Online Journal of Biological Sciences

Research Article

Molecular Identification and Antioxidant Profiling of *Penicillium heteringtonii* EUA-017 from Mangrove (*Avicennia alba*) in West Sumatra, Indonesia

Anthoni Agustien[1], Djong Hon Tjong[1], Yetria Rilda[2], Denny Bendrianis[1], Siti Zaharani Zalamah[3], Mifthahul Jannah[3] and Muhamad Hafidz Fadjri[4]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Andalas, Padang, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Indonesia*
[4]*Graduate School pf Agricultural Sciences, Doctoral Program, Faculty of Agriculture, Universitas Padjajaran, Indonesia*

Article history
Received: 28-12-2024
Revised: 14-02-2025
Accepted: 15-02-2025

Corresponding Author:
Anthoni Agustien
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia
Email: aagustien@gmail.com

Abstract: Mangrove ecosystems harbor diverse endophytic fungi that produce bioactive secondary metabolites with potential antioxidant activities. This study aimed to identify an endophytic fungal isolate (EUA-017) from *Avicennia alba* mangroves in West Sumatra, Indonesia, and characterize its antioxidant metabolites. Molecular identification was performed using internal transcribed spacer (ITS) region sequencing. Secondary metabolites from ethyl acetate extracts were characterized using Fourier-transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Phylogenetic analysis identified isolate EUA-017 as *Penicillium hetheringtonii*, showing high sequence similarity (≥98%) to *P. hetheringtonii* CBS 122392. FTIR analysis revealed 16 distinct absorption peaks representing various functional groups. GC-MS analysis identified 12 compounds, of which 10 exhibited predicted antioxidant activity based on structural analysis. LC-MS/MS detected 12 compounds, with 5 demonstrating potential antioxidant properties. The compound exhibiting the highest predicted antioxidant activity from GC-MS analysis was 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl- (nerolidol) with an activity score of 0.431. From LC-MS/MS analysis, 1,2,3,4-tetramethoxy-5-prop-2-enylbenzene showed the highest antioxidant potential (activity score 0.406). These findings demonstrate that *P. hetheringtonii* EUA-017 from Indonesian mangrove ecosystems produces diverse bioactive compounds with significant antioxidant potential. This study contributes to the discovery of natural antioxidants from mangrove-associated endophytic fungi and highlights the biotechnological potential of mangrove-derived fungal resources for pharmaceutical and nutraceutical applications.

Keywords: Penicillium hetheringtonii, Endophytic Fungi, Mangrove, Avicennia alba, Antioxidant Activity, Secondary Metabolites, Molecular Identification, Indonesia

Introduction

Mangrove is an ecosystem that has interaction between living creatures and their surroundings (Hutasoit et al., 2017). The mangrove community grows in lush waters with a wide range of salinity (Rozirwan et al., 2022). Mangrove plants are known to be one of the sources of endophytic fungi that produce a variety of bioactive metabolite compounds with a range of biological activities (Sari et al., 2022). An endophyte fungus is a fungus that lives in plant tissues and can mimic a secondary metabolite with the same pharmacological activity as its host through a genetic transfer process or co-evolution without damaging its host plant tissue (Ebube et al., 2023). Mangrove fungus potentially produces many compounds, including

bioactive metabolites such as flavonoids, phenolic, alkaloids, tannins, and saponins (Sobolewska et al., 2020; Satyavani et al., 2014)

The metabolite of the fungus endophyte has been found to have potential as a producer of antioxidant compounds that are effective in fighting free radicals (Rumidatul et al., 2020). Molecules with one or more unpaired electrons that are relatively unstable are called free radicals (Ridlo et al., 2017) and harm bodily cell tissue, including lipid, protein, carbohydrate, and DNA damage. These harms can result in acute and chronic illnesses, cancer, early aging, and other conditions. An antioxidant is a molecule that can provide free radicals with one or more electrons so they can be absorbed. Antioxidants help shield cells from oxidative damage, prevent oxidative stress, and shield the body from Reactive Oxygen Species (ROS) (Omodamiro et al., 2016).

Exploration of antioxidant sources from plants will have an impact on plant survival, and requires a lot of organic solvents and little production, so it requires high production costs, so the right solution is needed and utilizes endophytic microbes in plant tissues (Handayani et al., 2019; Agustien et al., 2017; Dao et al., 2023). This study used 10 collections of mangrove endophytic fungal isolates from the Andalas University Biota Laboratory, then tested for antioxidant potential, 4 potential isolates were obtained with the highest isolate, namely EUA-017, based on this, molecular identification was carried out on the EUA-017 isolate and identified the antioxidant compounds produced so that this study has the potential to contribute to various industries, as well as the development of drugs and food ingredients based on natural resources.

Materials and Methods

Materials

The tools used are a reaction tube, petri cup, measuring glass, vial bottle, oxy needle, spirits lamp, cotton, cloth, fabric, measur, oven, Erlenmeyer, Eppendorf, water bath, centrifuge, aluminum foil, autoclave, rotary evaporation, magnetic stirrer, hotplate, laminae water flow, GC-MS set, LC MSMS, and electrophoresis set. While the materials used are antioxidant-producing isolates with isolation numbers EUA-017, PDA (Merck Art. No. 1.10130.0500), aquadest, potatoes, dextrose, and ethyl acetate.

Molecular Identification of Endophyte Fungi Isolates

DNA Isolation of Endophytes Fungi

DNA isolation is done using the PhytopureTM Kit (Amersham). The finely stained miselia is inserted into a sterile microtube, then 600 µL of reagent 1 is added and mixed using a spatula. Then, reagent 2 is added 200 µL invested into homogeneity and then incubated for 10 minutes in a water bath at a temperature of 65°C. The sample is then incubated in a freezer for 20 minutes. The samples are then added to chlorophores that have previously been stored at -20°C of 500 µL. After adding and centrifuging for 10 minutes at a speed of 1300 xg, 100 µL of DNA extraction resin suspension is removed. Three phases will form in the centrifugation sample. After that, the samples were centrifuged for five minutes at a velocity of 4000 xg to create DNA pellets. After washing the pellet with 100 µL of 70% cold ethanol, it was centrifuged for 10 minutes at a speed of 4000xg. The pellets were left without the supernatant for an hour, or until the ethanol was no longer present. For resuspension, 50 µL of nuclease-free water was added to DNA pellets.

DNA Amplification

Using a 25 µL reaction mix volume, Polymerase Chain Reaction (PCR) is used to amplify DNA. The reaction is composed of gotaq green master mix 12,5 µL, nuclease-free water 8.5 µL and primary ITS4 (reverse) and ITS 5 (forward) 1 µL respectively, then added 2 µL DNA template. The mixture is then inserted into the Thermal Cycler device for DNA amplification of the ITS area of the endophyte fungi. The PCR machine's temperature adjustment is configured as follows: Initial denaturation takes place for 90 seconds at 95°C, and 35 cycles follow: 30 seconds at 95% for denaturing, 30 seconds at 55°C for annealing, 90 seconds at 72°C for extension, and 5 minutes at 72°C for final extension. The final phase's storage temperature is 15°C (Green et al., 2018)

Electrophoresis

DNA Visualization and Sequencing PCR amplification products need to go through a qualitative test stage to prove the presence of DNA accurately. After inserting the amplification product into a 1% (w/v) 3µL agarose gel well, the chamber is submerged in 1x TAE buffer. DNA markers of 100 bp of 2 µL are added to the agarose gel as well as a length measurement tool of the PCR product. After that, the gelose with the sample and DNA marker is electrophoresed for 25 minutes at a voltage of 220 volts. On the Doc XR Gel, samples that have already completed the electrophoresis step are visible through the use of UV light.

DNA Sequencing

The Cycle Sequencing Ready Reaction Kit is used to sequence DNA. The DNA that has been amplified is colored to detect the type of nitrogen base and then displayed in the form of an electroforegram. This study

only uses DNA sequence sequencing with the ITS 5 primary for further analysis.

The BLAST Analysis

The BioEdit software version 7.7.1 was utilized to assess the results of the electropherogram sequence alignment and BLAST analysis. Next, DNA sequences are subjected to Basic Local Alignment Search Tools (BLAST) analysis to determine how similar each base sequence is to the gene data stored in the GenBank at the National Centre for Biotechnology Information (NCBI). When the collected fungal isolates have a similarity rating of 99–100%, they belong to the same species. Clustal X ver. 2. 1 was then used to align the endophyte fungi sequence data set that was obtained from sequencing and BLAST results (Dao et al., 2023) Using phylogenetic analysis With MEGA11, a phylogenetic tree analysis was performed. (Genetic Analysis Using Molecular Evolution). Using bootstrap with 1000 repetitions, phylogenetic tree construction based on genetic proximity distance with Neighbor-Joining (NJ) testing (Gascuel et al., 2006).

Isolation and Analysis of the Secondary Metabolite Compounds

Production and Reproduction of Inoculum

Prepared 10 mL of PDB medium in a 100 mL Erlenmeyer, then inoculated 2-3 boxes of mycelium from the fungi into the medium. The medium containing mycelium fungus shaker for 24 hours at a speed of 100 rpm at a temperature of 30°C. After 24 hours the inoculum is mixed into 90 mL PDB media in a 250 mL Erlenmeyer and then differentiated using a 24-hour shaker at a rate of 100 RPM at 30° C.

Fermentation of Endophyte Fungus EUA-017

The cultivation of endophyte fungus is carried out culturally silent (static) in a liquid medium of PDB in a 1000 mL glass bottle. Cultivation is conducted for 14 days (Heirina et al., 2020; Novika et al., 2021). Endophyte fungi that have been cultivated and are not contaminated, are then harvested to separate between endophyte and media (Gakuubi et al., 2022)

Extraction of Fermentation Results

Following the fermentation process, the fungal material is removed, and the mixture is placed in an oven set at 40°C for three hours. Following the fungus's spraying with a 1:10 ratio of ethyl acetate16 for 2 days in a dark condition (Sukmawaty et al., 2021).

Identification of Secondary Metabolite Compounds with FTIR

A positive control, ascorbic acid, is used as the standard antioxidant and is handled in the same manner as the samples in an antioxidant test with DPPH before analyzing the antioxidant compounds. FTIR Analysis using the Perkin Elmer Frontier FTIR ALD Cambridge Nanotech Savannah S100 with the following tool settings, Preparation of the collecting glass: The collecting glass used must be clean and dry. Measurement: Place the endophytic fungus sample on the collecting glass and place it on the FTIR tool. Measurement mode: Use transmission or attenuated total reflectance (ATR) measurement mode. Wave range: the wave range used for organic compound analysis is 4000-400 cm-1. Resolution: the resolution used for organic compound analysis is 4 cm-1. Data processing: FTIR measurement results can be processed using special software for FTIR spectrum analysis.

Identification of Secondary Metabolite Compounds With GC-MS

GC MS analysis using the GC-MS Thermo Scientific Trace 1310 Gas Chromatograph with the following tool settings, Columns: Use polar columns such as the DB-5 column or the DB-1 column with a length of 30 meters and a diameter in the range of 0.25-0.32 mm. Column temperature: The column temperature starts at about 50°C for 2 minutes, then increases to about 10°C/min to a temperature of 280°C and isined for 10-15 minutes. Injector temperature: Injector temperature is around 250-280°C. Detector temperature: The detector's temperature is about 280-300°C. Injection Mode: Use splitless injection mode with a 1-2 µL volume. Carrier gas: Use helium at a flow rate of about 1 mL/min. Retention time: The retention time of the compound found on the sample of the endophyte fungi can be seen in the results of the GC-MS analysis.

Identification of Secondary Metabolite Compounds with LC-MS/MS

LC MSMS analysis was performed with LC system UPLC (Ultra Performance Liquid Chromatography) ACQUITY U PLC® H-Class System (waters, USA) and U PL C Column HSS (high Streght Silica) AC QUITY UPL C18 (1.8 µm 2.1x100 mm) (Watters, USA), and Mass Spectrometer Two Generation Stepwave Quadrupole time-of-flight mass spectrometry type Xevo G2-S QT of (waters, USA). Separation is LC System: Ultra Performance Liquid Chromatography (UPLC), Column: C18 (1.8 µm 2.1x100 mm) HSS, Temperature: 50°C (Column), 25°C(room), Mobile phase: Water + 5 mM Ammonium Formic (A) and Acetonitrile + 0.05% Formic acid (B), Flow rate: 0.2 ml/min (step gradients) running 23 minutes (see slide moving phase), Injection Volume: 5 µl sample filtered with 0.2 µm filter and Mass Spectrometry Conditions System: ES (electrospray

ionization), Mode: Positive Mode, Mass analysis range: 50 – 1200 m/z, Source Temperature: 100°C, Desolvation Temperature: 350°C, Gas Cone flow: 0 L/hr, Gas flow desolvations: 793 L/h, Collision energy: 4t (low energy voltages), Energy Ramp Ramp: 25 – 60 volt (high energy).

Data Analysis

The data obtained is a chromatogram that has peaks that can be analyzed with several programs, GC-MS analysis using NIST ver. 2.5 and LC-MSMS analysis can be analyzed using MassLynx ver. 4.1 and interpretation of results verified through several webs such as ChemSpider, MassBank, MoNA, HMDB, textbooks, articles, dissertations as well as other research results which can be used as references. The analysis is descriptive and presented in the form of tables, images, and graphs.

Result and Discussion

Molecular Identification of Endophyte Fungi Isolates

Amplification of the EUA-017 isolate was determined by electrophoresis using primary ITS5 (forward) (5'-GGAAGTAAAAGTCGTAACAAGG-3') and primary ISS4 (reverse) (5'-TCCTCCGCTTATTGATATGATGC-3'), at annealing temperatures of 45°C, as shown in Figure 1.

Further analysis of the sequence of the genes ITS rDNA isolated fungi endophyte mangrove *Rhizophora apiculata* producing antioxidants obtained results contig data forward and reverse DNA sequencing as follows:
AGTTTTAACTAATTTCGTTATAGGTCTCAGACTGCAACTTCAG
ACAGCGTTCAGGGGGGCCGTCGGCGGGCGCGGGGCCCGC
CGAGGCAACATAGGTTCGGGCAACACGGGTGGGAGGTTGGGC
CCCGAGGGGCCCGCACTCGGTAATGATCCTTCCGTAGGT
GAACCTGCGGAAGGATCATTACCGAGTGCGGGCCCCTCGGGG
CCCAACCTCCCACCCGTGTTGCCCGAACCTATGTTGCCTC
GGCGGGCCCCGCGCCCGCCGACGGCCCCCCTGAACGCTGTCT
GAAGTTGCAGTCTGAGACCTATAACGAAATTAGTTAAAAC
TTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGC
AGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGT
GAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTC
CGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAG
CCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGGGAC
GGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGA
GCGTATGGGGCTTCGTCACCCGCTCTAGTAGGCCCGGCCGGC
GCCAGCCGACCCCCAACCTTTAATTATCTCAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAA
GCGGAGGAA

The base sequence of nucleotides is then compared with the DNA sequence found on GenBank to determine the degree of similarity of DNA sequences through the BLAST (Basic Alignment Search Tools) program on the NCBI (National Center for Biotechnology Information) or website (http://blast.ncbi.nlm.nih.gov/Blast) presented in Table 1.

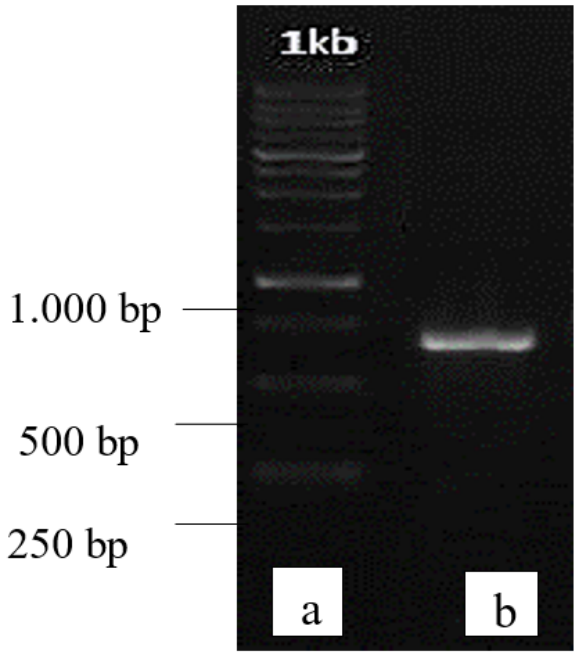


Fig. 1: Amplifikasi gen ITS rDNA isolate EUA-017 a Marker ladder b. Fragment PCR

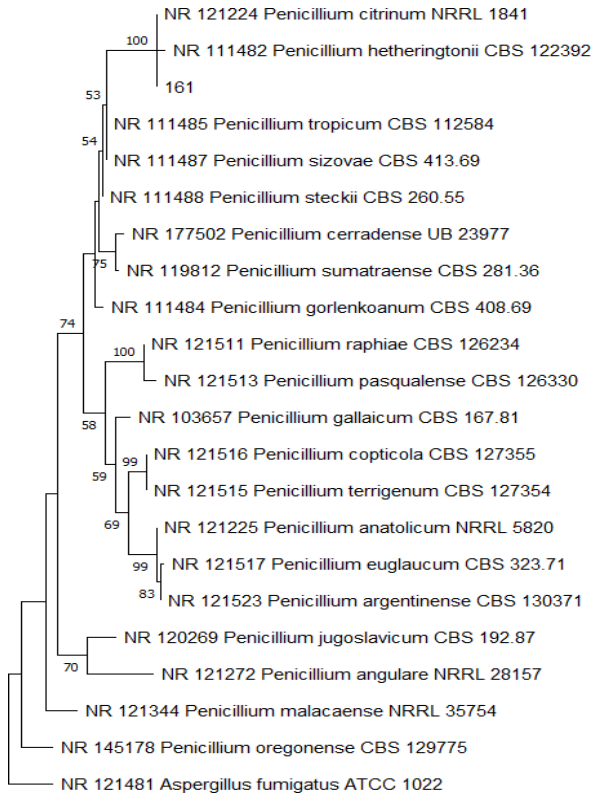


Fig 2: Phylogenetic tree isolation of fungus endophyte EUA-017

Table 1: Results of analysis of BLAST isolates EUA-017 with isolates found in GenBank

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Penicillium citrinum NRRL 1841	989	1276	97%	0.0	100%	NR_121224.1
Penicillium hetheringtonii CBS 122392	915	1178	91%	0.0	99.40%	NR_111482.1
Penicillium tropicum CBS 112584	737	833	73%	0.0	96.62%	NR_111485.1
Penicillium *sizovae* CBS 413.69	726	820	73%	0.0	96.40%	NR_111487.1
Penicillium steckii CBS 260.55	717	818	73%	0.0	95.96%	NR_111488.1
Penicillium cerradense UB 23977	737	969	89%	0.0	95.48%	NR_177502.1
Penicillium sumatraense CBS 281.36	699	798	73%	0.0	95.28%	NR_119812.1
Penicillium gorlenkoanum CBS 408.69	710	811	73%	0.0	95.73%	NR_111484.1
Penicillium raphiae CBS 126234	695	833	78%	0.0	93.97%	NR_121511.1
Penicillium pasqualense CBS 126330	682	818	78%	0.0	93.51%	NR_121513.1
Penicillium gallaicum	684	902	89%	0.0	93.74%	NR_103657.1
Penicillium copticola CBS 127355	702	921	89%	0.0	94.18%	NR_121516.1
Penicillium terrigenum CBS 127354	702	921	89%	0.0	94.17%	NR_121515.1
Penicillium anatolicum NRRL 5820	702	790	76%	0.0	94.22%	NR_121225.1
Penicillium euglaucum CBS 323.71	697	921	89%	0.0	94.00%	NR_121517.1
Penicillium argentinense CBS 130371	691	921	89%	0.0	93.80%	NR_121523.1
Penicillium jugoslavicum CBS 192.87	699	699	65%	0.0	93.96%	NR_120269.1
Penicillium angulare NRRL 28157	684	684	65%	0.0	93.52%	NR_121272.1
Penicillium malacaense NRRL 35754	771	771	73%	0.0	93.51%	NR_121344.1
Penicillium oregonense CBS 129775	678	678	65%	0.0	93.32%	NR_145178.1
Aspergillus fumigatus ATCC 1022	671	796	78%	0.0	92.95%	NR_121481.1

The result BLAST sequence of nucleotide isolate fungus endophyte mangrove producing antioxidants with 21 isolates found on GenBank has a similarity value of 92.95% - 100%. The E-value obtained is 0.0 which indicates an increasingly significant alignment with the BLAST result. Further phylogenetic analysis using the Neighbor-Joining (NJ) phylogenetic reconstruction as in Figure 2.

Phylogenetic analysis in Figure 2 is carried out using the Neighbour Joining method with a bootstrap value of 1000x to estimate the level of confidence of a phylogenetic tree in the MEGA (Molecular Evolutionary Genetic Analysis) software version 11. The antioxidant-producing endophyte fungus isolate EUA-017 has a very close affinity with the

Penicillium hetheringtonii. with a similarity value of 99.50% (Table 1), so the EUA-017 isolate is indicated as the fungus *Penicillium hetheringtonii*. Molecular identification serves as the basis for determining species with the potential to produce bioactive compounds, while

bioactivity tests confirm the function of the produced compounds. Both complement each other in the exploration of natural resources for biotechnology or pharmaceutical applications.

Identification of Secondary Metabolite Compounds With FTIR

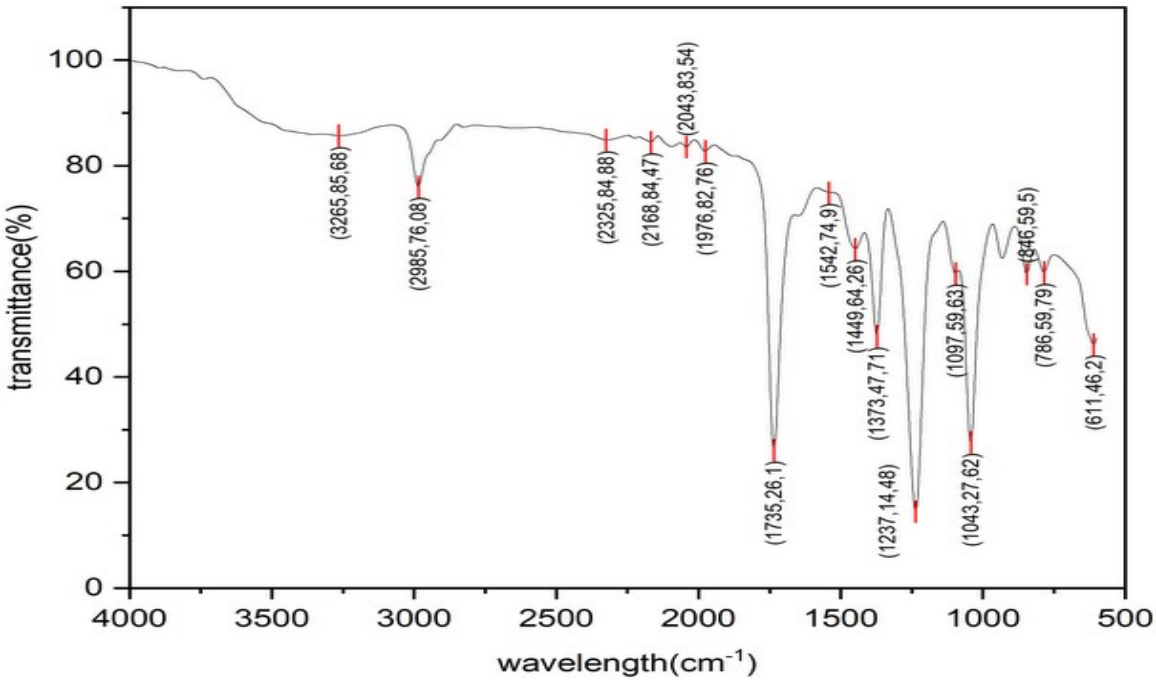
A chemical bond is detected as a band at a certain position that can be used to identify the type of chemical compound content.

The intensity of the band represents the amount of absorption that occurs after the infrared passes through the sample. FTIR-ATR has been widely used in the analysis of chemical compounds. Several studies have shown that FTIR-ATR is able to analyze secondary metabolite compounds (Alara *et al.,* 2021; Malacarne *et al.,* 2018) Secondary metabolite compounds such as phenolics, flavonoids, tannins, alkaloids, and terpenoids can also be identified by representing bands that appear at certain wave numbers. Based on the FTIR spectrum in Graph 1, there are several peaks at Wavelength. The results of the identification of the functional groups of the isolate are presented in Table 4.

Based on Table 2, the FTIR spectrum shows the absorption of the functional groups of the ethyl acetate extract of the EUA-111 endophytic fungus, the groups that are read include the presence of hydroxyl groups (O-H), the presence of deformed C-H bonds originating from lipids and cellulose, carbonyl groups C = O (aldehydes, ketones, carboxylic acids, esters), hydroxyl groups C-O (alcohols, esters, carboxylic acids, ethers) C-O bonds are a representation of alcohol groups indicating that the results of the sample analysis show the presence of alcohol groups. alcohol groups are most likely derived from phenolic compounds which have antibacterial properties (Kavanagh et al., 2019) and alkene groups C = C.

This fact is supported by several references to the analysis of secondary metabolite compounds that mangrove endophytic fungal extracts contain polyphenol compounds (Cadamuro et al., 2021). Compounds that have an aromatic ring structure with attached hydroxyl (OH) groups and generally have antioxidant activity (Hatiboruah et al., 2020)

Based on research (Jung et al., 2018) the C-H, O-H and NH3 groups are bonds that indicate carbohydrates, carboxylic acids, free amino acids, and phenolics, C = O and C = C which represent phenol molecules; while in the O–H, C–O, C-H, and C = C groups which symbolize flavonoids and phenolics and in the COH, C-C and C-O groups believe in the carbohydrate structure, and C-O slows down phenolics (Alara et al., 2021) In addition, the C—C and C—O groups include terpenoid compounds as functional bonds of terpenoids (Mashwani et al., 2016).Other research conducted an FTIR analysis of the endophytic fungus SKF 15 and obtained the functional groups O-H, C-H, C=O, C=C and C-O.



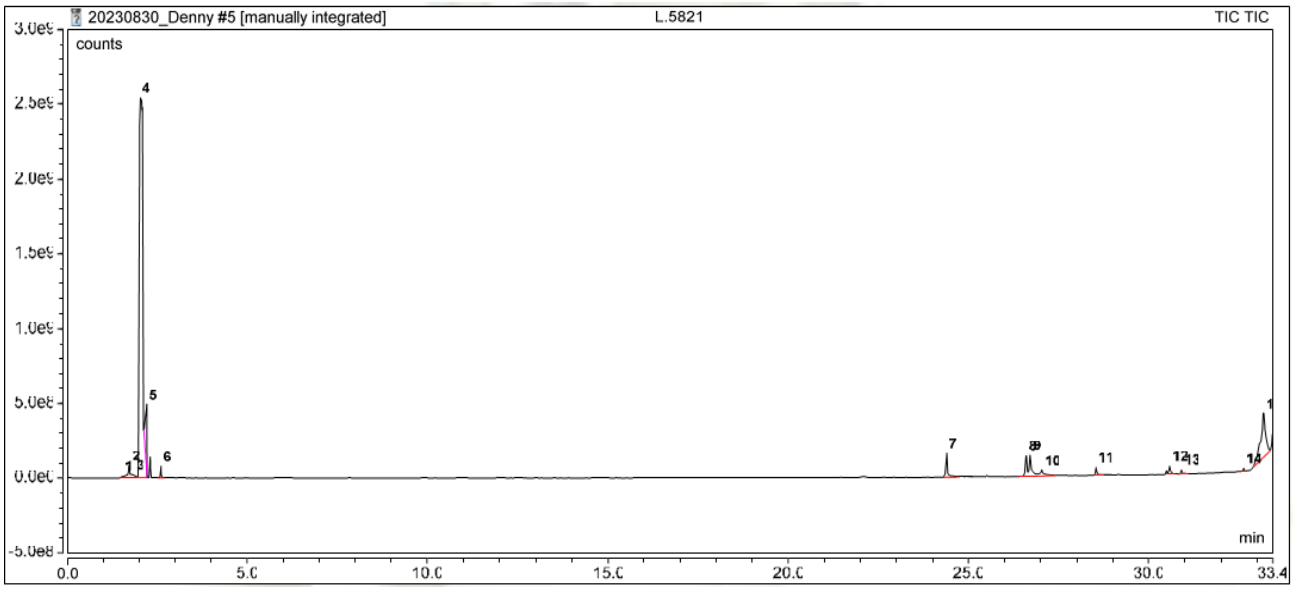
Graph 1: FTIR chromatogram of the secondary metabolite of the fungus endophyte EUA-017

Table 2: Results of web pass online representation of 17 compounds resulting from FTIR

No.	IR Spektrum	Wave length (cm^{-1})	Serapan	Group
1.	3263,17	3500 – 3200	O – H	Fenolik
2.	2985,08	2850 – 3000	C – H	Terpenoid
3.	2322,93	2850 – 3000	C – H	Terpenoid
4.	2168,03	2150 – 2260	C ≡ C	Flavanoid
5.	2042,35	1990 – 2140	N = C = S	Alkaloid
6.	1976,22	1990 – 2140	N = C = S	Alkaloid
7.	1734,93	1690 – 1760	C = O	Poliketida
8.	1448,48	1430 – 1460	--CH3	Poliketida
9.	1373,42	1375 – 1450	--CH3	Poliketida
10.	1236,56	1050 – 1300	C – O	Fenolik
11.	1096,98	1050 – 1300	C – O	Fenolik
12.	1043,15	1050 – 1300	C – O	Fenolik
13.	932,58	650 – 995	C – H	Terpenoid
14.	846,22	650 – 995	C – H	Terpenoid
15.	785,76	650 – 995	C – H	Terpenoid
16.	610,16	515 – 680	C – S	-

Identification of Secondary Metabolite Compounds With GC-MS

Based on Graph were obtained consisting of 12 compounds that can be seen in Table 3. All these compounds are further seen in their biological activity against antioxidants in the software passed online, as presented in Table 3.Based on Table 2, In the results of GC MS, compounds that come from several similar groups were found in Table 3, among them the pentacyclic terpenoid groups gibberellic acid, carboxylic acid acetic acid, methyl ester, and ethyl acetate; n-Hexadecanoic acids, 9-Octadecenoic acid (Z); methyl esters, Octadecanoic acid; 1,6,10- Dodecatrieln-3-ol, 3,7,11-trimethyl; dioxygen is digitoxin and ester is isopropyl and acetyl Glycerolate 1-palmitate. it can be seen that of the 12 compounds obtained only gibberellic acid and formic acid not detected have biological activity as an antioxidant. In Table 3, we can see that the compound with the highest potential activity is 1,6,10-Dodecatrieln-3-ol, 3,7,11-trimelthyl-. compounds that have antioxidant activity contain hydroxyl groups, which is consistent with the FTIR results in Table 2 that detect hydroxyl groups (OH) which are a group of secondary metabolites in the phenolic category.



Graph 2: GC-MS chromatogram of the secondary metabolite of the fungus endophyte EUA-017

DOI: 10.3844/ojbsci.2025.1099.1109

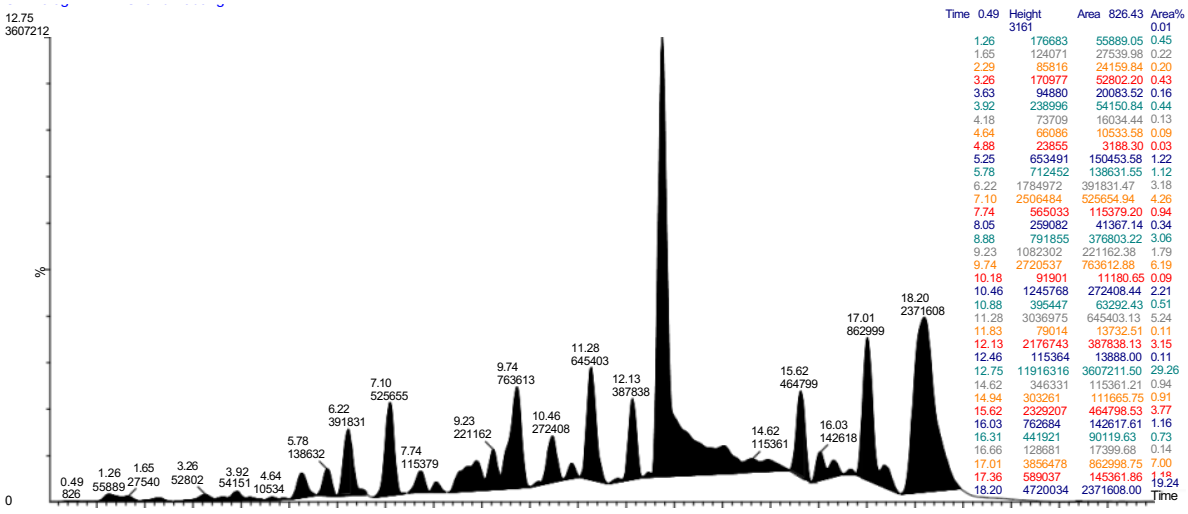
Table 3: Results of web pass online representation of 12 compounds resulting from GC-MS

No.	Name	Rt. (Min)	Arela (%)	Pa	Pi	Biology activity
1.	Gibberellic acid	1.520	0.16	-	-	-
2.	Acetic acid, methyl ester	1.724	0.41	0.221	0.045	Antioxidant
3.	Ethyl Acetate	2.037	71.57	0.206	0.052	Antioxidant
4.	Formic acid	2.609	5.14	-	-	-
5.	n-Hexadecanoic acid	24.400	2.25	0.222	0.045	Antioxidant
6.	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	27.029	2.64	0.431	0.010	Antioxidant
7.	Glycerol 1-palmitate	28.535	0.61	0.248	0.037	Antioxidant
8.	9-Octadecenoic acid (Z)-, methyl ester	26.600	1.72	0.269	0.030	Antioxidant
9.	Octadecanoic acid	30.903	0.28	0.222	0.045	Antioxidant
10.	Digitoxin	33.175	11.68	0.263	0.032	Antioxidant
11.	Oxacyclododecan-2-one	26.709	2.64	0.165	0.085	Antioxidant
12.	Cholest-5-en-3-ol (3ß)-, tetradecanoate	32.630	0.13	0.186	0.064	Antioxidant

Identification of Secondary Metabolite Compounds With LC-MSMS

The following are the results of the LC MSMS test conducted against the ethanol acetate extract of the endophyte mangrove fungi EUA-017.

Based on Graph 3 there are 12 compounds detected from a sample of ethanol acetate extract of endophyte mangrove fungus EUA-017, 3 of which are unknown compound names. All the compounds that are then obtained show their biological activity against antioxidants in the Passonline software, as presented in Table 4.



Graph 3. LC-MSMS secondary metabolite chromatogram of endophyte fungi EUA-01

Table 4: Results of web pass online representation against 16 compounds resulting from LC-MSMS

No	Compounds name	Formula	Arela (%)	Pa	Pi	Biology activity
1.	1-ethenylpyrrolidin-2- one;ethyl 2-methylprop-2- enoate;2-methylprop-2-enoic acid	$C_{16}H_{25}NO_5$	3.18	-	-	-
2.	2-(2-ethoxyphenoxy)ethanol	$C_{10}H_{14}O_3$	4.26	0.190	0.061	Antioxidant
3.	1,2,3,4-tetramethoxy-5-prop-2-enylbenzene	$C_{13}H_{18}O_4$	0.94	0.406	0.012	Antioxidant
4.	6-[[(11E)-11-[3-(dimethylamino)propylidene]-6H-benzo[c][1]benzoxepin-2- yl]oxy]-3,4,5-trihydroxyoxane-2- carboxylic acid	C25H29NO8	1.79	0.274	0.029	Antioxidant
5.	Unknown	C43H36N10	6.19	-	-	-
6.	(2S,3S,4R)-2-aminooctadecane-1,3,4-triol	C18H39NO3	2.21	0.160	0.091	Antioxidant
7.	5-ethyl-3-[2-methyl-6-(1H- 1,2,4-triazol-5-yl)pyridin-3- yl]-7,8-dihydropyrazino[2,3-b]pyrazin-6-one	C16H16N8O	5.24	-	-	-
8.	2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone	C22H25NO2	3.15	-	-	-
9.	methyl 5-(diaminomethylideneamino)-2-[3-(diaminomethylideneamino) propyl]-2-[[[(2S)-2-(diaminomethylideneamino)-3-(2,4,6-tritert-butyl-1H- indol-3- yl)propanoyl]amino]methyl] pentanoate	C36H63N11O3	3.77	0.134	0.122	Antioxidant
10.	[2-methyl-3-[2-(4methylcyclohexyl)acetyl]ox y-2-[[2-(4-methylcyclohexyl)acetyl]ox ymethyl]propyl] 3-[[3- hydroxy-3-[2-methyl-3-[2- (4-methylcyclohexyl)acetyl]ox y-2-[[2-(4-methylcyclohexyl)acetyl]ox ymethyl]propoxy]propyl]- [(2-methylpropan-2-yl)oxycarbonyl]amino]propanoate	C57H97NO14	1.16	-	-	-
11.	Unknown	C55H87N15O3	7.00	-	-	-
12.	Unknown	C55H39N15O2	19.24	-	-	-

Potential activity (Pa) and potential inhibitor (Pi) are concepts used in computerized biological analysis (CADD) to predict the biological activity of a molecule (Basanagouda *et al.,* 2010). In this context, Pa and Pi are used to determine the possible activity or inhibition of a compound against a particular biological function, such as an antioxidant. All of these compounds are further seen in their biological activity against antioxidants in the pass online software, as presented in Table 4 below. Functional groups in compounds that have antioxidant activity such as butyl groups, oxygen groups, ester functional groups, butyl groups, and hydroxyl groups

Conclusion

Based on this, it can be concluded that the molecular-based analysis of the endophyte mangrove fungus isolate EUA-017 has similarities with *Penicillium hetheringtonii* CBS 122392. Analysis of compounds using GC MS analyzes of 12 different compounds and 10 of them have biological activity as antioxidants; LC MSMS analyzes obtaining 12 compounds and 5 of them having biological activities as anti-oxidants. The highest antioxidant potential obtained from GC-MS is 1,6,10-Dodelcatrieln-3-ol, 3,7,11-trimethyl-. with a potential activity value of 0.431 and LC-MSMS anti-oxidant potential

composition with the highest potential activity value is 1,2,3,4- tetramethoxy-5-prop-2-enylbenzene of 0.406.

Acknowledgment

The authors would like to thank the Directorate of Research and Development, Ministry of Education, Culture, Research and Technology, and LPPM Universitas Andalas for their support.

Funding Information

The authors would like to thank the Ministry of Education, Culture, Research and Technology's Directorate of Research and Development. Thanks to the Ministry of Education and Culture Ristekdikti for funding the PFR scheme research with contract number: 041/E5/PG.02.00.PL/2024.

Author's Contributions

Anthoni Agustien: Developed the initial idea, wrote the manuscript, designed the research, and reviewed and approved the manuscript.
Yetria Rilda: Data analysis, experiment development.
Djong Hon Tjong: Designing research, methodology, data analysis, and manuscript write.
Feskaharny Alamsjah: Literature search and data analysis.
Denny Bendrianis: Materials and equipment involvement, and script write.
Miftahul Jannah: Materials and equipment involvement and monitoring research.
Siti Zaharani Zalamah: Data analysis and experiment development.
Muhamad Hafidz Fajri: Materials, equipment involvement and data analysis.

Ethics

The authors acknowledge the novelty of this article, affirming that it is an original contribution that has not been previously published. All participating authors have thoroughly reviewed and endorsed the content, ensuring there are no ethical issues.

References

Agustien, A., Santoso, P., Permata Sari, N., Annisa, F., Nasir, N., Rilda, Y., & Djamaan, A. (2017). Screening of Endophyte Piper betle Bacteria from the Forests of HPPB University Andalas as Antibiotics Producer. *International Journal of Current Microbiology and Applied Sciences*, *6*(12), 3970–3975. https://doi.org/10.20546/ijcmas.2017.612.457

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science*, *4*, 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Basanagouda, M., Shivashankar, K., Kulkarni, M. V., Rasal, V. P., Pate, H., Mutha, S. S., & Mohite, A. A. (2010). Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethyl coumarin. *European Journal of Medicinal Chemistry*, *45*(3), 1151–1157. https://doi.org/10.1016/j.ejmech.2009.12.022

Cadamuro, R. D., da Silveira Bastos, I. M. A., Silva, I. T., da Cruz, A. C. C., Robl, D., Sandjo, L. P., Alves, S., Lorenzo, J. M., Rodríguez-Lázaro, D., Treichel, H., Steindel, M., & Fongaro, G. (2021). Bioactive Compounds from Mangrove Endophytic Fungus and Their Uses for Microorganism Control. *Journal of Fungi*, *7*(6), 455. https://doi.org/10.3390/jof7060455

Dao, V. H. T., Do, K. P., Nguyen, H. V., Nguyen, C. M., Thi Tran, D. T., Thanh Dang, T. T., Nguyen, T. X., & Dinh, S. T. (2023). Identification and Evaluation of the Growth Promotion of Endophytic Bacteria on in vitro Potato Plants. *Pakistan Journal of Biological Sciences*, *26*(7), 371–379. https://doi.org/10.3923/pjbs.2023.371.379

Ebube I., S., Umeokoli, B. O., Obidiegwu, O. C., Okezie, U. M., Okolo, C. C., Akintayo, D. C., & Chiedu Okoye, F. B. (2023). Screening of secondary metabolites produced by a mangroove-derived Nigrospora species an endophytic fungus isolated from Rhizophora racemosa for antioxidant and antimicrobial properties. *GSC Advanced Research and Reviews*, *15*(2), 047–060. https://doi.org/10.30574/gscarr.2023.15.2.0133

Gakuubi, M. M., Ching, K. C., Munusamy, M., Wibowo, M., Liang, Z.-X., Kanagasundaram, Y., & Ng, S. B. (2022). Enhancing the Discovery of Bioactive Secondary Metabolites from Fungal Endophytes Using Chemical Elicitation and Variation of Fermentation Media. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.898976

Gascuel, O. (2006). Neighbor-Joining Revealed. *Molecular Biology and Evolution*, *23*(11), 1997–2000. https://doi.org/10.1093/molbev/msl072

Green, M. R., & Sambrook, J. (2018). The Basic Polymerase Chain Reaction (PCR). *Cold Spring Harbor Protocols*, *2018*(5), pdb.prot095117. https://doi.org/10.1101/pdb.prot095117

Handayani, D., Pratiwi, E. M. I., & Fajrina, A. (2019). Isolasi Senyawa Antimikroba dari Jamur Endofit Trichoderma koningiopsis SaKB1. *Jurnal Sains Farmasi and Klinis*, *6*(2), 78–84. https://doi.org/10.25077/jsfk.6.2.78-84.2019

Hatiboruah, D., Devi, D. Y., Namsa, N. D., & Nath, P. (2020). Turbidimetric analysis of growth kinetics of bacteria in the laboratory environment using smartphone. *Journal of Biophotonics*, *13*(4). . https://doi.org/10.1002/jbio.201960159

Heirina, A., Rozirwan, R., & Hendri, M. (2020). Isolasi dan aktivitas antibakteri jamur endofit pada mangrove Sonneratia alba dari Tanjung Carat Kabupaten Banyuasin Sumatera Selatan. *Jurnal Penelitian Sains*, *22*(1), 16–24. https://doi.org/10.56064/jps.v22i1.562

Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez Cecilia, V., Beers, K. L., Balazs, G. H., Jones, T. T., Work, T. M., Brignac, K. C., Royer, S.-J., Hyrenbach, K. D., Jensen, B. A., & Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin*, *127*, 704–716. https://doi.org/10.1016/j.marpolbul.2017.12.061

Kavanagh, S., Gunnoo, J., Marques Passos, T., Stout, J. C., & White, B. (2019). Physicochemical properties and phenolic content of honey from different floral origins and from rural versus urban landscapes. *Food Chemistry*, *272*, 66–75. https://doi.org/10.1016/j.foodchem.2018.08.035

Malacarne, M., Antoniolli, G., Bertoldi, D., Nardin, T., & Larcher, R. (2018). Botanical origin characterisation of tannins using infrared spectroscopy. *Food Chemistry*, *267*, 204–209. https://doi.org/10.1016/j.foodchem.2017.06.131

Mashwani, Z.-R., Khan, M. A., Khan, T., & Nadhman, A. (2016). Applications of plant terpenoids in the synthesis of colloidal silver nanoparticles. *Advances in Colloid and Interface Science*, *234*, 132–141. https://doi.org/10.1016/j.cis.2016.04.008

Novika, D. S., Ahsanunnisa, R., & Yani, D. F. (2021). Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L.) Terhadap Penghambatan Denaturasi Protein. *Stannum : Jurnal Sains Dan Terapan Kimia*, *3*(1), 16–22. https://doi.org/10.33019/jstk.v3i1.2117

Omodamiro, O. D., & Ikekamma, C. O. (2016). In vitro Study of Antioxidant and Anticoagulant Activities of Ethanol Extract of Pandanus tectorius Leaves. *International Blood Research and Reviews*, *5*(1), 1–11. https://doi.org/10.9734/ibrr/2016/22231

Ridlo, A., Pramesti, R., Koesoemadji, K., Supriyantini, E., & Soenardjo, N. (2017). Aktivitas Antioksidan Ekstrak Daun Mangrove Rhizopora mucronata. *Buletin Oseanografi Marina*, *6*(2), 110–116. https://doi.org/10.14710/buloma.v6i2.16555

Rozirwan, Nugroho, R. Y., Hendri, M., Fauziyah, Putri, W. A. E., & Agussalim, A. (2022). Phytochemical profile and toxicity of extracts from the leaf of Avicennia marina (Forssk.) Vierh. collected in mangrove areas affected by port activities. *South African Journal of Botany*, *150*, 903–919. https://doi.org/10.1016/j.sajb.2022.08.037

Rumidatul, A., Rahmawati, N., & Sunarya, S. (2020). Production of Secondary Metabolites and its Antibacterial and Antioxidant Activity During the Growth Period of Endophytic Fungi Isolated from Gall Rust Sengon Plants. *Pharmacognosy Journal*, *13*(2), 325–331. https://doi.org/10.5530/pj.2021.13.42

Sari, R. W. W., Jamarun, N., Arief, S., Pazla, R., Yanti, G., & Ikhlas, Z. (2022). Nutritional Analysis of Mangrove Leaves (Rhizophora apiculata) Soaking with Lime Water for Ruminants Feed. *IOP Conference Series: Earth and Environmental Science*, *1020*(1), 012010. https://doi.org/10.1088/1755-1315/1020/1/012010

Hutasoit, Y. S., Melki, M., & Sarno, S. (2017). Struktur Vegetasi Mangrove Alami di Areal Taman Nasional Sembilang Banyuasin Sumatera Selatan. *Maspari Journal*, *9*(1), 1-8.

Satyavani, K., Gurudeeban, S., Manigandan, V., Rajamanick, E., & Ramanathan, T. (2014). Chemical Compositions of Medicinal Mangrove Species Acanthus ilicifolius, Excoecaria agallocha, Rhizophora apiculata and Rhizophora mucronata. *Current Research in Chemistry*, *7*(1), 1–8. https://doi.org/10.3923/crc.2015.1.8

Sobolewska, D., Galanty, A., Grabowska, K., Makowska-Wąs, J., Wróbel-Biedrawa, D., & Podolak, I. (2020). Saponins as cytotoxic agents: an update (2010–2018). Part I—steroidal saponins. *Phytochemistry Reviews*, *19*(1), 139–189. https://doi.org/10.1007/s11101-020-09661-0

Sukmawaty, E., Hafsan, H., Masri, M., Shintia, I., Wahyuni, S., & Amir, U. N. A. (2021). Skrining fitokimia dan aktivitas antioksidan ekstrak etil asetat cendawan endofit aspergillus sp. *BIOTIK: Jurnal Ilmiah Biologi Teknologi Dan Kependidikan*, *8*(2), 218.