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Research Paper

Activity of Mangrove-Derived *Fusarium equiseti* 20CB07RF Extract Against Clinical, Antibacterial-Resistant *Pseudomonas aeruginosa*

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Abstract
Endophytic fungi originating from mangroves are potential sources of secondary metabolites with varying bioactivities. This research explores the bioactive metabolites produced by endophytes derived from mangrove plants. Endophytic fungi were collected from various parts of several mangrove plants (roots, stems, and leaves, as well as the surrounding mud). A total of 17 endophytics fungi were obtained. The isolates were derived from the leaves (1 isolate), stems (8 isolates), roots (5 isolates), and surrounding mud (3 isolates). A single fungal colony was cultured using solid-state fermentation for 14 days. The fermented fungal biomass was extracted using ethyl acetate (EtOAc) and evaluated for its antibacterial activity against clinical pathogenic bacteria. In the preliminary screening, the EtOAc extract of the CB07RF1 isolate exhibited notable growth-inhibitory effects against *Pseudomonas aeruginosa*. The isolate was verified by molecular identification using a study of the rDNA internal transcribed spacer (ITS) sequence, revealed that isolate CB07RF1 was very similar to *Fusarium equiseti* (99% similarity). Isolate 20CB07RF1, obtained by solid-state fermentation using a rice medium indicated as peptide compound group, and featured active components that exhibited potent growth-inhibitory activity against *Pseudomonas aeruginosa* at a concentration of 12.5 mg/mL. This study demonstrates, for the first time, that *Fusarium equiseti* extracts grown in a rice medium contain antimicrobial compounds that can inhibit the growth of *P. aeruginosa*, an important clinical pathogen known for its antibacterial resistance. These findings accent mangrove endophytic fungi as important sources of bioactive compounds and will advance related research in the fields of biotechnology, pharmacology, and life sciences.

Keywords
Antibiotic Resistance, Bacterial Pathogen, Endophytic Fungi, Mangrove

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1. INTRODUCTION

The surge in the number of health issues linked to bacterial infections underpins the significant threat of antimicrobial resistance (AMR) globally. The World Health Organization (WHO) has stipulated AMR as one of the threats to global public health and sustainable development. AMR can induce delayed healing, patient disability, or even death (Gajdács et al., 2021). Without bioactive, antibacterial compounds, the success of modern medicine in treating infectious diseases will decline significantly. To date, natural products and their structural analogs are still employed in drug development (Atanasov et al., 2021). However, when searching for new bioactive compounds, the same compounds often re-emerge. Consequently, the search for undiscovered bioactive compounds, particularly antibacterial compounds, from new sources and using new methods is of great importance.

Studies conducted over the past 20 years have revealed that the endophytic mangrove fungi are rich in bioactive compounds with various bioactivities (Cadamuro et al., 2021). Endophytic are fungi that colonize the intercellular or intracellular spaces of plant tissues during at least one stage of their life cycle. The microorganisms in mangrove ecosystems are exposed to unique conditions featuring variations in salinity, pH, temperature, and organic matter content. This ecosystem can indeed influence the diversity of living microorganisms, accounting for the diversity of the bioactive metabolites produced (Liao et al., 2020; Lai et al., 2022). Generally, fungi are decomposers

that can adapt to very extreme conditions, such as those under which mangrove plants grow. Certainly, this is related to the secondary metabolites, which they produce for self-defense. This phenomenon is one of the fundamental considerations for researchers studying bioactive fungal compounds (Boruah et al., 2024). Unlike bioactive metabolites from plants and animals, which can be obtained directly through extraction and isolation processes, bioactive compounds obtained from endophytic fungi can undergo stages of fermentation involving highly complex biochemical reactions (Zhang et al., 2024). Notably, fermentation technology is not new. Various household products, such as vinegar, soy sauce, and beverages, are produced by microbial fermentation. However, the extraction of bioactive compounds via the fermentation of microorganisms is considered less economical because of the relatively low yields. Nonetheless, one of the main merits of fermentation technology is its environmental friendliness and sustainability (Abu Yazid et al., 2017).

Currently, there have been research advances in various areas, including chemical analysis techniques, microbiological culture technology, solid-state fermentation, and genome analysis and mining. These have opened up new opportunities for advancing the search for bioactive compounds in mangrove fungal endophytes that can inhibit the growth of drug-resistant bacteria. Secondary metabolites derived from endophytic fungi have unique characteristics that distinguish them from synthetic molecules. Recently, Lu et al. (2024) successfully isolated indole compounds from mangrove fungus Aspergillus spinosus, demonstrating α-glucosidase inhibitory activity. Similarly, Li et al. (2024) obtained five previously unreported polyketide compounds from mangrove fungus Fusarium proliferatum NSD-1, showing bioactivity as cytotoxic agents.

Fungal metabolite compounds have diverse frameworks with complex structures. These metabolites can prove valuable in the search for antibacterial compounds. Their general structure includes many sp3-bonded carbon and oxygen atoms, although some of them feature nitrogen and halogen atoms. From a structural perspective, the molecules of these compounds have a higher number of hydrogen bond donors and acceptors and a lower the octanol–water partition coefficient (clog P value, indicating high hydrophilicity) than synthetic molecules in general. This difference makes it possible to exploit them in controlling the interactions between proteins (Hodges et al., 2019).

This research aims to explore the antibacterial activities of bioactive compounds sourced from mangrove endophytic fungi in inhibiting the growth of pathogenic bacteria. As a part of our ongoing research in exploring novel bioactive compound from endophytic microorganisms (Laila et al., 2023; Setiawan et al., 2022a; Setiawan et al., 2022b). This study highlight for the first time, the significant potential of the mangrove fungal endophyte from, *Fusarium equiseti* 20CB07RF isolated from stem of east Lampung Mangrove plant, as a source of bioactive compounds. The compounds were obtained through the OSMAC fermentation and used the dereplication approach that considered the use of novel sources of mangrove fungal endophytes, innovations in antibacterial-bioactivity screening using resistant clinical bacteria, and analysis of metabolite compound profiles. This foundational information will certainly be valuable for advancing researches in the pharmacology and biotechnology fields.

2. EXPERIMENTAL SECTION

2.1 Materials

Biomaterial fungi from parts of several mangrove plants (roots, stems, leaves), as well as the mud around the mangrove plants. Isolates CB07RF1 were collected from the mangrove area on the east coast of Sriminosari village, East Lampung, Indonesia (coordinates: 5° 9' 010" S: 105° 49' 19.8" E) in September 2021.

2.2 Chemical and Instrumental

The materials to conduct this study were Trypric Soy Broth (TSB) from Merck, alcohol 70%, from Onemed, TLC SiO_2 Gel plates (Merck kieselgel 60 GF254, 0.25 mm, 20 × 20 cm). The organic solvents used are n-hexane, ethyl acetate, methanol (Sigma-Aldrich). Autoclave (Tomy SX–700), analytical balance (Wigen Houser), rotary evaporator (Buchii/R210), laminar air flow, incubator (Memmert–Germany/INC–02), mikroskop cahaya axio (Zeiss A1), lampu UV (Kohler/SN402006). For characterization LC-MSMS analysis was equipped with the ACQUITY UPLC®H-Class System (Waters, Beverly, MA, USA), ACQUITY UPLC®HSS C18 column (1.8 μm 2.1 × 100 mm) (Waters, Beverly, MA, USA), and Xevo G2-S Qtof Mass Spectro (Waters, Beverly, MA, USA).

2.3 Methods

2.3.1 Susceptibility Test

Gram-negative clinical pathogenic bacteria, *Pseudomonas aeruginosa*, and gram-positive bacteria, *Staphylococcus aureus*, were obtained from Abdoel Moeloek General Hospital, Bandar Lampung, Indonesia. The disc diffusion method was applied to determine the pattern of bacterial resistance to several antibiotics according to the Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical Laboratory Standards Institute, 2017). In simple terms, bacteria are cultured on 2% (w/v) nutrient agar (NA). Each inoculum of isolate with OD 0.08-0.1 was incubated overnight and re-cultured (equivalent to 1.0 × 108 CFU/mL). The bacterial suspension was spread on an agar plate using sterile cotton. Susceptibility tests were carried out against several types of commercial antibiotics, including amoxicillin, erythromycin, ciprofloxacin, clindamycin, doxycycline, cefadroxil, chloramphenicol, lincomycin, and thiamphenicol. The plates were incubated at 37°C for 18 hours, after which the diameter of the observed inhibition zone was measured and interpreted according to CLSI provisions.

2.3.2 Preliminary Screening of Antibacterial Activity

Antibacterial activity tests for the fungal biomass extracts against the pathogenic bacteria were carried out in 96 assay wells using

resazurin as an indicator. Briefly, *S. aureus* and *P. aeruginosa* were cultured in a 2% (w/v) NA medium. The inoculum was adjusted to a turbidity standard of 0.5 Mc-Farland (OD 0.08-0.1), after which the sample extract test solution and positive control solution were added, each at a concentration of 0.5 mg/mL. The 96-well plate was incubated at 37°C for 18 h. Subsequently, the indicator, resazurin, was added, followed by incubation for 8 h. The absorbance (Abs) was measured at λ = 630 nm using a Hospitex plate reader (Italy). A minimum concentration barrier test was carried out on the active subfraction (Elshikh et al., 2016).

2.3.3 Morphological Analysis

Microscopic analysis of fungi was implemented by the coverslip method using a light microscope (Senanayake et al., 2020). Spores and sporulation were observed in each fungal culture that had been inoculated for 4 days using an Axio Imager M1 (Carl Zeiss AG, Germany) with 100× magnification. Next, the characteristics of the spores were identified by scanning electron microscopy (SEM). Briefly, the fungus sample was attached to a sheet of aluminum, which was then glued to a carbon tape. Thereafter, gold coating was carried out in approximately 20 minutes. The samples that had been coated with gold were subjected to SEM analysis at a voltage of 10 kV on a Carl Zeiss EVO MA10, Oberkochen, Germany (Vacelet and Donadey, 1977).

2.3.4 Phylogenetic Analysis

Fungal genomic deoxyribonucleic acid (DNA) was extracted using a QIAamp DNA Minikit (Qiagen, Germany) following the manufacturer's instructions. In this study, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA from target isolates was amplified using the forward primer, ITS1-F (5'-TCCGTAGGTGACCTGCGG-3'), and the reverse primer, ITS4-R (5'-TCCTCCGCTTATTGATA TGC-3') (White et al., 1990). The total final reaction volume of 20.5 mL consisted of 10 mL of NEXProTM PCR kit (PCR Biosystem, UK), 0.25 mL of the reverse primer, 0.25 mL of the forward primer, 5 mL of the genomic DNA template, and 5 mL of double distilled H2O. To verify the absence of contamination, the DNA was replaced with distilled water as a negative control. The PCR method was implemented using a Sensoquest Sensodirect Gradient Termo block 96 (Senso Quest, Germany) programmed for 5 minutes at 94°C; 35 cycles for 1 minute at 94°C, 1 minute at 52°C, and 1 minute at 72°C; then extended for 5 minutes at 72°C. The PCR products were separated on a 2% agarose gel using buffer TAE 1× (40 Mm Tris-acetate and 1 Mm EDTA, pH 8.0), stained with ethidium bromide (0.5 mg/mL), and documented using QIAxcel Advanced (Qiagen, German). Next, the PCR products were analyzed by direct bidirectional sequencing using ABIPRISM3730 ×1 Genetic Analysis (Applied Biosystem, USA) at the First BASE Laboratory Sdn., Bhd., Selangor, Malaysia.

2.3.5 Cultivation, Extraction, and Fractionation

A selected isolate, identified for its exceptional antibacterial activity, underwent further cultivation in various media to explore and optimize its bioactive potential. Briefly, single fungal isolates were grown in 10 mL of a mixture of malt extract and tryptic soy broth, followed by culturing for 2–4 days. The fungal inoculum was transferred to rice, shrimp shell, and potato medium, each containing 100 g medium and 110 mL of ASW in a 1-L Erlenmeyer flask. Fungal cultivation was carried out for 14 days at 30°C under static conditions. The fungal biomass was extracted three times using 100 mL of ethyl acetate (EtOAc) to obtain the active extract. Next, the extract solution was concentrated using a rotary evaporator at a low pressure of 95 mbar at 40°C until the crude extract was obtained. Afterward, the components of the extract were separated using thin-layer chromatography (TLC). Spots indicative of the extract components on the TLC plate were visualized under an ultraviolet lamp (λ = 254 nm), reacted with cerium sulfate ($CeSO_4$) reagent, and reacted with Dragendroff and Ninhydrin specific reagents (Setiawan et al., 2021).

2.3.6 Profile of the EtOAc SUb Extract

The profiles of the components in the active sub extract of EtOAc were analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) method, and the LC–MS/MS data were interpreted based on a combination of the Masslynk and Sirius databases. Each sub extract from the cultivation in different media was dissolved in methanol p.a. (Merck, KGaA, Germany) and analyzed using a UPLC–MS/MS system equipped with an H-Class ACQUITY UPLC® system (Waters, Beverly, MA). The components in the extract sample were separated using an ACQUITY UPLC®HSS C18 column (1.8 mm; 2.1 mm × 100 mm) (Waters, Beverly, MA) with a Xevo G2 Q-tof mass spectrometer (Yang et al., 2017).

2.3.7 Data Analysis

Microsoft Excel was employed to enter and retrieve the measurement data. The inhibitory activity of the fungal isolate extracts against pathogenic bacteria was analyzed using one-way analysis of variance (ANOVA). Statistical significance was defined as a $p < 0.05$. Further analysis was carried out using the Bonferroni–Nemenyi (BNT) test with a significance level of 5% representing real differences.

3. RESULTS AND DISCUSSION

3.1 Isolated Endophytic Fungi

Rhizophora apiculata is a species of mangrove commonly found in the mangrove area of Sriminosari village, eastern Lampung (see Figure 1). The roots of the *Rhizophora* plant grow out of the water to support the plant. This plant can grow quickly and reach a height of 30 m.

Furthermore, *Rhizophora* plants play an important role in the mangrove ecosystem; they provide shelter and food for various marine life. *Rhizophora* roots help stabilize shorelines and prevent erosion. The mangrove area of Sriminosari village is

Figure 1. *R. apiculata* Growing in the Mangrove Area of Sriminosari Village, East Lampung

located adjacent to the Way Kambas National Park Nature Reserve (5952.55 hectares). The area is relatively small, around 100 hectares. However, it is an important habitat for a variety of marine life, including fish, shrimp, crabs, and birds. Furthermore, the mangrove forest helps protect villages from floods and storms (Ardiansyah and Safe'i, 2021). The mangrove ecosystem is unique because it is found in the intertidal zone, which is where land meets the ocean. This exposes them to a variety of stress factors, including salt water, high temperatures, and strong winds. However, mangroves can survive these harsh conditions thanks to their various adaptations, including their specialized root systems and ability to produce salt-resistant compounds. In addition, mangrove endophytic fungi exhibit unique biodiversity, accounting for the various obtainable bioactive compounds that are valuable for pharmacological studies (Wen et al., 2022).

In this study, samples of the plant parts of *R. apiculate* (including leaves, stems, and roots) and the surrounding mud were taken from the mangrove area of Sriminosari village (coordinates: 5° 19' 01.0" LS; 105° 49' 19.8" E). After the enrichment and purification process, 17 fungi (5 from the roots, 8 from the stems, 1 from the leaves, and 3 from the surrounding mud) were isolated, as shown in Table 1. Various types of rare and common endophytic fungi have been isolated from *Rhizopora* species. The common general of fungi in R*hizophora* plants include *Aspergilus*, *Cladosporium*, *Penicillium*, and *Tricorderma*. Meanwhile, the rare genera of endophytic fungi include *Ampelomyces*, *Fusarium*, *Lasiodiplodia*, *Pestalostiopsis*, and *Phyllosticta*. Studies have demonstrated that endophytic fungi can produce bioactive metabolites. These bioactive metabolites are highly diverse, attributed to the biological relationship between the endophytic fungi and their host plants as well as their geographical environment. Endophytic fungi produce steroids, terpenoids, alkaloids, peptides, and polyketides (Hashem et al., 2023). The

Table 1. Isolated Endophytic Fungi Derived from Parts of Mangrove Plants and The Surrounding Mud

Samples Code	Mangrove Part	Fungsi Isolate	Color
20AA01	Root	AA01RF1	White
20AA02	Root	AA02RF1	White
20AA03	Root	AA03RF1	White
20AB01	Stem	AB01RF1	White
20AB02	Stem	AB02RF1	Greyish
		AB03RF2	Greyish
20AB03	Stem	AB03RF1	White–Greyish
20AB04	Root	BA04RF1	Greyish
20AB05	Root	BA05RF1	White
20BB04	Stem	BB04RF1	Greyish–Black
20BB05	Stem	BB05RF1	White
20CB06	Stem	CB06RF1	White
20CB07	Stem	CB07RF1	White–Greyish
20CD01	Leaf	CD01RF1	White–Orange
20CL01	Mud	CL01RF1	White
		CL01RF2	White
20CL02	Mud	CL02RF1	White

diversity of the endophytic fungi found in *Rhizophora* plants is likely due to the unique environmental conditions of the mangrove ecosystem. Interestingly, research on the endophytic fungi found in *Rhizophora* plants is still ongoing, as there is still much to learn about valuable bioactive metabolites.

3.2 Antibacterial Action

3.2.1 Susceptibility Test

In this study, two isolates of clinical pathogenic bacteria *S. aureus* (Gram-negative) and *P. aeruginosa* (Gram-positive) were employed for bioassay. Sensitivity tests of the two bacteria against the antibiotics showed that *S. aureus* was sensitive to doxycycline hyclate and cefadroxil, but resistant to clindamycin, ciprofloxacin, erythromycin, lincomycin, and amoxicillin. Meanwhile, *P. aeruginosa* was resistant to amoxicillin, chloramphenicol, erythromycin, clindamycin, lincomycin, and thiamphenicol, as shown in Figure 2.

Overall, the results of this test highlighted that both pathoge nic bacteria are resistant to more than three types of antibiotics and, thus, can be classified as multidrug-resistant (MDR) bacteria. There are several reasons for the resistance of both *S. aureus* and *P. aeruginosa* isolates to amoxicillin, erythromycin, and lincomycin, including antibiotic overuse/misuse, horizontal gene transfer, mutation, and biofilm formation.

3.2.2 Preliminary Screening

Initial screening is a crucial first step in the search for bioactive metabolites, such as antimicrobial agents. The agar diffusion test is a common method implemented as part of the preliminary tests. In this study, the bioactivities of the extracts from the fungal isolates were tested on two clinical pathogenic bacteria: *S. aureus* and *P. aeruginosa*. These bacteria are known to be MDR. The results of the initial screening showed that two extracts of fungi, BB05RF1 and CB07RF1, significantly inhibited the growth of both *S. aureus* and *P. aeruginosa*. The

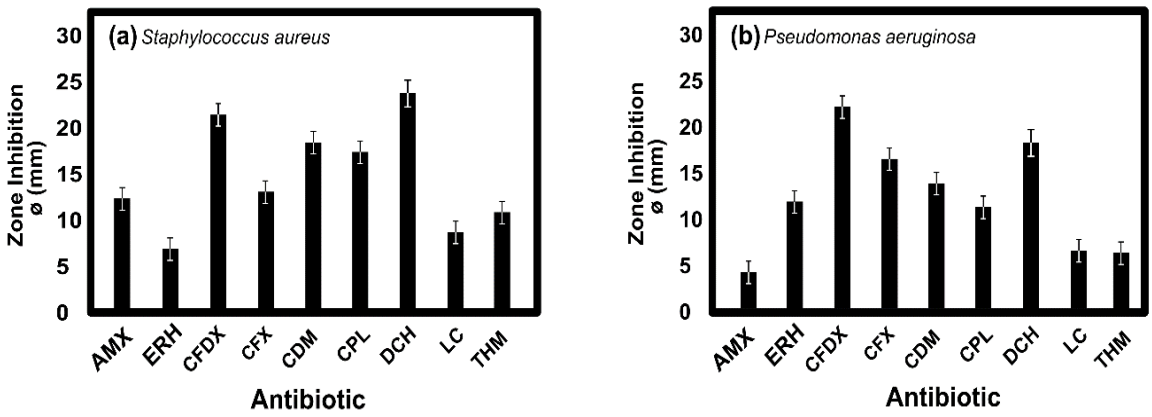

Figure 2. Antibiotic Susceptibility Testing of Clinical Bacteria to Multiple Antibiotics: (a) *Staphylococcus aureus* and (b) *Pseudomonas aeruginosa*, Abbreviations: Doxycycline Hyclate (DCH), Cefadroxil (CFDX), Clindamycin (CDM), Ciprofloxacin (CFX), Erythromycin (ERH), Lincomycin (LC), Amoxicillin (AMX), Chloramphenicol (CPL), and Thiamphenicol (THM).

Table 2. Antibacterial Activities of Isolated, Mangrove-Derived Endophyte Fungi

Fungi Isolate	EtOAc Extract	Host	Inhibition Zone (mm)	
			S. aureus	*P. aeruginosa*
AA01RF1	AA01RF11	Root	6	0
AB01RF1	AB01RF11	Stem	6	0
BA04RF1	BA04RF11	Root	0	6
BA05RF1	BA05RF11	Root	6	0
BB05RF1	BB05RF11	Stem	8	8
CB07RF1	CB07RF11	Stem	8	16
CL01RF2	CL01RF21	Mud	6	0

other five extracts of fungi exhibited weak inhibitory activity, as shown in Table 2. Based on these results, isolate CB07RF1 was selected for further study.

3.3 Characterization of the Selected Fungus

3.3.1 Morphological Analysis

The screening results showed that the CB07RF1 isolate can inhibit drug-resistant bacteria. Further morphological analysis showed that a single CB07RF1 isolate on agar media at 30°C formed white colonies with abundant mycelia that turned pink on the fourth day (Figure 3a)

Microscopic analysis revealed the presence of fine, branched, cylindrical, and septate mycelia (Figure 3b), confirming that isolate CB07RF is a fungus. The conidiophores were simple, short, and septate. The microconidia were oval, hyaline, and 0-1 septate, whereas the macroconidia were hyaline, 2-5 septate, and curved with tapered and elongated apical cells and prominent foot cells (Figure 3c). SEM observations revealed that the mycelia consist of branching, septate, and smooth-walled hyphae (1.3–2.7 mm wide; mean: 2.1 mm). Furthermore, the conidial heads were radiate, usually dividing into two to four dense columns with age. The stipe had a rough wall and was bright-orange pigmented. The vesicles were round and biseriate.

The morphological analysis of isolate CB07RF showed that it had some similarities with *F. equiseti*. Both fungi have fine, branched, cylindrical, and septate mycelia. The conidiophores are also cylindrical, short, simple, and septate. However, there are some key differences between the two fungi. The microconidia of CB07RF1 are oval, hyaline, and 0–1 septate, whereas the microconidia of *Fusarium equiseti* are ellipsoidal, hyaline, and 1–3 septate. The macroconidia of CB07RF are hyaline, 2-5 septate, and curved with tapered, elongated apical cells and prominent foot cells, whereas the macroconidia of *F. equiseti* are 3–5 septate and straight, with rounded apical cells. Further molecular analysis of the isolates was carried out to confirm the identity of the fungus.

3.3.2 Phylogenetic Analysis

The sequencing of the 18S rRNA, ITS1-5.8S-ITS4, 28S rRNA sequence region (517 base pair, accession number LC682 289 of strain 20CB07RF) was amplified by PCR, and the genome sequence indicated the genus, *Fusarium*. Strain 20CB07 RF was identified as a member of the *F. equiseti* species, with a sequence identity of 99%, as shown in Figure 4.

The phylogenetic tree analysis of *F. equiseti* 20CB07RF was conducted in MEGA 11 (Tamura et al., 2021). The maximum likelihood method and the Jukes and Cantor (1969)'s model was employed for this analysis. Clade stability was assessed via 1000 bootstrap replications, and *Fusarium polyphialidicum* NRRL 1359 was used as an outgroup (Wang et al., 2019). The properties of the culture and the morphology of strain 20CB07RF were consistent with those of *F. equiseti*. Its phylogenetic analysis confirmed that strain 20CB07RF belongs to *F. equiseti*, and it was designated as *F. equiseti* 20CB07RF. *F. equiseti* is a common fungus that is native to mangrove habitats. Although this fungal species can produce active metabolites, its structure and properties have been poorly studied. Although *Fusarium* is widely distributed in mangroves and many fungi species are present, there is limited information on the antibac-

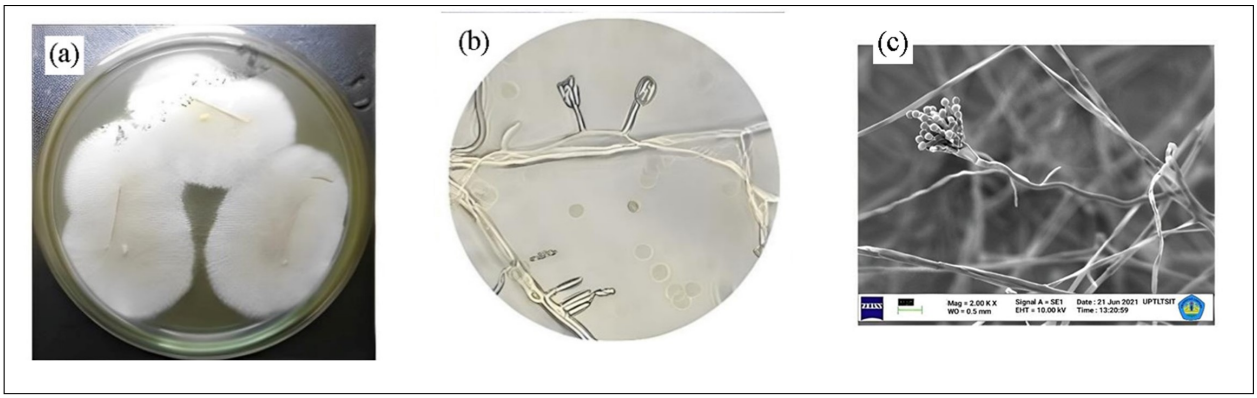

Figure 3. CB07RF1 Isolate in NA Media: (a) Single Isolate; (b) Visualization with a Light Microscope at 400×; (c) SEM Visualization at 200 K

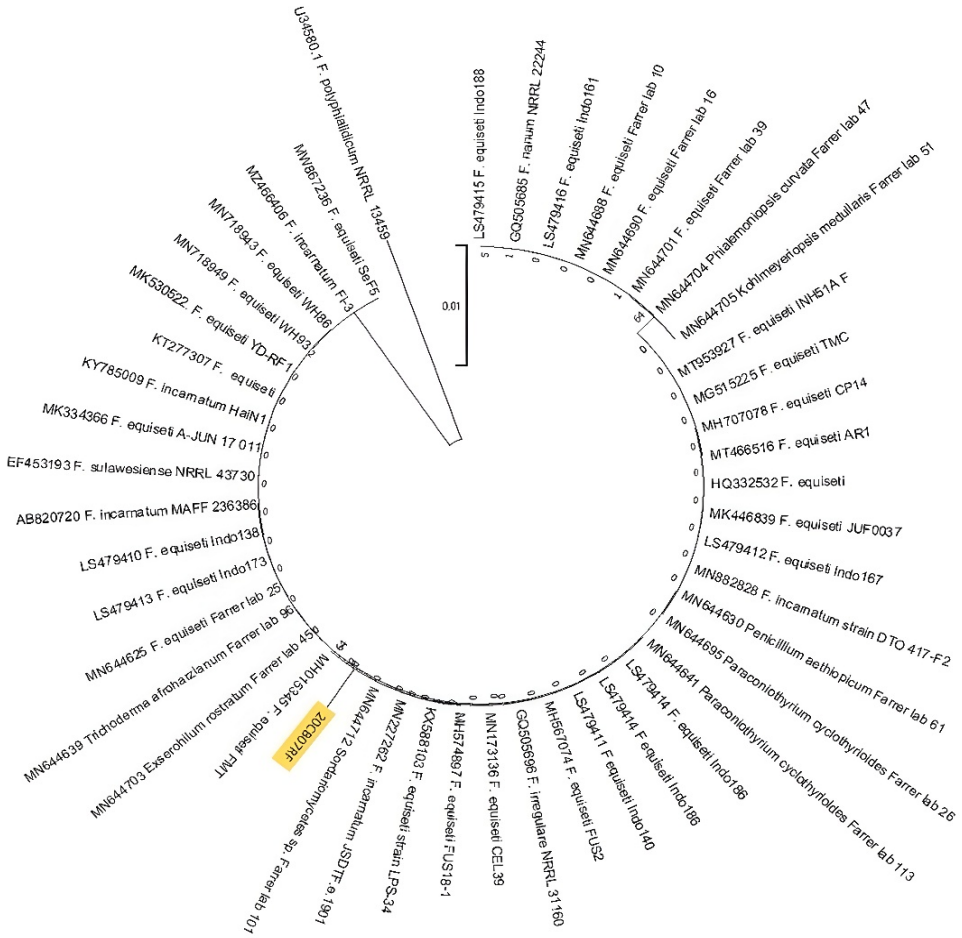

Figure 4. The Phylogenetic Tree of *F. equiseti* 20CB07RF.

terial properties of their natural products, particularly against drug-resistant bacteria (Imhoff, 2016).

3.3.3 Cultivation of a Selected Fungus in Various Media

The cultivation medium is one of the factors that can affect the metabolism of fungi. This information can certainly be

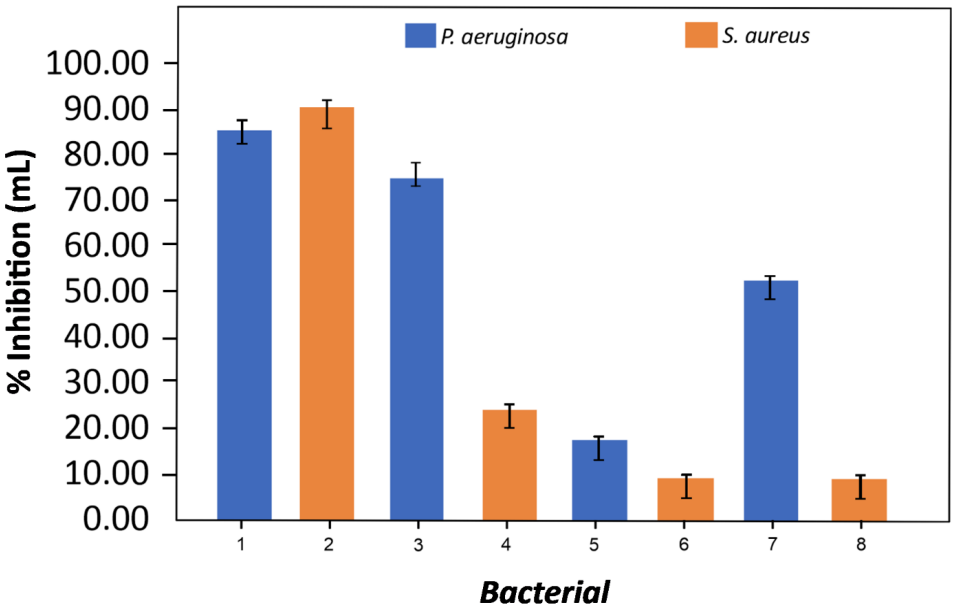

Figure 5. Medium 1: Control (+), 2: Rice, 3: Potato, and 4: Shrimp Shell

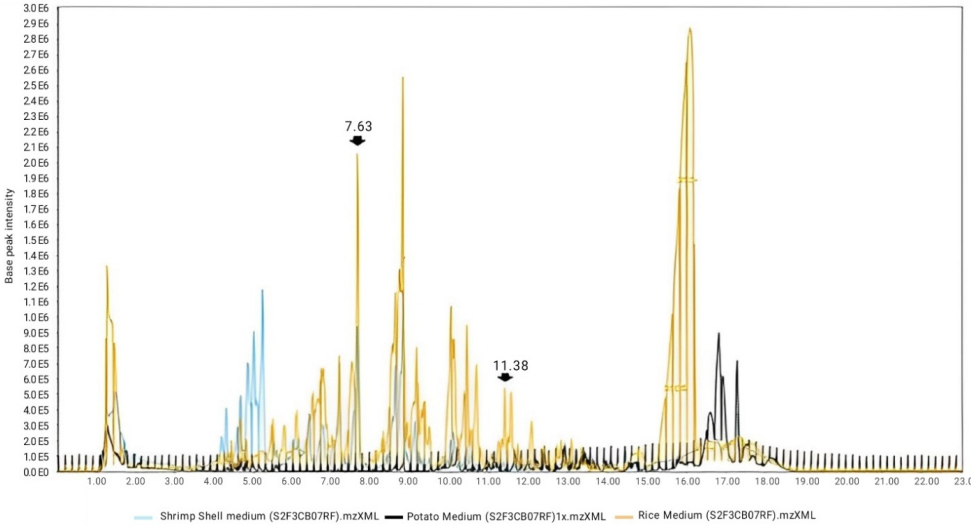

Figure 6. Chromatogram of the CB07RF11 Extract in (a) Rice (orange), (b) Shrimp Shell (black), (c) Potato Media (blue)

employed to optimize the production of bioactive compounds for potential application in medicine or agriculture. In this study, isolate 20CB07RF was cultured in three different media: rice, shrimp shell, and potato media. After cultivation and extraction, each extract were tested for activity against *S. aureus* and *P. aeruginosa*. The results are shown in Figure 5.

The antibacterial test demonstrated that the choice of medium influences the production of bioactive compounds by *F. equiseti* CB07RF (see Figure 5). Rice is a good source of carbohydrates, which are essential for fungal growth. It is also a good source of other nutrients, such as amino acids and vitamins. One-way ANOVA test at a significance level of $\alpha = 0.05$ ($p \leq 0.05$) during data processing for *P. aeruginosa* and *S. aureus* yielded significant p-values of 0.000 and 0.001, respectively. The one-way ANOVA results are considered robust, with the p-value being ≤ 0.05. Additionally, the BNT test at a 5% significance level revealed significant differences among the negative control (K(-)), rice group (R), potato group (P), shrimp shell group (SS), and positive control (K(+)), as well as the blank group (N). Notably, group R exhibited similar effects to K(-), suggesting a potentially negative impact. Conversely, group P exhibited similar effects to both K(-) and K(+), indicating no significant difference. The shrimp shell group demonstrated similar effects to K(+), implying comparable efficacy. For *S. aureus*, the BNT test at a 5% significance level highlighted significant differences between K(+) and groups R, SS, K(-), P,

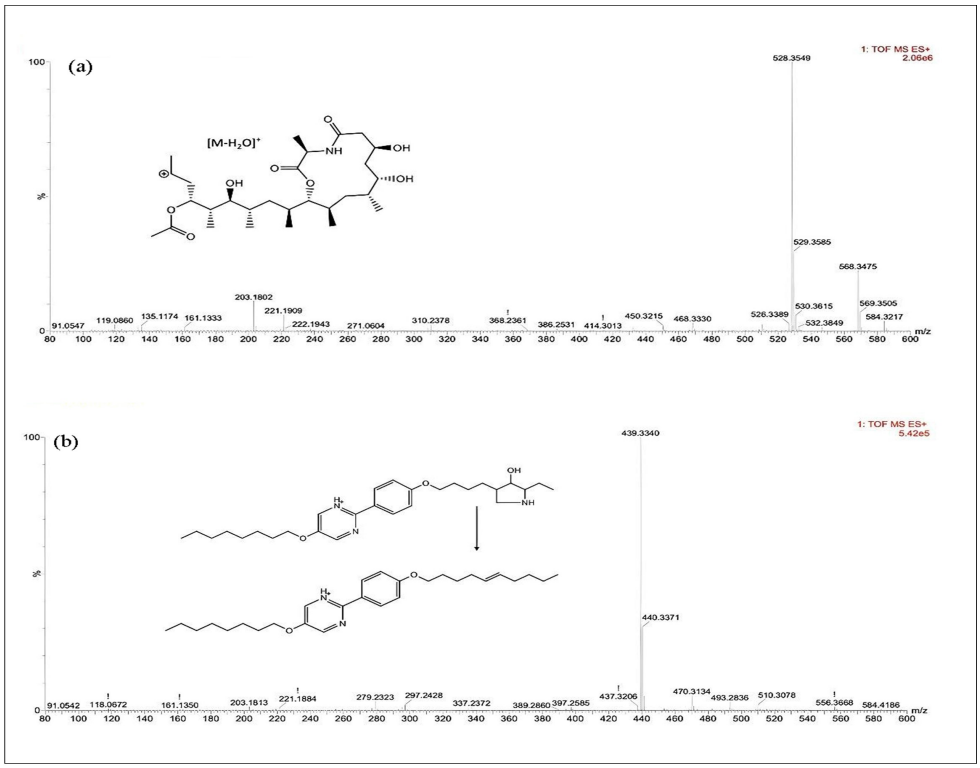

Figure 7. Molecular Ion Peak [M+H]+ of Compounds (a) 1 and (b) 2 at m/z 528.3514 and m/z 439.3340, Respectively

and SS. This suggests that the rice and shrimp shell groups have similar efficacy, as indicated by their comparable notations. Group K(-) differs significantly from the other groups, including the R, SS, and P groups. This information is very important in further analyses aimed at utilizing the bioactive compounds from the 20CB07RF isolate.

3.4 LC-MS/MS Analysis

Table 3. Bonding Energy of the Active Compound with 3U59 Protein

Bond Energy (kJ/mol)	Bond Type	Binding Amino Acids	Bond Length (Å)
	Hydrogen	HIS237	2.64
	Pi-Donor Hydrogen	PHE191	3.45
	Hydrogen	LYS238	1.80
	Hydrogen	ASP196	2.87
-6.44	Pi-Alkyl	HIS19	5.07
	Alkyl	ALA214	3.51
	Alkyl	VAL216	4.04
	Alkyl	ILE197	4.27
	Alkyl	ALA206	4.31

To determine the characteristics of the active compounds in the three extracts (in rice, shrimp shell, and potato media), further analysis was carried out using LC–MS/MS, as shown in Figure 6. Significant differences in the chromatogram patterns were noted at certain retention times. In other words, different chromatogram shapes reflect different components in each extract. As explained previously, the extract from *F. equiseti* 20CB07RF, which was cultured on the rice medium, exhibited higher antibacterial activities than the extracts cultured on the shrimp shell and potato media (see Figure 5).

To ensure that compounds with antibacterial properties are present in the active extract, further analysis was needed. The LC–MS/MS spectra of the extract using the rice medium show two different chromatogram peaks. The peak at the retention time of 7.63 minutes is assigned to compound 1, whereas the peak at the retention time of 11.38 minutes is assigned to compound 2 (Figure 7). Further interpretation shows that compound 1 has a [M+H]+ molecular ion peak at m/z 528.3514, corresponding to a peptide hybrid compound. Meanwhile, compound 2 has a molecular ion peak [M+H]+ at m/z 439.3340, corresponding to a terpene alkaloid compound. Further analysis related to the bioactivity properties of compound 1, which has a probability of 60%, was conducted.

The results of pharmacokinetics analysis based on the datab ase, according to the nature of the extract activity from isolate CB07RF1 cultured in the rice medium, are shown in Figure 8. Computational pharmacokinetic tests using the Swiss ADME and Protox programs showed that the isolated compounds are antibacterial drug candidates. All computational data show that the compounds fulfill the requirements for use as a drug (absorption, distribution, metabolism, and excretion) and are

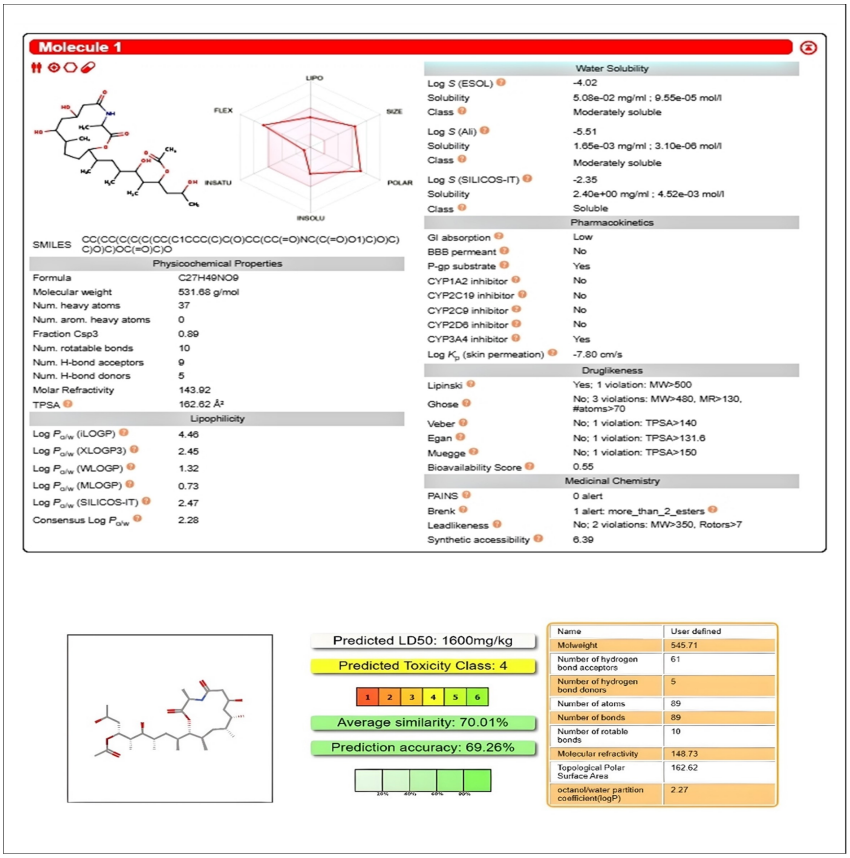

Figure 8. Pharmacokinetic Test Using Swiss ADME and Protox

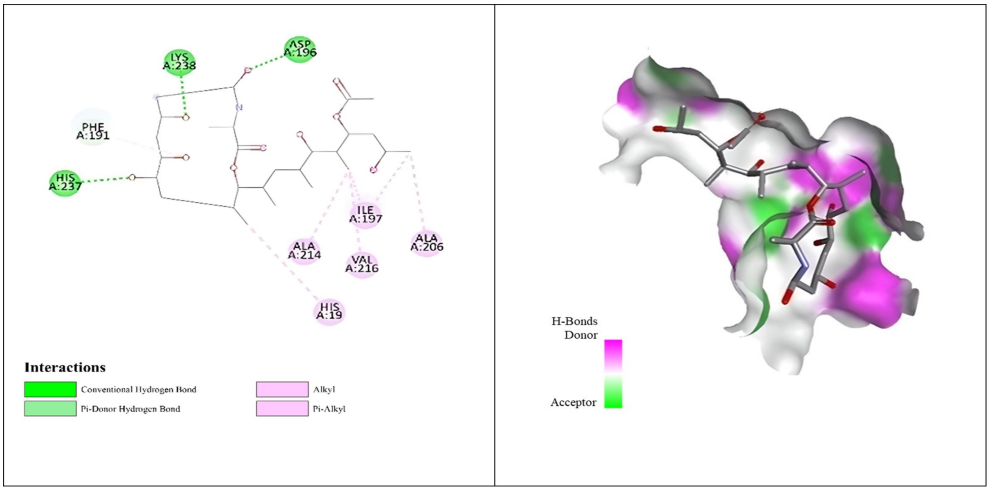

Figure 9. Molecular Docking of Active Compound 1 with Protein 5U39

safe for use, with a toxicity value of 4.

Further analysis using an in silico approach showed that the compound with a retention time of 7.63 min interacted with *P. aeruginosa* LpxC. The docking results (protein 5U39: *P. aeruginosa* LpxC in complex with CHIR-090) are shown in Figure 9 and Table 3. The docking of the active compound with 3U59 protein had a bonding energy of -6.44 kJ/mol, indicating good interaction as an inhibitor of *P. aeruginosa*. The interaction involved four hydrogen bonds; therefore, it can be concluded that the interaction and the inhibitory activity were

excellent (Piizzi et al., 2017).

4. CONCLUSION

The extract of *F. equiseti* 20CB07RF, which was isolated from the stem of *R. apiculata*, is a valuable source of bioactive metabolites. Through a solid-state fermentation process using a rice medium, the active sub-faction of *F. equiseti* 20CB07RF was derived, and it exhibited strong growth-inhibitory activity against the pathogenic bacteria, *P. aeruginosa*, at a concentration of 12.5 mg/mL. Comparative analysis of the profiles of the *F. equiseti* 20CB07RF extract obtained using LC–MS/MS indicated that there is a correlation between the medium and the antibacterial activity of compound 1. This is also supported by the results of pharmacokinetic and molecular docking studies. This foundational information is certainly valuable for advancing related research in the fields of life sciences, biotechnology, and pharmacology.

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REFERENCES

Abu Yazid, N., R. Barrena, D. Komilis, and A. Sánchez (2017). Solid-State Fermentation As a Novel Paradigm for Organic Waste Valorization: A Review. *Sustainability*, **9**(2); 224

Ardiansyah, F. and R. Safe'i (2021). Analysis of Changes in Health of Coastal Mangrove Forest on the East Coast of Lampung. In *IOP Conference Series: Earth and Environmental Science*, volume 755. IOP Publishing, page 012028

Atanasov, A. G., S. B. Zotchev, V. M. Dirsch, and C. T. Supuran (2021). Natural Products in Drug Discovery: Advances and Opportunities. *Nature Reviews Drug Discovery*, **20**(3); 200–216

Boruah, J. L. H., D. Das, P. Gogoi, N. Kumar, B. Borah, M. Borah, P. Talukdar, C. Lulu, P. Gogoi, and A. Yadav (2024). Fungal Endophytes As an Alternative Natural Resource for the Discovery of Bioactive Compounds of Pharmacological Importance. In *Endophytic Fungi: The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries*. Springer, pages 57–78

Cadamuro, R. D., I. M. A. da Silveira Bastos, I. T. Silva, A. C. C. da Cruz, D. Robl, L. P. Sandjo, S. Alves Jr, J. M. Lorenzo, D. Rodríguez-Lázaro, and H. Treichel (2021). Bioactive Compounds from Mangrove Endophytic Fungus and Their Uses for Microorganism Control. *Journal of fungi*, **7**(6); 455

Clinical Laboratory Standards Institute (2017). *M-100 Performance Standards for Antimicrobial Susceptibility Testing*, volume 43 of *33rd*. CLSI Suplement, pages 106–112

Elshikh, M., S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant, and I. M. Banat (2016). Resazurin-Based 96-Well Plate Microdilution Method for the Determination of Minimum Inhibitory Concentration of Biosurfactants. *Biotechnology letters*, **38**(March); 1015–1019

Gajdács, M., E. Urbán, A. Stájer, and Z. Baráth (2021). Antimicrobial Resistance in the Context of the Sustainable Development Goals: A Brief Review. *European Journal of Investigation in Health, Psychology and Education*, **11**(1); 71–82

Hashem, A. H., M. S. Attia, E. K. Kandil, M. M. Fawzi, A. S. Abdelrahman, M. S. Khader, M. A. Khodaira, A. E. Emam, M. A. Goma, and A. M. Abdelaziz (2023). Bioactive Compounds and Biomedical Applications of Endophytic Fungi: A Recent Review. *Microbial Cell Factories*, **22**(1); 107

Hodges, G., C. Eadsforth, B. Bossuyt, A. Bouvy, M. H. Enrici, M. Geurts, M. Kotthoff, E. Michie, D. Miller, and J. Müller (2019). A Comparison of Log Kow (n-Octanol-Water Partition Coefficient) Values for Non-Ionic, Anionic, Cationic and Amphoteric Surfactants Determined Using Predictions and Experimental Methods. *Environmental Sciences Europe*, **31**(1); 1–18

Imhoff, J. F. (2016). Natural Products from Marine Fungi—Still an Underrepresented Resource. *Marine Drugs*, **14**(1); 19

Jukes, T. H. and C. R. Cantor (1969). Evolution of Protein Molecules. *Mammalian Protein Metabolism*, **3**(24); 21–132

Lai, J., W. Cheah, K. Palaniveloo, R. Suwa, and S. Sharma (2022). A Systematic Review of the Physicochemical and Microbial Diversity of Well-Preserved, Restored, and Disturbed Mangrove Forests: What Is Known and What Is the Way Forward? *Forests*, **13**(12); 2160

Laila, A., F. Setiawan, W. Widyastuti, M. R. Fadhilah, A. Setiawan, N. L. G. R. Juliasih, W. A. Setiawan, E. Apriliana, P. Ahmadi, and M. Arai (2023). Exploration and Biorefinery Antimicrobial Agent through Solid State Fermentation from Indonesia's Marine Actinomycetes. *Fermentation*, **9**(4); 334

Li, T., W. Yang, T. Chen, H. Ouyang, Y. Liu, B. Wang, H. Yu, and Z. She (2024). Five Secondary Metabolites from Mangrove Endophytic Fungus Fusarium. Proliferatum NSD-1. *Journal of Molecular Structure*, **1302**(April); 137434

Liao, S., Y. Wang, H. Liu, G. Fan, S. K. Sahu, T. Jin, J. Chen, P. Zhang, L. Gram, and M. L. Strube (2020). Deciphering the Microbial Taxonomy and Functionality of Two Diverse Mangrove Ecosystems and Their Potential Abilities to Produce Bioactive Compounds. *Msystems*, **5**(5); 10–1128

Lu, P., Y. Shi, J. Zhang, K. Hong, Y. Xue, and L. Liu (2024). New Prenylated Indole-Benzodiazepine-2, 5-Diones with α-Glucosidase Inhibitory Activities from the Mangrove-Derived Aspergillus Spinosus. *International Journal of Biological Macromolecules*, **257**(Part 2); 128808

Piizzi, G., D. T. Parker, Y. Peng, M. Dobler, A. Patnaik, S. Wattanasin, E. Liu, F. Lenoir, J. Nunez, and J. Kerrigan (2017).

Design, Synthesis, and Properties of a Potent Inhibitor of Pseudomonas Aeruginosa Deacetylase LpxC. *Journal of Medicinal Chemistry*, **60**(12); 5002–5014

Senanayake, I., A. Rathnayaka, D. Marasinghe, M. Calabon, E. Gentekaki, H. Lee, V. Hurdeal, D. Pem, L. Dissanayake, and S. Wijesinghe (2020). Morphological Approaches in Studying Fungi: Collection, Examination, Isolation, Sporulation and Preservation. *Mycosphere*, **11**(1); 2678–2754

Setiawan, A., R. Lutfiah, N. L. G. R. Juliasih, W. A. Setiawan, J. Hendri, and M. Arai (2022a). Antibacterial Activity of EtOAc Extract from Marine-Derived Fungus Aspergillus Nomiae A12-Rf against Clinical Pathogen Bacteria, Staphylococcus Aureus. *AACL Bioflux*, **15**(3); 1413–1421

Setiawan, A., F. Setiawan, N. L. G. R. Juliasih, W. Widyastuti, A. Laila, W. A. Setiawan, F. M. Djailani, M. Mulyono, J. Hendri, and M. Arai (2022b). Fungicide Activity of Culture Extract from Kocuria Palustris 19C3A1 against Fusarium Oxysporum. *Journal of Fungi*, **8**(3); 280

Setiawan, A., W. Widyastuti, A. Irawan, O. S. Wijaya, A. Laila, W. A. Setiawan, N. L. G. R. Juliasih, K. Nonaka, M. Arai, and J. Hendri (2021). Solid State Fermentation of Shrimp Shell Waste Using *Pseudonocardia carboxydivorans* 18S13O1 to Produce Bioactive Metabolites. *Fermentation*, **7**(4); 247

Tamura, K., G. Stecher, and S. Kumar (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, **38**(7); 3022–3027

Vacelet, J. and C. Donadey (1977). Electron Microscope Study of the Association between Some Sponges and Bacteria. *Journal of experimental marine Biology and Ecology*, **30**(3); 301–314

Wang, M., Q. Chen, Y. Diao, W. Duan, and L. Cai (2019). Fusarium Incarnatum-Equiseti Complex from China. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, **43**(1); 70–89

Wen, J., S. K. Okyere, S. Wang, J. Wang, L. Xie, Y. Ran, and Y. Hu (2022). Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. *Journal of Fungi*, **8**(2); 205

White, T. J., T. Bruns, S. Lee, and J. Taylor (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, **18**(1); 315–322

Yang, S., L. Shan, H. Luo, X. Sheng, J. Du, and Y. Li (2017). Rapid Classification and Identification of Chemical Components of *Schisandra chinensis* by UPLC-Q-TOF/MS Combined with Data Post-Processing. *Molecules*, **22**(10); 1778

Zhang, Y., L. Feng, X. Hemu, N.-H. Tan, and Z. Wang (2024). OSMAC Strategy: A Promising Way to Explore Microbial Cyclic Peptides. *European Journal of Medicinal Chemistry*, **268**(March); 116175

