA-3) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Manter and Vivanco 2007) with a reaction mixture composed of: 12 µL nuclease-free water (NFW), 20 µL GoTaq DNA Polymerase solution, 2 µL ITS-1F primer solution, 2 µL ITS-4 primer solution, 4 µL DNA template solution with a total volume of 40 µL in Eppendorf tube. The PCR was conditioned as: 95 °C for 3 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, and final extension at 72 °C for 7 min. The ITS-DNA amplicons were commercially sequenced to Macrogen, Inc. Korea. The molecular identity from isolate Zg15SU was compared with other fungal ITS sequences obtained from databases of the National Centre for Biotechnology Information (NCBI). The sequences were searched using the Basic Local Alignment Search Tool for nucleotide (BLASTn) (Zhang et al., 2000; Morgulis et al. 2008). Furthermore, the sequence data were aligned using MUSCLE in software MEGA ver.7.0 (Edgar 2004; Kumar et al. 2016). A phylogenetic tree is constructed based on the neighbour-joining method with bootstrap replication 1000x (Felsenstein 1985; Saitou and Nei 1987).

2.5. Fermentation condition

An endophytic fungus, *Hypomontagnella monticulosa*, which exhibited the strongest and most wide-spectrum antibacterial activity during preliminary screening was subjected to liquid-state fermentation (Marcellano et al. 2017). Three pieces of the mycelial plug were inoculated into each 100 mL Potato Dextrose Broth (PDB) and incubated at an ambient temperature for 4 weeks without agitation. Biomass or mycelial mats were washed using sterile distilled water, collected, and dried at room temperature after the incubation period. Mycelial mats were manually macerated using 100 mL EtOAc for 3 days under agitation. The EtOAc extract was separated using a buchner funnel placed with a Whatman # 1 filter paper. The extract was then concentrated *in vacuo* and stored at 4 °C for further experimentation.

2.6. Metabolite extraction of the *Zingiber griffithii* Baker rhizome

The extraction of metabolites from *Z. griffithii* rhizome used a similar solvent, EtOAc by maceration (Harborne 1987; Altemimi et al. 2017). Five-hundred grams of rhizome powder obtained from air-dried rhizome parts were submerged and macerated using EtOAc (w/v) for 3 days under agitation. The macerates were filtered and separated using a separatory

funnel and filter paper Whatman # 1. The extract was concentrated using a rotary evaporator at *in vacuo* and stored in 4 °C for further experiments.

2.7. Antibacterial assay

The EtOAc extracts from *H. monticulosa* mycelium and *Z. griffithii* rhizome were assayed for their antibacterial activities based on the standard disk diffusion assay (Balouiri et al. 2016). Suspensions of bacteria were prepared from 24 h old culture. The suspensions were prepared ($OD_{600} = 0.08\text{--}0.10$) approximately 1.5×10^8 CFU/mL based on the 0.5 McFarland solution. The cell suspensions were inoculated on top of Mueller-Hinton Agar (MHA). The dried crude extracts were diluted into 10% (v/v) dimethyl sulfoxide (DMSO), and then 10 μ L of extracts were pipetted to a 5-mm sterile disk (Whatman Paper no. 1). A paper disk eluted with chloramphenicol (30 μ g) was included as a positive control in the MHA plates. All plates were incubated at room temperature for 24 h and any visible zones of inhibition were measured using regular millimetre-unit caliper (mm).

2.8. GC-MS analysis

The EtOAc extracts of *H. monticulosa* mycelium and *Z. griffithii* rhizome were determined for its chemical composition using GCMS-QP2010 (Shimadzu Europa GmbH, Dusseldorf, Germany) instrument fitted with RTX-5MS (30 \times 0.25 \times 0.10 m) column. The volume of injection was 1 μ L. The oven temperature was maintained at 80.0 °C for 1 min and programmed to increase until 250 °C at the rate of 5 °C/min. The

total flow was 6.0 mL/min within the pressure of 102.5 kPa. Helium was used as the carrier gas with a flow rate of 1.50 mL/min at a linear speed of 45.1 cm/s. The samples were injected in splitless mode. The scanning range of the spectral mass was set at 30 to 600 (m/z). The identification of potentially bioactive compounds present in the extract was done by comparison to the library of the National Institute of Standard and Technology, NIST, US. All identified compounds were summarized in terms of the percentage relative peak area.

2.9. Structural elucidation

Optical rotations were recorded on a polarimeter Jasco DIP-1000. The IR spectra were measured on the spectrophotometer of Hitachi T-2001. HRESIMS-ESI spectra was recorded on a Jeol Mass spectrometer. The ^1H , ^{13}C NMR, DEPT, COSY, HMBC, and HSQC were recorded on a Bruker 500 MHz. Column chromatography was carried out on Merck silica gel 60. HPLC was conducted in reversed phase on a Hitachi L-4000H apparatus.

2.10. HPLC purification

Crude ethyl acetate extract of *Z. griffithii* rhizome was partitioned using EtOAc: H₂O (v/v). The EtOAc layer (24.2 mg) was separated by open column chromatography on silica gel using *n*-hexane: EtOAc gradient to produce 9 fractions. The major fraction was purified by HPLC RP-C₁₈ using MeOH: H₂O (10:1) yielded the novel compound (1). Meanwhile, the crude extract of *H. monticulosa* Zg15SU partitioned following a similar procedure to yield the EtOAc portion (16.3 mg) was



Figure 1. Wild *Zingiber griffithii* Baker in Tahura Tongkoh Bukit Barisan, Karo regency, North Sumatra, Indonesia. Scale bar: 10 cm.

separated using open column chromatography producing 4 fractions. The major fraction was purified by HPLC NP-SiO₂ using *n*-hexane: EtOAc (6:2) yielded the known compound (2).

2.11. Cytotoxicity assay

The cytotoxic effect of compounds 1 and 2 was determined by 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay using TACS™ MTT Cell Proliferation Assay (MTT-CPA) kit (Trevigen®, USA) in different human tumour cell lines viz. Panc-1 (pancreatic cancer), NBT-T2 (bladder cancer), and HCT116 (colon cancer). Briefly, 100 μL cells of known concentration (1.0 × 10⁴ cell density) were placed into triplicate wells for each diluted concentration of compounds. Ten microlitres of MTT reagent was added to each well and incubated for 2–4 h at 37 °C. Subsequently, 100 μL of detergent were added into each well following the presence of purple precipitate. The plate was incubated in the dark at 18–24 °C from 2 h to overnight. Negative control was prepared from untreated cells, MTT, and solubilizing buffer (10% SDS in 0.1 HCl). The absorbance was then determined by an ELISA reader at 570 nm. The inhibitory concentration (IC₅₀) value was extrapolated from the concentration-response plot using linear regression analysis.

3. Results

3.1. Antagonistic activity of endophytic fungi

The endophytic fungi isolated from *Z. griffithii* rhizome were sampled from a forest area of Tahura Tongkoh Bukit Barisan, Karo regency, North Sumatra. The forest was categorized as a montane secondary forest (>1,000 masl) harbouring diverse flora and fauna. The sample was collected by exploring the entire forest (Figure 1). A total of 17 endophytic fungal isolates were isolated from *Z. griffithii* rhizome and subjected to preliminary antagonism assay or dual culture assay (Figure 2). All isolates were found to inhibit the growth of pathogenic bacteria to at least one of the tested pathogens (Table 1). From those 17 antagonistic strains, all of them inhibited *S. aureus* by 100% (17/17) while the majority of them inhibited *E. coli* by 94% (16/17). MRSA was inhibited by 3 strains, namely Zg03SU, Zg14SU, and Zg15SU by 17% (3/17) while EPEC was inhibited by only two strains, namely Zg10SU and Zg15SU by

11% (2/17). Therefore, an isolate encoded Zg15SU showed a wide spectrum activity, inhibiting both Gram-positive/negative bacteria and was subjected to further assays.

3.2. Molecular identification of isolate Zg15SU

The potential isolate, Zg15SU was identified for its species identity using the ITS-rDNA region. The ITS sequence was then checked for similarity using the nucleotide database retrieved from NCBI. Based on the similarity percentage, the isolate Zg15SU was recognized as *Hypomontagnella monticulosa*, an Ascomycetous fungus with a percentage of 99% (data not shown). The ITS sequence was submitted into GenBank and given an accession code, MN718862.1. The phylogenetic relationship among members in Hypoxylaceae was confirmed by constructing a phylogenetic tree (Figure 3). The phylogenetic tree was constructed using the neighbour-joining method and analysed using the Kimura 2-parameter method with the gamma distribution model as the best DNA model predictor in MEGA7.0.

3.3. Antibacterial assay

Both the *Z. griffithii* rhizome and *H. monticulosa* mycelium were extracted using an organic solvent, EtOAc. The EtOAc extracts were tested for its antibacterial activity against the same pathogens using standard disk diffusion assay (Table 2). Chloramphenicol as positive control produced antibacterial activity to all bacteria, except MRSA. A crude extract of *H. monticulosa* was observed to inhibit *E. coli* EPEC and *S. aureus* with no inhibition against MRSA, indicating that the extract has a considerably wide spectrum of antibacterial activity.

3.4. GC-MS analysis

The mass spectra profiles from the EtOAc extract of *Z. griffithii* rhizome and *H. monticulosa* mycelium were analysed using GC-MS. The peak constituting different chemicals were detected and identified according to their relative peak area, RT-based on NIST library databases (Figure 4). The chemical composition was summarized and discussed descriptively to point out the similarity among chemicals produced by both the host and endophyte (Table 3). Oleic acid, Cyclononasiloxane, octadecamethyl-, and Eicosanoic acid were detected as the highest proportion of chemicals from both extracts while indicating a relationship between organisms through co-synthesis.

3.5. Structural elucidation and cytotoxicity properties

Compound 1 was isolated as an amorphous powder, [α]_D²⁵ = +65.6° (CHCl₃, *c* 0.65). Compound was isolated as a yellowish/reddish powder and given a molecular formula of C₂₃H₃₈N₁NaO₃ (*m/z* = 376.54380 with = Δ-0.09 mmu). IR absorptions were observed at 3389, 1731 and 1374 cm⁻¹, suggesting the presence of hydroxyl, ester and aromatic tertiary amine. Analysis of 2D NMR data (COSY, HMQC, HMBC) revealed that compound 1 is a Terpenoid-Alkaloid which has a skeleton of scalarane but having nitrogen atom in the cyclic skeleton that has double bond with C16. Since the carbonyls and C=N account for two out of the six degrees of unsaturation required by the formula, compound 1 must be tetracyclic. The proton NMR spectrum of compound 1 that measured in CDCl₃ (C₆D₆) revealed the presence of typical spectrum of steroid/terpenoid, i.e. five singlet methyl groups (sp³), at 0.80, 0.82, 0.86, 0.87, 0.94 (Table 4).

The signal at δ 6.84 ppm (1H, d, 4.9; 3.5 Hz) was assigned to an olefinic proton of the unsaturated carbon (C=C) at C-16. The ¹³C NMR and DEPT spectra indicated the presence of 23 carbon signal including a carbonyl carbon at 169.9 ppm; six olefinic carbons at 41.9, 33.28, 56.4, 17.9, 135.5, 51.0; five quaternary carbon at 33.28, 17.9, 36.8, 37.6, 169.9; five methine carbons at 56.4, 52.3, 74.4, 49.5 and 135.5. The COSY and HSQC analysis led to assignment of the following spin systems:

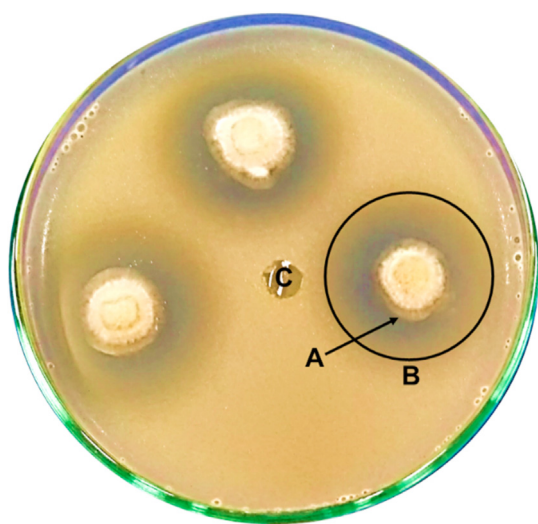


Figure 2. Dual culture antagonistic test on PDA+1% (w/v) yeast extract agar medium. Three agar plugs of isolate Zg15SU (A) was inoculated on top of a lawn of Methicillin-resistant *Staphylococcus aureus* ATCC® 43300™ (±1.2 × 10⁸ CFU/mL). Following incubation at 25–28 °C, a clear zone around fungal plugs indicated a presence of inhibition (B) against bacterial lawn. This is compared to control agar plug without inoculation (C).

Table 1. Antagonistic activity of endophytic fungi isolated from *Zingiber griffithii* rhizome.

No	Isolate code	Inhibition zone (mm)*, ^a			
		<i>S. aureus</i>	<i>E. coli</i>	MRSA	EPEC
1	Zg01SU	20.00 ± 1.20	n.d	n.d	n.d
2	Zg02SU	22.27 ± 1.71	14.67 ± 1.04	n.d	n.d
3	Zg03SU	21.77 ± 2.87	13.33 ± 1.17	12.43 ± 0.49	n.d
4	Zg04SU	19.13 ± 0.55	15.07 ± 1.72	n.d	n.d
5	Zg05SU	19.90 ± 0.00	21.20 ± 4.08	n.d	n.d
6	Zg06SU	30.60 ± 2.45	18.47 ± 1.56	n.d	n.d
7	Zg07SU	24.73 ± 3.49	11.13 ± 1.86	n.d	n.d
8	Zg08SU	25.67 ± 2.06	17.53 ± 0.32	n.d	n.d
9	Zg09SU	21.07 ± 1.85	18.33 ± 2.90	n.d	n.d
10	Zg10SU	22.50 ± 1.64	22.07 ± 2.35	n.d	20.46 ± 0.95
11	Zg11SU	17.40 ± 2.86	16.77 ± 2.97	n.d	n.d
12	Zg12SU	23.73 ± 1.20	17.27 ± 3.98	n.d	n.d
13	Zg13SU	24.70 ± 1.30	17.70 ± 3.59	n.d	n.d
14	Zg14SU	26.50 ± 2.82	19.13 ± 3.30	9.70 ± 5.29	n.d
15	Zg15SU	24.00 ± 3.96	26.83 ± 1.56	13.63 ± 1.17	18.23 ± 0.12
16	Zg16SU	24.03 ± 2.39	24.53 ± 1.50	n.d	n.d
17	Zg17SU	23.10 ± 0.87	15.20 ± 5.00	n.d	n.d

n.d: no detectable inhibition zone.

This is to highlight the potential of our isolate for the next experimentation.

* mean diameter of inhibition zone ±S.D. (n = 3).

^a agar plug diameter of 6 mm.

CH₂-CH₂-CH₂ (C-1-C-3), CH-CH₂-CH₂ (C-5-C-7), CH-CH₂-CHOH (C-9-C-12), CH-CH₂ (C-14-C-15), CH-CH (C-16-C-17). The attachment of the hydroxy group at C-17 was deduced from the proton signal at δ 3.14 (1H, brq, 8.6; 4.8 Hz). The acetoxy group at C-12 was assigned from the proton signal (H-12) at δ 4.87 in coupling with H-11 by COSY, as well as from HMBC correlations to C-9 (52.3) and C-17 (51.0). Furthermore, the presence of nitrogen moiety was confirmed at C-15 (2H, 2.36; 2.13, m), the COSY signal indicated that the C-15 cross peak at C-14/C-15 (see supplementary Fig S1-S7).

Upon further analysis of compound **2**, the signal at δ 4.87 ppm (1H, brt, 2.9 Hz) was typical of the proton geminal to an acetoxy moiety at C-

12, indicating an axial orientation for this functionality and suggesting the configuration at this carbon position as scalarial (2) (Figure 5). The difference in ¹H NMR data of **1** was the presence of a hydroxy group at δ 3.14 ppm (1H, brq, 8.6; 4.8 Hz) at C-17 and were confirmed by HMBC and COSY with the cross peak at C-22/C-17, C-12/C-17, and C-16/C-17, respectively. Furthermore, the presence of nitrogen moiety as detected by HRESIMS was confirmed at C-15 (2H, 2.36; 2.13, m). The COSY signal indicated that the C-15 cross peak at C-14/C-15 indicated compound **1** as an unknown compound.

The IC₅₀ or cytotoxicity activities from compound **1** against cancer cell lines: Panc-1, NBT-T2, and HCT116 were 0.05, 0.75, and 0.05 ppm,

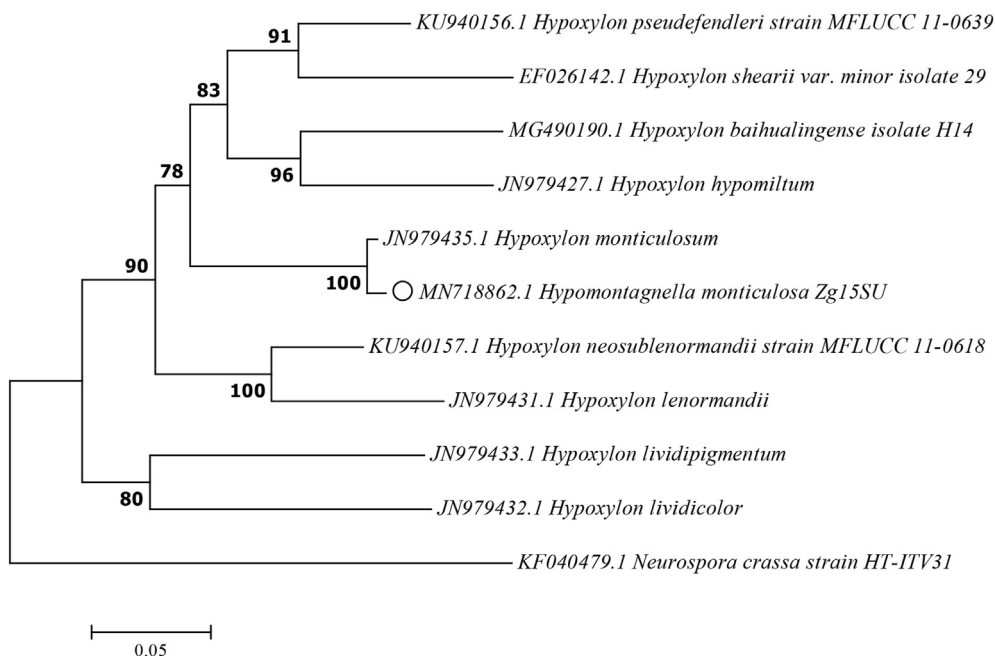


Figure 3. Phylogenetic tree showing the relationship between an isolate *Hypomontagnella monticulosa* Zg15SU (O) and related Xylarian species bootstrap value (BV) ≥70%; the tree was inferred using neighbour-joining and Kimura 2-parameter method, modelled with a gamma distribution.

Table 2. Antibacterial activity of EtOAc extracts from *Zingiber griffithii* rhizome and *Hypomontagnella monticulosa* Zg15SU mycelial extracts.

No	Extract	Inhibition zone (mm) ^{*,a}			
		<i>S. aureus</i>	<i>E. coli</i>	MRSA	EPEC
1	<i>H. monticulosa</i>	10.25 ± 1.18	20.90 ± 0.93	n.d	12.75 ± 3.50
2	<i>Z. griffithii</i>	n.d	10.35 ± 2.10	n.d	n.d
3	Chloramphenicol	25.10 ± 2.56	40.90 ± 0.60	n.d	17.92 ± 1.52

n.d: no detectable inhibition zone.

* mean diameter of inhibition zone ±S.D. (n = 3).

^a agar plug diameter of 6 mm.

respectively. Furthermore, the IC₅₀ value for compound 2 was 0.71, 0.30, and 0.67 ppm against respective cell lines.

4. Discussion

Endophytic fungi isolated from the medicinal plant have drawn much attention from researchers. Through exhaustive isolation and genome mining, some natural compounds may be yielded from the laboratory assay by using the diversity of endophytic fungi. Moreover, the synthesis of novel compound may be initiated by modifying the growth condition

of an isolate or so called OSMAC (One Strain, Many (VanderMolen et al. 2013)). Prior to a deeper investigation of compound analysis, we conduct a preliminary investigation upon selecting potential endophytic fungal isolates.

Members of Zingiberaceae have been reported to be colonized by beneficial endophytes which may co-synthesize a similar metabolites or bioactive compounds from its host as reported from previous study, mostly as antioxidant due to its medicinal nature. An endophytic fungus, *F. oxysporum* isolated from *Zingiber zerumbet* was reported to synthesize a high antioxidant compound in its culture extract possessing similar

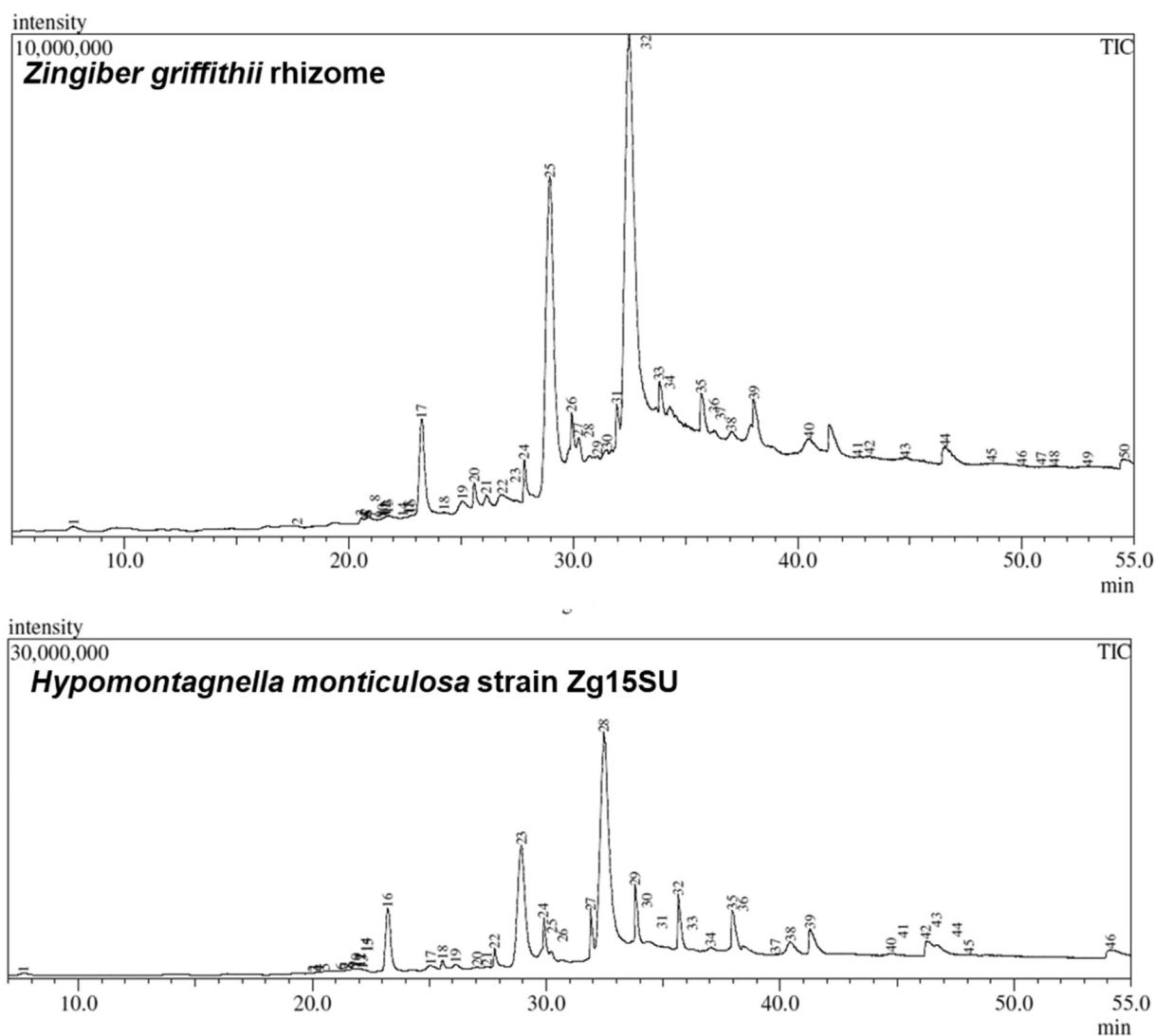


Figure 4. The GC-MS diagram of EtOAc extract from *Zingiber griffithii* rhizome and *Hypomontagnella monticulosa* Zg15SU mycelium.

Table 3. The identified compounds and their relative peak area percentage of EtOAc extracts of *Zingiber griffithii* rhizome and *Hypomontagnella monticulosa* Zg15SU mycelial extracts.

No.	Identified compounds	Peak area percentage (%)	
		<i>Z. griffithii</i> rhizome	<i>H. monticulosa</i> Zg15SU
1	1,2-Benzenedicarboxylic acid, ditridecyl ester	8.81	2.23
2	Cyclononasiloxane, octadecamethyl-	2.32	30.44
3	Diethyl Phthalate	1.92	4.56
4	Eicosanoic acid	1.12	14.09
5	Heptasiloxane, hexadecamethyl-	21.18	1.20
6	Isopropyl Palmitate	0.17	0.08
7	Nonanal	0.08	0.14
8	Octasiloxane	1.09	0.02
9	Oleic acid	22.89	34.00
10	Oleoyl chloride	3.10	2.61
11	Tridecanoic acid, methyl ester	0.04	0.26

properties as its host extract (Nongalleima et al. 2013). The mutualistic relationship exposed that plants have their own population structuring organization to certain fungal species, conferring benefits to both organisms (Aly et al. 2011; Jia et al. 2016).

Based on our results, we may also expect the wide spectrum of biological activities possessed by Zingiberaceae species to be the antimicrobials or at least antagonistic to certain pathogenic microbes. Previous studies have reported that different Zingiberaceae species may harbour different endophytic fungal isolates (Uzma et al. 2016; Munir et al.

2019). Moreover, antagonistic fungi have been isolated from *Hedychium coronarium* healthy rhizome, yielding 12 fungal morphotypes, which were strong antagonists to *Staphylococcus aureus* ATCC® 29213™ (Lutfia et al. 2019a). In a similar study, endophytic fungal isolates from *Globba pendula* were also strong antagonists to the same tested pathogen. In this study, we evaluated the antibacterial properties of antagonistic fungi through screening with clinical and resistant pathogenic bacteria (Lutfia et al. 2019b). In addition, five endophytic fungi isolated from a wild ginger *Amomum centrocephalum*, e.g. *Aspergillus aculeatus*, *Clonostachys*

Table 4. The NMR data of compound 1 at ^1H (500 MHz) and ^{13}C (125 MHz).

No	^{13}C (CDCl ₃)	^1H (CDCl ₃)	Mult in <i>J</i>	DEPT				HSQC	HMBC	COSY
				CH ₃	CH ₂	CH	C			
1	39.7	1.56	2H, m	X		X	X	CH2		2
		0.68								
2	18.4	1.58	2H, m	X		X	X	CH2		1–3
		1.35								
3	41.9	1.33	2H, m	X		X	X	CH2	5	2
		1.14								
4	33.28			X	X	X		C		
5	56.4	0.85	1H, m	X	X		X	CH		6
6	17.9	1.39	2H, m	X		X	X	CH2	8–10	5–7
		1.58								
7	41.4	1.70	2H, m					CH2	9	6
		1.12								
8	36.8		1H, m	X	X	X		C		
9	52.3	1.25	1H, m	X	X		X	CH		11
10	37.7			X	X	X		C		
11	22.3	1.79	2H, m	X		X	X	CH2		9
		1.66								
12	74.4	4.87	1H, br t; 2.9 Hz	X	X		X	CH	22	11
13	37.6			X	X	X		C		
14	49.5	1.40	1H, m	X	X		X	CH		15
15	24.0	2.36	2H, m	X		X	X	CH2		14
		2.13								
16	135.5	6.84	1H, d; 4.9; 3.5 Hz	X	X		X	CH		17
17	51.0	3.14	1H, br q; 8.6, 4.8 Hz	X	X		X	CH		16
18	21.33	0.80	3H, s		X	X	X	CH3	3-4-5-19	
19	33.2	0.86	3H, s		X	X	X	CH3	3-4-5-18	
20	16.2	0.82	3H, s		X	X	X	CH3	7-8-9	
21	15.0	0.87	3H, s		X	X	X	CH3	12-13-14-17	
22-OAc	169.9									
23	21.39	1.96	3H, s		X	X	X	CH3	23	

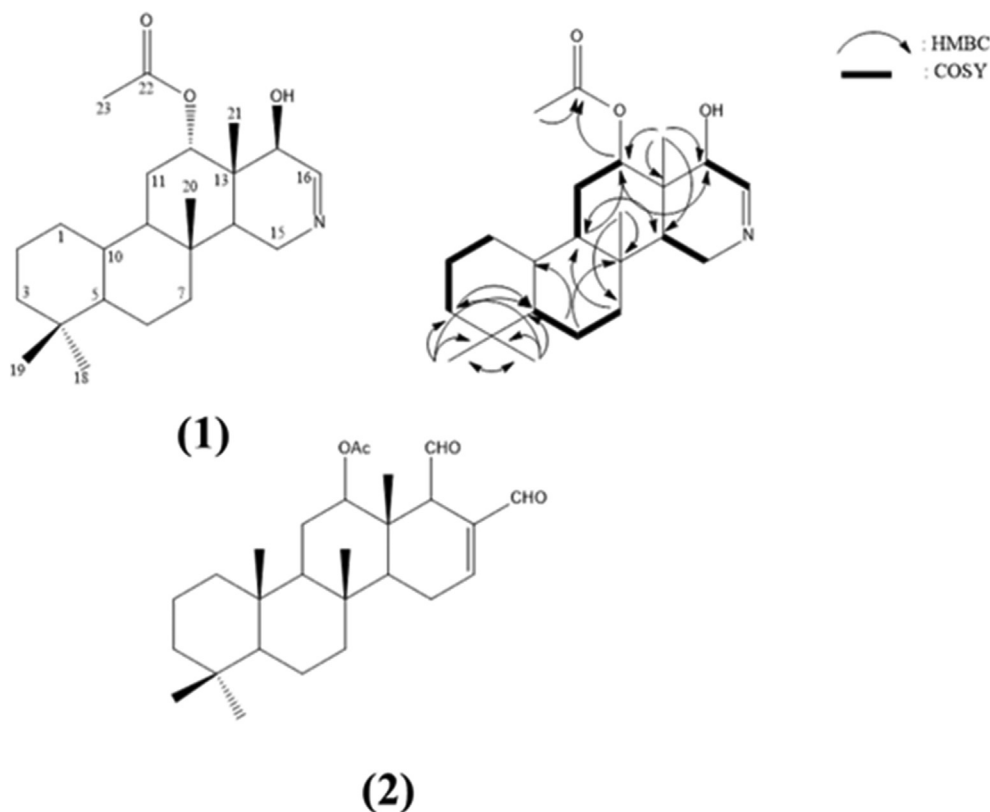


Figure 5. The HMBC, ^1H - ^1H COSY correlation and chemical structure of Compound (1) and (2).

rosea, *Daldinia caldariorum*, and two strains of *Trichoderma* were potential antagonists against bacterial pathogens and phytopathogenic fungi (Lutfia et al. 2019c).

The potential isolate, Zg15SU is then identified as *Hypomontagnella monticulososa*, an ascomycetous fungus with the former name *Hypoxyylon monticulosum* in an on-going study of its natural compounds. *Hypomontagnella monticulososa* isolated from marine origin was reported to synthesize a class of chemicals called Sporothriolide along with other minor constituents (Leman-Loubière et al., 2017a; Leman-Loubière et al. 2017b). The endophytic *Hypoxyylon* has also been reported from various plant species that can synthesize oil-based or volatile organic compounds. Another study reported that an endophytic *Hypoxyylon* sp. isolated from *Persea americana* was known to synthesize monoterpene, 1, 8-Cineole, which may be utilized and developed as fuel additives (Tomscheck et al. 2010). The terpenoid compound also possessed antifungal properties against phytopathogenic fungi, i.e., *Botrytis cinerea*, *Phytophthora cinnamoi*, *Cercospora beticola*, and *Sclerotinia sclerotium*. Other studies also supported the finding of VOCs produced by *Hypoxyylon* to be used as a preservative agent. Endophytic *H. anthochroum* isolated from Burseraceae, Fabaceae, Celastraceae, and Euphorbiaceae were reported to produce sesquiterpene and monoterpene compounds with potential anti fungi to *Fusarium oxysporum*, causing infection in cherry tomatoes (Macías-Rubalcava et al. 2018). Other findings also reported the mycodiesel potential of *Hypoxyylon* sp. CI-4 due to its abundant VOCs produced at a laboratory scale (Ul-Hassan et al. 2012).

To date, isolation efforts of *Hypoxyylon* spp. are still limited to certain medicinal plant species. A bioprospection study of *H. investiens* isolated from stems of *Litsea akoensis* var. *chitouchiaoensis*, a Taiwanese species, reported that BuOH extract of fungus exhibits biological activities, i.e., Nitric Oxide (NO) and Interleukin-6 (IL) inhibitory activities with medical importance (Chang et al. 2014). In addition, Taiwanese *Ilex formosana* harbored endophytic *Hypoxyylon* sp. 12F0687 with similar biological properties as reported previously (Chen et al.

2015). Hence, the *Hypomontagnella monticulososa* strain Zg15SU isolated from *Z. griffithii* may provide valuable information through the study of its natural products and bioactivities. The confirmation of natural product co-synthesis between *H. monticulosum* and *Z. griffithii* rhizome was analysed based on the composition percentage of similar compounds detected in GC-MS. Oleic acid was detected as the highest proportion of compounds produced by both endophytic fungus and its host. Other studies also supported our findings. Endophytic fungi isolated from four ginger cultivars (*Zingiber officinale*) were known to produce a myriad of bioactive fatty acids, one of which was oleic acid. It was assumed that a different composition of fatty acids among ginger cultivars was structured by native endophytic fungi (Anisha and Radhakrishnan, 2017). Oleic acid along with linoleic acid and sciadonic acid were the shared components identified by GC-MS between lipid-producing endophytic fungus and its host, the Chinese *Torreya grandis* (Yang et al. 2015).

The GC-MS analysis of an Indian medicinal plant, *Ocimum sanctum*, and its endophytic fungus *Macrophomina phaseolina* revealed a high proportion of Oleic acid in crude extracts from both organisms. The extract was known to possess antifungal activity against phytopathogenic fungi (Chowdhary and Kaushik 2015). We also tested the antibacterial activity of the crude extract against clinical pathogenic bacteria. The results showed minor differences in inhibition with greater and broader antibacterial activity by *H. monticulososa* extract than the *Z. griffithii* rhizome extract. It was proposed that antibacterial activity possessed by oleic acid and other unsaturated fatty acids initiated a disruption of fatty acid synthesis by bacteria led by the expression of FabI (Zheng et al. 2005).

Although oleic acid was detected in the highest portion of compounds from both extracts, other constituents may also contribute to the antibacterial activity or even cross-inhibit the effectiveness of extracts. The other portion, e.g. Cyclononasiloxane, octadecamethyl- was also detected during a GC-MS analysis between endophytic fungus,

Nigrospora sphaerica and *Parthenium hysterophorus* with other derivatives of polysiloxane compounds. Cyclononasiloxane, octadecamethyl- was also reported to act as antifungal according to the previous study of *Argemone ochroleuca* latex against pathogenic fungi (Moustafa et al. 2013).

Based on the identification of purified compounds and NMR data, we conclude that the purified bioactive compound (2) derived from our endophytic strain belongs in the group of terpenoids or scalaradial. Scalaradial is a member of scalarane sesterterpenoid which is a ubiquitous secondary metabolite synthesized across organismal taxa, from fungi, marine organisms, and plants (Dewick 2009). Scalarane sesterterpenoids are currently being used as chemotaxonomic markers among sponge species which also possessed potential anticancer activities based on laboratory evidences (Zhang et al. 2009; González, 2010; Chang et al. 2012). Endophytic fungi produce various forms of terpenoids, but mainly the sesquiterpenoids which displayed multifarious biological activities, such as anticancers, antimicrobials, antitumors, and enzyme inhibitions (de Souza et al. 2011). Majority of sesterterpenoids produced by fungi were reported as ophiobolin-type derivatives in the Chinese region dominantly produced by *Aspergillus* and *Bipolaris* which also showed diverse biological activities (Zhao et al. 2020). Aspergilloidins A-K were isolated from a mangrove fungus, *Aspergillus* sp. ZJ-68 which displayed inhibition against *Mycobacterium tuberculosis* and LPS-induced nitric oxide production (Cai et al. 2019). *Bipolaris* A-H were isolated from an endophytic fungus in wheat plant, *Bipolaris* sp. TJ403-B1 which displayed inhibition against *Enterococcus faecalis* (Liu et al. 2019). In a more recent study, five sesterterpenes were isolated from an endophytic fungus *Aplosporella javeedii* which exhibit a range from moderate to strong-level anticancer activities against cell lines through apoptotic cell death (Gao et al. 2020). In addition, fungal sesterterpenoids were also reported as phytotoxic compounds involved in the chemical defense by allelopathic plants (Cimmino et al., 2014).

Although sporothriolides have been assigned as chemotaxonomic markers for the species of *Hypoxylon* or *Hypomontagnella* spp; here, we report that different strains may possess different capability in metabolite synthesis during fermentation (Surup et al. 2014). Moreover, we assumed previously that we would find novel chemical compounds produced by our endophytic strain but instead we found an unknown compound of terpenoid-alkaloid (1) from its host, *Z. griffithii* which was also described as an uncommon Zingiberaceous species. The compound was then proposed as a novel compound since only a few thousand molecules originating from terpenoid-alkaloids reported so far, especially those derived from Zingiberaceous sources (Cherney and Baran 2011). The compound may be an interesting subject and motivation to reveal the significance of finding other terpenoid-alkaloids from uncommon Zingiberaceous species which may be potential in bioprospective study. Therefore, we claim that this is the first report on the identified scalaradial compound produced by *Hypomontagnella monticulosa* strain Zg15SU as well as the unknown terpenoid-alkaloid from *Zingiber griffithii* from North Sumatra, Indonesia.

5. Conclusions

Isolation of endophytic fungi residing within the rhizome of *Zingiber griffithii* from North Sumatran forest has revealed an endophytic fungus, identified as *Hypomontagnella monticulosa* producing bioactive metabolites as an antagonistic strain. The antimicrobial activity of both the fungus and rhizome extract were potential against human pathogenic bacteria. Chemical composition based on GC-MS analysis revealed that some portion of bioactive chemicals were observed from both fungus and plant MeOH extracts, indicating a possible co-synthesis between endophyte and its host. Structural elucidation of the highest portion chemicals from both extracts showed that the purified compounds were members of Terpenoid-Alkaloid (1) and Sesterterpenoid (2) with bioactive properties as anticancer against Panc-1, NBT-T2, and HCT116 cell lines.

Declarations

Author contribution statement

Anisa Lutfia: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Erman Munir: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yurnaliza Yurnaliza: Analyzed and interpreted the data.

Mohammad Basyuni: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

- Aly, A.-H., Debbab, A., Proksch, P., 2011. Fungal endophytes: unique plant inhabitants with great promises. *Appl. Microbiol. Biotechnol.* 90 (6), 1829–1845.
- Altamimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.-G., Lightfoot, D.-A., 2017. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* 6 (4), E42.
- Anisha, C., Radhakrishnan, E.-K., 2017. Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale* Rosc. identifies myriad of bioactive compounds including tyrosol. *3 Biotech* 7 (2), 1–10.
- Balouiri, M., Sadiqi, M., Ibsouda, S.-K., 2016. Methods for in vitro evaluating antimicrobial activity: a review. *J. Pharm. Anal.* 6 (2), 71–79.
- Cai, R., Jiang, H., Mo, Y., Guo, H., Li, C., Long, Y., Zang, Z., She, Z., 2019. Ophiobolin-type sesterterpenoids from the mangrove endophytic fungus *Aspergillus* sp. ZJ-68. *J. Nat. Prod.* 82 (8), 2269–2278.
- Chang, C.-W., Chang, H.-S., Cheng, M.-J., Liu, T.-W., Hsieh, S.-Y., Yuan, G.-F., Chen, I.-S., 2014. Inhibitory effects of constituents of an endophytic fungus *Hypoxylon investiens* on nitric oxide and interleukin-6 production in RAW264.7 macrophages. *Chem. Biodivers.* 11 (6), 949–961.
- Chang, Y.-C., Tseng, S.-W., Liu, L.-L., Chou, Y., Ho, Y.-S., Lu, M.-C., Su, J.-H., 2012. Cytotoxic sesterterpenoids from a sponge *Hippospongia* sp. *Mar. Drugs* 10 (5), 987–997.
- Chen, Y.-S., Cheng, M.-J., Hsiao, Y., Chan, H.-Y., Hsieh, S.-Y., Chang, C.-W., Liu, T.-W., Chang, H.-S., Chen, I.-S., 2015. Chemical constituents of the endophytic fungus *Hypoxylon* sp. 12F0687 isolated from Taiwanese *Ilex formosana*. *Helv. Chim. Acta* 98 (8), 1167–1176.
- Cherney, E.-C., Baran, P.-S., 2011. Terpenoid-Alkaloids: their biosynthetic twist of fate and total synthesis. *Isr. J. Chem.* 51 (3-4), 391–405.
- Chowdhary, K., Kaushik, N., 2015. Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn. *PLoS One* 10 (11), 1–25.
- Cimmino, A., Andolfi, A., Evidente, A., 2014. Phytotoxic terpenes produced by phytopathogenic fungi and allelopathic plants. *Nat. Prod. Comm.* 9 (3), 401–408.
- Deshmukh, S.-K., Verekar, S.-A., Bhawe, S.-V., 2015. Endophytic fungi: a reservoir of antibacterials. *Front. Microbiol.* 5, 1–43.
- de Souza, J.-J., Vieira, I.-J.-C., Rodrigues-Filho, E., Braz-Filho, R., 2011. Terpenoids from endophytic fungi. *Molecules* 16 (12), 10604–10618.
- Dewick, P.-M., 2009. *Medicinal Natural Products - A Biosynthetic Approach*. John Wiley & Sons Ltd, Chichester.
- Edgar, R.-C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32 (5), 1792–1797.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39 (4), 783–791.
- Fernando, O., Nursyahra, Rizki, 2008. Studi pemanfaatan tumbuhan familia Zingiberaceae yang berkhasiat obat di Kecamatan luhak nan duo kabupaten pasaman barat. *Pendidik. Biol.* 4 (2), 1–5.

- Gao, Y., Stuhldreier, F., Schmitt, L., Wesselborg, S., Wang, L., Muller, W.-E.-G., Kalscheuer, R., Guo, Z., Zuo, K., Liu, Z., Proksch, P., 2020. Sesterterpenes and macrolide derivatives from the endophytic fungus *Aptosporella javeedii*. *Fitoterapia* 146, 104652.
- Ginting, R.-C.-B., Sukarno, N., Widyastuti, U., Darusman, L.-K., Kanaya, S.-K., 2013. Diversity of endophytic fungi from red ginger (*Zingiber officinale* Rosc.) plant and their inhibitory effect to *Fusarium oxysporum* plant pathogenic fungi. *HAYATI J. Biosci.* 20 (3), 127–137.
- González, M.-A., 2010. Scalarane sesterterpenoids. *Curr. Bioact. Comp.* 6, 178–210.
- Harborne, J.-B., 1987. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman & Hall, Inc, London.
- Hartanto, S., Fitmawati Sofiyanti, N., 2014. Studi etnobotani famili Zingiberaceae dalam kehidupan masyarakat lokal di Kecamatan pangean kabupaten kuantan singingi, Riau. *Biosaintifika* 6 (2), 98–108.
- Hastuti, U.-S., Rahmawati, D., Sari, R.-Y., Fitri, R.-D., Al Asna, P.-M., 2008. Antimicrobial activity of endophytic fungi isolated from a medicinal plant, *Hedychium acuminatum* Roscoe. *AIP Conf. Proc.* 50002, 1–5.
- Jia, M., Chen, L., Xin, H.-L., Zheng, C.-J., Rahman, K., Han, T., Qin, L.-P., 2016. A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Front. Microbiol.* 7, 1–14.
- Juwita, T., Puspitasari, I., I.M., Levita, J., 2018. Torch ginger (*Etilingera elatior*): a review on its botanical aspects, phytoconstituents and pharmacological activities. *Pak. J. Biol. Sci.* 21 (4), 151–165.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33 (7), 1870–1874.
- Lambert, C., Wendt, L., Hladki, A.-I., Stadler, M., Sir, E.-B., 2019. *Hypomontagnella* (Hypoxylaceae): a new genus segregated from *Hypoxylon* by a polyphasic taxonomic approach. *Mycol. Prog.* 18 (1–2), 187–201.
- Leman-Loubière, C., Goff, G.-L., Debitus, C., Ouazzani, J., 2017a. “Sporochartines A–E, a new family of natural products from the marine fungus *Hypoxylon monticulosum* isolated from a *Sphaerocladina* sponge. *Front. Mar. Sci.* 4 (399), 1–9.
- Leman-Loubière, C., Goff, G.-L., Retailleau, P., Debitus, C., Ouazzani, J., 2017b. Sporothriolide-related compounds from the fungus *Hypoxylon monticulosum* CLL-205 isolated from a *Sphaerocladina* sponge from the Tahiti coast. *J. Nat. Prod.* 80 (10), 2850–2854.
- Liu, M.-T., He, Y., Shen, L., Hu, Z.-X., Zhang, Y.-H., 2019. Bipolarins A-H, eight new ophiobolin-type sesterterpenes with antimicrobial activity from fungus *Bipolaris* sp. TJ403-B1. *Chin. J. Nat. Med.* 17 (12), 935–944.
- Lutfia, A., Munir, E., Yurnaliza, 2019a. Antagonistic endophytic fungi of *Hedychium coronarium* Roxb. From hutan sibayak and Taman hutan Raya, North Sumatra against *Staphylococcus aureus* ATCC® 29213™. *IOP Conf. Ser. Earth Environ. Sci.* 305, 012002.
- Lutfia, A., Munir, E., Yurnaliza, 2019b. Antagonistic endophytic fungi of *Globba pendula* Roxb. From Taman hutan Raya, North Sumatra against *Staphylococcus aureus* ATCC® 29213™. *IOP Conf. Ser. Earth Environ. Sci.* 305, 012003.
- Lutfia, A., Munir, E., Yurnaliza Hartanto, A., 2019c. Antagonistic fungal endophytes colonizing rhizome of *Amomum centrocephalum* A.D. Poulsen from North Sumatera, Indonesia. *Int. J. Adv. Sci. Eng. Inf. Technol.* 9 (4), 1427–1433.
- Macías-Rubalcava, M.-L., Sánchez-Fernández, R.-E., Roque-Flores, G., Lappe-Oliveras, P., Medina-Romero, Y.-M., 2018. Volatile organic compounds from *Hypoxylon anthochroum* endophytic strains as postharvest mycofumigation alternative for cherry tomatoes. *Food Microbiol.* 76, 363–373.
- Manter, D.-K., Vivanco, J.-M., 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods* 71 (1), 7–14.
- Marcellano, J.-P., Collanto, A.-S., Fuentes, R.-G., 2017. Antibacterial activity of endophytic fungi isolated from the bark of *Cinnamomum mercadoi*. *Pharmacogn. J.* 9 (3), 405–409.
- Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.-L., Agarwala, R., Schäffer, A.-A., 2008. Database indexing for production MegaBLAST searches. *Bioinformatics* 24 (16), 1757–1764.
- Moustafa, M.-F.-M., Alamri, S.-A., Taha, T.-H., Alrumman, S.-A., 2013. In vitro antifungal activity of *Argemone ochroleuca* sweet latex against some pathogenic fungi. *Afr. J. Biotechnol.* 12 (10), 1132–1137.
- Munir, E., Lutfia, A., Yurnaliza, 2019. Records of culturable endophytic fungi inhabiting rhizome of *Eleocharis* in hutan sibayak, North Sumatera. *IOP Conf. Ser. Earth Environ. Sci.* 305, 012004.
- Nongalleima, K., Dey, A., Deb, L., Singh, C.-B., Thongam, B., Devi, H.-S., Devi, S.-I., 2013. Endophytic fungus isolated from *Zingiber zerumbet* (L.) Sm. inhibits free radicals and cyclooxygenase activity. *Int. J. PharmTech Res.* 5 (2), 301–307.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4 (4), 406–425.
- Sanchez-Ballesteros, J., González, V., Salazar, O., Acero, J., Portal, M.-A., Julián, M., et al., 2007. Phylogenetic study of *Hypoxylon* and related genera based on ribosomal ITS sequences. *Mycologia* 92 (5), 964–977.
- Stadler, M., Fournier, J., Laessle, T., Lechat, C., Tichy, H.-V., Piepenbring, M., 2008. Recognition of hypoxylid and xylarioid *Entonaema* species and allied *Xylaria* species from a comparison of holomorphic morphology, HPLC profiles, and ribosomal DNA sequences. *Mycol. Prog.* 7 (1), 53–73.
- Stadler, M., Quang, D.-N., Tomita, A., Hashimoto, T., Asakawa, Y., 2006. Changes in secondary metabolism during stomatal ontogeny of *Hypoxylon fragiforme*. *Mycol. Res.* 110 (7), 811–820.
- Strobel, G.-A., 2003. Endophytes as sources of bioactive products. *Microb. Infect.* 5 (6), 535–544.
- Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.* 67 (4), 491–502.
- Strobel, G., 2018. The emergence of endophytic microbes and their biological promise. *J. Fungi* 4 (2), 57.
- Surup, F., Kuhnert, E., Lehmann, E., Heitkamper, S., Hyde, K.-D., Fournier, J., Stadler, M., 2014. Sporothriolide derivatives as chemotaxonomic markers for *Hypoxylon monticulosum*. *Mycology* 5 (3), 110–119.
- Tomscheck, A.-R., Strobel, G.-A., Booth, E., Geary, B., Spakowicz, D., Knighton, B., Floerchinger, C., Sears, J., Liarzi, O., Ezra, D., 2010. *Hypoxylon* sp., an endophyte of *Persea indica*, producing 1,8-cineole and other bioactive volatiles with fuel potential. *Microb. Ecol.* 60 (4), 903–914.
- Ul-Hassan, S.-R., Strobel, G.-A., Booth, E., Knighton, B., Floerchinger, C., Sears, J., 2012. Modulation of volatile organic compound formation in the mycodiesel-producing endophyte *Hypoxylon* sp. CI-4. *Microbiology* 158 (2), 465–473.
- Uzma, F., Konappa, N.-M., Chowdappa, S., 2016. Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. *Egypt. J. Basic Appl. Sci.* 3 (4), 335–342.
- VanderMolen, K.-M., Raja, H.-A., El-Elimat, T., Oberlies, N.-H., 2013. Evaluation of culture media for the production of secondary metabolites in a natural products screening program. *AMB Express* 71 (3), 1–7.
- Yang, Y., Jin, Z., Jin, Q., Dong, M., 2015. Isolation and fatty acid analysis of lipid-producing endophytic fungi from wild Chinese *Torreya grandis*. *Microbiology* 84 (5), 710–716.
- Yurnaliza Artyantha, I.-N.-P., Esyanti, R.-R., Susanto, A., 2014. Antagonistic activity assessment of fungal endophytes from oil palm tissues against *Ganoderma boninense* Pat. *Plant Pathol. J.* 13 (4), 257–267.
- Zhang, H.-J., Tang, H.-F., Yi, Y.-H., Lin, H.-W., 2009. Scalarane sesterterpenes from the Chinese sponge *Phyllospongia foliascens*. *Helv. Chim. Acta* 92 (4), 762–767.
- Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* 7 (1–2), 203–214.
- Zhao, Y., Cui, J., Liu, M., Zhao, L., 2020. Progress on terpenoids with biological activities produced by plant endophytic fungi in China between 2017 and 2019. *Nat. Prod. Commun.* 15 (7), 1–18.
- Zheng, C.-J., Yoo, J.-S., Lee, T.-G., Cho, H.-Y., Kim, Y.-H., Kim, W.-G., 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Lett.* 579 (23), 5157–5162.