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Molecular Analysis And Bioactive Compounds Of Endophytic Fungus IFATL-05 Isolate From *Helminthostashys Zeylanica* Linn. Roots As Antibacterial

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Abstract
Objective: *This study aimed to examine the compound chemical activity and molecular analysis of endophytic fungus IFATL-05 isolate from Helminthostashys zeylanica Linn. root against gastrointestinal infection bacteria.*
Material and Methods: *Molecular analysis of endophytic fungus IFATL-05 isolate by polymerase chain reaction (PCR), antibacterial activity by Bioautography-TLC and gar Diffusion, analisys of fungal groups by FTIR and analisys of secondary methabolites by GC-MS spectrophotometry.*
Results: *The result of molecular analysis showed that endophytic fungus IFATL-05 isolate is the species Talaromises sp. Isolation bioactive compounds of ethyl acetate extract of IFATL-05 obtained 3 pure isolate (TL-1, TL-2, and TL-3 isolates) and antibacterial activity by Bioautography-TLC showed that Rf value of 0.98 (TL-1), Rf 0.75 (TL-2) and Rf 0.43 (TL-3) were active to E. coli, S. thypi, and S. disenteriae. Analysis by FTIR spectrophotometry of TL-1, TL-2 and TL-3 compound contain an OH group, aliphatic C-H, C=O carboxylic acid/ether/ester, C-O/phenol, C=C streaching/aromatic, C-OH cyclic and aromatic substitution group. Based on GC-MS spectroscopy analysis of the ethyl acetate extract of endophytic fungus IFATL-05 isolate contains chemical compounds 2-pentanone, 4-hydroxy-4-methyl- ($C_6H_{12}O_2$; 116), diethyl phthalate ($C_{12}H_{14}O_4$; 222) 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- ($C_{15}H_{26}O$; 222), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$; 278), Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$; 390) and 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester) ($C_{24}H_{38}O_4$; 390).*
Conclution: *Endophytic fungus isolate IFATL-05 is the species Talaromises sp and contains seven main chemical compounds activity to against E. coli, S. typhi, and S. dysenteriae bacteria.*
Keywords: *Endophytic Fungus, Antibacterial, FTIR and GC-MS*

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I. Introduction

Gastrointestinal infections are common and are typically linked to dietary practices and hand hygiene. Various bacteria responsible for gastrointestinal infections include *E. coli*, *S. dysenteriae*, *S. typhi*, and *V. cholerae* [1]. Bacterial gastrointestinal infections from food pose a significant and unresolved health issue. Gastrointestinal infections commonly cause diseases such as typhoid fever, paratyphoid, diarrhea, and gastroenteritis [2]. Salmonella bacteria, including *S. typhi* and *S. paratyphi*, are responsible for typhoid and paratyphoid fever.

Bacterial resistance to antimicrobial agents results from three primary mechanisms: 1) The drug has not reached its target. 2) The drug has become inactive. 3) The target has changed [3]. Drug resistance resulting from antibacterial use is on the rise, leading researchers to seek new antibacterial alternatives, including those derived from endophytic microbes [4–6]. This situation highlights the increasing significance of efforts to secure affordable antibacterial agents, which are consistently available in large quantities and include essential components for their production, including those derived from endophytic fungi [5].

Helminthostachys zeylanica Linn, known as Tunjuk Langit, is a medicinal fern belonging to the Opiglossaceae family[7]. It is traditionally used in medicine and commonly found in tropical open forests with clay and moist soil [8]. The production of secondary metabolites aims to extract bioactive compounds from the *H. zeylanica* root for use as antibacterial agents against gastrointestinal infection-causing bacteria. The health-beneficial parts of the *H. zeylanica* plant used in traditional medicine include the roots, stems, leaves, tubers, and sap [9–12]. The root rhizome of *H. zeylanica* is used medicinally for dysentery, cataracts, early-stage tuberculosis, syphilis cough, malaria, and as a laxative and tonic. The plant roots contain various ugonin-type flavonoids. Fermentation of the endophytic fungus from the roots produced 8 isolates, with IFATL-05 showing significant antibacterial potential against gastrointestinal bacteria, particularly inhibiting *Shigella dysentriae* with a diameter of 29.13 mm [13].

II. Material And Methods

Material

The research tools include an autoclave, incubator (memmert type UM200), Laminar Air Flow, UV-Vis Spectrophotometry, FTIR, PCR, GCMS spectrophotometry. And the materials are *E. coli* (ATTC 25922), *Salmonella thypi*, and *Shigella dysenteriae* (NCTC 786) bacteria, IFATL-05 isolate, Nutrient Agar medium, Potato Dextrose Agar medium, Potato Dextrose Agar Chloramphenicol medium, Yeast Broth Maltose medium.

Preparation of Samples

The IFATL-05 culture derived from *H. zeylanica* was purified via the quadrant streak method and incubated at 25°C–30°C for 3 days to achieve a single isolate. The isolate was inoculated onto slant agar medium for storage [14].

PCR Analysis of the IFATL-05 Isolate

DNA extraction to separate the DNA genome from other molecules in cells. using the Geneaid PrestoTM Mini Gdna Tissue kit. The next stage is amplification using ITS5 Primer Forward: (5'GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 Primer Reverse: (5'-TCCTCCGCTTATTGATATGC-3'). The sequencing process was carried out by 1st Base through PT Genetica Indonesia using "Single Pass DNA Sequencing" using the same primers as gene amplification in the PCR process. The sequencing results are processed using the Bioedit application, then species identification using BLAST and phylogenetic analysis using the Maximum Likelihood method [15].

Production of the Secondary Metabolite

Endophytic fungus were fermented using a Maltose Yeast Broth (MYB) medium. The active isolate (IFATL-05 isolate) was inoculated into 250 mL of MYB medium, and fermentated using a rotary shaker with 200 rpm at room temperature for 21 days. The 10 g of supernatant was extracted with 500 mL of ethyl acetate and then, isolated using Preparative TLC [14,16].

Characterization of IFATL-05 Isolate

Macroscopic Characterization of IFATL-05 Isolate

One loop of IFATL-05 isolate was inoculated into PDAC medium, and incubated at 25^{o}C using an incubator for 3x24 jam. The results of the macroscopic examination were observed including the shape, elevation, edge, and colony color [14].

The Antibacterial Activity by TLC – Bioautography

The chromatogram was tested for antibacterial activity by TLC-Bioautography in a petri dish with 10 mL of Nutrient Agar (NA) and 20 µL of bacterial suspension (McFarland standard 1x10^{8} cells/mL). The chromatogram plate was placed on the surface of the Nutrient Agar (NA) medium for 1 hour. The plate was removed and incubated for 24 hours (37^{0}C). Then, observe the spots that inhibit the test bacteria[17]

The Antibacterial Activity by Agar Diffusion

The culture was diluted in 0.9% NaCl (1:9) to attain a bacterial concentration of 1.5x10^{8} CFU/mL. Subsequently, 20 µL of the bacterial culture was combined with 15 mL of Nutrient Agar (NA) and transferred into a petri plate. Discs immersed in the supernatant of fungal isolate were evaluated in conjunction with a positive control (Ampicillin, 10 mg/mL) and a negative control (distilled water). The discs were positioned on the agar surface and incubated at 37°C for 24 hours [18].

UV-Vis and FTIR Analysis

The pure compounds were dissolved in methanol pro-analysis to determine their maximum wavelength and absorbance values [19]. The bioactive compounds from the endophytic fungus IFATL-05 were analyzed using a Nicolet iS10 FTIR spectrophotometer with a DTGS detector and OMNIC® software [20]. Samples were placed on the ATR crystal at 20°C, with measurements recorded at a resolution of 4 cm^{-1} over 32 scans (4000–400 cm^{-1}) [21–23].

GC-MS Analysis

The ethyl acetate extract from endophytic fungal isolate IFATL-05 was analyzed using an Agilent 6980N GC-MS system with an Agilent 5973 inert MSD detector (70 eV). A 2 µL sample was injected into a J&W Scientific HP-5MS capillary column (30 mm × 0.25 mm × 0.25 µm). Helium served as the carrier gas at a flow rate of 1 mL/min with a split ratio of 1:10. The oven was set to 50°C for 5 minutes, then increased by 10°C per minute to 280°C, and maintained for 15 minutes. The injector port temperature was set to 290°C; the interface temperature was set to 230°C. Chemical compounds were identified using the Willey database version 7.0 through mass spectra and fragmentation pattern comparison [17,24].

III. Results

PCR Analisis Endophytic Fungus Isolate IAFTL-05

DNA sequencing results showed that the assembly sequence was 564 bp which was analyzed using the BLAST program via NCBI (National Center for Biotechnology Information). The Tree-Maximum Likelihood phylogenetic results of IFATL-05 endophytic fungal isolates show that isolates using the Kimura method with two parameters. The phylogenetic scheme of the IFTAL-05 isolate in Figure 1 belongs to the same species as *Talaromises sp.*

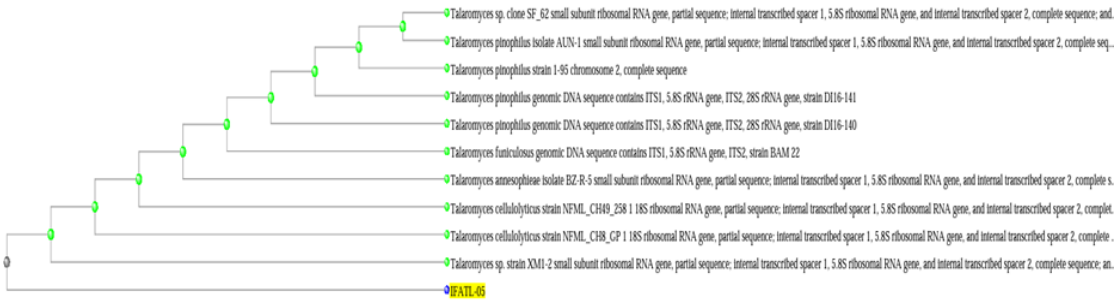


Figure 1. Tree-Maximum Phylogenetic Tree of IFATL-05 Isolates

Production of the Secondary Metabolite from Endophytic Fungus Isolate

The IFATL-05 ethyl acetate extract in preparative thin layer chromatography (TLC-Preparative) with n-hexane : ethyl acetate (1:1) eluent obtained three isolates : TL-1, TL-2, and TL-3.

The Antibacterial activity by TLC–Bioautography and Agar Diffusion

Secondary metabolite compounds TL-1, TL-2, and TL-3 were tested using TLC-Bioautography using n-hexane eluent: ethyl acetate (1:4). The Rf values obtained for the isolates were 0.98 for TL-1, 0.75 for TL-2, and 0.43 for TL-3 all three showed antibacterial activity against *S. typhi, E. coli*, and *S. dysenteriae*. The in vitro antibacterial activities were assayed through the diffusion method. It indicated that TL-1 obtained from the endophytic fungus IFATL-05 had an inhibition zone of 12 mm against *S. dysenteriae*, *E. coli*, and *S. typhi* at a concentration of 10%, with additional tests conducted at 0.1%, 0.5%, and 1%. (table 2).

Table 2. Test results of antibacterial activity TL-1 isolate from endophytic fungus IFATL-05

Bacteria	Inhibition Zone Diameter IFATL-05 (Isolate TL-1) (mm)				Average	Standard Deviation	Ampicillin (10 mg/mL^{-1})
	0.1%	0.5%	1%	10%			
S. dysenteriae	0	7	9	12	7	5.09	20
E. coli	0	0	8	12	5	6	14
S. typhi	0	0	9	12	5.25	6.18	11

UV-Vis and FTIR Analysis

The analysis of bioactive compounds of endophytic fungus IFTAL-05 isolate by UV-Vis spectrophotometry shows compound isolates with maximum absorptions, namely TL-1 = 254.18 nm (0.784 abs),

TL-2 = 252.62 nm (0.327 abs), and TL-3 = 257.10 nm (0.134 abs). To determine the wavelength based on the interpretation of the functional groups of TL-1, TL-2, and TL-3 were analyzed by FTIR (Thermo Scientific Nicolet iS10) spectrophotometry (figure 2).

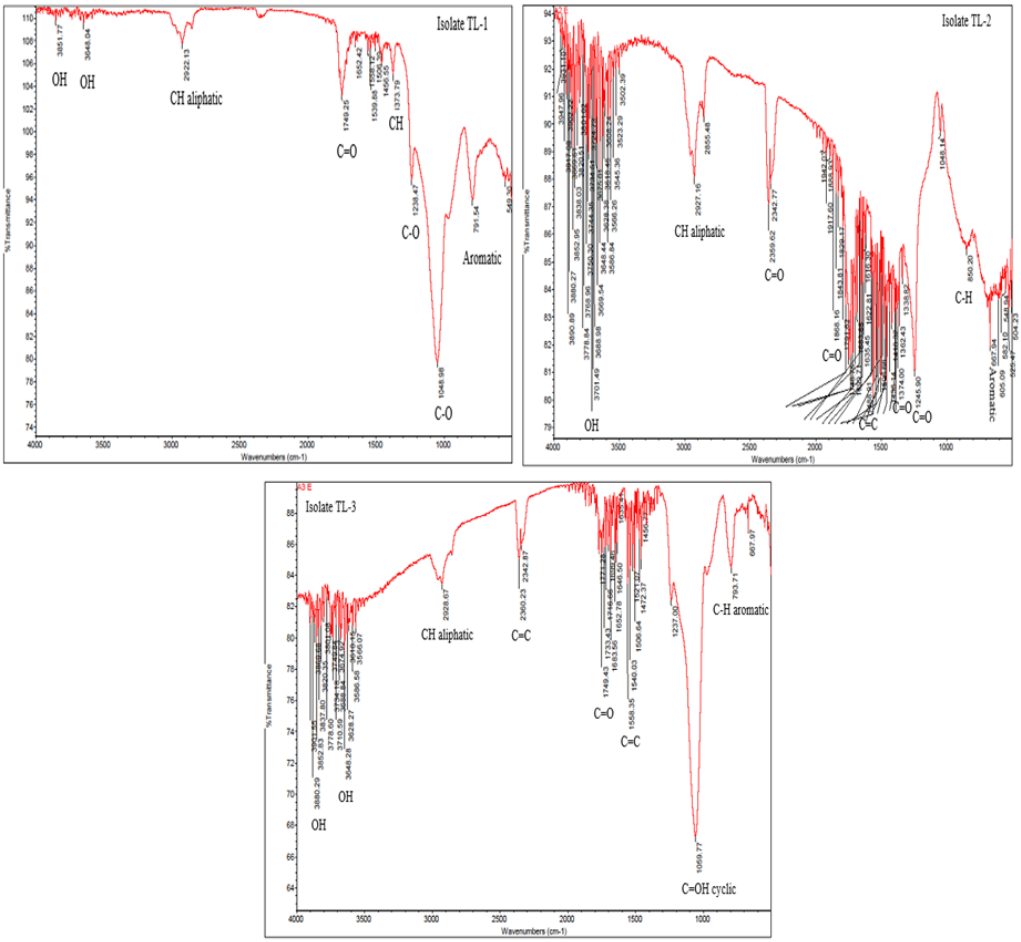


Figure 2. Spectrum of TL-1, TL-2, and TL-3 Isolate by FTIR spectrophotometry

The FTIR spectrophotometry analysis showed that TL-1, TL-2, and TL-3 had several distinctive absorption bands due to the absorption emerged from the FTIR spectrum. The appearance of each wavelength absorption band was due to the FTIR absorption caused by the functional group's vibration. The results of the absorption analysis of each compound can been seen in table 3 below.

Table 3. The analysis of bioactive compounds TL-1, TL-2, and TL-3 pure isolate

Isolate Code	Wavelength (cm^{-1})	Functional Group	Intensity
TL-1	3851.77, 3648.04	O-H	Weak
	2922,13	aliphatic C-H	Weak
	1749.25	C=O /carboxylic acid	currently
	1373.79	C-H and O-H	Weak
	1238,47	C-O/ether	Weak
	1048.98	C-O	Strong
	791.54	aromatic substitution	Currently
TL-2	3852.95, 3768.96, 3566.35	O-H	Currently
	2927.16	C-H aliphatic	Currently
	2359.62, 2342.77	-C=C-	Weak
	1868.16	C=O/ether	Weak
	1488.91	-C=C-/aromatic	Currently
	1374.00	C=O ether	Strong
	667.94	substituted aromatic bond	Weak
TL-3	3880.29, 3586.58	O-H	Weak
	2928.67	C-H aliphatic	Weak
	2360.23, 2342.87	-C=C-/aromatic	Weak
	1771.28, 646.50	C=O	Weak

	1558.35, 1456.77	-C=C-/aromatic	Weak
	1059.77	C-OH cyclic	Strong
	793.71, 667.97	C-H aromatic	Currently

GC-MS Analysis

Gas Chromatography-Mass Spectrophotometry (GC-MS) is a spectroscopic tool used to analyze chemical compounds in medicinal plants such as alkaloids, steroids, flavonoids, and others. It separates the mixed and identifies bioactive compounds that can be useful forc medicine. The results of GCMS analysis of the ethyl acetate extract of isolate IFATL-05 showed that 50 chemical compounds were obtained (table 4; figure 3).

Table 4. Results of GC-MS Spectroscopy Analysis of IFATL-05 Isolate Ethyl Acetate Extract

Peak	Real Time	Nama Senyawa	Area%	Molecular Formula	Molecular Weight
1	3.066	2-pentanone, 4-hydroxy-4-methyl-	6.70	$C_6H_{12}O_2$	116
20	13.585	Diethyl Phthalate	4.34	$C_{12}H_{14}O_4$	222
28	15.080	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	6.19	$C_{15}H_{26}O$	222
39	17.071	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	16.15	$C_{16}H_{22}O_4$	278
40	17.134	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	7.98	$C_{16}H_{22}O_4$	278
48	30.876	Bis(2-ethylhexyl) phthalate	3.65	$C_{24}H_{38}O_4$	390
50	34.344	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1.85	$C_{24}H_{38}O_4$	390

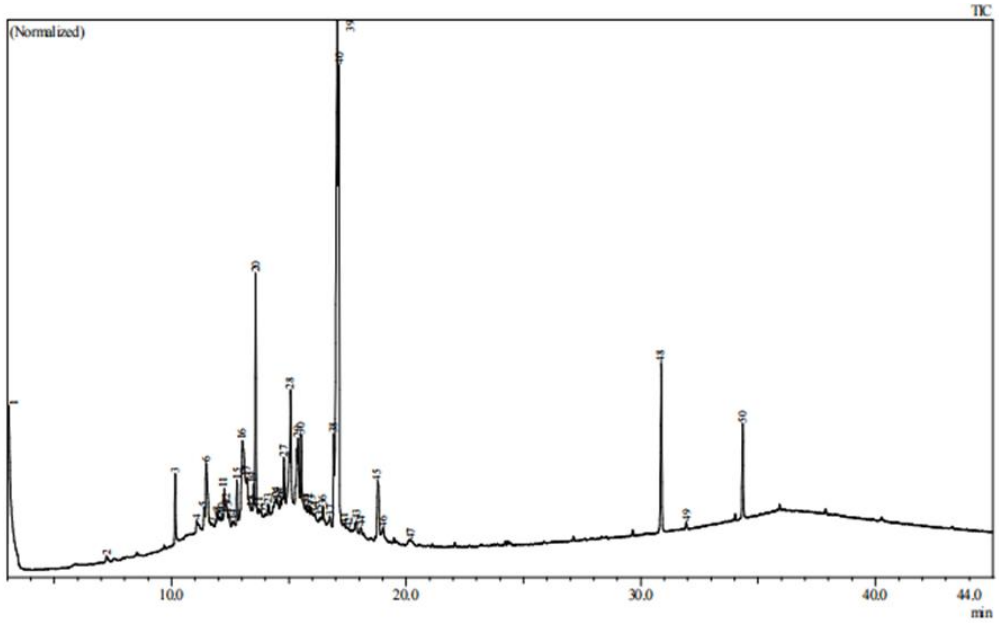


Figure 3. Chromatogram Spectroscopy GC-MS Ethyl Acetate Extract Isolate IFATL-05

IV. Discussion

Endophytic microorganisms inhabit plant tissues (hosts) without causing damage to the plants[25]. Endophytic microorganisms such as endophytic fungi are recognized for their ability to produce bioactive compounds that may serve as pharmaceutical raw materials, including anticancer agents and antibiotics[20]. The isolation and structural elucidation compounds from *Helminthostachys zeylanica* Linn. via NMR revealed the structures of neougonin A (5,7,3′,4′-tetrahydroxy-6-(2,6-dimethyl-6-vinyl-2-hexenyl)- flavanoneneurogonin B (4″,5″-dihydro-3,5,3′,4′-tetrahydroxy-4″,4″,5″- trimethylfurano[2″,3″:6,7] flavonol)[26]. The compounds exhibit inhibitory activity againts on bacterial neuraminidase (BNA) and antibiofilm[27].

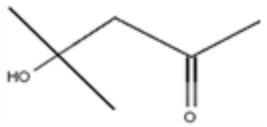
Molecular identification of isolate IFATL-05 by extraction of endophytic fungus DNA genes using the Quick-DNA fungus Miniprep KIT (Zymo research, D6005). The extracted DNA served as a template for fungal rDNA amplification, utilizing the ITS5 Forward primer (5'-GGAAGTAAAAGTCGTAACAAGG-3') and the ITS4 Reverse primer (5'-TCCTCCGCTTATTGATATGC-3')[28]. The PCR product DNA analysis revealed ITS fragments, with electrophoresis on a 1% agarose gel indicating sizes of approximately 564 bp. No non-specific priming was observed, as evidenced by a single DNA band from the isolate IFATL-05. The next stage of PCR product sequencing aims to determine the nitrogen base sequence of a DNA sample[29].

DNA sequencing indicated an assembly sequence of 564 bp, confirming the IFATL-05 isolate as the same species as *Talaromyces sp*. (Figure 3). This fungus belongs to the ascomycetous group and produces various secondary metabolites, such as terpenoids, diphenyl ether derivatives, ergosterol analog lactones, and polyketide derivatives [30–32]. Bioassay tests showed that compounds from *Talaromyces sp.* exhibited α-glucosidase inhibitory activity, with an IC50 of 16.6 ± 0.9 to 57.3 ± 1.3 μM[33].

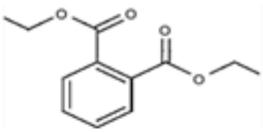
The antibacterial activity analysis of TL-1, TL-2, and TL-3 isolates via TLC-Bioautography revealed Rf values of 0.98 for TL-1, 0.75 for TL-2, and 0.43 for TL-3, demonstrating effectiveness against *S. typhi*, *E. coli*, and *S. dysenteriae*. In diffusion testing, TL-1 at 0.1%, 0.5%, 1%, and 10% exhibited a 12 mm inhibition zone at 10% concentration, indicating medium activity. Higher concentrations of TL-1 resulted in larger inhibition zones, classifying it as a potent inhibitory agent. The inhibition zone diameters was S. dysenteriae = 5.09 mm, E. coli = 6 mm, and S. typhi = 6.18 mm. Based on inhibitory zone size, endophytic fungus activity is classified as weak (<5 mm), medium (5-10 mm), strong (10-20 mm), or very strong (>20 mm)[34].

The UV-Vis analysis of bioactive compounds from the endophytic fungus IFTAL-05 isolate reveals maximum absorption at TL-1 = 254.18 nm, TL-2 = 252.62 nm, and TL-3 = 257.10 nm. The maximum absorption at that wavelength by TL-1, TL-2, and TL-3 isolates results from electronic transitions from n – π*, indicating the presence of chromophore groups like conjugated OH, C-H, C=O, and C=C bonds. Literature studies indicate that compounds with UV-Vis spectra at TL-1, TL-2, and TL-3, exhibiting λmax in the 250-280 nm range, are classified as flavonoids[35]. The absorption spectrum from the TL-1, TL-2 and TL-3 isolates suggest the absorbption range of flavonoid compounds. The absorption at 254.18 nm, 252.10 nm, and 257.10 nm in the isolates indicates n→π* transitions due to O-H auxochromes and π→π* transitions from C=O chromophores. The hydroxy group's position on the flavonoid core was established using a shear reagent. Hydroxylation of rings A, B, and C. Hydroxylation is influenced by bathochromic shift, whereas methylation and glycosylation result in a hypsochromic band shift to lower wavelengths[36]. FTIR spectrophotometry analysis revealed distinctive absorption bands in TL-1, TL-2, and TL-3 isolates from the FTIR spectrum. The FTIR absorption bands correspond to the vibrations of functional groups (Table 3).

Gas Chromatography-Mass Spectrometry (GCMS) analyzes chemical compounds in medicinal plants, i ncluding alkaloids, steroids, and flavonoids[37]. GC-MS spectroscopy analysis of the ethyl acetate extract from IFATL-05 isolate identified 50 compounds. Seven compound components exhibited the highest peak,notably the first compound, 2-pentanone, 4-hydroxy-4-methyl- ($C_6H_{12}O_2$, 116) (figure 4a). The 20th identified is diethyl phthalate (figure 4b), and the 28th was 2,6,10-Dodecatrien-1-]ol,3,7,11 trimethyl (figure 4c). The 39th and 40th compounds were identified 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (figure 4d and 4e). The 48th compound was Bis(2-ethylhexyl) phthalate (figure 4f) and the 50th compound was 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester) (figure 4g).



4a



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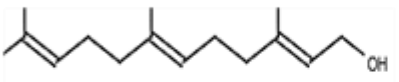

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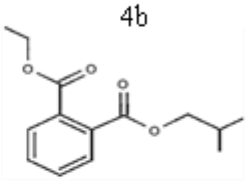
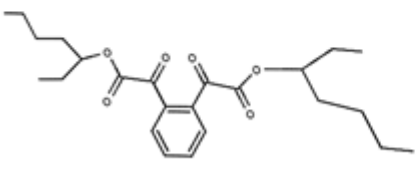
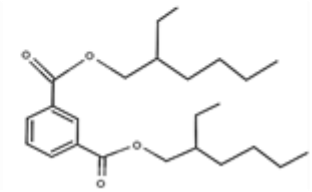


4d; 4e



4f



4g

V. Conclusion

The result of molecular analysis of endophytic fungus of *Helminthostachys zeylanica* Linn. (IFATL-05 isolate code) is the species *Talaromises sp* and based on GC-MS spectroscopy analysis of the ethyl acetate extract of endophytic fungus isolate IFATL-05 contains chemical compounds 2-pentanone, 4-hydroxy-4-methyl- ($C_6H_{12}O_2$; 116), diethyl phthalate ($C_{12}H_{14}O_4$; 222) 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- ($C_{15}H_{26}O$; 222), 1,2-

Benzenedicarboxylic acid, bis(2-methylpropyl) ester ($C_{16}H_{22}O_4$; 278), Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$; 390) and 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester) ($C_{24}H_{38}O_4$; 390) having activity as antibacterial against *E. coli, S. typhi,* and *S. dysenteriae* bacteria.

References

[1] Conlon CP. Gastrointestinal Tract: Bacterial Infections. In: Encyclopedia Of Life Sciences. Wiley. 2010. DOI: 10.1002/9780470015902.A0002219.Pub2

[2] Farrar J Et Al. Manson's Tropical Infectious Diseases. Elsevier Health Sciences. 2023

[3] Rante H Et Al. Isolation Of Sponge Bacterial Symbionts From Kodingareng Keke Island-Makassar Indonesia Which Is Potential As A Producer Of Antimicrobial Compounds. J Pure Appl Microbiol.; 16(1). DOI: 10.22207/JPAM.16.1.79

[4] Acar J, Röstel B. Antimicrobial Resistance: An Overview. Rev Sci Tech. 2002; 20:797–810

[5] Tiwari P, Bae H. Endophytic Fungi: Key Insights, Emerging Prospects, And Challenges In Natural Product Drug Discovery. Microorganisms. 2022; 10(2):360

[6] Bhore S, Christina A, Christapher V. Endophytic Bacteria As A Source Of Novel Antibiotics: An Overview. Pharmacogn Rev. 2013; 7(1):11

[7] Mitra A, Herwin H, Nuryanti S. Isolation Of Endophytic Fungi From Patchouli Leaves (Pogostemon Cablin Benth) As Antibacterial Against Pathogenic Bacteria By Bioautography And Agar Diffusion. Journal Microbiology Science. 2023; 3(1):45–56

[8] Hartini S, Sumanto. Study Of The Potential Of Helminthostachys Zeylanica (L.) Hook. As Medicinal Plants And Study Of Their Occurrence In Nature. DRAFT VERS. RIN Dataverse. DOI: Hdl:20.500.12690/RIN/4FBV7A

[9] Ridhasya FE, Teruna HY, Hendra R, Almurdani M. Natural Antidiabetic Of Tunjuk Langit (Helminthostachys Zeylanica) Rhizome Extracts. Pharmacology And Clinical Pharmacy Research.; 4(3). DOI: 10.15416/Pcpr.V4i3.24897

[10] Liou C-J Et Al. Water Extract Of Helminthostachys Zeylanica Attenuates LPS-Induced Acute Lung Injury In Mice By Modulating NF-KB And MAPK Pathways. J Ethnopharmacol. 2017; 199:30–38

[11] Suja SR, Latha PG, Pushpangadan P, Rajasekharan S. Evaluation Of Hepatoprotective Effects Of Helminthostachys Zeylanica (L.) Hook Against Carbon Tetrachloride-Induced Liver Damage In Wistar Rats. J Ethnopharmacol. 2004; 92(1):61–66

[12] Army MK Et Al. Antibacterial In Vitro Screening Of Helminthostachys Zeylanica (L.) Hook. Root Extracts. J Pharm Pharmacogn Res. 2023; 11(2):291–296

[13] Rahim N, Yuda Teruna H. Isolation And Characterization Of Secondary Metabolite Compounds From Methanol Extract Of Tunjuk Langit Plant Root (Helminthostacshys Zeylanica). Jurnal Penelitian Farmasi Indonesia. 2017; 5(2):62–66

[14] Asnita A, Herwin H, Kosman R, Nurung A. Isolation And Identification Of Endophytic Fungi Sesuru Stem (Euphorbia Antiquorum L.) As Antibacterial Producers Using Bioautography-TLC Method. As-Syifaa Jurnal Farmasi. 2020; 12(2):2085–4714

[15] Hasyyati NS, Suprihadi A, Raharjo B, Dwiatmi K. Isolation And Characterization Of Endophytic Mold From Gotu Kola (Centella Asiatica (L.) Urban). Jurnal Akademika Biologi. 2017; 6(2):66–74

[16] Variani YA Et Al. Analysis Of Bioactive Compounds In Secondary Metabolite Extracts Of Serratia Marcescens Strain MBC1. Indonesian Journal Of Chemical Analysis. 2021; 4(2):64–71

[17] Suhendar U, Fathurrahman M, Sogandi S. Antibacterial Activity And Mechanism Of Action Of Methanol Extract From Kasturi Mango Fruit (Mangifera Casturi) On Caries-Causing Bacterium Streptococcus Mutans. Jurnal Kimia Sains Dan Aplikasi. 2019; 22(6):235–241

[18] Bonev B, Hooper J, Parisot J. Principles Of Assessing Bacterial Susceptibility To Antibiotics Using The Agar Diffusion Method. Journal Of Antimicrobial Chemotherapy. 2008; 61(6):1295–1301

[19] Nurlaila E, Tukiran T. UV-VIS And FT-IR Spectrophotometric Analysis Of Isolated Compounds From Chloroform Extract Of Indonesian Bay Plant Bark (Syzygium Polyanthum). UNESA Journal Of Chemistry.; 6(1)

[20] Kartika R. Inhibition Test Of Endophytic Fungi Of Rhizophora Apiculata Mangrove Roots Against Staphylococcus Aureus And Escherichiae Coli Bacteria. Ebiomedik.; 2(1)

[21] Tejamukti EP Et Al. Application Of FTIR Spectroscopy And HPLC Combined With Multivariate Calibration For Analysis Of Xanthones In Mangosteen Extracts. Sci Pharm. 2020; 88(3):35

[22] Made SSN, Wayan SI. Analysis Of The Functional Groups Of Cough Syrup By Fourier Transform Infrared Analysis. Emasains: Jurnal Edukasi Matematika Dan Sains. 2021; 10(2):339–345

[23] Radiena MSY, Dompeipen EJ. Identification Of Triterpenoid Active Compounds From Silpau Green Marine Algae Extract (Dictyosphaeria Versluysii) With FTIR Spectrophotometer. Indonesian Journal Of Industrial Research. 2019; 15(1):33–40

[24] John GE Et Al. GC-MS Analysis And Antibacterial Susceptibility Pattern Of Crude Extracts Of Gongronema Latifolium And Allium Sativum On Selected Multidrug-Resistant Clinical Isolates. Malays J Microbiol. 2023; 19(2):182–190

[25] Sanchez S, Demain AL. Secondary Metabolites. In: Comprehensive Biotechnology. Elsevier. 2011, Pp. 155–167

[26] Su L-H Et Al. Anti-Inflammatory Prenylated Flavonoids From Helminthostachys Zeylanica. Chem Pharm Bull (Tokyo). 2016; 64(5):497–501

[27] Shah AB Et Al. Inhibition Of Bacterial Neuraminidase And Biofilm Formation By Ugonins Isolated From Helminthostachys Zeylanica (L.) Hook. Front Pharmacol. 2022; 13:890649

[28] Jayus J, Rini AWE, Sugiharto B, Rahmanto DE. Isolation And Identification Of Alcohol Tolerant Yeast From Molasses. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati. 2022; 13:1–10

[29] Tasma IM. Utilization Of Genome Sequencing Technology To Accelerate Plant Breeding Programs. Jurnal Penelitian Dan Pengembangan Pertanian. 2015; 34(4):30950

[30] Lei L-R Et Al. Research Advances In The Structures And Biological Activities Of Secondary Metabolites From Talaromyces. Front Microbiol.; 13. DOI: 10.3389/Fmicb.2022.984801

[31] Nicoletti R, Bellavita R, Falanga A. The Outstanding Chemodiversity Of Marine-Derived Talaromyces. Biomolecules. 2023; 13(7):1021

[32] Song F Et Al. New Antibacterial Secondary Metabolites From A Marine-Derived Talaromyces Sp. Strain BTBU20213036. Antibiotics. 2022; 11(2):222

[33] Yang W Et Al. Secondary Metabolites With A-Glucosidase Inhibitory Activity From Mangrove Endophytic Fungus Talaromyces Sp. CY-3. Mar Drugs. 2021; 19(9):492

[34] Panangi AN, Sakka L. Training On Making Moringa Leaf Simplisia (Morinaga Oliefera) For The Mangeloreng Village Community, Bantimurung District, Maros Regency. Jurnal Pengabdian Farmasi Dan Sains. 2022; 1(1):36–39

[35] Dewi N, Gunawan IW, Puspawati NM. Isolation And Identification Of Flavonoid Antioxidant Compounds From Ethyl Acetate Extract Of Pranajiwa Leaves (Euchresta Horsfieldii Lesch Benn.). Cakra Kimia (Indonesian E-Journal Of Applied Chemistry). 2017;

5(1):26
[36] Rita WS, Suprapta DN, Swantara IMD, Sudana IM. Antifungal Activity Of Phenolic Compounds From Samanea Saman Leaves Against Stem Rot Disease On Dragon Fruits Caused By Fusarium Solani. Kne Life Sciences. 2022; :621–635
[37] Adem Endris Y, Abdu KY, Abate SG. Investigation Of Bioactive Phytochemical Compounds Of The Ethiopian Medicinal Plant Using GC-MS And FTIR. Heliyon. 2024; 10(15):E34687