BIODIVERSITAS
Volume 26, Number 8, August 2025
Pages: 3745-3757

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260808

Bioactivity and chemical composition of *Penicillium griseofulvum* from estuarine soil in East Java, Indonesia

AFRIAN ROSYADI[1], MOCHAMAD RAZAQ SETYANTO[5], BAWON TRIATMOKO[5], JOKO TRI WIBOWO[2], LESTYO WULANDARI[3], EVI UMAYAH ULFA[4], ARI SATIA NUGRAHA[5,♥]

[1]Graduate Program of Pharmacy, Faculty of Pharmacy, Universitas Jember. Jl. Kalimantan I No. 2, Jember 68121, East Java, Indonesia
[2]Research Center for Biotechnology, Life Sciences Research Organization, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km 46, Bogor 16911, West Java, Indonesia
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Jember. Jl. Kalimantan I No. 2, Jember 68121, East Java, Indonesia
[4]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Jember. Jl. Kalimantan I No. 2, Jember 68121, East Java, Indonesia
[5]Drug Utilization and Discovery Research Group, Faculty of Pharmacy, Universitas Jember. Jl. Kalimantan I No. 2, Jember 68121, East Java, Indonesia. Tel.: +62-331-324736, ♥email: arisatia@unej.ac.id

Manuscript received: 17 April 2025. Revision accepted: 5 August 2025.

Abstract. *Rosyadi A, Setyanto MR, Triatmoko B, Wibowo JT, Wulandari L, Ulfa EU, Nugraha AS. 2025. Bioactivity and chemical composition of* Penicillium griseofulvum *from estuarine soil in East Java, Indonesia. Biodiversitas 26: 3745-3757.* Fungi have been a notable source for new antibiotic discovery, yet bioprospecting on Indonesian micro fungi remains scarce. The previous study has collected several soil fungi from understudied estuarine areas, a unique environment where the coastal region has provided a fertile environment for fungi to grow and adapt. This has left the soil fungi as a promising source of antibacterial compounds. This study aimed to isolate antibiotic-producing fungi from these unique estuarine habitats as well as evaluate their secondary metabolite. Soil samples were collected from Kendit Village, Situbondo, East Java, Indonesia. Antibacterial activities were measured based on the CLSI microdilution method. Species identification of fungi was based on the DNA barcoding technique using ITS region-specific primers. A secondary metabolite was produced using solid-state fermentation using rice as substrate. Metabolite profiling relied on the GC-MS technique. Seven fungal isolates were obtained and tested for antibacterial activity against *Staphylococcus aureus*. All isolates exhibited positive inhibition, with isolate IS-IB-T2 showing the highest activity, forming an inhibition zone of 18.37 ± 0.31 mm. IS-IB-T2 was identified as *Penicillium griseofulvum* isolate CF00049 (GenBank accession no. OQ076449.1). It demonstrated significant antibacterial activity against *S. aureus* (30.5 ± 3.9% inhibition) and *Pseudomonas aeruginosa* (65.6 ± 3.1% inhibition). GC-MS analysis revealed 18 bioactive compounds, with *n*-Hexadecanoic acid, Hexadecanoic acid ethyl ester, and Vinyl *trans*-cinnamate as major constituents. These compounds are known to disrupt bacterial metabolic processes, redox balance, and membrane integrity. In summary, the study suggested that the estuarine habitat was a notable source for antibiotic-producing fungal isolates. Isolated *P. griseofulvum* from the Situbondo estuarine environments holds potential as a natural source for the development of new antimicrobial agents. Further research is necessary to isolate the bioactive compound as well as obtain the Minimum Inhibitory Concentration (MIC) value of the single compound.

Keywords: Antibacterial, GC-MS compounds, microdilution, *Penicillium griseofulvum*, soil fungi, solid state fermentation

INTRODUCTION

Infection is a disease caused by microorganisms like bacteria, viruses, fungi, or parasites, and it poses a significant health concern. These microorganisms can enter the human body through various routes, including inhalation, contaminated food, the bloodstream, or sexual activity. Pathogenic microorganisms, in particular, are a major cause of infectious diseases, significantly affecting human mortality and morbidity (Rosana 2023). According to the World Health Organization (WHO), in 2021, three infectious diseases COVID-19, tuberculosis, and diarrheal diseases were among the top ten causes of death in Indonesia. The global impact of infectious diseases is evident in the severity of the COVID-19 pandemic, caused by the SARS-CoV-2 virus.

Antibiotics play a crucial role in treating bacterial infections. However, their misuse has led to increasing resistance (Rosana 2023). *Staphylococcus aureus* is one of the gram-positive pathogenic bacteria that has shown rising resistance to antibiotics (Lade and Kim 2021). Although it is part of the normal flora on the skin, *S. aureus* can cause infections, especially in damaged tissue, and may lead to sepsis if it enters the bloodstream (Lade and Kim 2021). In addition, *Pseudomonas aeruginosa*, Gram-negative bacterium, is also resistant to multiple antibiotic classes. Studies in several Indonesian hospitals reported it as the cause of 3.3 - 30.8% of infectious cases (Elfadadny et al. 2024). This resistance drives the search for new antibacterial agents. Natural sources, especially fungi, have historically yielded effective antibiotics. However, resistance to fungal-derived drugs like penicillin and cephalosporins is now emerging. Therefore, continued exploration of fungi is essential, as they remain a rich source of antimicrobial compounds (Keller 2019). Soil fungi have demonstrated pharmacological antibacterial activity (Fatimah et al. 2022), including compounds like indoloquinoline derivatives active against MRSA (Stan et al. 2021).

Estuarine soils transitional areas between river and sea are rich in microbial life due to plant roots releasing organic

compounds, which enhance fungal diversity (Palit et al. 2022; Haqqa et al. 2023). These conditions support mycelial growth and metabolite production (Madusanka et al. 2024). Additionally, estuarine fungi have adapted to fluctuating salinity and sandy soils, producing metabolites to survive tides (Zhang et al. 2021). These dynamic ecosystems support interactions between microbes, nutrients, and environmental factors (Crump and Bowen 2023), offering great potential for biotechnological applications (Akaniro et al. 2023). Despite the biodiversity of marine fungi, research on their metabolites remains limited (Gonçalves et al. 2022; Nugraha et al. 2023), partly due to challenges in culturing, environmental shifts, and climate change (Gonçalves et al. 2022).

Penicillium griseofulvum is known for producing bioactive metabolites, including griseofulvin an antifungal agent used to treat infections in humans and animals. It also generates other bioactive compounds such as alkaloids, terpenes, and phenolics, which show antimicrobial, antifungal, and antioxidant properties (Conrado et al. 2022; Zhang et al. 2022). Estuarine environments, where freshwater and seawater meet, exhibit unique physicochemical features like fluctuating salinity, temperature, and nutrients. These dynamic conditions support diverse microorganisms, including fungi adapted to thrive in such ecosystems. As a result, estuarine fungi likely produce diverse metabolites, making them valuable for bioprospecting. These environments are microbial hotspots, with fungi forming complex interactions with plants, animals, and other microbes, potentially leading to novel bioactive compounds. Exploring these habitats allows researchers to discover new fungal strains and metabolites with applications in medicine, agriculture, and other sectors (Orfali and Perveen 2019; Palit et al. 2022; Hu and Qin 2025).

Although Indonesia is an archipelagic country with the third-longest coastline globally (54,716 km), its potential as a source of antibacterials remains underutilized. Our previous research evaluated several antibacterial-producing fungal isolates from estuarine soil. In the study by Rosyadi et al. (2022), fungal extracts from estuary soil where river water meets seawater were analyzed. Most of the identified compounds were terpenoids, natural substances common in plants and fungi, many of which possess antibacterial properties. Because terpenoids were found in high concentrations, researchers suspect these compounds contribute to the antibacterial activity of the fungi. In essence, the fungi likely inhibit bacterial growth through terpenoid production. This finding is significant as it suggests that fungi from estuarine soils could be a valuable source of new antibiotic compounds, especially relevant in addressing the global rise of antibiotic resistance.

In this study, we isolated *P. griseofulvum* from estuarine soil and evaluated its antimicrobial activity and metabolites. Samples were collected from Kendit Village, Situbondo, East Java, Indonesia. The study included fungal isolation, identification, metabolite profiling using gas chromatography, and antibacterial testing against *S. aureus* and *P. aeruginosa* using a microdilution method.

MATERIALS AND METHODS

Study area

The picture below shows the location of the estuary soil sampling in Kendit Village, Situbondo District, East Java, Indonesia (Figure 1).

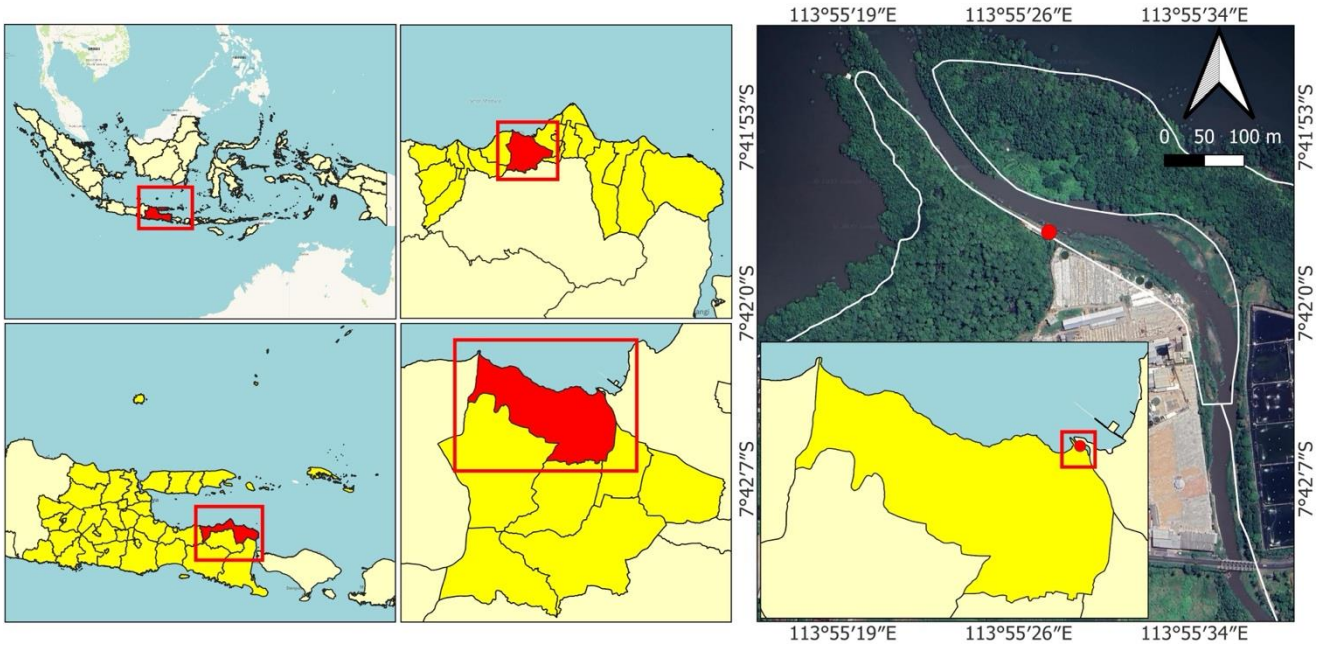

Figure 1. Location of sampling site of estuary soil at Kendit Village, Situbondo, East Java, Indonesia (red dot: 7.70°S, 113.92°E)

Procedures

Collection and preparation of the estuary soil sample

Soil samples were taken from the estuary in Kendit Village, Situbondo District, East Java, Indonesia, at 7.70°S latitude and 113.92°E longitude during the dry season (Figure 1). The ambient temperature of the estuarine ecosystem where soil samples were collected ranges from 26℃ to 29℃. Soil samples were taken using a soil bore at 0-40 cm depth and then put in a sterile plastic bag.

Soil samples were collected from the top, middle, and bottom parts of the estuary using a pipe. Next, samples were transferred to centrifuge tubes and mixed with 10 mL of sterile water using a vortex. The mixture was then centrifuged, and the supernatant was collected with a micropipette. The supernatant was cultured on Potato Dextrose Agar (PDA) media, which was then covered with plastic wrap and incubated at room temperature, about 28℃, for 7 days. The soil samples were planted on the media in aseptic conditions to prevent contamination.

Fungal isolation and antagonist test

The soil samples were carefully suspended in sterile distilled water and then subjected to the precise and reliable pour plate method for inoculation into Potato Dextrose Agar with Chloramphenicol (PDA w/chloramphenicol). The samples were then incubated for one week at 28°C. The process of isolating soil fungi involves replanting to obtain single fungi based on morphological differences after growth.

The isolated fungi are then tested for antagonism to determine their antibacterial potential. This study, which could potentially lead to the discovery of new antibiotics, begins with the inoculation of the test bacteria onto new media and incubation, and the preparation of a bacterial suspension. The turbidity of the suspension is measured using a UV-Vis spectrophotometer. In the direct contact test, the bacterial suspension is spread on the media, and the fungal isolate is placed on the media in contact with the bacteria. The media is then incubated, and the presence of a clear zone around the fungus, a visually striking result, indicates antibacterial activity. This process helps in the identification of soil fungi with potential antibacterial properties, potentially contributing to the development of new antibiotics.

Molecular identification

Molecular identification was performed according to a standard protocol described by Vu et al (Vu et al. 2016, 2019). The DNA barcoding process for mold begins with the DNA extraction stage, which is carried out using the Quick-DNA Magbead Plus Kit from Zymo Research (product code D4082). The extraction procedure followed the manufacturer's instructions to obtain pure DNA from the mold sample. After the DNA was successfully extracted, the next step was DNA amplification using the PCR method.

PCR reaction was performed using MyTaq HS Red Mix (2×) from Bioline (BIO-25048). The total PCR reaction volume was 25 µL, consisting of 12.5 µL MyTaq HS Red Mix, 1 µL ITS1 primer (10 µM), 1 µL ITS4 primer (10 µM), and template DNA with a concentration between 1.5 ng and 75 ng. The remaining volume was filled with DNase/RNase-free water (ddH_2O) until it reached 25 µL. The primers used had the following sequences: ITS1 - 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4 - 5'-TCC TCC GCT TAT TGA TAT GC-3'.

Thermal conditions for PCR include an initial denaturation step at 95℃ for 1 minute, followed by 35 cycles each consisting of denaturation at 95℃ for 10 seconds, annealing at 52°C for 15 seconds, and extension at 72℃ for 15 seconds. After the cycle is complete, the reaction is terminated with a hold stage at 4℃ indefinitely. The final stage of this process is bi-directional sequencing of PCR products, which aims to obtain DNA sequence data from both directions to ensure the accuracy of mold species identification (Vu et al. 2019).

After that, Agarose Gel Electrophoresis Visualization and bidirectional sequencing using the Sanger DNA Sequencing by using Capillary Electrophoresis. Bioinformatics Analysis using the Sanger Sequencing and Phylogenetic Tree - Neighbor-Joining (Unrooted Tree) by NCBI Blast Tree Methods (Vu et al. 2016).

Solid-state fermentation and extract preparation of Penicillium griseofulvum

In this study, we undertook a significant research endeavor: Fermenting soil fungal isolates in Potato Dextrose Broth (PDB) media and conducting solid-state fermentation on rice media. The fungal isolates were placed in the PDB media and incubated for 14 days to reach the equilibrium phase. For the solid-state fermentation, rice was mixed with water, sterilized, and then inoculated with liquid fermentation. The rice media were then incubated for 40 days at 28℃, contributing to our understanding of fermentation processes (Abdel-Motleb et al. 2022; Ezeonuegbu et al. 2022).

After the fermentation process, a meticulous extraction of secondary metabolite compounds was performed. The fermented samples were carefully mixed with ethyl acetate, stirred, and macerated for 24 hours. The mixture was filtered, and the residue was further macerated until the solvent became clear. Then, the filtered extract was concentrated using a rotary evaporator, and the concentrated extract was collected and weighed. This process was designed to retrieve the secondary metabolite compounds produced during the fermentation, demonstrating the thoroughness of our research (Naher et al. 2021; Ezeonuegbu et al. 2022).

Antibacterial activity of the extract of Penicillium griseofulvum *against* Staphylococcus aureus ATCC 6538 *and* Pseudomonas aeruginosa ATCC 27853

All test steps refer to the Clinical and Laboratory Standards Institute M07-A8 standard protocol in 2009 (Clinical and Laboratory Standards Institute 2009). The work steps performed are as follows:

Bacterial suspension in Cation Adjusted Mueller Hinton Broth (CAMHB) was diluted 100 times using CAMHB until the concentration of bacteria ranged from 1×10^8 CFU/mL to 1×10^6 CFU/mL. Each well contains 50 µL of bacterial suspension in CAMHB, then 50 µL of test solution, positive control, or negative control is added.

The final concentration of bacteria per well was 5×10^4 CFU/mL. The treatment group consisted of a mixture of 50

µL of 200 µg/mL concentration extract solution in 1% Dimethyl Sulfoxide (DMSO) and 50 µL of bacteria (5×10^5 CFU/mL) in CAMHB media with a final concentration of 100 µg/mL. The extract control is a mixture of 50 µL of 200 µg/mL concentration extract solution in 1% DMSO and 50 µL of CAMHB media. Negative control extract is a mixture of 50 µL of 1% DMSO in CAMHB media and 50 µL of bacteria in CAMHB media. The 1% DMSO control is a mixture of 50 µL of 1% DMSO in CAMHB media and 50 µL of CAMHB media. Positive control is a mixture of 50 µL gentamicin concentrations of 2 µg/mL and 50 µL bacteria in CAMHB media with a final concentration of 1 µg/mL. The gentamicin negative control consists of a mixture of 50 µL CAMHB media and 50 µL bacteria in CAMHB, while the media control is 100 µL CAMHB media. All treatments were replicated three times. All antibacterial test procedures were carried out in an LAF.

GC-MS analysis

Crude ethyl acetate extracts of *P. grisefulvum* fungus were further analyzed for GC-MS, which is used for compound detection. GC-MS was carried out by using a Thermo Trace 1300GC coupled with a Thermo TSQ 800 Triple Quadrupole MS with a column (30∗0.25 mM, 0.25µM). Next, the samples were injected in split mode as 10:1. Different steps are involved in the reaction to carry out different compounds present in the extracts; the initial temperature is 60°C for 3 minutes, the oven temperature is 280°C at an increased rate of 15°C for 19 minutes, injection port temperature is 260°C for 1 minute, helium used for 1 mL, the flow rate is 1 min, and ionization voltage is 70eV. MS scans at speeds ranging from 50 to 650m/z. The identification of each compound was computer-matched with standard reference databases NIST Ver. 2.1 MS based on the comparison of the Mass Spectra (MS) (Kumari et al. 2022).

Data analysis

In addition, the results of compound identification will provide data on the class of compounds contained in the soil fungi extract. From the measurement of absorbance, the inhibition of bacterial growth can be calculated based on the following formula: Abs: absorbance, A: negative control isolate/gentamicin, B: 1% DMSO control or CAMHB media, C: isolate/gentamicin test solution, and D: isolate/gentamicin control.

$$\% \text{ inhibition} = 1\text{-Abs C-Abs DAbs A-Abs B} \times 100\%$$

One-way ANOVA analysis was used to analyze the significant differences in which was used to see significant differences between groups, followed by an LSD test to determine the significance value. If it was found that the data was not normally distributed and not homogeneous, then a non-parametric test was used, namely the Kruskal-Wallis test. A p-value of <0.05 with a 95% confidence level indicates that the test results are significantly different.

RESULTS AND DISCUSSION

Result of antagonistic test activity and fungal isolation

Seven fungal isolates were obtained from the sampling sites coded IS-IB-A1, IS-IB-A2, IS-IB-A3, IS-IB-T1, IS-IB-T2, IS-IB-B1, and IS-IB-B2. The seven isolates have diverse macroscopic characteristics. The seven fungal isolates were then tested for antagonism with *S. aureus* test bacteria to determine the initial potential for antibacterial activity. The results of the initial screening of antibacterial activity are shown in Table 1. All fungal isolates inhibited antibacterial activity, as evidenced by clear zones around the isolates. This initial screening of antibacterial bioactivity indicated the potential of these fungi as a source of antibacterial compounds. Further research was conducted on the fungal isolate, IS-IB-T2, which demonstrated the strongest antagonistic activity.

Furthermore, the IS-IB-T2 fungal isolate was purified to obtain a fungal isolate without impurities. In general, the macroscopic character of IS-IB-T2 fungal colonies is small, dry, wrinkled, fibrous, or velvety on their surface. In addition, the mycelium of IS-IB-T2 is grey with filamentous white edges (Figure 2).

The antagonistic test results demonstrated the ability of various isolates to inhibit microbial growth, as indicated by the presence of clear zones around the test samples. All tested isolates showed positive inhibition activity, as marked by the "+" symbol, indicating the formation of inhibition zones. The diameter of the inhibition zones varied among the isolates, with IS-IB-T2 showing the highest inhibition zone diameter at 18.37±0.31 mm, indicating strong antagonistic activity. This was followed by IS-IB-T1 (14.63±0.21 mm) and IS-IB-A1 (13.80±0.40 mm). The lowest inhibition was observed in IS-IB-A3, with a diameter of 10.50±0.26 mm. The consistent presence of inhibition zones across all samples confirms the potential antimicrobial activity of the tested isolates, although the degree of effectiveness varied among them.

Table 1. Results of the inhibition zone between the fungal isolate against *Staphylococcus aureus*

Code	Result	Zone of inhibition (mm)
IS-IB-A1	+	13.80 ± 0.40^{bc}
IS-IB-A2	+	13.20 ± 0.20^{c}
IS-IB-A3	+	10.50 ± 0.26^{e}
IS-IB-T1	+	14.63 ± 0.21^{b}
IS-IB-T2	+	18.37 ± 0.31^{a}
IS-IB-B1	+	11.85 ± 0.05^{d}
IS-IB-B2	+	11.82 ± 0.10^{d}

Note: +: Clear zone present. Statistical analysis using One-Way ANOVA followed by LSD Post Hoc test confirmed that the differences in inhibition zones were statistically significant at the 0.05 level, as indicated by the different superscript letters assigned to each value. Least Significant Difference (LSD) post hoc test results showing pairwise comparisons of inhibition zone diameters among fungal isolates. Significant differences ($p<0.05$) are observed between several isolate pairs, indicating varied antibacterial activity levels. The mean plot visualizes these differences, highlighting isolate IS-IB-T2 as having the highest inhibitory effect

Molecular identification of the fungal isolate based on ITS region-specific primers

The growth of the fungus was observed after 4-5 days of inoculation. For molecular identification, total genomic DNA was isolated according to the previously described protocol. PCR was performed, and the resulting DNA bands (PCR products) on the gel are shown in Figure 3. The results showed that molecular confirmation of *P. griseofulvum* using universal primers ITS1 and ITS4 yielded an amplification product with a sequence assembly of 542 bp (Figure 4). After BLAST analysis, the sample showed 100% similarity with *P. griseofulvum* isolate CF00049 with accession number OQ076449.1. Further phylogenetic analysis was performed using MEGA. Figure 5 presents the phylogenetic relationship of the fungus.

Solid state fermentation and extract preparation of *Penicillium griseofulvum*

Prior to extraction, the fungal isolate was first fermented to increase fungal biomass, so that it could increase the secondary metabolite production. Fermentation in this study used liquid fermentation followed by solid-state fermentation. The results showed that *P. griseofulvum* can grow and produce secondary metabolites with potential antibacterial properties through solid-state fermentation. Solid-state fermentation was performed on sterilized rice substrate under incubation conditions of 28°C temperature, 70% humidity, and pH 5.5 for 40 days. Secondary metabolite extraction was carried out using ethyl acetate solvent with a maceration method involving agitation (Figure 6), which was then concentrated using a rotary evaporator until a thick extract was obtained. The ethyl acetate solvent was observed to change color to pink, indicating the transfer of secondary metabolites from the rice substrate to the ethyl acetate solvent.

GC-MS analysis

GC-MS analysis was conducted by the Chemistry Laboratory of the National Research and Innovation Agency in Tangerang, Banten, Indonesia. The presence of bioactive compounds in fermented ethyl acetate extract of *P. griseofulvum* was identified by GC-MS, which showed the presence of a number of compounds with peaks at different retention times, as shown in Figure 7. The compounds detected through GC-MS are shown in Table 2. Analysis of bioactive compounds from the ethyl acetate extract of *P. griseofulvum* via our study, the major compounds detected in the ethyl acetate extract are shown in Table 2. Bioactive compounds contained in the ethyl acetate extract of *P. griseofulvum* have been analyzed using GC-MS, which shows the presence of several compounds that may be responsible for bioactivity.

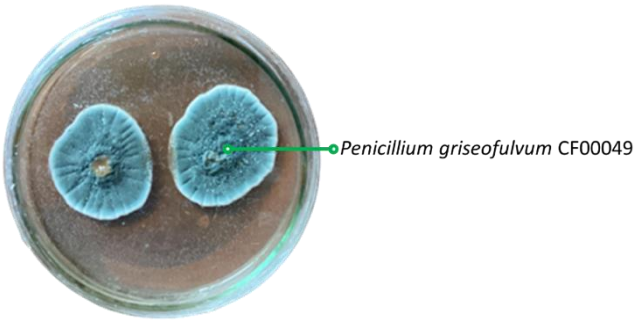

Figure 2. Fungal isolate of IS-IB-T2 from estuary soil from Kendit Village, Situbondo, East Java, Indonesia

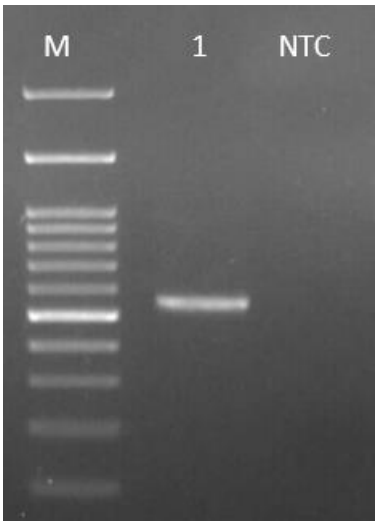

Figure 3. Electrophoresis image - Amplification product (1 µL of the amplified product was resolved on a 1% agarose gel in TBE buffer, with a 100 bp DNA ladder (2.5 µL) as a reference, NTC: No Template Control (Negative amplification control)

```
1    TCCGTAGGTG AACCTGCGGA AGGATCATTA CCGAGTGCGG GCCCCTCGGG GCCCAACCTC
61   CCCACCCGTG TTGCCCGAAC CTATGTTGCC TCGGCGGGCC CCGCGCCCGC CGACGGCCCC
121  CCTGAACGCT GTCTGAAGTT GCAGTCTGAG ACCTATAACG AAATTAGTTA AAACTTTCAA
181  CAACGGATCT CTTGGTTCCG GCATCGATGA AGAACGCAGC GAAATGCGAT AACTAATGTG
241  AATTGCAGAA TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCT CTGGTATTCC
301  GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG TGTTGGGCCC
361  CGTCCCCCCC GCCGGGGGGA CGGGCCCGAA AGGCAGCGGC GGCACCGCGT CCGGTCCTCG
421  AGCGTATGGG GCTTCGTCAC CCGCTCTAGT AGGCCCGGCC GGCGCCAGCC GACCCCCAAC
481  CTTTAATTAT CTCAGGTTGA CCTCGGATCA GGTAGGGATA CCCGCTGAAC TTAAGCATAT
541  CA
```

Figure 4. ITS sequence data of isolate IS-IB-T2 from estuary soil from Kendit Village, Situbondo, East Java, Indonesia

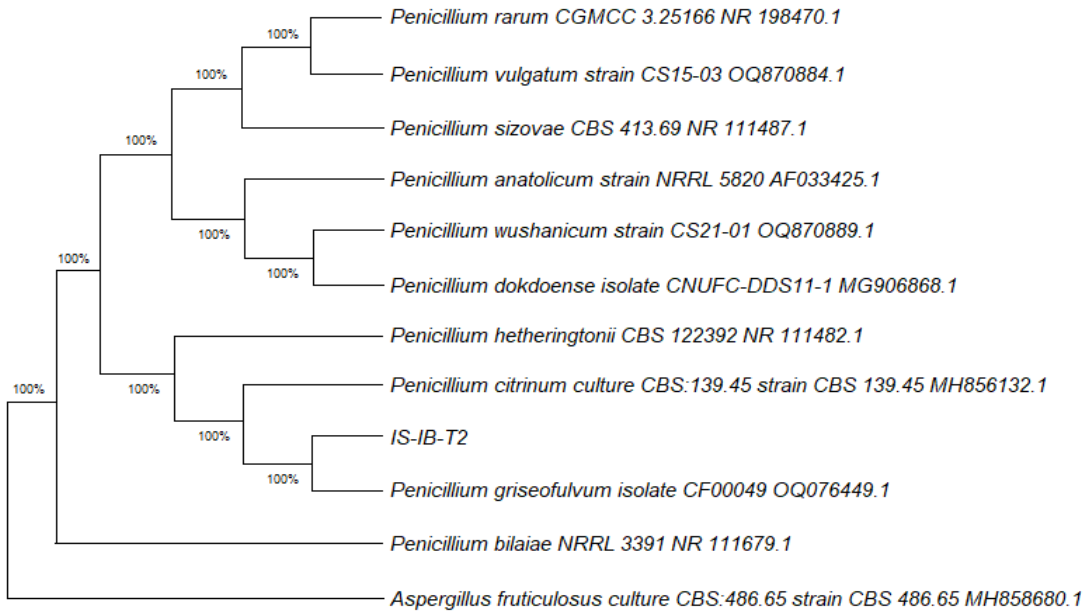

Figure 5. Phylogenetic tree of fungal isolate IS-IB-T2 constructed by MEGA (Tamura et al. 2021)

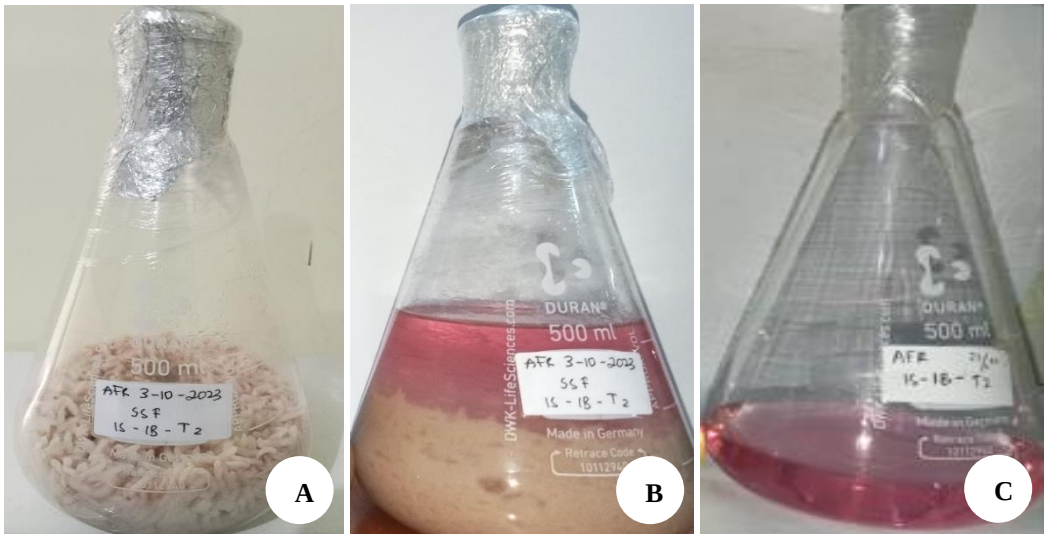

Figure 6. A. The initial color of SSF media after SSF 40 days, B. The extraction process with ethyl acetate after SSF 40 days, C. The extraction result of ethyl acetate was observed to change color into pink

The GC-MS analysis of the ethyl acetate extract of *P. griseofulvum* revealed the presence of various bioactive compounds, each with different molecular structures and potential biological activities. A total of 18 compounds were identified, with match scores ranging from 52.76 to 81.47, indicating moderate to high confidence in the compound identification. These compounds include a diverse range of chemical classes such as aromatic compounds (e.g., benzaldehyde and vinyl *trans*-cinnamate), phenolic derivatives (e.g., benzaldehyde, 2,4-dihydroxy-6-methyl-), fatty acids and their esters (e.g., *n*-hexadecanoic acid, linoleic acid ethyl ester, and hexadecanoic acid ethyl ester), as well as heterocyclic and nitrogen-containing compounds (e.g., indole-2-carboxylic acid methyl ester and 1*H*-pyrrolo[2,3-b]pyridine, 2-ethyl-). Three compounds are the main components contained in the extract, with *n*-Hexadecanoic acid and Hexadecanoic acid, ethyl ester being fatty acid and ester compounds. At the same time, Vinyl *trans*-cinnamate is an ester compound with potential biological activity.

Table 2. The presence of different bioactive compounds in the ethyl acetate extract of *Penicillium griseofulvum* was detected through GC-MS

Name of compound	Class compounds	Molecular formula	Match score	RT
Vinyl *trans*-cinnamate	Esters	$C_{11}H_{10}O_2$	76.47	4.76
3-Hydroxybutan-2-one	Ketones	$C_4H_8O_2$	69.63	6.42
Benzaldehyde	Aldehydes	C_7H_6O	77.81	6.92
Pentanoic acid, 4-oxo-, methyl ester	Esters	$C_6H_{10}O_3$	64.93	7.22
3-hydroxy-2-methylbutanoic acid	Carboxylic acids	$C_5H_{10}O_3$	55.84	8.27
Indole-2-carboxylic acid methyl ester	Pyridine	$C_{10}H_9NO_2$	63.28	8.80
1*H*-Pyrrolo[2,3-b]pyridine, 2-ethyl-	Pyridine	$C_9H_{10}N_2$	58.81	8.96
1-methylcyclopent-1-ene	Cycloalkenes	C_6H_{10}	60.21	9.44
5-(2-amino-1-hydroxyethyl)benzene-1,3-diol	Phenolics	$C_6H_{10}F_3NO$	81.47	9.45
oxalic acid	Carboxylic acids	$C_2H_2O_4$	62.36	11.89
Benzaldehyde, 2,4-dihydroxy-6-methyl-	Phenolics	$C_8H_8O_3$	78.33	14.11
1-Undecanol	Alcohols	$C_{11}H_{24}O$	74.99	18.57
Pentadecanoic acid, 14- methyl-, methyl ester	Esters	$C_{17}H_{34}O_2$	52.76	19.39
n-Hexadecanoic acid	Carboxylic acids	$C_{16}H_{32}O_2$	78.69	19.74
Hexadecanoic acid, ethyl ester	Esters	$C_{18}H_{36}O_2$	81.40	20.05
6*H*-Dibenzo(b,d)pyran-1-ol, 3-hexyl-7,8,9,10-tetrahydro-6,6,9-trimethyl-	Phenolics	$C_{22}H_{32}O_2$	76.35	20.53
Linoleic acid ethyl ester	Esters	$C_{20}H_{36}O_2$	59.58	21.64
2-(3,4-dihydroxyphenyl)-2-hydroxyacetic acid	Phenolics	$C_8H_8O_5$	67.83	25.17

Antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

The antibacterial activity test in this study showed that the ethyl acetate extract of fermented estuarine soil fungi isolate IS-IB-T2 had inhibitory activity against the growth of *S. aureus*, with an inhibition percentage of 30.5%, and *P. aeruginosa* bacteria, with an inhibition percentage of 65.6%. The percentage inhibition of the positive control and the extract is listed in Table 3.

The antibacterial activity of the ethyl acetate extract from the fermented fungal isolate IS-IB-T2 showed significant variation between *S. aureus* and *P. aeruginosa*. As presented in Table 3, the mean inhibition percentage against *S. aureus* was 30.5±3.9%, whereas it was notably higher against *P. aeruginosa*, at 65.6±3.1%. Statistical analysis using one-way ANOVA confirmed that these differences were significant, as indicated by the different superscript letters. This result suggests that the extract exhibits selective antibacterial activity, with greater effectiveness against the Gram-negative *P. aeruginosa* compared to the Gram-positive *S. aureus*. Interestingly, this is contrary to the general trend where natural products tend to show stronger inhibition against Gram-positive bacteria due to differences in cell wall structure. These findings align with studies such as Stan et al. (2021), which reported similar differential activities of fungal-derived compounds. The significant inhibition of *P. aeruginosa* by IS-IB-T2 highlights its potential as a source of bioactive compounds effective against resistant Gram-negative pathogens, inspiring further research and potential breakthroughs in the field.

The one-way ANOVA results indicate a statistically significant difference in the inhibition percentage of *P. aeruginosa* between groups (Table 4). The analysis yielded an F-value of 45.984 with a corresponding *p*-value of 0.002, which is less than the significance threshold of 0.05. This suggests that there is a significant difference in inhibition levels between the tested groups. Particularly, the sum of squares between groups was 1,771.602 with 1 degree of freedom, while the within-group variation was 154.107 with 4 degrees of freedom. The ANOVA was considered an appropriate test, since the data align with the assumptions of normality and homogeneity, and post hoc analysis (e.g., LSD test) can be applied to determine which specific groups differ significantly. The large F-value indicates that the difference between treatment groups is a highly significant effect on the measured variable. The significant difference between treatment groups can be caused by several factors, such as differences in extract concentration, differences in bacterial species used, or differences in experimental conditions (Montgomery 2017). Previous studies have shown similar results, namely significant differences between treatment groups. For example, a study by Sebe et al. (2023) showed that ANOVA analysis can be used to determine significant differences between treatment groups.

Table 3. Percentage inhibition of gentamicin and ethyl acetate extract of fermented fungal isolate IS-IB-T2 against gram-positive bacteria *S. aureus* and gram-negative bacteria *P. aeruginosa*

Entry	Mean inhibition (%)	
	S. aureus	***P. aeruginosa***
Positive control (Gentamicin)	100.1±2.5^a	100.0±8.2^a
IS-IB-T2	30.5±3.9^c	65.6±3.1^b

Note: Concentration of gentamicin is 1 µg/mL, and extract is 100 µg/mL. Different letters indicated significant differences based on one-way ANOVA analysis

Table 4. ANOVA statistical result of *P. aeruginosa*

	Sum of squares	df	Mean square	F	Sig.
Between groups	1771.602	1	1771.602	45.984	.002
Within groups	154.107	4	38.527		
Total	1925.708	5			

Note: ANOVA statistical analysis results for *P. aeruginosa* indicate a significant difference between treatment groups (F = 45.984, p = 0.002)

Table 5. ANOVA statistical result of *S. aureus*

	Sum of squares	df	Mean square	F	Sig.
Between groups	7,259.282	1	7,259.282	681.196	.000
Within groups	42.627	4	10.657		
Total	7,301.908	5			

Note: ANOVA statistical analysis results for *S. aureus* showing a significant difference between treatment groups (F = 681.196, p<0.001)

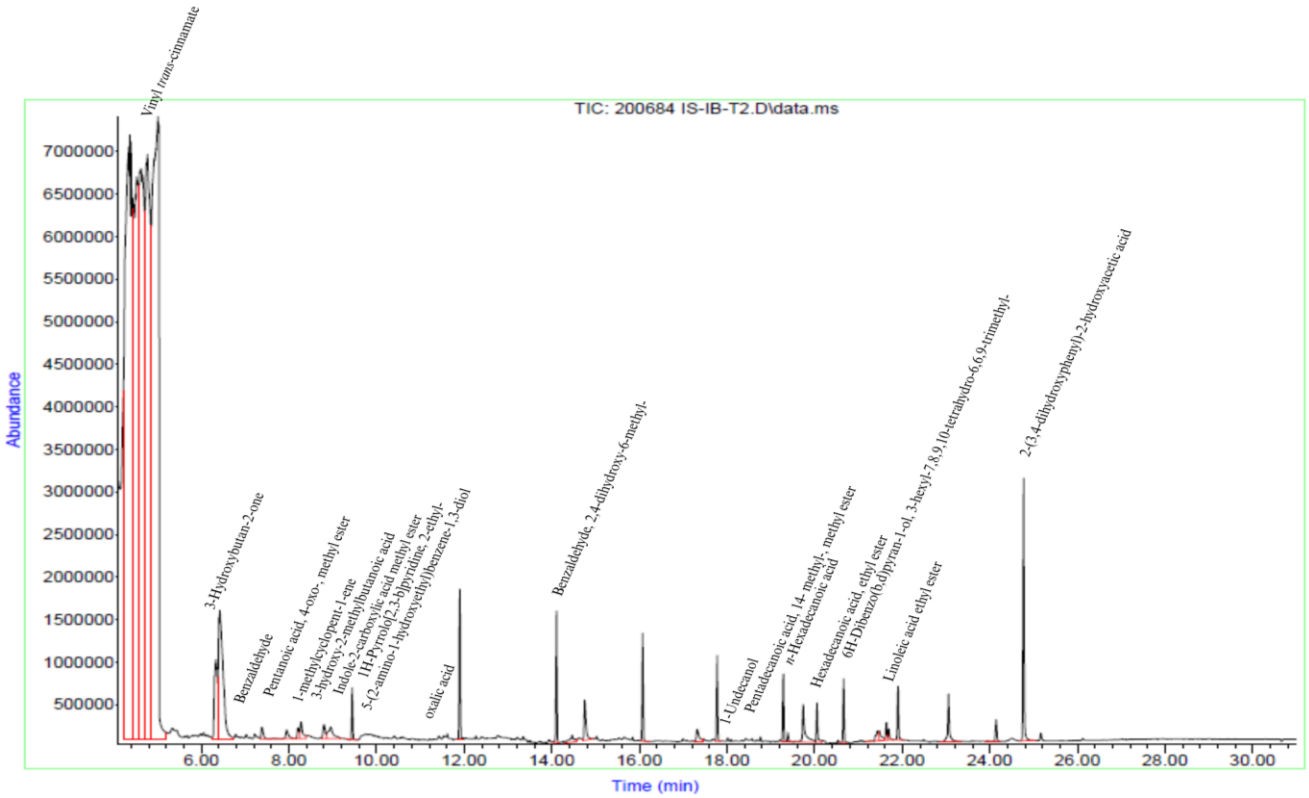

Figure 7. The chromatogram of the ethyl acetate extract of *Penicillium griseofulvum* indicated major peaks of metabolites

The One-Way ANOVA analysis of the percent inhibition of *S. aureus* revealed a statistically significant difference between the treatment groups (Table 5). The F-value was 681.186 with a p-value of 0.000, which is well below the standard significance level of 0.05. This indicates a highly significant effect of treatment on the inhibition of *S. aureus*. The sum of squares between groups was 7,259.282 with 1 degree of freedom, while the within-group sum of squares was only 42.827 with 4 degrees of freedom, indicating low variability within groups compared to between groups. These results confirm that the treatments had a pronounced and statistically significant impact on the inhibition level of *S. aureus*, and post hoc testing (such as the LSD test) can be used to determine which specific groups differed. The large F-value indicates that the difference between treatment groups is highly significant. Therefore, it can be concluded that the treatment given has a significant effect on the measured variable. The low variability within groups can be caused by several factors, such as sample uniformity, good control over external variables, and accurate measurement methods (Krzywinski and Altman 2013). A study by Saha et al. (2023) showed that ANOVA analysis can be used to determine significant differences between treatment groups with low variability within groups.

Discussion

This study harnessed the potential of estuary soil as a source of soil fungi, which were identified as promising antibacterial agents from natural materials. The soil sampling was specifically conducted at the estuary of Kendit Village, Situbondo District, a location chosen for its rich microbial environment, particularly in the rhizosphere of mangrove plants. These plants' roots provide a nutrient-rich habitat for microorganisms in the soil. The estuary soil's varying salinity gradients caused by tidal fluctuations create an environment that encourages microorganisms to produce secondary metabolites for survival. The chemical and physical components of the soil were crucial in shaping the

fungi diversity. Several fungi that produce antimicrobial compounds utilize different carbon sources as energy for their growth (Fatimah et al. 2022). The optimum growth temperature for fungi ranges from 25-30°C; this study revealed that the optimal mesophilic growth of fungi occurs within this temperature range. Fungi can grow in a wide range of pH values, from acidic to alkaline, about 3.0-8.5, though the ideal pH depends on the species and other environmental factors (Mustafa et al. 2023). In addition, soil moisture and salinity are vital causes of spatial variation in specific microorganisms associated with mineralization and humification processes. In contrast, plant-induced dominant microorganisms exert minimal influence on the spatial differences of soil humification intensity (Dong et al. 2025).

Only fungal isolates that exhibited significant antibacterial activity were identified in this research. The identified fungal isolate, namely *P. griseofulvum*, belongs to the phylum Ascomycota. Similarly, previous studies have disclosed the prevalence of Ascomycetes in marine environments. The isolate is classified under the class Eurotiomycetes, family Aspergillaceae, and order Eurotiales. These findings following the previous studies conducted in hypersaline environments that report *Penicillium* as a fungal genus with species that have been repeatedly isolated from marine ecosystems (Mavoa et al. 2024). Marine fungi have gained recognition as potential sources of bioactive secondary metabolites with diverse properties, including antimicrobial, antifungal, antioxidant, antifouling, and anti-inflammatory activity. These bioactive metabolites play a crucial role in enhancing the survival of marine fungi in their extreme environment (Devi and Jayaseelan 2020). Thus, estuarine habitats, located at the interface of marine and terrestrial ecosystems, are dynamic and unique environments that can support an extraordinary diversity of microorganisms. Microbes inhabiting these ecosystems have adapted to extreme conditions, such as fluctuating salinity, temperature, and nutrient levels, which can trigger the production of unique bioactive compounds with potential applications in medicine, agriculture, and industry (Mitra and Zaman 2016).

Estuaries can harbor a wide range of microbial taxa, including bacteria, archaea, and fungi, that have evolved to thrive in these environments. These microorganisms can produce bioactive compounds, such as antibiotics, antivirals, and antifungals, to protect themselves from environmental stressors and compete with other microorganisms. Some examples of unique microbial taxa found in estuaries include halophilic bacteria that produce bioactive compounds with potential applications in medicine and industry, and Fungal strains that produce enzymes and bioactive compounds with potential applications in biotechnology (Anderson and Harvey 2022). Conservation of microbial diversity in estuaries is crucial for several reasons; microorganisms in estuaries can be a rich source of novel bioactive compounds with potential applications in medicine, agriculture, and industry. Microbial diversity plays a key role in maintaining ecosystem balance and resilience against environmental changes; loss of microbial diversity can have negative impacts on ecosystem health and human well-being. The study of microorganisms in estuaries can lead to the discovery of novel natural products with potential applications in various sectors. Some examples of bioactive compounds discovered from estuarine microorganisms include antibiotics and antivirals with potential applications in medicine, enzymes and other bioactive substances for biotechnology, and compounds for agriculture, such as biopesticides and biofertilizers (Amin et al. 2024).

P. griseofulvum is a species of fungus that belongs to the genus *Penicillium*. This fungus is known to produce bioactive compounds, including antibiotics, griseofulvin, and other compounds with potential applications in medicine and industry. *P. griseofulvum* can grow on various substrates, including rice and wheat bran, and can produce a range of bioactive compounds through solid-state fermentation. These compounds have potential applications in several fields, including as antibiotics, antifungals, and anti-inflammatory agents. Research on *P. griseofulvum* has shown that this fungus has the ability to produce unique bioactive compounds with potential uses in various industries (Corbu et al. 2023).

Fermentation is a process of fungal biomass multiplication and secondary metabolite production for fungi. The secondary metabolite produced by fungi can be maximized after undergoing the fermentation process. There are several types of fermentation processes, including batch process, continuous batch process, and fed-batch process. Among these methods, the batch process was chosen for this research because it is considered easy, efficient, and can minimize contaminants. Fermentation was carried out in PDB liquid medium for 14 days at a controlled temperature with shaking assistance (Molelekoa et al. 2021). The 14-day fermentation period was chosen because the stationary phase of the fungus occurs within the 7-14-day range, and this phase is crucial in fermentation as the production of secondary metabolites from fungi reaches its maximum. A solid-state fermentation process was followed for several weeks. During the fermentation process, events such as changes in the color of the fermentation medium, changes in fungal morphology, and an increase in fungal growth and development will occur. These events are expected to happen as they indicate the fungal process of producing secondary metabolites (Šelo et al. 2021).

Solid-State Fermentation (SSF) of *P. griseofulvum* is a process that offers significant benefits. The fungus that grows on a solid substrate, such as rice or wheat bran, to produce bioactive compounds (Abdel-Motleb et al. 2022). This allows for the optimal growth of the fungus and the production of secondary metabolites, including antibiotics and other compounds with potential applications in medicine and industry. SSF is a cost-effective and environmentally friendly approach to producing these valuable compounds, and *P. griseofulvum* has been shown to produce a range of bioactive compounds under these conditions. The fermentation process involves inoculating the substrate with the fungus, followed by incubation under controlled conditions to allow for the growth and production of the desired compounds (Naher et al. 2021). The resulting fermented material can then be extracted and processed to isolate the bioactive compounds. Extraction of the fermentation broth using ethyl acetate was performed to extract both polar and non-polar compounds. Ethyl acetate was chosen because it is semi-polar, non-toxic, and volatile to heat (Poojar et al. 2020).

The antibacterial activity test in this study showed that the ethyl acetate extract of *P. griseofulvum* had inhibitory activity against *P. aeruginosa* and *S. aureus* bacteria growth. The antibacterial activity results were shown in percent inhibition. The percent inhibition value of the extract on *P. aeruginosa* was greater than on *S. aureus*, which might be due to several factors, including the structure of the *S. aureus* cell wall. It has a more complex and thicker cell wall structure compared to *P. aeruginosa*. The *S. aureus* cell wall is composed of a tetrapeptide chain consisting of L-alanyl-D-isoglutaminyl-L-lysyl-D-alanine and an interpeptide bridge consisting of five glycine units, making it more resistant to antibacterial compounds. In addition, the active compounds in the extracts may have chemical properties that are more suitable for inhibiting *P. aeruginosa* than *S. aureus* (Trizna et al. 2020; Indraningrat et al. 2024). The results of antibacterial activity tests can vary depending on the type of extract, concentration, and method used.

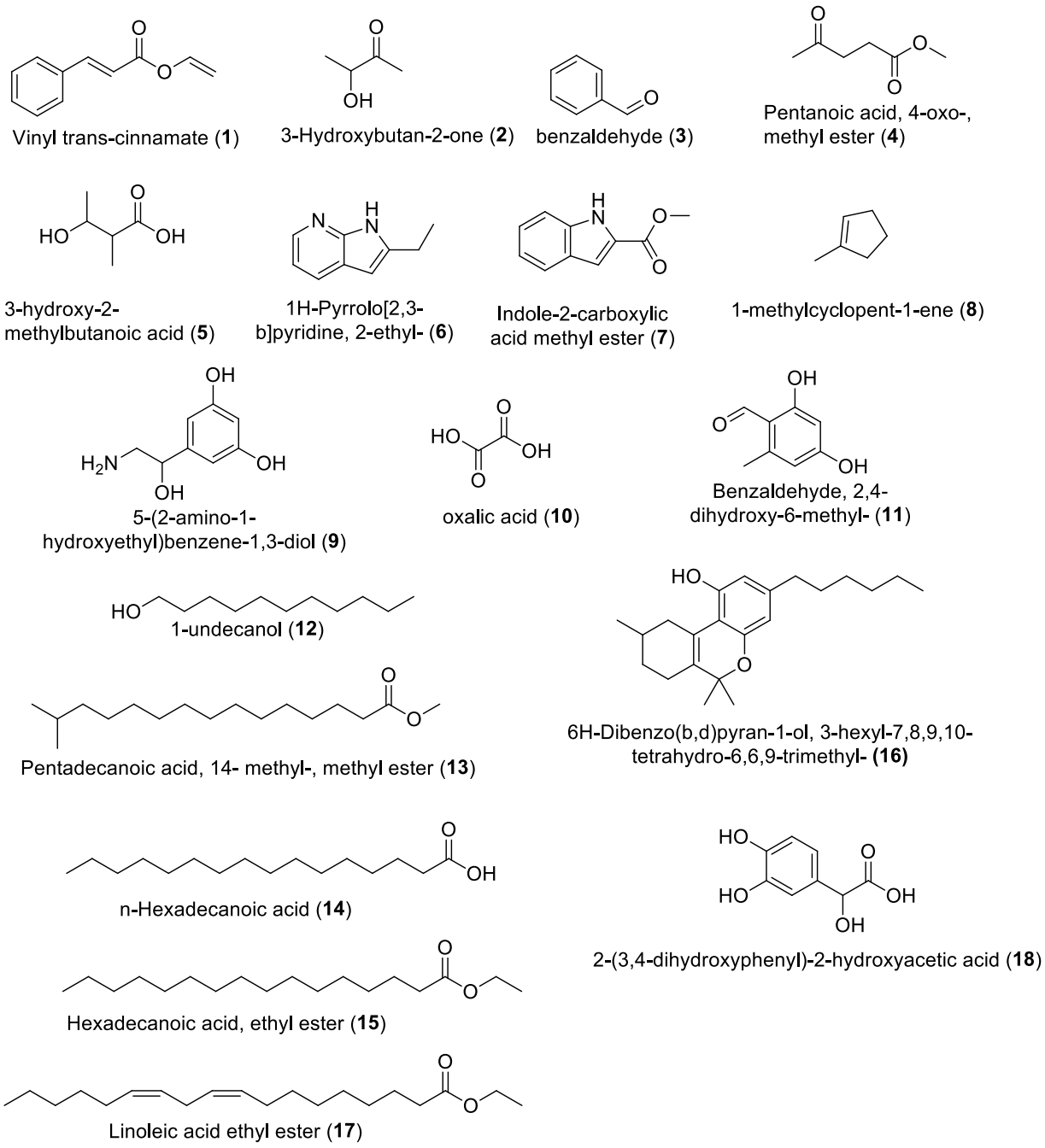

Figure 8. Several compounds from the ethyl acetate extract of *Penicillium griseofulvum* were identified through GC-MS analysis

Gas chromatography tandem with mass spectrometry has been one of the reliable protocols for generating metabolite profiles of micro fungal extracts (Samirana et al. 2023; Soni and Bagaria 2024; Cunha et al. 2025). The GC-MS analysis of the ethyl acetate extract of *P. griseofulvum* identified 18 different bioactive compounds, including a variety of organic acids, esters, aldehydes, and heterocyclic molecules (Figure 8). GC-MS has been a robust protocol in generating chemical components information of the micro fungi extracts (Octarya et al. 2021; Sopalun et al. 2021; Chakraborty et al. 2023).

The identified compounds in the extract of *P. griseofulvum*, including *n*-Hexadecanoic acid, Vinyl trans-cinnamate, and Hexadecanoic acid, ethyl ester, are known to possess antimicrobial properties. *n*-Hexadecanoic acid, a fatty acid, has been reported to exhibit antibacterial and antifungal activities, while Vinyl trans-cinnamate has been shown to possess antimicrobial and antioxidant properties. Hexadecanoic acid, ethyl ester, a derivative of palmitic acid, may also contribute to the observed bioactivity (Zhang et al. 2022). Cinnamic acid derivatives, including vinyl *trans*-cinnamate, have been reported to inhibit both Gram-positive and Gram-negative bacteria (Annuur et al. 2024). The antibacterial activity against *S. aureus* of long-chain fatty alcohols was investigated, with a focus on normal alcohols. The antibacterial activity varied with the length of the aliphatic carbon chain and not with the water/octanol partition coefficient; 1-Undecanol had bactericidal activity and membrane-damaging activity with an MIC of 1-Undecanol about 16 µg/mL (Togashi et al. 2007). Their antibacterial activity against Gram-positive bacteria like *S. aureus* involves disruption of cellular metabolism, redox balance, and damage to bacterial membranes, while against Gram-negative bacteria such as *P. aeruginosa*, they interfere with sulphate assimilation and glutathione redox homeostasis (Wang et al. 2021; Wang et al. 2024). Additionally, it shows antifungal activity against *Candida albicans* by disrupting enzymatic reactions within fungal cells (Osuntokun and Cristina 2019; Huang et al. 2021; Showkat et al. 2025). Together, these findings highlight the potential of *P. griseofulvum* extracts as sources of natural antimicrobial agents with diverse bioactive properties. In addition, several microbial organic volatile compounds, including alcohol derivatives, are important for organisms to adapt to abiotic stress, such as salinity, in an estuary environment (Poveda 2021). Other important secondary metabolite, oxalic acid, also evolved in response to both biotic and abiotic stresses, including defense and heavy metals detoxification (Li et al. 2022). Benzoic class compound was also reported as a metabolite released in response to biotic stress, including from pathogenic organisms (Lv et al. 2024).

The observed antimicrobial activity of *P. griseofulvum* can be attributed to the synergistic effects of these compounds. The combination of fatty acids, esters, and other bioactive metabolites may enhance the antimicrobial properties of the extract, making it effective against a range of microorganisms. Studies on *Penicillium* spp. from other coastal or marine environments have also reported the isolation of bioactive compounds with antimicrobial properties. For example, *Penicillium chrysogenum* isolated from marine sediments has been reported to produce compounds with antibacterial and antifungal activities. Similarly, *Penicillium citrinum* isolated from coastal sediments has been shown to produce compounds with antimicrobial and antioxidant properties (Conrado et al. 2022; Zhang et al. 2022). The findings of this study are consistent with previous reports on the bioactive potential of *Penicillium* spp. from coastal and marine environments. However, the specific compounds identified in this study, particularly the combination of *n*-Hexadecanoic acid, Vinyl *trans*-cinnamate, and Hexadecanoic acid, ethyl ester, may be unique to *P. griseofulvum* from estuarine environments. Further studies are needed to fully elucidate the bioactive properties of these compounds and their potential applications. Overall, this study highlights the potential of *P. griseofulvum* from estuarine environments as a source of novel antimicrobial agents and underscores the importance of exploring these ecosystems for fungal bioprospecting.

In conclusion, this study highlights the potential of *P. griseofulvum* isolated from estuarine soil as a source of antimicrobial agents. Estuarine habitats with their rich sources of microbial diversity and bioactive compounds play a crucial role in the discovery of natural products. The conservation of these ecosystems is essential for maintaining ecosystem health and promoting the discovery of natural products. This study demonstrated that estuarine soil from the Kendit Village, Situbondo, particularly in the rhizosphere of mangrove plants, serves as a promising source of microorganisms, especially fungi, capable of producing antibacterial compounds. The fungal isolate *P. griseofulvum* was successfully identified and exhibited significant antibacterial activity against *S. aureus* and *P. aeruginosa*. Fermentation in both liquid and solid-state media effectively induced the production of secondary metabolites, which were extracted using ethyl acetate. GC-MS analysis revealed the presence of several compounds that could be responsible for the antibacterial activities. Therefore, *P. griseofulvum* isolated from estuarine environments holds potential as a natural source for the development of new, environmentally friendly antimicrobial agents for medical and industrial applications. The current study was limited to antibacterial evaluation on crude extract instead of purified compounds and was performed at a single concentration. Further research is necessary to isolate and characterize the active compounds, in which antibacterial activities are evaluated based on the Minimum Inhibitory Concentration (MIC) value of single compounds, and to evaluate their cytotoxicity. In addition, an action mechanism study of potent compounds is necessary to ensure the feasibility of discovering a new class of compounds, as well as in vivo validation. Additionally, further research is necessary to investigate the microbial diversity of estuaries and harness the potential of these ecosystems for the discovery of novel bioactive compounds.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding support from Universitas Jember, Indonesia, and Indonesia National

Research and Innovation Agency, and extend their appreciation to all parties who contributed to this research.

REFERENCES

Abdel-Motleb A, Ghareeb MA, Abdel-Aziz MS, El-Shazly MAM. 2022. Chemical characterization, antimicrobial, antioxidant and larvicidal activities of certain fungal extracts. J Adv Biotechnol Exp Ther 5 (3): 456-472. DOI: 10.5455/jabet.2022.d128.

Abubakar AR, Haque M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci 12 (1): 1-10. DOI: 10.4103/jpbs.JPBS_175_19.

Akaniro IR, Chibuike IV, Onwujekwe EC, Gbadamosi FA, Enyi DO, Onwe ON. 2023. *Penicillium* species as chassis for biomanufacturing and environmental sustainability in the modern era: Progress, challenges, and future perspective. Fungal Biol Rev 46: 100326. DOI: 10.1016/j.fbr.2023.100326.

Amin MHF, Kim H-W, Kim K, Wahyudianto FE, Dianbudiyanto W, Oktavitri NI. 2024. Analyzing microbial communities and metabolic functions in estuaries affected by human activity to uncover bioelectrochemical system potential. Environ Adv 16: 100537. DOI: 10.1016/j.envadv.2024.100537.

Anderson SR, Harvey EL. 2022. Estuarine microbial networks and relationships vary between environmentally distinct communities. PeerJ 10: e14005. DOI: 10.7717/peerj.14005.

Annuur RM, Triana D, Ernawati T, Murai Y, Aswad M, Hashimoto M, Tachrim ZP. 2024. A review of cinnamic acid's skeleton modification: Features for antibacterial-agent-guided derivatives. Molecules 29 (16): 3929. DOI: 10.3390/molecules29163929.

Chakraborty B, Shashiraj KN, Basavarajappa DS, Bhat MP, Nayaka S, Kumar RS, Almansour AI, Perumal K. 2023. Biological activities and GC-MS based chemical profiling of polymolecular methanol extract of *Alternaria alternata* KUDB15. J King Saud Univ Sci 35 (8): 102884. DOI: 10.1016/j.jksus.2023.102884.

Clinical and Laboratory Standards Institute. 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, Document M07-A8, 8th eds. Clinical and Laboratory Standards Institute, Wayne, PA.

Conrado R, Gomes TC, Roque GSC, De Souza AO. 2022. Overview of bioactive fungal secondary metabolite: Cytotoxic and antimicrobial compounds. Antibiotics 11: 1604. DOI: 10.3390/antibiotics11111604.

Corbu VM, Gheorghe-Barbu I, Dumbravă AȘ, Vrâncianu CO, Șesan TE. 2023. Current insight in fungal importance-A comprehensive review. Microorganisms 11: 1384. DOI: 10.3390/microorganisms11061384.

Crump BC, Bowen JL. 2023. The microbial ecology of estuarine ecosystems. Ann Rev Mar Sci 16: 335-360. DOI: 10.1146/annurev-marine-022123-101845.

Cunha MB, Jorge AF, Nunes MJ, Sousa JR, Lança MJ, da Silva MG, Gaudêncio SP. 2025. GC/MS fatty acid profile of marine-derived actinomycetes from extreme environments: Chemotaxonomic insights and biotechnological potential. Mar Drugs 23 (1): 1. DOI: 10.3390/md23010001.

Devi PB, Jayaseelan R. 2020. Natural medicinal compounds from marine fungi towards drug discovery: A review. In: Parikesit AA (eds). Drug Design-Novel Advances in the Omics Field and Applications. IntechOpen, London. DOI: 10.5772/intechopen.94137.

Dong H, Mei W, Qian L, Gao X, Liu H, Fu X, Wang L. 2025. The combined effects of soil moisture and salinity on the spatial differences of soil humification intensity in the Yangtze River estuarine wetlands. J Soils Sediments 25: 1227-1240. DOI: 10.1007/s11368-025-04019-5.

Elfadadny A, Ragab RF, AlHarbi M, Badshah F, Ibáñez-Arancibia E, Farag A, Hendawy AO, De Los Ríos-Escalante PR, Aboubakr M, Zakai SA, Nageeb WM. 2024. Antimicrobial resistance of *Pseudomonas aeruginosa*: Navigating clinical impacts, current resistance trends, and innovations in breaking therapies. Front Microbiol 15: 1374466. DOI: 10.3389/fmicb.2024.1374466.

Ezeonuegbu BA, Abdullahi MD, Whong CMZ, Sohunago JW, Kassem HS, Yaro CA, Hetta HF, Mostafa-Hedeab G, Zouganelis GD, Batiha GE-S. 2022. Characterization and phylogeny of fungi isolated from industrial wastewater using multiple genes. Sci Rep 12 (1): 2094. DOI: 10.1038/s41598-022-05820-9.

Fatimah, Suroiyah F, Solikha N, Rahayuningtyas ND, Surtiningsih T, Nurhariyati T, Ni'matuzahroh, Affandi M, Geraldi A, Thontowi A. 2022. Antimicrobial activity of actinomycetes isolated from mangrove soil in Tuban, Indonesia. Biodiversitas 23 (6): 2957-2965. DOI: 10.13057/biodiv/d230622.

Gonçalves MFM, Esteves AC, Alves A. 2022. Marine Fungi: Opportunities and Challenges. Encyclopedia 2 (1): 559-577. DOI: 10.3390/encyclopedia2010037.

Gonçalves MFM, Santos L, Silva BMV, Abreu AC, Vicente TFL, Esteves AC, Alves A. 2019. Biodiversity of *Penicillium* species from marine environments in Portugal and description of *Penicillium lusitanum* sp. nov., a novel species isolated from sea water. Intl J Syst Evol Microbiol 69 (10): 3014-3021. DOI: 10.1099/ijsem.0.003535.

Haqqa ZA, Rupaedah B, Handayani I, Wahid A, Sugianto M. 2023. Screening and characterization of soil fungal isolates to inhibit the growth of *Ganoderma boninense*. Jurnal Bioteknologi Biosains Indonesia 10 (1): 59-67. DOI: 10.29122/jbbi.v10i1.5669.

Hu J, Qin D. 2025. Diversity and biological activity of secondary metabolite produced by the endophytic fungus *Penicillium ochrochlorae*. Fermentation 11 (7): 394. DOI: 10.3390/fermentation11070394.

Huang L, Zhu X, Zhou S, Cheng Z, Shi K, Zhang C, Shao H. 2021. Phthalic acid esters: Natural sources and biological activities. Toxins 13 (7): 495. DOI: 10.3390/toxins13070495.

Indraningrat AAG, Purnami PPCP, Damayanti E, Wijaya MD, Masyeni DAPS, Sari NLPEK. 2024. Antibacterial potential of *P. aeruginosa* isp1rl4 isolated from seaweed *Eucheuma cottonii* against multidrug-resistant bacteria. Biomed Pharmacol J 17 (4): 2341-2354. DOI: 10.13005/bpj/3029.

Keller NP. 2019. Fungal secondary metabolism: Regulation, function and drug discovery. Nat Rev Microbiol 17: 167-180. DOI: 10.1038/s41579-018-0121-1.

Krzywinski M, Altman N. 2013. Importance of being uncertain. Nat Methods 10 (9): 809-810. DOI: 10.1038/nmeth.2613.

Kumari S, Kumari S, Attri C, Sharma R, Kulshreshtha S, Benali T, Bouyahya A, Gürer ES, Sharifi-Rad J. 2022. GC-MS analysis, antioxidant and antifungal studies of different extracts of *Chaetomium globosum* isolated from *Urginea indica*. Biomed Res Intl 2022: 1388850. DOI: 10.1155/2022/1388850.

Lade H, Kim J-S. 2021. Bacterial targets of antibiotics in methicillin-resistant *Staphylococcus aureus*. Antibiotics 10 (4): 398. DOI: 10.3390/antibiotics10040398.

Li P, Liu C, Luo Y, Shi H, Li Q, PinChu C, Li X, Yang J, Fan W. 2022. Oxalate in plants: Metabolism, function, regulation, and application. J Agric Food Chem 70: 16037-16049. DOI: 10.1021/acs.jafc.2c04787.

Lin W, Liu X, Gong L, Liu R, Ling M, Guo C, Meng H, Luo Z, Du X, Guo Y, Xu W. 2023. Impact of environmental factors on diversity of fungi in sediments from the Shenzhen River Estuary. Arch Microbiol 205 (3): 96. DOI: 10.1007/s00203-023-03438-7.

Lv M, Zhang L, Wang Y, Ma L, Yang Y, Zhou X, Wang L, Yu X, Li S. 2024. Floral volatile benzenoids/phenylpropanoids: Biosynthetic pathway, regulation and ecological value. Hortic Res 11 (10): uhae220. DOI: 10.1093/hr/uhae220.

Madusanka C, Udayanga D, Nilmini R, Rajapaksha S, Hewawasam C, Manamgoda D, Vasco-Correa J. 2024. A review of recent advances in fungal mycelium based composites. Discov Mater 4: 13. DOI: 10.1007/s43939-024-00084-8.

Mavoa BA, Kassuwi SAA, Hussein JM. 2024. Antibacterial activity and phylogeny of griseofulvin producing *Aspergillus* and *Penicillium* species from kunduchi mangrove sediments, Tanzania. Tanzania J Sci 50 (3): 454-467. DOI: 10.4314/tjs.v50i3.4.

Mitra A, Zaman S. 2016. Estuarine ecosystem: An overview. In: Basics of Marine and Estuarine Ecology. Springer, New Delhi. DOI: 10.1007/978-81-322-2707-6_1.

Molelekoa TBJ, Regnier T, da Silva LS, Augustyn W. 2021. Production of pigments by filamentous fungi cultured on agro-industrial by-products using submerged and solid-state fermentation methods. Fermentation 7 (4): 295. DOI: 10.3390/fermentation7040295.

Montgomery DC. 2017. Design and Analysis of Experiments. John Wiley & Sons, New York.

Mustafa HK, Anwer SS, Zrary TJ. 2023. Influence of pH, agitation speed, and temperature on growth of fungi isolated from Koya, Iraq. Kuwait J Sci 50 (4): 657-664. DOI: 10.1016/j.kjs.2023.02.036.

Naher L, Fatin SN, Sheikh MAH, Azeez LA, Siddiquee S, Zain NM, Karim SMR. 2021. Cellulase enzyme production from filamentous fungi *Trichoderma reesei* and *Aspergillus awamori* in submerged fermentation with rice straw. J Fungi 7 (10): 868. DOI: 10.3390/jof7100868.

Nugraha AS, Firli LN, Rani DM, Hidayatiningsih A, Lestari ND, Wongso H, Tarman K, Rahaweman AC, Manurung J, Ariantari NP, Papu A,

Putra MY, Pratama ANW, Wessjohann LA, Keller PA. 2023. Indonesian marine and its medicinal contribution. Nat Prod Bioprospect 13 (1): 38. DOI: 10.1007/s13659-023-00403-1.

Octarya Z, Novianty R, Suraya N, Saryono. 2021. Antimicrobial activity and GC-MS analysis of bioactive constituents of *Aspergillus fumigatus* 269 isolated from Sungai Pinang Hot Spring, Riau, Indonesia. Biodiversitas 22 (4): 1839-1845. DOI: 10.13057/biodiv/d220429.

Orfali R, Perveen S. 2019. New bioactive metabolites from the thermophilic fungus *Penicillium* sp. isolated from Ghamiqa Hot Spring in Saudi Arabia. J Chem 2019 (1): 7162948. DOI: 10.1155/2019/7162948.

Osuntokun OT, Cristina GM. 2019. Bio-guided isolation, chemical purification, identification, antimicrobial and synergistic efficacy of extracted essential oils from stem bark extract of *Spondias mombin* (Linn). Intl J Mol Biol: Open Access 4 (4): 135-143. DOI: 10.15406/ijmboa.2019.04.00110.

Palit K, Rath S, Chatterjee S, Das S. 2022. Microbial diversity and ecological interactions of microorganisms in the mangrove ecosystem: Threats, vulnerability, and adaptations. Environ Sci Pollut Res Intl 29 (22): 32467-32512. DOI: 10.1007/s11356-022-19048-7.

Poveda J. 2021. Beneficial effects of Microbial Volatile Organic Compounds (MVOCs) in plants. Appl Soil Ecol 168: 104118. DOI: 10.1016/j.apsoil.2021.104118.

Rosana Y. 2023. Microbiology becomes the answer to prevent high cases of infectious disease in Indonesia. https://www.ui.ac.id/en/microbiology-becomes-the-answer-to-prevent-high-cases-of-infectious-disease-in Indonesia.

Rosyadi A, Triatmoko B, Nugraha AS. 2022. Isolation of estuary soil fungi and screening antibacterial activity against *Staphylococcus aureus*. Indones J Pharm Sci Technol 9: 17-25. DOI: 10.24198/ijpst.v1i1.28834. [Indonesian]

Saha S, Al Amin GM, Khan MS, Goswami B, Afroz F, Habib MA, Akter S, Banu TA. 2023. Assessment of antibacterial activity and cytotoxic effects of in vitro and in vivo plant parts of a medicinal plant *Gynura procumbens* (Lour.) Merr. Heliyon 9 (12): e22954. DOI: 10.1016/j.heliyon.2023.e22954.

Samirana PO, Murti YB, Jenie RI, Setyowati EP. 2023. GC-MS metabolomic approach to study antimicrobial activity of the marine sponge-derived fungi *Trichoderma reesei* TV221. J Appl Pharm Sci 13 (7): 159-173. DOI: 10.7324/japs.2023.124424.

Sebe GO, Oghenerhoro SO, Jonathan OE, Anyaogu EV, Adebowale AD, Ntomchukwu RC. 2023. Enhancing accumulation and penetration efficiency of next-generation antibiotics to mitigate antibiotic resistance in *Pseudomonas aeruginosa* PAO1. J Biomed Sci Eng 16 (8): 107-120. DOI: 10.4236/jbise.2023.168008.

Šelo G, Planinić M, Tišma M, Tomas S, Komlenić DK, Bucić-Kojić A. 2021. A comprehensive review on valorization of agro-food industrial residues by solid-state fermentation. Foods 10 (5): 927. DOI: 10.3390/foods10050927.

Showkat S, Dharumadurai D, Kumar TS. 2025. Phytochemical profiling, spectroscopic identification of active compounds, and mechanism of the anticandidal properties of *Datura stramonium* L. using SwissADMET prediction and molecular docking analysis. Microb Pathog 198: 107104. DOI: 10.1016/j.micpath.2024.107104.

Soni K, Bagaria A. 2024. GC-MS based identification of anti-microbial bioactive compounds, isolated from *Bacillus halotolerans* of marine sediment. J Exp Mar Biol Ecol 577: 152026. DOI: 10.1016/j.jembe.2024.152026.

Sopalun K, Laosripaiboon W, Wachirachaikarn A, Iamtham S. 2021. Biological potential and chemical composition of bioactive compounds from endophytic fungi associated with thai mangrove plants. S Afr J Bot 141: 66-76. DOI: 10.1016/j.sajb.2021.04.031.

Stan D, Enciu A-M, Mateescu AL, Ion AC, Brezeanu AC, Stan D, Tanase C. 2021. Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. Front Pharmacol 12: 723233. DOI: 10.3389/fphar.2021.723233.

Tamura K, Stecher G, Kumar S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Togashi N, Shiraishi A, Nishizaka M, Matsuoka K, Endo K, Hamashima H, Inoue Y. 2007. Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. Molecules 12 (2): 139-148. DOI: 10.3390/12020139.

Trizna EY, Yarullina MN, Baidamshina DR, Mironova AV, Akhatova FS, Rozhina EV, Fakhrullin RF, Khabibrakhmanova AM, Kurbangalieva AR, Bogachev MI, Kayumov AR. 2020. Bidirectional alterations in antibiotics susceptibility in *Staphylococcus aureus-Pseudomonas aeruginosa* dual-species biofilm. Sci Rep 10 (1): 14849. DOI: 10.1038/s41598-020-71834-w.

Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM. 2019. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92: 135-154. DOI: 10.1016/j.simyco.2018.05.001.

Vu D, Groenewald M, Szöke S, Cardinali G, Eberhardt U, Stielow B, de Vries M, Verkleij GJM, Crous PW, Boekhout T, Robert V. 2016. DNA barcoding analysis of more than 9 000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. Stud Mycol 85: 91-105. DOI: 10.1016/j.simyco.2016.11.007.

Wang B-L, Ge Z-K, Qiu J-R, Luan S-Q, Hao X-C, Zhao Y-H. 2024. *Sedum aizoon* L.: A review of its history, traditional uses, nutritional value, botany, phytochemistry, pharmacology, toxicology, and quality control. Front Pharmacol 15: 1349032. DOI: 10.3389/fphar.2024.1349032.

Wang H, Xu F, Zhang X, Shao X, Wei Y, Wang H. 2021. Transcriptomic analysis reveals antibacterial mechanism of flavonoids from *Sedum aizoon* L. against *Pseudomonas fragi*. Food Control 134: 108755. DOI: 10.1016/j.foodcont.2021.108755.

WHO [World Health Organization]. 2021. Top 10 Causes of Death in Indonesia for Total Aged Total Years (2021). https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death

Zhang W, Han J, Wu H, Zhong Q, Liu W, He S, Zhang L. 2021. Diversity patterns and drivers of soil microbial communities in urban and suburban park soils of Shanghai, China. PeerJ 9: e11231. DOI: 10.7717/peerj.11231.

Zhang X, Yin Q, Li X, Liu X, Lei H, Wu B. 2022. Structures and bioactivities of secondary metabolite from *Penicillium* genus since 2010. Fitoterapia 163: 105349. DOI: 10.1016/j.fitote.2022.105349.