Marine-Derived Matairesinol: A Potential Antimicrobial Agent Against *Klebsiella pneumoniae* from Sponge-Associated *Trichoderma longibrachiatum*

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Abstract

The fungus Trichoderma sp., which is associated with marine sponges, produces various antibacterial compounds that can be used as lead compounds for new antibiotics. The ethyl acetate extract of the sponge-associated fungus Trichoderma longibrachiatum contains an active compound that inhibits the growth of Klebsiella pneumoniae. This study aimed to identify the sponge host and determine the active compound produced by this fungus with anti-K. pneumoniae activity. This study began with a sample of fungal cultures and the identification of the host sponge. The extract samples were obtained using a partition method between methanol and ethyl acetate. Determination of antibacterial compounds in ethyl acetate extracts using disc diffusion and bioautography. Active compounds were identified using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Nuclear Magnetic Resonance Spectroscopy (NMR). The results showed that the host sponge of T. longibrachiatum is Axinella sp. The ethyl acetate extract exhibited antibacterial activity against K. pneumoniae, with inhibition zones of 10.12 and 14.45 mm at doses of 250 and 500 µg discs^{-1}, respectively. The bioautography test revealed that the active fraction had a retardation factor (Rf) of 0.14. The active compound was isolated and anti-K. pneumoniae was identified as matairesinol ($C_{20}H_{22}O6$), also known as (3R,4R)-3,4-bis[(4-hydroxy-3-methoxyphenyl) methyl]oxolan-2-one). Matairesinol is a polyphenol from the lignan group with a gamma (γ)-lactone ring. The isolation of matairesinol as an active antibacterial compound against K. pneumoniae provides valuable insights into the potential of marine-derived fungi as a source of novel antimicrobial agents.

Keywords*: Trichoderma longibrachiatum, associated fungus, Axinella sp., matairesinol*

Introduction

The issue of bacterial resistance to antibiotics is a significant health concern in various countries, including Indonesia, Timor Leste, Papua New Guinea, Singapore, Myanmar, Thailand, Cambodia, and India (Lo *et al*., 2019). The main causes of infection in Indonesia are *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter* spp., and *Staphylococcus aureus* (Dahesihdewi *et al*., 2019). *A. baumannii* showed 66.0% resistance to meropenem, whereas *Klebsiella* spp. exhibited 67.14% resistance to cefotaxime in a hospital setting (Sokolović *et al*., 2023). *S. epidermidis*, the most common gram-positive isolate, showed high resistance rates of 90% to penicillin G and roxithromycin and 88% to several other antibiotics, including cefotaxime, imipenem, and piperacillin-tazobactam (Tessema *et al*., 2021).

K. pneumoniae produces a protective polysaccharide capsule to survive environmental pressure (Adwan *et al.,* 2020). Bacteria can spread through raw or fast food and are resistant to some antibiotics. The discovery of MDR *K. pneumoniae* strains in the food chain has contributed to the increased transmission of pathogens between humans, leading to the spread of infectious diseases (Hartantyo *et al*., 2020). In Indonesia, *K. pneumoniae* is resistant to several cephalosporins (cefazolin, ceftazidime, ceftriaxone, cefepime) and monobactams. This is because *K. pneumoniae* has extended-spectrum β-lactamase (ESBL) enzymes that can hydrolyze β-lactam class antibiotics (penicillin, first, second, and third generations), such as cephalosporins and monobactams (Elmawati *et al.,* 2021). MDR *K. pneumoniae* often infects chickens (Permatasari *et al*., 2020) and pets, such as cats (Ramadhan *et al*., 2021). The increasing prevalence of diseases caused by MDR *K. pneumoniae* can endanger public health because it allows transmission between humans and animals.

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https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.31.2.159-168
Received : 13-01-2026
Accepted : 22-05-2026

The discovery of new antibacterial compounds that can kill resistant bacteria is crucial for addressing this problem. Natural resources, including marine resources, are alternative sources for new antibacterial agents (Jakubczyk and Dussart, 2020; Arumawati *et al.,* 2026). Marine organisms contain both primary and secondary metabolites. Several secondary metabolites possess antibacterial properties, which is the first step in discovering new antibacterial compounds.

Trichoderma spp. produce many secondary metabolites with diverse biological activities. This fungus is found in several terrestrial and marine ecosystems globally (Li *et al*., 2019). Representing terrestrial regions, *Trichoderma* spp. are widespread in the soils of the rhizosphere in many regions and are mainly composed of *T. harzianum* and *T. longibrachiatum* (Almashhadani and Qassim, 2025b). *T. harzianum* from the soil is potent as a biocontrol agent against the pathogen of *Alternaria alternata*. The production of hydrolytic enzymes is an essential aspect for the development of antagonism (Qassim *et al*., 2026). Other studies have shown that the mycelial aqueous extract of *T. longibrachiatum* from Mosul (Iraq) exhibits potent antibacterial activity against MDR pathogens (*Pseudomonas aeruginosa, S. aureus, E. coli, and K. pneumoniae*) (Almashhadani and Qassim, 2025a).

Moreover, this equivalence extends to fungi derived from marine ecosystems. Ethyl acetate extracts from *Trichoderma* spp.-associated mangrove ecosystems have been reported to exhibit antibacterial activity against *Bacillus cereus*, *E. coli*, *P. aeruginosa*, and *S. aureus* (Basiriya *et al*., 2017). An ethyl acetate extract derived from the fungus *T. longibrachiatum*, isolated from soft corals in the waters of Panjang Island, Jepara (Central Java, Indonesia), inhibited the growth of MDR *S. haemolyticus.* The inhibition zone diameter was 12.2 mm at a concentration of 300 µg.disc^{-1} (Sabdaningsih *et al*., 2017). Previously, the ethyl acetate extract of *T. longibrachiatum* associated with the sponge *Haliclona* sp. from Sulawesi (Indonesia) contained vertinoid polyketides, such as epoxysorbicillinol (Sperry *et al*., 1998). Other researchers have identified several antibacterial compounds produced by *Trichoderma* sp., including trichodermaquinone ($C_{14}H_{12}O_2$), which can inhibit Methicillin-resistant *S. aureus* (MRSA) (Khamthong *et al*., 2012); trichodin A ($C_{21}H_{25}NO_3$), B ($C_{26}H_{33}NO_7$); and pyridoxatin ($C_{15}H_{21}NO_3$), which have antibacterial activity against *S. aureus* and *S. epidermidis* (Wu *et al*., 2014).

The fungus *T. longibrachiatum*, associated with a sponge (code TE-F-03) collected from Falajava Beach, Ternate Island, North Maluku Province (Indonesia), produces metabolites that inhibit the growth of the MDR pathogen *K. pneumoniae* (Sedjati *et al*., 2020). These bioactive metabolites have been identified in the ethyl acetate extract of this fungus (Sedjati *et al*., 2022). Given this background, the present study aimed to identify the host sponge and isolate an active compound against K. *pneumoniae*, produced by *T. longibrachiatum*. Furthermore, this study is expected to contribute to the discovery of novel marine-derived antibacterial compounds with potential pharmaceutical applications.

Materials and Methods

Sponge sample TE-F-03

A sponge with the code TE-F-03 (host of *T. longibrachiatum*) was collected and preserved in alcohol 95% as in a previous study by Sedjati *et al*. (2020). The sponge was collected from the waters of Falajava Beach, Ternate Island, North Maluku, Indonesia, in April 2019.

Sponges were identified using the method described by Hooper (2003). Macroscopic observations were performed by examining the color and shape of the sponge surface, and histological tests were performed to observe the morphology of the spicules. Small sponge fragments were cut and treated with sodium hypochlorite (NaClO) to remove the organic components. After only the mineral skeleton remained, it was washed several times with an ethanol solution. The spicules were placed on glass slides and observed under a microscope.

Trichoderma longibrachiatum isolate and ethyl acetate extract preparation

The isolate was identified with 99.84% homology to *T. longibrachiatum* strain IHB F 539 (Accession Number HM461859.1). Macroscopically and microscopically, it is characterized by rapidly growing green colonies on a plate, covered with white-cottony mycelia, long, thin, branched conidiophores with closely packed green globose conidia on the tips of phialides, formed in chains (Sedjati *et al*., 2020). The ethyl acetate extract derived from *T. longibrachiatum* associated with the sponge (code TE-F-03) was extracted and collected at the Marine Natural Product Laboratory, Diponegoro University, as described in a previous study by Sedjati *et al*. (2022). The fungus was cultured using Malt Extract Agar (MEA, Merck) medium for 9 days (24 h of dark, static conditions, pH 5.6, 60‰ salinity, and 27 °C temperature). After nine days of culture in MEA, *T. longibrachiatum* was harvested. The biomass and medium were macerated using methanol, partitioned with ethyl acetate, and added until the two fractions were completely separated. The ethyl acetate fraction

was further concentrated using a rotary evaporator to obtain an ethyl acetate extract.

Metabolite profiling of the extract

Metabolite profiling was performed using thin layer chromatography (TLC). The ethyl acetate extract was spotted on the baseline surface of a TLC plate (Merck, silica gel 60 F254) and eluted with the appropriate mobile phase, resulting in perfectly separated spots. The mobile phases used were mixtures of n-hexane and ethyl acetate (4:1, 3:2, 2:3, 1:4, and 0:5) in order of increasing polarity. The spots were visualized under 365 nm UV light and with 2% vanillin-H_2SO_4. Spot identification was based on the retardation factor (Rf= migration distance of the metabolite fraction/migration distance of the mobile phase).

Klebsiella pneumoniae pathogen

The pathogen was a multidrug-resistant (MDR) strain of *K. pneumoniae* resistant to at least five types of antibiotics (Ampicillin, Ceftazidime, Cefotaxime, Ceftriaxone, and Ciprofloxacin). Pure fungal cultures were obtained from the Microbiology Laboratory of Diponegoro National Hospital (RSND) in Semarang, Central Java Province.

Anti-Klebsiella pneumoniae test using disc diffusion and bioautography

An *anti-K. pneumoniae* was performed using a disc diffusion assay, as described by Trianto *et al*. (2017). The pathogenic bacteria were subsequently recultivated in Mueller-Hinton Broth (MHB Merck). A pathogen suspension with a density of 0.5 McFarland (1.5×10^8 CFU.ml^{-1}) was inoculated onto Mueller-Hinton Agar (MHA Merck) using the swab method for the antagonist test. Approximately 10 µL of the extract solution (in DMSO) was dripped onto the surface of disc paper (Oxoid, 6 mm) to obtain final concentrations of 250 and 500 µg discs^{-1}. The extract-impregnated disc paper was placed in a culture medium containing pathogens and incubated at 37°C for 24 h. The appearance of a clear zone indicated the presence of *anti-K. pneumoniae* compound.

Bioautography was performed using contact techniques (contact bioautography), as described by Sedjati *et al*. (2022). The extract in ethyl acetate was spotted on the baseline surface of the TLC plate and eluted with a mobile phase of n-hexane: ethyl acetate (2:3). The TLC plate was positioned with its surface facing downward onto MHA media, which had been inoculated with *K. pneumoniae*, and left there for 6 min. Subsequently, the TLC plate was removed from the test bacterial medium, and the Petri dish was sealed. Incubation was conducted for 24 h at 37 °C, and a clear zone was observed around the TLC spot.

Isolation of the anti-Klebsiella pneumoniae compound

The active compound was isolated using open-column chromatography (using silica gel 60–120 mesh) with the mobile phase referring to the TLC profile using the technique described by Sedjati *et al*. (2022). The extract was eluted using a solvent sequence based on increasing polarity. Finally, the active compound was identified, and its name and chemical structure were determined.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) Analysis

Identification of anti-*K. pneumoniae* compound isolate, and the molecular weight was determined using an LC-MS/MS instrument (Electro Spray Ionization–Mass Spectrometry; ESI-MS/ tandem MS1 and MS2) at the Research Center for Chemistry, National Research and Innovation Agency (BRIN), Serpong, Banten. Indonesia. The LC-MS/MS analysis results were referenced from Pitt (2009).

Nuclear Magnetic Resonance (NMR) spectroscopy analysis

Isolates of the anti-*K. pneumoniae* compounds were analyzed at the Research Center for Chemistry, BRIN, using a JEOL NMR spectrometer (JNM-ECZ500R series) with deuterated methanol (CD_3OD) as the solvent. The chemical structure of the isolate was determined based on the results of spectroscopic tests of the atomic nucleus of the proton ^{1}H-NMR and carbon ^{13}C-NMR, based on the chemical shift value (δ ppm), as well as the breakdown of the proton signal (singlet, doublet, doublet of doublets, and multiplet) and its J coupling. This spectral pattern was subsequently confirmed by Shoeb *et al*. (2004), Smeds *et al*. (2012), and Jenie *et al*. (2014), and assisted by the ChemDraw application.

Data analysis

Data analysis was conducted using descriptive methods and visual representations, such as graphs and tables. Chemical structure prediction and illustration served as analytical tools for interpreting LC-MS/MS and NMR spectral profiles with the aid of comparative references and the ChemDraw software application.

Result and Discussion

Trichoderma longibrachiatum isolate and host sponge

The fungus associated with the sponge (code TE-F-03) exhibited *anti-K. pneumoniae* compound was identified as *T. longibrachiatum* in a previous study by Sedjati *et al*. (2020). The Identification was performed molecularly and morphologically to ensure

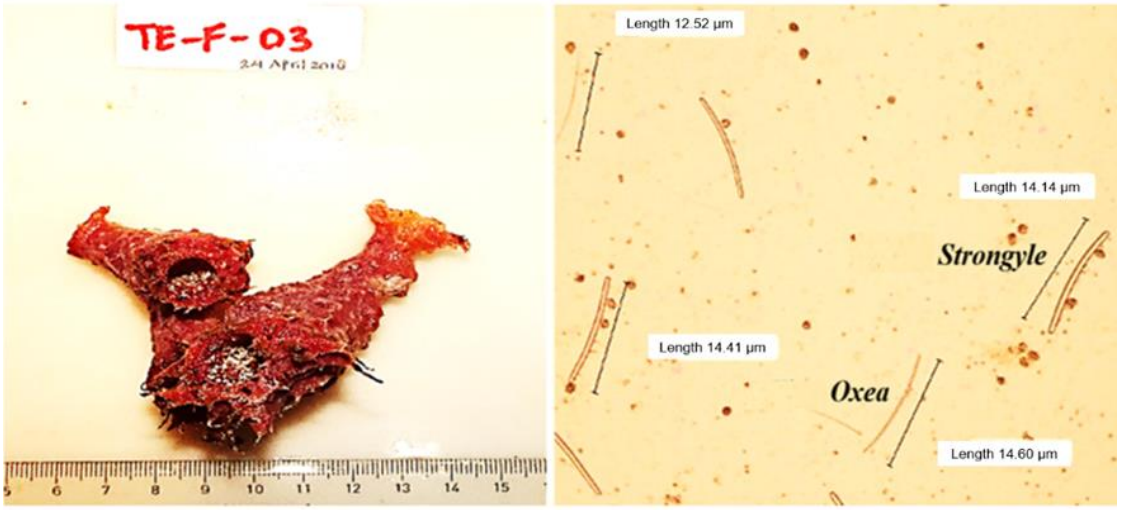
that the isolate was *T. longibrachiatum*. According to Almashhadani and Qassim (2025b), phenotypic and molecular-based approaches with the integration of results are the most suitable for the identification of *Trichoderma* spp., as morphology frequently changes with different environmental conditions and culture media. In a recent study, the host sponge associated with the fungus was identified.

According to Hooper's guidelines (2003), *Axinella* sp. is the host of *T. longibrachiatum*. The shapes, colors, and spicules of the sponges are shown in Figure 1. The identification results, according to the statement by Qu *et al*. (2012), *Axinella* (Demospongiae; Halichondrida; Axinellidae) is a genus of sponges having an orange or yellow color and spicules of various types. The Axinellidae family has three types of spicules: oxea, style, and strongyle. The style is a spicule with one pointed end and the other (head or base) blunt, with both ends sharp, whereas a strongyle has both ends blunt. Alvarez *et al*. (2016) added that the shape of the spicules of *Axinella* sp. is generally oxea 11.8–19.8 µm long. Therefore, the morphological observations in this study are consistent with previous descriptions of *Axinella* sp.

Active compound as an anti-Klebsiella pneumoniae

The ethyl acetate extract derived from *T. longibrachiatum* exhibited inhibitory effects against MDR *K. pneumoniae*. Disc diffusion assays revealed inhibition zones of 10.12 and 14.45 mm in diameter at concentrations of 250 and 500 µg discs^{-1}, respectively (Figure 2a). Bioautographic analysis further substantiated these findings by identifying active spots with anti-*K. pneumoniae* infection (Figure 2b). The antibacterial compound was detected at an Rf value of 0.14, exhibiting reactivity to both 365 nm ultraviolet light and a 2% vanillin-H2S04 staining reagent. A low Rf value indicates a polar compound, suggesting potential implications for its antimicrobial properties. Nichols (2022) reports that visualization under ultraviolet light demonstrates particular efficacy for aromatic compounds and extensively conjugated systems. The versatility of vanillin stains is evident in their broad applicability to various nucleophiles of both high and low strengths, encompassing alcohols and amines, as well as their utility in detecting a diverse array of aldehydes and ketones.

The first step in anti-*K. pneumonia* compound identification process in the current study was based on the results of LC-MS/MS analysis. This analysis was used to determine the molecular profiles of the secondary metabolites present in the active extract of *T. longibrachiatum*. The chromatogram profile of the LC results obtained using tandem MS/MS is shown in Figure 3a. Only the compounds with the highest peaks were analyzed owing to the impurities in the isolates of the active compounds. In this study, dilute formic acid gave rise to a molecular ion, $[M+H]^+$, which was observed at m/z=359.14 in the MS1 spectrum (Figure 3b). If the mass spectrogram peak value is assumed to be $[M+H]^+$, then the actual m/z value of the peak is 358.14. The anti-*K. pneumoniae* was identified as matairesinol.



a b

Figure 1. The host sponge of *T. longibrachiatum* produces anti-*K. pneumoniae* compounds: (a) Sponge shape and color, (b) Shape of spongy spicules

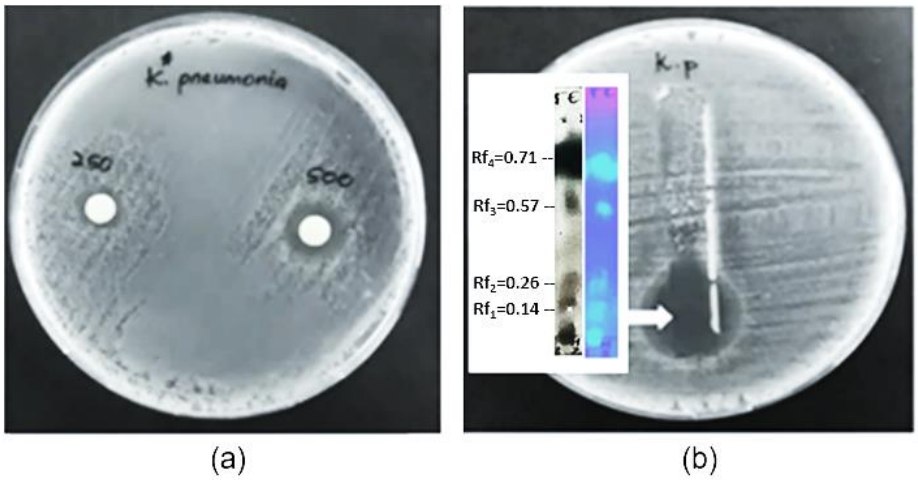
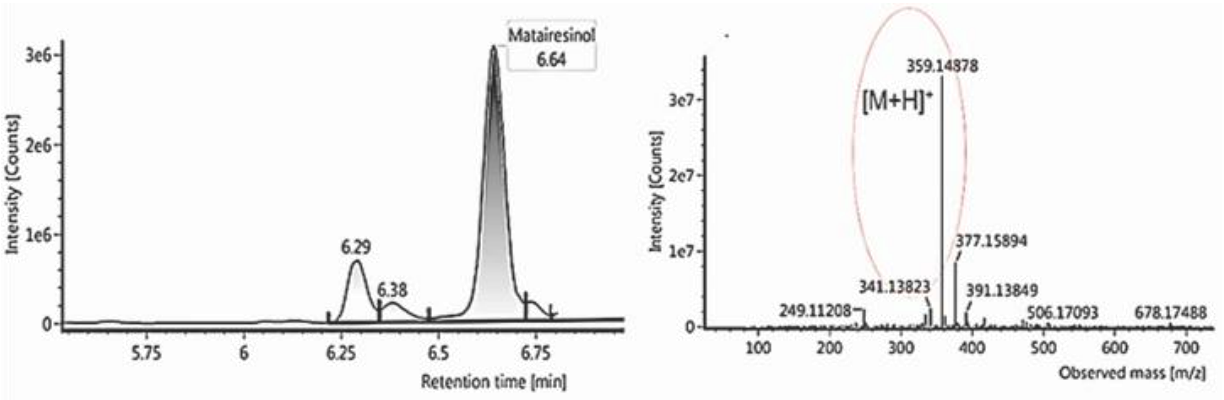


Figure 2. *Anti-K. pneumonia* test of ethyl acetate extract of *T. longibrachiatum*: (a) inhibitory zone at doses of 250 and 500 µg discs^{-1}, (b) bioautographic profile of the active isolate (TLC's mobile phase is n-hexane: ethyl acetate 2:3, visualized with 2% vanillin-H_2SO_4 and UV 365 nm)



	Expected m/z	Status	Observed m/z
1	95.04914	Theoretical	95.04918
2	107.04914	Theoretical	107.04890
3	125.05971	Theoretical	125.05914
4	205.08592	Theoretical	205.08599
5	207.10157	Theoretical	207.10301
6	219.10157	Theoretical	219.10214
7	221.08084	Theoretical	221.08027
8	329.13835	Theoretical	329.13748
9	341.13835	Theoretical	341.13794

	Detector counts	Observed RT (min)	Formula
1	1287246	6.64356	C6H7O
2	41888	6.63936	C7H7O
3	59592	6.64741	C7H9O2
4	369333	6.64441	C12H13O3
5	167876	6.64292	C12H15O3
6	140248	6.64431	C13H15O3
7	177725	6.64432	C12H13O4
8	165174	6.63600	C19H21O5
9	1251270	6.64457	C20H21O5

(c)

Figure 3. LC-MS/MS profiles of isolates of antibacterial compounds: (a) LC chromatogram (gradient system; mobile phase 0.1% formic acid in water and 1% formic acid in acetonitrile), (b) The molecular spectrum of the ion $[M+H]^+$ in MS1, (c) Fragment ion at MS2, MS/MS

The accuracy of the identification of matairesinol in the active compound using NMR can be applied to determine the arrangement and number of atoms in an isolated organic molecule. According to Jenie *et al*. (2014), the chemical shift region of the proton or ^{1}H-NMR (δH) has a value range between 13-0 ppm. The carboxylic group protons (-COOH) appear in the downfield region between 13-10 ppm, aldehydes (-CHO) between 10-9 ppm, aromatics between 8.5-7 ppm, amides (-$CONH_2$) between 8-5 ppm, double bonds (HC=CH) between 7-4.5 ppm, methoxy (-O-CH_3) between 4-3.5 ppm, methine (-CH),

and methylene (-CH_2) in the upfield area between 3.2-2 ppm. Meanwhile, the ^{13}C-NMR (δC) chemical shift region ranges from 210-0 to ppm. The area of δC was between 210-0 ppm. The carbon signal of the carbonyl compounds (ketones and aldehydes) appeared at approximately 200 ppm. The carbonyl carbon is highly deshielded; thus, it has a high chemical shift value. The following groups appeared as esters and carboxylates: the aromatic carbon group appears between 160-110 ppm, methoxy between 62-32 ppm, and methine and methylene between 50-30 ppm.

The compound arrangement or number of atoms was analyzed by tracing the δH and δC values. Similar to the depiction of the atomic positions in matairesinol in Figure 4, the chemical shift was determined by Shoeb *et al*. (2004) and assisted by the prediction of proton and carbon nucleus signals using the ChemDraw application. The δH and δC values were complemented by the signal shape and proton coupling J (Hz) of the isolated anti-K. *pneumoniae* compounds are listed in Table 1. Matairesinol compounds contain several functional groups that facilitate signal detection in the NMR spectrum. The functional group consists of two aromatic groups, two methoxy groups (O-CH_3), two methylene groups (CH_2), two methane groups (CH), and one ester bond in the gamma (γ)-lactone ring. The observed outcome aligns with the TLC profile of the compound (Figure 2b), demonstrating the characteristics of a polar substance that responds to UV visualization (suggesting the existence of aromatic moieties and conjugated systems) and vanillin staining (indicating the presence of alcohol and ketone functionalities).

Based on the compatibility of the signals from the results of ^{1}H and ^{13}C-NMR spectroscopy, as reported by Basir and Eliza (1999), Shoeb *et al*. (2004), Smeds *et al*. (2012), Jenie *et al*. (2014), and anti-*K. The pneumonia* compound isolated in this study was matairesinol. The δH signal of matairesinol has a value of less than 7 ppm, as well as the J coupling signal splitting of the proton on the aromatic group in the meta (m) position, approximately 1-3 Hz, and ortho (o) around 6-9 Hz. Meanwhile, the δC signal of matairesinol had the highest value at approximately 180 ppm. ^{1}H and ^{13}C-NMR spectra of the compounds isolated in this study. These identification results were supported by the LC-MS/MS test results shown in Figure 3. The peak in the mass spectrogram of the molecular ion indicates the presence of a compound with a molecular weight of 358.14 g.mol^{-1}. This corresponds to the molecular weight of maireresinol with the chemical formula $C_{20}H_{22}O_6$.

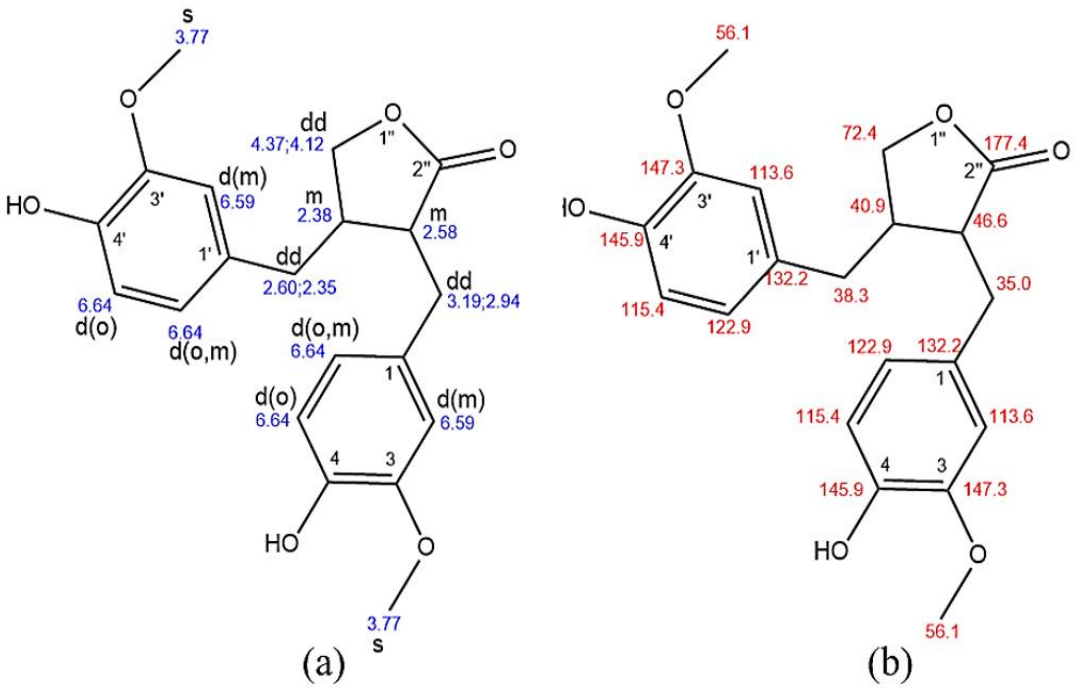


Figure 4. Matairesinol chemical structure and NMR signal characteristics (using the ChemDraw application): (a) Prediction of the value of the chemical shift (δ) of the ^{1}H proton, the shape of the proton signal (s=single, d=doublet, dd=doublet of doublets, m= multiplet) and its position to the nearest H (o=ortho, m=meta), (b) Prediction of the value of the chemical shift (δ) of the ^{13}C

Table 1. Chemical shift (δ) of the ^{1}H proton, ^{13}C NMR, and coupling constant J of the matairesinol compound

Carbon no.	Chemical shift (δ) in ppm and coupling constant J (Hz) in parentheses			
	Anti-*K.pneumoniae* isolate		*Matairesinol as a comparison	
	δ_H	δ_C	δ_H	δ_C
1	-	129.95	-	130.2
2	6.89 (d, 2 Hz)	112.00	6.63 (d, 2 Hz)	112.7
3	-	146.50	-	147.8
4	-	142.00	-	145.0
5	6.94 (d, 8.5 Hz)	114.00	6.67 (d, 8.4 Hz)	114.9
6	6.71 (dd, 8.5; 2 Hz)	121.00	6.54 (dd, 8.4; 2 Hz)	121.8
7	2.78 (dd, 14.5;6 Hz)	33.14	2.81 (dd, 14.0; 5.6 Hz) 2.78 (dd, 14.0; 6.8 Hz)	34.1
1'	-	129.95	-	129.5
2'	6.89 (d, 2 Hz)	112.00	6.51 (d, 1.6 Hz)	112.1
3'	-	146.50	-	147.8
4'	-	142.00	-	145.2
5'	6.94 (d, 8.5 Hz)	114.00	6.64 (d, 8 Hz)	115.0
6'	6.71 (dd, 8.5; 2 Hz)	121.00	6.46 (dd, 8; 1.6 Hz)	121.0
7'	2.51 (m)	36.54	2.50 (m)	37.7
2"	-	178.15	-	180.4
3"	2.62 (m)	46.50	2.61 (m)	46.5
4"	2.46 (m)	40.21	2.48 (m)	41.3
5"	4.21 (m); 3.66 (m)	69.20	4.11 (dd, 9,2;7.6 Hz) 3.82 (dd, 8.8; 7.6 Hz)	71.7
O-CH_3(3)	3.60 (s)	57.50	3.70 (s)	55.1
O-CH_3(3')	3.60 (s)	57.50	3.70 (s)	55.2

Notes: - Proton signal forms: s = singlet, d = doublet, dd = doublet of doublets, m= multiplet
*) Spectrum of matairesinol in CD_3OD according to Shoeb *et al*. (2004)

LC-MS/MS analysis revealed that the compound identified as matairesinol was cis-matairesinol ((3R,4S) -3,4-bis[(4-hydroxy-3-methoxy phenyl)methyl] oxolan-2-one). cis-Matairesinol is a lignan in which the C3 and C4 positions are substituted with a 4-hydroxy-3-methoxybenzyl group (NCBI, 2021). These compounds have a dibenzylbutyractone skeleton (polyphenol, lignan, and γ-lactone families). Lactone is a cyclic ester of hydroxycarboxylic acid and is called γ because it is composed of five atoms in the ring. Matairesinol, an antibacterial compound, has been studied by Akiyama *et al*. (2007) and has bioactivity against *S. aureus.* There is a relationship between the stereoisomeric structure of matairesinol and its antibacterial activity. Stereoisomers or optical isomers occur because of chiral C atoms in organic compounds such as lignans. Two chiral C atoms in matairesinol are bound to the γ-lactone group (C3 and C4). According to various studies, matairesinol can be synthesized by higher plants, including grain-producing plants (Zanella *et al*., 2017; Zaki *et al*., 2023), vegetables, spices, fruits, and edible mushrooms (Lee *et al*., 2024). Matairesinol is a secondary metabolite belonging to the polyphenol group, produced by organisms to serve specific purposes, such as deterring predation by herbivores and insects (Singh *et al*., 2021) and providing chemical protection for grains (Tanase *et al*., 2019). Sharma *et al*. (2019) noted that polyphenols also function as a biochemical defense mechanism against abiotic stresses, including drought, salinity, heavy metals, extreme temperatures, and ultraviolet radiation.

One of the functions of matairesinol is its chemical defense against bacterial pathogens. According to Rempe *et al*. (2017), the antibacterial activity of most phenolic compounds is a membrane disruptor in both Gram-positive and Gram-negative bacteria. However, the exact mechanisms of action of most naturally derived phenolic compounds are not specific. The targets of phenolic compounds are diverse and include DNA, DNA gyrase, efflux pumps, carrier proteins, protein kinases, and helicases. Miklasińska-Majdanik *et al*. (2018) and Kauffmann and Castro (2023) revealed that polyphenols interact with proteins in biomembranes. As an antibacterial compound, the hydroxyl group (-OH) of the phenol ring can interact with the functional groups in the proteins that make up the bacterial cell membrane through ionic and hydrogen bonds. This interaction disrupts the stability and integrity of the cell wall, causing lysis and plasma leakage in pathogenic cells.

The newly identified aqueous extract of terrestrial *T. longibrachiatum* mycelia also exhibited antibacterial activity against MDR *K. pneumoniae*. Based on the Fourier Transform Infrared (FTIR) spectra, the presence of hydroxyl (–OH), carbonyl

(C=O), amine ($-NH_2$), and amide ($-CONH_2$) groups was observed (Almashhadani and Qassim, 2025a). Hydroxyl and carbonyl moieties were also identified in the matairesinol. The efficacy of anti-K. pneumoniae activities of aqueous and ethyl acetate extracts of *T. longibrachiatum* in the current study are predicted to be related to the hydroxyl group, which can cause damage to pathogenic cells through lysis.

Conclusion

The sponge, the host of the fungus *T. longibrachiatum* (code TE-F-03.1), collected from Falajava Beach waters, Ternate Island, Province of North Maluku, was identified as *Axinella* sp. The anti-*K. pneumoniae* compound isolated from *T. longibrachiatum* extract is matairesinol ($C_{20}H_{22}O_6$)/ (3R,4R)-3,4-bis[(4-hydroxy-3-methoxyphenyl) methyl] oxolan-2-, which has a dibenzylbutirolactone skeleton (a lignan and γ-lactone group of the polyphenol family).

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