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Exploration and Antimicrobial Activity Test of Secondary Metabolites from Endophytic Fungi of *Curcuma Zedoaria* from West Nusa Tenggara

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ABSTRACT

Antibiotic resistance has become a significant public health issue in Indonesia, prompting the search for alternative antimicrobial agents from natural sources. Endophytic fungi symbiotically living inside medicinal plants such as *Curcuma zedoaria* (*temu putih*) have the potential to produce bioactive secondary metabolites that could serve as new antibacterial agents. This study aimed to isolate endophytic fungi from the rhizome of temu putih originating from West Nusa Tenggara (NTB), evaluate the antibacterial activity of isolates grown on Potato Dextrose Broth (PDB) and Yeast Malt Broth (YMB), and identify as well as elucidate the structure of the secondary metabolite compounds produced. This experimental comparative study involved isolation of endophytic fungi by surface sterilization, culturing on PDB and YMB media, extraction with ethyl acetate, and antibacterial testing against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 using the microdilution method. Metabolite profiling was performed using HPLC-DAD, preparative HPLC for fractionation, LC-HRMS for molecular mass identification, and NMR spectroscopy for structural elucidation. Five endophytic fungal isolates were successfully obtained from the temu putih rhizome. Antibacterial activity was stronger in isolates cultured on PDB compared to YMB. From isolate TP 3 cultured in PDB, several secondary metabolite fractions were isolated, with fraction 4 (F4) containing linoleic acid as the pure compound. F4 demonstrated a Minimum Inhibitory Concentration (MIC) of 200 µg/mL against *S. aureus*, comparable to the TP 3 PDB isolate, while against *E. coli*, F4 showed MIC > 200 µg/mL and TP 3 PDB isolate showed an MIC of 100 µg/mL.

Keywords: *Curcuma zedoaria*, endophytic fungi, secondary metabolites, antimicrobial activity, West Nusa Tenggara

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INTRODUCTION

Antibiotic resistance is a significant public health problem in Indonesia, with various studies highlighting alarming statistics in diverse healthcare settings. A cross-sectional study on urinary tract infections (UTIs) revealed that 35% of bacterial isolates are resistant to many drugs, with *Escherichia coli* being the most common pathogen and high levels of resistance to ampicillin (60%) and cotrimoxazole (50%) (Prastiyanto et al., 2024).

Natural materials are emerging as a promising alternative in the fight against antimicrobial resistance (AMR), a global public health crisis exacerbated by the indiscriminate use of antibiotics. The rich diversity of secondary metabolites in medicinal herbs, such as alkaloids, tannins, and polyphenols, offers antimicrobial and resistance-modifying properties (Mohammed, Mukhles et al., 2023).

The diversity of Indonesia's ecosystems—such as tropical rainforests, savannas, coral reefs, and mountains—also supports high biodiversity. Indonesia is one of the countries with very high biodiversity, especially for natural medicinal ingredients. Indonesia's biodiversity, including flora and fauna, makes it a potential source for the development of traditional and modern medicines. This great potential can be further developed to support the growth of the pharmaceutical industry, especially through the acceleration of research in the field of herbs (BPOM, 2021).

Historically, drug discovery has made phenomenal advances, such as in the discovery of anticancer drugs like Taxol, isolated from the plant *Taxus brevifolia* and its endophytic fungus *Taxomyces andreanae*. Similarly, various compounds from endophytic fungi of diverse plants show bioactivity, including fumigaclavine C from the fungus *Aspergilus* sp. (antimicrobial), solamargine from *A. flavus* (cytotoxic), aspernidine from *Emericella* sp. (H1N1 antiviral), extracts from *Talaromyces* sp. (anti-inflammatory), and epoxycytochalasin H from *Diaporthe* sp. (antimalarial) (Fadiji & Babalola, 2020).

Fungal endophytes are a promising source for discovering new bioactive compounds due to their symbiotic relationship with plants, which allows them to produce unique secondary metabolites that protect their hosts from threats such as pests and diseases. These metabolites have shown potential for treating various diseases, including microbial, viral, and parasitic infections, as well as cancer (Singh et al., 2024).

A study isolated 73 endophytic bacteria from *Curcuma zedoaria*, with 16 showing antimicrobial activity against pathogens such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, including *Citrobacter freundii*, *Bacillus subtilis*, *Pseudomonas otitidis*, and *Burkholderia cenocepacia*, demonstrating prominent effectiveness (Sulistiyani et al., 2019). Additionally, endophytic bacteria from the white Temu rhizome showed potential as antibacterial agents against *Klebsiella pneumoniae* (Indrawati et al., 2018).

Derivatives of pyranone compounds from the leaf endophytic fungus *Curcuma zedoria* exhibit antibacterial properties against *E. coli*, *S. dysentriae*, *S. aureus*, and *B. subtilis*, with the strongest effects observed against *S. aureus*. The study showed an inhibition zone of 11 mm at a concentration of 250 ppm. This compound also exhibited antioxidant activity with a half-maximal inhibitory concentration (IC50) value of 16.05 µg/mL (Muharni et al., 2016).

Based on these data, the research potential on endophytic fungi remains very broad. In this study, endophytic fungi were isolated from white Temu rhizomes originating from West Nusa Tenggara. In West Nusa Tenggara, the influence of the Wallace Line is evident in the region's biodiversity and ecological characteristics. These lines act as a barrier to the spread of various species, influencing macroorganisms and microorganisms, forming the unique biogeographic identity of the area (Mazaheri & Moosavi-Movahedi, 2021). This research

focuses on the exploration of bioactive compounds produced by endophytic fungi from the white Temu rhizome, which has great potential as an antimicrobial agent, as well as on exploring herbal medicinal ingredients from Eastern Indonesia.

The antibacterial activity test used *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739. This approach enables the determination of the effectiveness of endophytic fungal isolates and metabolite compounds from these isolates as antimicrobials under diverse conditions.

This study aims to explore the potential of endophytic fungal isolates and metabolite compounds produced by these fungi in their role as antimicrobial agents. It involves characterization, identification, and antimicrobial testing of compounds isolated from endophytic fungi. The research is expected to provide a strong preliminary basis for testing the antibiotic potency of new natural materials, potentially contributing to the development of more effective and sustainable antibiotic drugs from natural resources.

This study is based on an exploration of the potential of endophytic fungi found in the rhizome of white turmeric (*Curcuma zedoaria*) originating from West Nusa Tenggara (NTB), Indonesia. The research problem consists of four main aspects: (1) isolation of endophytic fungi from white turmeric rhizomes to obtain potential strains capable of producing bioactive compounds; (2) evaluation of the antibacterial activity of these isolates to determine their effectiveness in inhibiting bacterial growth; (3) identification, isolation, and elucidation of the structure of secondary metabolite compounds derived from selected fungal isolates; and (4) assessment of antibacterial and antibiofilm potency of these secondary metabolites to evaluate their effectiveness as potential antimicrobial agents.

Based on this problem formulation, the main objective of this research is to explore the potential of secondary metabolite compounds from endophytic fungi of white turmeric rhizomes (*Curcuma zedoaria*) from NTB and to evaluate their activity as antimicrobial agents. Specifically, the study aims to: (1) isolate endophytic fungi from white turmeric rhizomes; (2) assess the antibacterial activity of the fungal isolates; (3) identify, isolate, and elucidate the structure of secondary metabolite compounds from selected isolates; and (4) examine the antibacterial and antibiofilm potency of these selected compounds.

The anticipated benefits of this study are both scientific and practical. Scientifically, it contributes to advancing knowledge in pharmaceutical microbiology, particularly regarding the role of endophytic fungi as potential sources of bioactive compounds with medicinal properties. Practically, fungal isolates may be further developed for application in herbal medicine production. If the isolated compounds demonstrate strong pharmacological activity, they may serve as the basis for developing new products in pharmaceuticals, functional foods, or health supplements. Moreover, this research could provide added economic value for farmers and industries involved in the cultivation and processing of white turmeric from NTB.

METHOD

This study used a comparative experimental research design to test the differences and influence of culture media on the production of secondary metabolites and the antibacterial

activity of endophytic fungi. The research activities involved isolating endophytic fungi, culturing them in two different media, extracting metabolites, identifying compounds, and testing their bioactivity both quantitatively and qualitatively.

The study population consisted of white turmeric rhizomes (*Curcuma zedoaria*) obtained from West Nusa Tenggara (NTB), while the sample included successfully isolated endophytic fungal isolates selected based on purity and optimal growth. The fungi were cultivated using two types of culture media: Potato Dextrose Broth (PDB) and Yeast Malt Broth (YMB). The independent variables were the types of culture media, the intermediate variables were the ethyl acetate extracts and secondary metabolite compounds derived from the fungi, and the dependent variables were antibacterial activities tested against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739.

Materials used for isolation and cultivation included Potato Dextrose Agar (PDA), Yeast Malt Agar (YMA), Potato Dextrose Broth (PDB), Yeast Malt Broth (YMB), chloramphenicol, 70% ethanol, 7.5% sodium hypochlorite, and distilled water.

Materials for antibacterial activity tests included dimethyl sulfoxide (DMSO), Mueller Hinton Broth (MHB), chloramphenicol discs (30 µg), and nystatin discs (10 µg). Test microorganisms were *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739.

Materials for compound extraction and separation included n-hexane, ethyl acetate, dichloromethane, methanol, cyclohexane, chloroform, and acetone, all analytical grade.

Equipment used included laminar air flow cabinets, micropipettes, autoclave, analytical balance, magnetic stirrer, fume hood, Erlenmeyer flasks, orbital shaker, shaker incubator, rotary evaporators, HPLC-DAD, preparative HPLC, LC-HRMS, NMR spectrometers, UV lamp, 96-well microplates, LC-MS/MS, filter paper, microcentrifuge tubes, and low-temperature freezers.

The study sample consisted of rhizome parts of white turmeric obtained from NTB. The bacterial strains *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 were used for antibacterial testing. Sample preparation was carried out in January 2025.

RESULTS AND DISCUSSION

Preparation of Plant Materials

The plant material used in this study is the white Temu rhizome (*Curcuma zedoaria*) which comes from West Nusa Tenggara. The rhizome samples of *Curcuma zedoaria* used in this study are official collections and assets belonging to the National Research and Innovation Agency (BRIN).

Isolation of Endophytic Fungi from White Temu Rhizomes

Endophytic microbial isolation is the process of identifying and acquiring endophytic microorganisms, which live within plant tissues. Endophytes include both *bacteria* and *fungi*, which can provide a variety of benefits to plants, such as promoting growth, resistance to stress, and producing bioactive compounds. This process generally begins with the sampling of plants from various parts, such as roots, stems, or leaves, followed by specific isolation

techniques that resemble microbial cultures. In this study, isolation was intended for endophytic fungi carried out from the rhizome *Curcuma zedoaria*.

Isolation of endophytic fungi uses chloramphenicol as the antibiotic of choice because chloramphenicol has a broad spectrum that can inhibit the growth of various Gram-positive and Gram-negative *bacteria* that are often contaminants in the culture of microorganisms. In addition, the bacteriostatic properties of chloramphenicol allow these antibiotics to inhibit bacterial growth without damaging fungal metabolism or inhibiting the growth of the fungus itself. due to chloramphenicol due to its extensive bactericidal influence and its bacteriostatic properties that do not inhibit fungi (Blachowicz et al., 2022). Isolation of endophytic fungi from white temu rhizomes resulted in five endophytic fungal isolates. The five mushrooms obtained were coded with TP 1, TP 2, TP 3, TP 4, and TP 5. For comparison, there is a study on the isolation of endophytic fungi from the roots and rhizomes of white temu originating from the Pingbian Regency, Yunnan Province, China. The endophytic fungi are Penicillium frequentans and Penicillium baarnense (Xuan et al., 2011).

Cultivation and Extraction of Endophytic Mushroom Metabolites

The cultivation of endophytic fungal isolates is carried out in stages to facilitate the transition from small-scale culture to adequate scale production for the purification process. In the initial stage (seed culture), three pieces of agar (Ø 6 mm) from a 7-day-old petri dish were inoculated into 100 mL of liquid media of Yeast Malt Broth (YMB) and in parallel with a different medium of Potato Dextrose Broth (PDB). Seed cultures were incubated for 7–10 days at 27 °C with orbital agitation of 120 rpm.

For the next scale-up stage, 10–20% (v/v) of the seed culture is transferred to a larger flask (containing 500 mL of YMB or GDP media in a 1 L flask) and cultivated for 14 days under the same conditions, in order to adapt growth to larger volumes. The final stage is performed by inoculating 10% (v/v) of the seed culture into an experimental flask containing 500 mL of media (PDB or YMB) on a 1 L flask, or into a small bioreactor if required. Production fermentation is carried out at 27 °C and agitation at 120 rpm for up to 28 days,

Biomass separation is carried out by aseptic filtration or centrifugation; Mycelium can be rinsed quickly when needed to reduce the contaminants of the medium. The entire treatment was carried out in biological triplicats, and control of the medium without inoculum was used to monitor possible contamination as well as the background of the media components. If the production of target metabolites at the pumpkin scale is still low, the process can be continued to the liter scale-up (1–5 L) while maintaining the key parameters (temperature, agitation/aeration, and harvest time) that have been optimized at the pumpkin stage.

Cultivation is carried out with 2 different media aimed at distinguishing the growth ability and morphological characteristics of the incubated mushrooms. Research shows that Potato Dextrose Broth (PDB) may be more effective for the growth of certain fungi, including Alternaria brassicicola, which shows better vegetative growth in GDP than Malt Extract (ME) (Khadka & Acharya, 2023; Nur-E-Nasreen et al., 2017). For the purpose of screening for

antibacterial activity, extraction is carried out in combination by mixing mycelium and fermented liquid media. The extraction process uses the liquid–liquid method with ethyl acetate solvent at a ratio of 1:1 (v/v), i.e. 100 mL of sample is mixed with 100 mL of ethyl acetate, then shaken using a shaker at 250 rpm at room temperature for 2 hours. The organic phase formed is separated, then evaporated with a rotary evaporator until a crude ethyl acetate extract is obtained, which is then used in the microdilution test.

Endophytic fungal isolates that show antibacterial activity at the screening stage are then cultured on a larger scale for the purpose of isolation and compound elusion. At this stage, the mycelium and supernatant are separated, then extracted separately. Mycelium is extracted with ethanol:ethyl acetate (1:1, v/v) mixture with a total volume according to the solvent-to-biomass ratio, while supernatant is extracted with ethyl acetate at a ratio of 1:1 (v/v). The extraction results of each fraction are evaporated to dry to obtain a coarse extract. The weight of the ethyl acetate extract of the three endophytic mushrooms on 2 different cultivation media can be seen in the table.

Table 1. Weight results of the fifth ethyl acetate extract of the endophytic mushroom isolate *Curcuma zedoaria*

	Weight (grams)				
Extract	**TP 1**	**TP 2**	**TP 3**	**TP 4**	**TP 5**
PDB	7.5	5.3	11	6.4	6.1
PDB — Mycelium	3.4	2.3	7.1	2.9	2.5
PDB — Supernatant	2.5	1.2	3.1	1.9	1.7
YMB	5.9	5.3	3.1	5.6	5.4

Based on the table above, it is known that the largest weight of the endophytic mushroom crude ethyl acetate extract is produced in TP 3 endophytic mushroom extract in the PDB medium. Different media can also affect pigmentation and secretion of secondary metabolites from fungi. For example, Shimizuomyces paradoxus incubated in MEA exhibits darker colony pigment compared to fungi incubated in GDP (Sung et al., 2010). Research on Monascus shows that potato extract-containing media such as PDB can support the production of pigments and other metabolites better than malt-based media, although a combination of the two can be done to optimize yields (Seo et al., 2012).

Antibacterial Activity Test Raw Ethyl Acetate Extract White Temu Endophytic Mushroom

The antibacterial activity test was carried out on white temu endophytic mushroom extract that had been overgrown with 2 media, namely PDB and YME. The method used for the determination of antibacterial activity is a MIC-based microtiter dilution test. The microdilution test is the best method to quantitatively, accurately, and efficiently determine the antibacterial potential of endophytic mushroom extracts and is internationally recognized. The test was carried out in microtiter plates by making a serial dilution of the extract ranging from the highest concentration (200 µg/mL) to the lowest (1.5625 µg/mL). Each well is

incubated with a standardized test bacterial suspension. After incubation, bacterial growth was observed (both visually and spectrophotometrically), and the lowest concentrations capable of inhibiting full bacterial growth were recorded as MIC values (N. P. D. P. Sari et al., 2021; Sasebohe et al., 2023). The following is a table of the results of the microdilution test of white temu endophytic mushroom extracts.

Table 2. Results of the Minimum Concentration Inhibitor (MIC) Test of *Curcuma zedoaria* mushroom isolate extract in PDB and YM media

		MIC (Minimum Inhibitor Concentration	
SAMPLE		**EC (µg/mL)**	**SA (µg/mL)**
PDB	TP 1	25	50
	TP 2	>200	25
	TP 3	>200	50
	TP 4	12.5	6.25
	TP 5	12.5	12.5
YMB	TP 1	100	50
	TP 2	200	200
	TP 3	100	200
	TP 4	200	100
	TP 5	25	100
Tetracycline		25	100

Antibacterial activity testing was conducted on two test *bacteria*, *Escherichia coli* (EC) and *Staphylococcus aureus* (SA), using the microdilution method to determine the minimum inhibitory concentration (MIC) of the five rhizome endophytic mushroom extracts *Curcuma zedoaria*, cultivated on Yeast Malt Broth (YMB) and Potato Dextrose Broth (PDB) media. Tetracycline is used as a positive control.

Tetracycline was chosen as a positive control in the microdilution test for several key reasons. First, tetracycline has a broad antibacterial spectrum, is effective against a wide range of Gram-positive and Gram-negative *bacteria*, making it ideal for use as a benchmark standard in antibacterial sensitivity testing. Second, tetracycline's bacteriostatic properties mean that it inhibits the growth of *bacteria* without killing them directly, making the test results more consistent and reliable. Third, tetracycline has long been used and tested in a variety of antibiotic sensitivity test methods, making it a commonly accepted standard and can be used for the validation of microdilution test results. Fourth, the stability of tetracycline under laboratory conditions also supports its use as an effective positive control. Thus, tetracycline is often selected as a positive control to ensure the validity and reproducibility of microdilution test results in microbiological research and diagnosis (Grossman, 2016).

The results of the microdilution test showed a significant difference in the antibacterial potential of *Curcuma zedoaria* endophytic fungal isolate cultivated in GDP media compared to YM. In the PDB media, some isolates showed very strong activity against *Escherichia coli*,

particularly TP 4 and TP 5 with a MIC value of 12.5 µg/mL, which was even lower than the tetracycline positive control (25 µg/mL). TP 1 in GDP also showed a tetracycline-equivalent MIC (25 µg/mL) against *E. coli*, while TP 2 and TP 3 had a MIC value of >200 µg/mL, indicating weak activity.

Against *Staphylococcus aureus*, TP 4 isolates at GDP showed a MIC value of 6.25 µg/mL, which showed very strong antibacterial activity and exceeded the effectiveness of tetracycline (100 µg/mL). TP 5 isolate also has high activity (12.5 µg/mL) against *S. aureus*. Meanwhile, YM media generally produced higher (weaker) MIC values in both test *bacteria*. Only TP 5 showed fairly good activity against *E. coli* (25 µg/mL) and *S. aureus* (100 µg/mL), but still lost to the GDP results.

This difference suggests that the nutrient composition of the GDP media favors the biosynthesis of secondary metabolites with high antibacterial activity, especially compounds that are effective against *S. aureus* and *E. coli*. Considering the potential for very strong activity, particularly in TP 4 and TP 5, as well as the MIC profile that goes beyond positive control in some cases, the GDP medium was chosen for the advanced stage of cultivation.

Other studies have also mentioned that GDP is optimal in supporting the growth of certain types of fungi, which produce more secondary metabolites compared to YMB (Javaid et al., 2017). A concrete example of this is the production of extracts from Aspergillus in which the culture filtrate made in the GDP shows greater herbicide activity compared to that produced in YMB (Javaid et al., 2017).

In addition, GDP media has been shown to be more effective in promoting the radical growth and sporulation of fungi. Several studies have shown that the growth of fungi Alternaria spp. and Rhizoctonia spp. shows better results on GDP than other media such as malt extract (Rex & Rajasekar, 2021; Sharma et al., 2023). These observations are similar to the results of other studies showing that the use of PDB can increase the production of metabolites, as in the case of toxin production by Alternaria eichhorniae which shows better effectiveness on dextrose-rich media (Shabana et al., 2001).

Based on these results, extracts from cultivated isolates in PDB media will be used for further analysis using HPLC and LC-HRMS for separation, identification, and elution of active compound structures that act as antibacterial agents. One study showed that GDP not only supports fungal growth, but also increases the production of bioactive compounds such as antibacterial metabolites (Hamed et al., 2018).

Isolation and Elusion of Secondary Metabolite Compounds of White Temu Rhizotic Endophytic Fungi

After obtaining antibacterial activity from the five mushroom extracts. The research continued with the detection of the presence of bioactive compounds that have the potential to be antibacterial agents. The initial stage is carried out with High Performance Liquid Chromatography (HPLC) using diode array detector (DAD) to obtain the chromatographic profile of the compounds in the extract. This technique allows the separation of compound mixtures based on their polarity differences and retention times, as well as providing UV-Vis

spectrum information that is useful in detecting aromatic compounds or conjugated compounds (Gerasimova et al., 2019). The resulting chromatogram profile is used as a reference to select the dominant fraction which is then retested for antibacterial activity. In general, the 210 nm wavelength is often used in HPLC-DAD because many organic compounds, including carboxylic acids and aromatic compounds, exhibit significant absorption ability at these wavelengths.

The use of HPLC-DAD to analyze components in plant extracts has been effectively documented, supporting the relevance of 210 nm wavelengths in the identification of various phytochemicals (Tuzimski, 2010). Additionally, 210 nm waves are particularly useful when the compound lacks strong aromatic chromophores (which are typically detected at 254, 280, or >300 nm) (Gerasimova et al., 2019). HPLC-DAD uses a Waters Sunfire C18, 19 mm (250 x 5 µm) column, flow, with a 210 nm detector. The phase used is the reverse phase, and with 2 eluents namely H2O and Acetonitril.

The five endophytic fungal isolates cultivated with PDB media were extracted separately between mycelium and supernatants aiming for fractionation. In addition, each extract is identified with HPLC-DAD. The HPLC condition is arranged in such a way that it includes time, flow rate, percentage of motion phase gradient composition, HPLC-DAD chromatogram have been interpreted in table 3.

Table 3. HPLC-DAD chromatogram results

Substance	Peak EP (min)	Information
Blanko	2, 3, 42.7	Noise/contaminant
TP 1 PDB Mycelium	2, 3, 42.7	Noise/contaminant
TP 1 PDB Supernatant	2, 3, 42.7	Noise/contaminant
TP 2 PDB Mycelium	2, 3, 42.7	Noise/contaminant
TP 2 PDB Supernatant	2, 3, 42.7	Noise/contaminant
TP 3 PDB Mycelium	2, 3, 42.7, 45, 46.2, 53	Noise/contaminants, there are 4 fractions in 42.7, 45, 46.2, and 53
TP 3 PDB Supernatant	2, 3, 42.7	Noise/contaminant
TP 4 PDB Mycelium	2, 3, 42.7	Noise/contaminant
TP 4 PDB Supernatant	2, 3, 42.7	Noise/contaminant
TP 5 PDB Mycelium	2, 3, 42.7	Noise/contaminant
TP 5 PDB Supernatant	2, 3, 29, 42.7	Noise/contaminant

Based on the results of the HPLC-DAD chromatogram, only TP-3 mycelium extract in PDB media that has active compounds was seen in HPLC-DAD and it was seen that TP-3 mycelium extract with PDB media had 4 dominant fractions. From the results of the chromatogram, TP-3 mycelium extract in PDB media can be grouped into four dominant fractions. The four narrowest fractions are coded F1, F2, F3, and F4. From observations, fraction 4 appears at a retention time of more than 40 minutes. It can be assumed that the fraction tends to be non-polar which makes it easier for the compound to penetrate the cell membrane. After that, the four fractions of TP 3 mycelium extract in PDB media were fractionated with HPLC Preparative.

Fraction 4 of the TP-3 mycelium extract in PDB media was then selected for further analysis using *Liquid Chromatography–High Resolution Mass Spectrometry* (LC-HRMS). LC-HRMS analysis provides accurate data on exact molecular mass and ion fragmentation patterns, which is useful in determining empirical molecular formulas and enabling dereplication processes, i.e. comparing data with known natural compound databases (Wei et al., 2014). From the results of LC-HRMS, there are 15 compounds produced from the F4 fraction. The examination is followed by *Nuclear Magnetic Resonance* (NMR) which is a spectroscopic technique that functions to study the properties and structure of molecules (Kaunitz, 2018). Readings from NMR, the ^{13}C NMR spectrum shows a number of signals representing different types of carbon in the molecule. In the range of 13–34 ppm, peaks are seen indicating the presence of long-saturated alkyl carbon (CH_3 and CH_2). In the range of 127–130 ppm, a carbon sp^2 signal appears indicating the presence of a double bond. The quaternary carbon signal at 174.6 ppm is typical for carbonyl ester or carboxylic acid groups.

The DEPT-135 spectrum helps distinguish the types of carbon based on the number of protons bonded. Positive signals appear for CH_3 and CH, negative for CH_2, and the quaternary carbon signal appears to be missing. In this way, the identification of alkyl fragments and other functional groups becomes clearer. The ^1H NMR spectrum records proton signals that bind directly to carbon in HSQC. Terminal methyl protons (CH_3) appear as triplets in δ_H at about 0.90 ppm, while methylene protons (CH_2) in saturated alkyl chains appear as multiplets between 1.3 to 1.6 ppm. Vinyl fragments (alkene protons) appear as multiplets at around 5.3–5.4 ppm, supporting the presence of terminal double bonds.

The HMBC spectrum reveals the long-distance linkage between protons and carbon (2–3 bonds), thus strengthening the mapping of connections between molecular fragments. Protons in carbon α to carbonyl (about 2.0–2.3 ppm) are associated with quaternary carbonyl carbons at 174.6 ppm, confirming a terminal carboxylic acid group. Allylic protons at around 1.4–1.6 ppm and olefinic at 5.3 ppm are associated with olefinic carbon in the range of 26–34 ppm, marking the location of double bonds in the carbon chain. From the results of the integration and correlation of the NMR spectrum, the molecules analyzed are suspected to be carboxylic acid compounds with long saturated alkyl chains, containing carbonyl groups of carboxylic acid at δ_C 174.6 ppm and alkene/vinyl fragments at δ_H 5.3–5.4 ppm and δ_C 127–130 ppm. The signal pattern of protons and carbon is consistent with the structure of long-chain carboxylic acids. The following is attached a table of the results of NMR integration with LC-HRMS.

Table 4. Results of Interpretation of Secondary metabolite compounds in F4 based on their readability in NMR

No.	Compound Name	Molecule Formula	Character/ Type	Readability in NMR	Signal Sign (Expected NMR Signal)
1	Stearamide (Octadecanamide)	$C_{18}H_{37}NO$	Alkyl Amide	No	N–H signal (6–8 ppm, width ^1H), C=O amide (^{13}C 165–180 ppm), CH_2 α-N (^1H 2.5–3.0 ppm, ^{13}C 30–60 ppm); *Invisible*
2	*Methyldiol (Methynodiol)*	$C_{21}H_{30}O_2$	Alcohol/diol	No	O–CH (^1H 3.0–4.5 ppm; ^{13}C 60–80 ppm); *Invisible*

No.	Compound Name	Molecule Formula	Character/ Type	Readability in NMR	Signal Sign (Expected NMR Signal)
3	Quindecamine	$C_{30}H_{38}N_4$	Polyamine	No	N–H (^{1}H 2.5–4.5 ppm, width/multipleth), CH_2 α-N; *Invisible*
4	7-Morpholinomethyltheophylline	$C_{12}H_{17}N_5O_3$	Heterocyclic N	No	Heterocyclic, aromatic (^{1}H 6–8 ppm, ^{13}C >120 ppm); *Invisible*
5	Famciclovir	$C_{14}H_{19}N_5O_4$	Nucleoside N	No	Sugar O–H (^{1}H 3–5 ppm), aromatic (6–8 ppm); *Invisible*
6	Pipemidic acid	$C_{14}H_{17}N_5O_5$	Heterocyclic N	No	N–H, aromatic (^{1}H 6–8 ppm), O–H acid (10–13 ppm); *Invisible*
7	Indoline	C_8H_9N	Aromatics N	No	Aromatic multiplex (^{1}H 6–8 ppm, ^{13}C 120–160 ppm); *Invisible*
8	4-Hydroxy amphetamine glucuronide	$C_{15}H_{21}NO_7$	Glucuronide N	No	O–H sugars (^{1}H 3–5 ppm), anomeric (^{1}H 4.5–5.5 ppm, ^{13}C 100–105 ppm); *Invisible*
9	Esculinamide	$C_{15}H_{19}NO_6$	Kumarin N	No	Aromatic/olefinic (6–8 ppm), amide N–H; *Invisible*
10	2-Naphthylamine	$C_{10}H_9N$	Aromatics N	No	Aromatic multiplex (^{1}H 6–8 ppm); *Invisible*
11	N-[3-(Dimethylamino)propyl] dodecanamide	$C_{17}H_{36}N_2O$	Amida-N	No	C=O amide (^{13}C 165–180), CH_2 α-N (^{1}H 2.5–3 ppm); *Invisible*
12	Myristamine oxide	$C_{16}H_{35}NO$	Amine oxide	No	N–O (^{1}H 2.0–3.5 ppm), CH_2/CH α–N; *Invisible*
13	4-Dodecylphenol	$C_{18}H_{30}O$	Phenol, alkyl	No	Aromatic H (^{1}H 6–8 ppm), aromatic C (^{13}C 120–160 ppm), Ar–OH (width/variable); only *minor signal potential*
14	Alfaxalone	$C_{21}H_{32}O_3$	Steroid	No	Cyclopentanoperhydrophenanthhrene (steroid ring, many singlets/multiplets 0.8–2.5 ppm); *Invisible*
15	*Linoleic acid*	$C_{18}H_{32}O_2$	Asam lemak	Very clear	CH_3 (^{1}H 0.9 ppm, triplet), CH_2 (1.2–1.6 ppm, multiplet), C=O acid (^{13}C 174 ppm), CH=CH (^{1}H 5.3–5.4 ppm, ^{13}C 127–130 ppm); All the main signals appear

Based on the results of compound identification using LC-HRMS, a total of 15 potential candidate compounds were obtained in the F4 fraction of the raw ethyl acetate extract TP-3 mycelium extract in PDB media. However, because the NMR spectrum is obtained from these fractions which are mixed and impure, correlation analysis of the presence of compounds in the fraction is important to evaluate which compounds are detected through NMR signals. Based on the analysis of the NMR data obtained, and comparing with the LC-HRMS chromatogram data, it is known that the linoleic acid (C18H32O2) compound is the main and pure compound which is very clearly proven to exist and is dominant in the fraction based on

the NMR signal. This is indicated by the appearance of all the corresponding major signals, namely terminal methyl protons (δ_H ~0.9 ppm, triplets), methylene protons in the alkyl chain (δ_H 1.2–1.6 ppm multiplet), carbonyl groups of carboxylic acid at δ_C about 174 ppm, and vinyl protons at δ_H 5.3–5.4 ppm with sp^2 carbon at δ_C 127–130 ppm. This pattern and integration of signals reflect the structure of long unsaturated fatty acids with two double bonds, consistent with the characteristics of linoleic acid.

In contrast, other compounds containing nitrogen groups such as stearamide, myristamine oxide, quindecamine, and various heterocyclic compounds or amides present in the LC-HRMS list, are not clearly detected in the fractional NMR spectrum. This is because the fraction does not show a proton N–H signal, a carbon signal α to nitrogen, nor an amide pattern typical of the expected chemical shift range (e.g. a 6–8 ppm wide signal for N–H protons, a C=O amide carbon signal at 165–180 ppm). Therefore, the presence of these compounds is likely to be in very low concentrations or obscured by the signal of the dominant compound. In addition, compounds with aromatic structures, such as 4-dodecylphenol, indoline, and 2-naphthylamine, also do not show a prominent aromatic signal in the region of 6–8 ppm protons or 120–160 ppm carbon. Thus, the aromatic signal of the fractional NMR spectrum is also very minimal or insignificant, which indicates that most of the content in the fraction is dominated by long-chain aliphatic structures.

Compounds with polyhydroxyl, sugar, or nucleoside structures such as famciclovir and glucuronide are not detected in NMR, such as anomeric protons, wide hydroxyl groups, or carbon signals in the range of 60–100 ppm, which confirms their absence in these fractions. Overall, only linoleic acid compounds strongly respond to all the characteristics of the NMR spectrum obtained from fractions. This confirms that in the fraction it is already pure and the main compound whose chemical structure can be analyzed with NMR technique is linoleic acid, while the other compounds recorded in the LCMS are likely to be minor and do not contribute significant NMR signals.

The linoleic acid found in endophytic mushroom extracts from the rhizome of *curcuma zedoaria* mostly comes from the metabolism of the endophytic fungus itself as well as metabolic interactions with the host plant. In general, linoleic acid is an omega-6 polyunsaturated fatty acid that can be produced by microorganisms, including fungi, through lipid biosynthesis pathways involving several desaturases and elongase enzymes (Shi et al., 2019). The mechanism of linoleic acid formation in endophytic fungi involves the synthesis of fatty acids starting from acetyl-CoA which then undergoes a series of condensation, reduction, dehydration, and desaturation reactions. The delta-9 desaturase enzyme first introduced a single double bond resulting in oleic acid (18:1), which was then by the delta-12 desaturase enzyme converted to linoleic acid (18:2) by the addition of a second double bond at the 12th position of the carbon of the fatty acid chain (Jiang et al., 2020). The production of linoleic acid is important for fungal cell membranes as a phospholipid component that improves membrane fluidity and function and plays a role in environmental stress response. In addition to being produced by the fungus itself, linoleic acid can also be obtained through host plant metabolites, which can then be modified by endophytic fungi,

suggesting the presence of symbiotic metabolic interactions in white encounter rhizomes. This relationship allows for the accumulation of linoleic acid in endophytic mushroom extracts, which then contributes to typical biological activities such as antioxidants and antimicrobials (Kumar et al., 2021).

F4 Fraction Antibacterial Activity Test

The research was continued with a microdilution test of the F4 fraction which was concluded to be a single compound of linoleic acid against bacterial inhibition. Testing of antibacterial activity was still carried out on two test *bacteria*, *Escherichia coli* (EC) and *Staphylococcus aureus* (SA). The following are the results of the F4 fraction microdilution test.

Table 5. F4 Fraction Microdilution Test Results

Sample	EC (µg/mL)	SA (µg/mL)
TP 3 (PDB)	100	200
Fraction 4	>200	200
Tetracycline	25	100

F4 obtained from the purification of TP 3 mycelium isolate extract on PDB media and identified as containing linoleic acid as the main component showed selective antibacterial activity. In the microdilution test, F4 (Fraction 4) had a MIC against *Staphylococcus aureus* of 200 µg/mL equivalent to TP 3 (PDB) which had a MIC of 200 µg/mL. In contrast, activity against *Escherichia coli* decreased (MIC > 200 µg/mL for fraction 4 versus 100 µg/mL for crude extract), indicating the loss of components that play synergistic roles against Gram-negative *bacteria* during the purification process.

In comparison, tetracycline (positive control) showed a MIC of 25 µg/mL against *E. coli* and 100 µg/mL against *S. aureus*, confirming the antimicrobial potential of F4 against *S. aureus* although it is not yet equivalent to the standard antibiotic. F4 is able to exert antibacterial effects mainly because its main content is linoleic acid, a long-chain unsaturated fatty acid that has been shown to have strong antibacterial activity especially against Gram-positive bacteria such as *Staphylococcus aureus* and Bacillus subtilis. The antibacterial activity of linoleic acid is related to its ability to inhibit the biosynthesis of fatty acids in bacterial membranes and interfere with energy metabolism, so its effectiveness in inhibiting bacterial growth is quite significant.

Studies have shown that linoleic acid has significant antibacterial activity against some pathogenic *bacteria*. It is reported that LA is bactericidal against *Staphylococcus aureus*, one of the common causes of skin infections and foodborne illnesses (Joujou et al., 2024). The underlying mechanism of this activity is primarily attributed to LA's ability to disrupt bacterial cell membranes, thereby causing cell lysis (King et al., 2012). The role of LA as an antimicrobial agent has been validated through studies that examined the minimum inhibitory

concentration of various bacterial strains, including both Gram-positive and Gram-negative species. Skalicka-Woźniak et al. conducted a comprehensive assessment of the antimicrobial activity of various fatty acids, including linoleic acid, and highlighted their effects on various strains of *bacteria*, although further specifications regarding their effectiveness against specific strains are still needed (Skalicka-Woźniak et al., 2010). In addition to linoleic acid, F4 also contains several other compounds such as 4-dodecylphenol and myristamine oxide that contribute to the antibacterial effect. 4-Dodecylphenol is known to have extensive activity against Gram-positive and Gram-negative *bacteria* (Imazato et al., 2002). Myristamine oxide, which is a surfactant amine oxide, exhibits a strong antibacterial effect with a bacterial cell membrane disruption mechanism, increasing the effectiveness of the fraction in fighting microorganisms (Ndesendo et al., 2008).

Thus, the antibacterial effect of fraction 4 is due to the presence of linoleic acid as the main bioactive compound that works to inhibit bacterial growth, supported by other compounds that also have antibacterial activity. This combination strengthens the potential of fraction as a natural antibacterial source that can be utilized for pharmaceutical or *biotechnology* applications.

CONCLUSION

Endophytic fungi were successfully isolated from white turmeric rhizomes (*Curcuma zedoaria*) from NTB, confirming the presence of cultivable endophytes with antibacterial activity against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739. Stronger antibacterial effects were observed in isolates cultured in Potato Dextrose Broth (PDB) compared to Yeast Malt Broth (YMB). Secondary metabolite fractions were isolated from the TP 3 extract cultured in PDB, with linoleic acid identified as the main compound in Fraction 4 (F4), which showed an MIC of 200 µg/mL against *S. aureus*, similar to the TP 3 isolate itself, but demonstrated less activity against *E. coli*. Future research could explore the synergistic effects of compound mixtures and assess additional endophytic fungi from diverse regions to identify more potent antimicrobial agents.

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REFERENCES

Adisasmito, A. W. (2016). Penggunaan Antibiotik Pada Terapi Demam Tifoid Anak Di RSAB Harapan Kita. In Sari Pediatri. https://doi.org/10.14238/sp8.3.2006.174-80

Aizawa, S.-I. (2014). Chapter 4 - Bacillus subtilis — The Representative of Gram-Positive Bacteria. In S.-I. Aizawa (Ed.), The Flagellar World (pp. 22–23). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-417234-0.00004-9

Alam, A. (2016). Pola Resistensi Salmonella Enterica Serotipe Typhi , Departemen Ilmu Kesehatan Anak RSHS, Tahun 2006–2010. In Sari Pediatri. https://doi.org/10.14238/sp12.5.2011.296-301

Aprinuha, R. L., Ariastuti, R., & Khusna, K. (2023). Tingkat Pengetahuan Masyarakat RW 02 Desa Menang Kabupaten Ponorogo Terhadap Penggunaan Antibiotik. In Sehatmas Jurnal Ilmiah Kesehatan Masyarakat. https://doi.org/10.55123/sehatmas.v2i1.1338

Ardenkjær-Larsen, J. H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M. H., Servin, R., Thaning, M., & Golman, K. (2003). Increase in Signal-to-Noise Ratio of ≫ 10,000 Times in Liquid-State NMR. In Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.1733835100

Bieri, M., Kwan, A. H., Mobli, M., King, G. F., Mackay, J. P., & Gooley, P. R. (2011). Macromolecular NMR Spectroscopy for the Non-spectroscopist: Beyond Macromolecular Solution Structure Determination. In Febs Journal. https://doi.org/10.1111/j.1742-4658.2011.08005.x

Dewi, N. P. K. C., Hidayati, A. R., & Hanifa, N. I. (2023). Aktivitas Antibakteri Senyawa Fenolik Dari Fraksi Kulit Buah Kopi Robusta (Coffea Canephora L.). In Unram Medical Journal. https://doi.org/10.29303/jku.v12i3.952

Fadiji, A. E., & Babalola, O. O. (2020). Elucidating Mechanisms of Endophytes Used in Plant Protection and Other Bioactivities With Multifunctional Prospects. 8(May), 1–20. https://doi.org/10.3389/fbioe.2020.00467 bakteri

Fan, Y., Fu, Y.-H., Fu, Q., Cai, J., Xin, H., Dai, M., & Jin, Y. (2016). Purification of Flavonoids From Licorice Using an Off-Line Preparative Two-Dimensional Normal-Phase Liquid Chromatography/Reversed-Phase Liquid Chromatography Method. In Journal of Separation Science. https://doi.org/10.1002/jssc.201501393

Faramayuda, F., Riyanti, S., Pratiwi, A. S., Mariani, T. S., Elfahmi, E., & Sukrasno, S. (2021). Isolasi Sinensetin Dari Kumis Kucing (Orthosiphon Aristatus Blume Miq.) Varietas Putih. In JPSCR Journal of Pharmaceutical Science and Clinical Research. https://doi.org/10.20961/jpscr.v6i2.48084

M. Y. Hindiyeh, D. Liu, A. Sails, P. Spearman, & J.-R. Zhang (Eds.), Molecular Medical Microbiology (Third Edition) (Third Edit, pp. 655–679). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-818619-0.00026-5

Frydman, L., Scherf, T., & Lupulescu, A. (2002). The Acquisition of Multidimensional NMR Spectra Within a Single Scan. In Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.252644399

Garcí, M., Sarabia, F., Dí, A., & López-romero, J. M. (2023). Approved antibacterial drugs in the last 10 years : from the bench to the clinic. 180–209. https://doi.org/10.37349/eds.2023.00013

Garcia, J. (2012). Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant sapindus saponaria l. Journal of Applied Pharmaceutical Science. https://doi.org/10.7324/japs.2012.21007

GERASIMOVA, V. V, Kaloyanov, N., Karsheva, M., Peycheva, M. F., & Stoilova, N. (2019). HPLC-DAD Method for Simultaneous Determination of Natural Polyphenols. https://doi.org/10.17352/ojabc.000009

Gummadi, S., Siyyadri, D. S., & Gummadi, S. (2020). NUCLEAR MAGNETIC RESONANCE AND HYPHENATIONS – A REVIEW. In International Journal of Pharmaceutical Sciences and Research. https://doi.org/10.13040/ijpsr.0975-8232.11(12).5932-50

Harmileni, Saragih, G., Hidayani, T. R., Mirnadaulia, M., Ginting, C. N., & Fachrial, E. (2023). Mikroba Endofit dalam Dunia Kesehatan: Manfaat dan Aplikasi.

Hermiaty, H., Karim, M., Latief, S., Hidayati, P. H., & Ramadhani, S. (2021). Perbedaan Uji Efektivitas Ekstrak Buah Pepaya Dan Mentimun Terhadap Bakteri Salmonella Thypi. In Fakumi Medical JournalJurnal Mahasiswa Kedokteran. https://doi.org/10.33096/fmj.v1i1.6

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Asif, M., Hussain, T., Ali, M., Rafiq, M., & Atif, M. (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1), 7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Kogel, K. H., Franken, P., & Hückelhoven, R. (2006). Endophyte or parasite - what decides? Current Opinion in Plant Biology, 9(4), 358–363. https://doi.org/10.1016/j.pbi.2006.05.001

Li, B., Yuling, Z., Changting, L., Zhenhong, C., & Dongsheng, Z. (2014). Molecular pathogenesis of Klebsiella pneumoniae. November. https://doi.org/10.2217/fmb.14.48

Mazaheri, M., & Moosavi-Movahedi, A. A. (2021). Biodiversity and Drug Discovery Approach to Natural Medicine BT - Rationality and Scientific Lifestyle for Health (A.

A. Moosavi-Movahedi (ed.); pp. 61–74). Springer International Publishing. https://doi.org/10.1007/978-3-030-74326-0_4

Mulat, D., Latva-Mäenpää, H., Koskela, H., Saranpää, P., & Wähälä, K. (2014). Rapid Chemical Characterisation of Stilbenes in the Root Bark of Norway Spruce by Off-Line HPLC/DAD-NMR. In Phytochemical Analysis. https://doi.org/10.1002/pca.2523

Neves, P. R., McCCulloch, J. A., Mamizuka, E. M., & Lincopan, N. (2014). PSEUDOMONAS | Pseudomonas aeruginosa. In Encyclopedia of Food Microbiology (Second Edition) (pp. 253–260).

Parikesit, Okubo, S., Husodo, T., Takeuchi, K., & Muhamad, D. (2012). Biodiversity Issues in Indonesia, with Special Reference to Biodiversity in Human-Dominated Landscapes BT - The Biodiversity Observation Network in the Asia-Pacific Region: Toward Further Development of Monitoring (S. Nakano, T. Yahara, & T. Nakashizuka (eds.); pp. 93–110). Springer Japan. https://doi.org/10.1007/978-4-431-54032-8_8

Permana, P. B. D., Widodo, A. D. W., Setyaningtyas, A., & Wahyunitisari, M. R. (2024). Factors Associated With Culture-proven Neonatal Sepsis and Resistance to First-line

Antibiotics in Indonesia. The Pediatric Infectious Disease Journal 43(1):p 5. https://doi.org/10.1097/INF.0000000000004108

Petrosyan, K., Thijs, S., Piwowarczyk, R., Rurаż, K., Vangronsveld, J., & Kaca, W. (2022). Characterization and diversity of seed endophytic bacteria of the endemic holoparasitic plant Cistanche armena (Orobanchaceae) from a semi-desert area in Armenia. Seed Science Research, 32(4), 264–273. https://doi.org/DOI: 10.1017/S0960258522000204

Prastiyanto, M. E., Iswara, A., Khairunnisa, A., Sofyantoro, F., Siregar, A. R., Mafiroh, W. U., Setiawan, J., Nadifah, F., Wibowo, A. T., & Putri, W. A. (2024). Clinical Infection in Practice Prevalence and antimicrobial resistance profiles of multidrug-resistant bacterial isolates from urinary tract infections in Indonesian patients : A cross-sectional study. Clinical Infection in Practice, 22(March), 100359. https://doi.org/10.1016/j.clinpr.2024.100359

Primasari, F. S., Puspitasari, I., & Nuryastuti, T. (2022). Prevalensi Bakteri Resisten Karbapenem Di RSUP Dr. Sardjito Periode Januari-Agustus 2020. In Majalah Farmaseutik. https://doi.org/10.22146/farmaseutik.v18i3.65823

Priyanto, J. A., Prastya, M. E., Sinarawadi, G. S., Datu, W., Yolanda, T., Ika, A., Yanuar, A., Azizah, E., Tachrim, Z. P., & Mozef, T. (2022). The antibacterial and antibiofilm potential of Paederia foetida Linn . leaves extract. 12(10), 117–124. https://doi.org/10.7324/JAPS.2022.121012

Rismarini, R., Anwar, Z., & Merdjani, A. (2016). Perbandingan Efektifitas Klinis Antara Kloramfenikol Dan Tiamfenikol Dalam Pengobatan Demam Tifoid Pada Anak. In Sari Pediatri. https://doi.org/10.14238/sp3.2.2001.83-7

Rocha, F. S., Gomes, A. J., Lunardi, C. N., Kaliaguine, S., & Patience, G. S. (2018). Experimental Methods in Chemical Engineering: Ultraviolet Visible Spectroscopy—UV-Vis. In The Canadian Journal of Chemical Engineering. https://doi.org/10.1002/cjce.23344

Sari, R., Maryam, M., & Yusmah, R. A. (2023). Penentuan C-Organik Pada Tanah Untuk Meningkatkan Produktivitas Tanaman Dan Keberlanjutan Umur Tanaman Dengan Metoda Spektrofotometri Uv Vis. In Jurnal Teknologi Pertanian. https://doi.org/10.32520/jtp.v12i1.2598

Singh, S., Kumar, K., Rao, A., & Kumar Prajapti, V. (2024). Bioinformatics Approaches in Studying the Fungal Endophyte-Derived Bioactive Compounds with Pharmacological Relevance. Fungal Biology Endophytic Fungi, 191–208. https://doi.org/https://doi.org/10.1007/978-3-031-49112-2_9

Toxicity Activity of Endophytic Bacteria Associated With *Curcuma Zedoaria*. In Iop Conference Series Earth and Environmental Science. https://doi.org/10.1088/1755-1315/308/1/012034

Tanaka, M., Sukiman, H., Takebayashi, M., Saito, K., Suto, M., Prana, T. K., Prana, M. S., & Tomita, F. (1999). Isolation, Screening and Phylogenetic Identification of Endophytes from Plants in Hokkaido Japan and Java Indonesia. Microbes and Environments, 14(4), 237–241. https://doi.org/10.1264/jsme2.14.237

Venter, A., Joubert, E., & Beer, D. de. (2013). Characterisation of Phenolic Compounds in South African Plum Fruits (Prunus Salicina Lindl.) Using HPLC Coupled With Diode-Array, Fluorescence, Mass Spectrometry and on-Line Antioxidant Detection. In Molecules. https://doi.org/10.3390/molecules18055072

Widodo, A., Permana, P., Setyaningtyas, A., & Wahyunitisari, M. (2024). Multidrug-Resistant Acinetobacter and Enterobacterales Causing Neonatal Sepsis at a Tertiary Healthcare Facility in Indonesia. Journal of Neonatology. https://doi.org/10.1177/09732179231218825

Windasari, W., & Sari, E. M. (2021). Kandungan Nitrit (No2) Dan Nitrat (No3) Dalam Air Minum Di Desa Ciketing Udik Kecamatan Bantar Gebang Kota Bekasi. In Jurnal Mitra Kesehatan. https://doi.org/10.47522/jmk.v3i2.79

Wurarah, M., & Mokosuli, Y. S. (2022). Inhibition of Bacterial Growth of Leilem Leaf Extract (Clerodendrum Minhassae Teijsm. &Amp;amp; Binn). In Jurnal Biologi Tropis. https://doi.org/10.29303/jbt.v22i2.3394

Xu, H. (2023). Emnpd: a comprehensive endophytic microorganism natural products database for prompt the discovery of new bioactive substances. Journal of Cheminformatics, 15(1). https://doi.org/10.1186/s13321-023-00779-9

Yuniar, I., Karyanti, M. R., Tambunan, T., & Rizkyani, N. A. (2016). Evaluasi Penggunaan Antibiotik Dengan Kartu Monitoring Antibiotik Gyssens. In Sari Pediatri. https://doi.org/10.14238/sp14.6.2013.384-90

Zhao, X., Hou, D., Xu, J., Wang, K., & Hu, Z. (2022). Antagonistic Activity of Fungal Strains against Fusarium Crown Rot. Plants (Basel, Switzerland), 11(3). https://doi.org/10.3390/plants11030255