

Isolation of endophytic fungi with antibacterial activity from medicinal plant *Jatropha multifida* L.

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ABSTRACT

The need for new antimicrobial agents is driven by the worldwide health crisis caused by bacteria resistant to existing ones. *Jatropha multifida* L. is an ethnomedicinal plant widely used in West Sumatra, Indonesia. This research examines the antibacterial properties of compounds extracted from the endophytic fungi isolated from the plant's roots, barks, and leaves, including identifying proficient fungi and their secondary metabolites' LC/MS-MS profiles. Fourteen endophytic fungi were successfully isolated from different parts using direct planting and pouring methods. Following 4–6 weeks of cultivation in rice medium, the fungus isolate was extracted using ethyl acetate. The antibacterial activity of each ethyl acetate extract of the fungus was evaluated using the Kirby–Bauer diffusion method against *Staphylococcus aureus*, *Escherichia coli*, and methicillin-resistant *S. aureus*. The JMB4 and JMD3 isolates of endophytic fungi showed promise as an antibacterial agent and showed notable inhibitory diameters, according to the inhibition zone measurement results. At a concentration of 5%, JMB4 and JMD3 exhibited inhibition zones measuring 21.79 ± 0.31 mm and 23.19 ± 0.58 mm against *S. aureus* and 22.55 ± 0.7 mm and 13.37 ± 0.77 mm against MRSA, respectively. The fungi JMB4 and JMD3 have been classified as *Fusarium incarnatum* and *Fusarium oxysporum* through macroscopic, microscopic, and molecular identification methods. Liquid chromatography–mass spectrometry/mass spectrometry analysis of the JMD3 extract revealed that the predominant peak corresponded to the molecular formula $C_{22}H_{31}NO_4$ and was tentatively identified as equisetin. The findings demonstrate that *J. multifida* L. plants harbor endophytic fungi that can synthesize antibacterial compounds. Additional research is required to determine the antibacterial compounds responsible. Those compounds will promote the advancement of novel antibacterial agents.

INTRODUCTION

The incidence of antibiotic resistance is recognized as a significant global public health threat. In 2020, the prevalence of cephalosporin resistance in *Escherichia coli* reached 47%, while methicillin resistance in *Staphylococcus aureus* was observed at 30% [1]. The conditions highlight the necessity for novel antibiotic agents, particularly from medicinal plants,

representing a promising new therapeutic compound source, such as *Jatropha multifida* (commonly known as the betadine plant) [2]. *Jatropha multifida*, widely referred to as the coral plant, coralbush, and physic nut (a designation it shares with other species in its genus), is a *Jatropha* indigenous to Mexico and the Caribbean. This garden plant has been introduced to Florida and several regions in South America, Africa, the Indian subcontinent, China, and Southeast Asia. Consumption leads to gastrointestinal distress as a result of mild toxicity [3]. The castor-root plant, belonging to the Euphorbiaceae family, has a historical application in treating bleeding wounds, swelling, bone fractures, and tooth decay. Numerous authors

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have reported using latex in treating gastrointestinal parasitic diseases in humans and animals (anthelmintic), severe wounds linked to microbial infections, and skin inflammation [4–6].

Additionally, Das *et al.* [3] reported the cytotoxic and antiproliferative effects of compounds extracted from the stem of *J. multifida* on tumor cells [3]. The research of Dah-Nouvlessounon *et al.* [4] identified various phenolic and flavonoid compounds, such as 2-Hydroxybenzoic acid, o-Coumaroylquinic acid, Apigenin-apiosyl-glucoside, and luteolin-galactoside. The extracts of *J. multifida* exhibited bactericidal effects against various pathogens, with concentration minimally bactericidal values between 22.67 mg/ml for *S. aureus* and *Candida albicans* and 47.61 mg/ml for *E. coli* [4].

The necessity for substantial biomass frequently constrains the identification of bioactive compounds in plants, while these compounds are typically present in minimal quantities within plant tissues [5]. The intricate structure of these compounds can complicate chemical synthesis, resulting in high costs and inefficiencies in their production. Endophytic fungi present an alternative method for producing bioactive compounds, as they are recognized for their capacity to synthesize secondary metabolites with notable bioactivities [6].

Endophytic fungi contain various metabolites that have been identified to have antimicrobial properties in laboratory studies. These compounds include steroids, alkaloids, quinones, phenolic acids, and polyketides. Previous studies [7–10] demonstrate that endophytic fungi derived from various medicinal plants can produce antibacterial and cytotoxic compounds.

Research on the bioactive compounds produced by the endophytic fungus of *J. multifida* L. is scarce in the scientific literature, especially compared to other medicinal plants thoroughly investigated for their bioactivities. *Jatropha multifida* L. is noted for its unique secondary metabolites demonstrating diverse biological activities. This unique biochemical profile suggests the potential for novel antimicrobial compounds not found in other species. This plant has been utilized in traditional medicine, especially for treating infections; this historical application may substantiate the hypothesis that its endophytic fungi also possess antimicrobial properties. This plant is readily available and proliferates extensively in West Sumatra, Indonesia. The location of growth and the environmental conditions of this plant can directly influence the diversity of its endophytic fungi.

The urgent need for new antibiotics to treat MRSA arises from the growing issue of antibiotic-resistant bacteria and the challenges associated with their management. This study sought to identify endophytic fungi in *J. multifida* L. plants and assess their ability to produce antimicrobial compounds effective against harmful and drug-resistant bacteria, such as *S. aureus*, MRSA, and *E. coli*.

MATERIALS AND METHODS

Identification of sample material

Jatropha multifida L. specimens were gathered in August 2023 from Durian Taruang, Kuranji District, Padang City, West Sumatra, located at 0°55'35.4" S and 100°24'25.3"

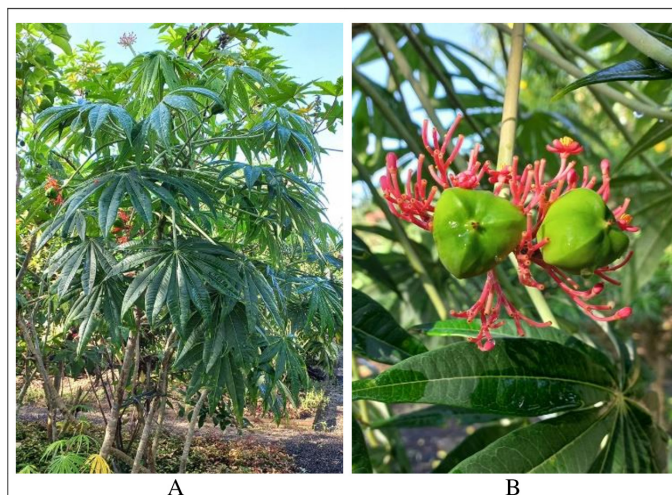


Figure 1. The pictures of *J. multifida* L. (A) Leaves. (B) Fruits and flowers.

E. The collected plant parts comprised roots, stems, leaves, and fruits (Fig. 1), with a total sample weight of approximately 100 g each. After a successful identification, the specimen was preserved at the Herbarium of Universitas Andalas in Padang, Indonesia. The voucher number for plant authentication was RSM002, and the letter number was 579/K-ID/ANDA/I/2023.

Sample preparation

Surface sterilization was conducted on healthy plant samples (roots, stems, leaves, and fruits) free from microbial infection and insect damage. The samples were washed with distilled water until all external contaminants were eliminated. The plant materials were then segmented into small pieces measuring approximately 1 × 1 cm. Surface sterilization of these segments was performed by immersing them in 70% ethanol for 30 seconds to 1 minute, followed by rinsing with sterile distilled water. The final few drops of rinse water were collected as a control. All sterilization procedures were performed under aseptic conditions. The sterilized plant segments were positioned on Sabouraud dextrose agar (SDA) plates with the damaged portion in contact with the media surface. The plates were incubated at ambient temperature (20°C–25°C) for 2–14 days. Fungal colonies exhibiting distinct morphologies were isolated and purified until a pure isolate was achieved [11].

The cultivation of pure fungi isolates in rice medium and the preparation of extracts

Pure isolates of endophytic fungi cultivated on SDA were sectioned into segments measuring 1 × 1 cm. The segments were aseptically transferred to sterile Erlenmeyer flasks containing autoclaved rice medium. The cultures were maintained at 20°C–25°C for 6 weeks, facilitating the fungal mycelium's complete colonization of the rice medium. After incubation, the fungal cultures were macerated with ethyl acetate as the extraction solvent. The extract was concentrated with a rotary evaporator at 40°C to yield a viscous crude extract [12].

Antimicrobial activity screening

The bacterial strains utilized for screening antibacterial activity consisted of *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and methicillin-resistant *S. aureus* (MRSA), obtained from the Microbiology Laboratory at the Faculty of Medicine, Universitas Andalas. The test of antibacterial activity was conducted utilizing the agar diffusion method. Bacterial suspensions were prepared to a turbidity standard of 0.5 McFarland and uniformly spread on the surface of nutrient agar plates. Sterile paper discs were infused with a 5% solution of endophytic fungal extract in dimethyl sulfoxide (DMSO). Chloramphenicol (Oxoid) was used as the positive control at 30- μ g/disc concentration. Discs containing DMSO served as negative controls, as DMSO can solubilize all compounds without affecting bacterial cell proliferation. The discs were positioned on the surface of the pre-inoculated nutrient agar medium within Petri dishes. The plates underwent incubation at 37°C for 24 hours. Clear zones surrounding the discs were quantified to ascertain the diameter of the inhibition zones, reflecting the antibacterial activity of bioactive metabolites generated by the endophytic fungi [13].

Initial phytochemical analysis of extracts from endophytic fungi

Phytochemical screening was performed on the ethyl acetate (EtOAc) extract of the endophytic fungi exhibiting the highest antibacterial activity to ascertain the presence of diverse classes of secondary metabolites. This qualitative method sought to identify the presence of alkaloids, flavonoids, steroid-terpenoids, and phenolic compounds. The alkaloid test was conducted with Dragendorff's reagent, resulting in a positive outcome signified by the emergence of an orange-yellow precipitate, further confirmed by Mayer's reagent, generating a white precipitate. The flavonoid test employed cyanidin reagent, producing a positive pink hue, which was confirmed by the citroborate reagent, generating fluorescent spots under UV light at 366 nm. The steroid-terpenoid assay was performed utilizing Liebermann-Burchard reagent, yielding positive results indicated by a green-blue hue for steroids and a brown coloration for terpenoids. The phenolic test was conducted using a ferric chloride reagent, yielding a green-violet coloration indicative of a positive result [14–16].

DNA extraction and polymerase chain reaction (PCR) amplification

Both macroscopic (including colony morphology, color, and margin characteristics) and microscopic analyses were performed to characterize endophytic fungi. Molecular identification was conducted utilizing PCR with primers Internal Transcribed Spacer (ITS)-1 and ITS-4. The PCR procedure commenced with a pre-denaturation phase at 95°C for 1 minute, succeeded by denaturation at 95°C for 10 seconds, annealing at 52°C for 15 seconds, and extension at 72°C for 15 seconds. The three stages were reiterated for 35 cycles, after which the temperature was decreased to 4°C for final stabilization. The resultant amplification products were visualized via 1% agarose gel electrophoresis. The collected base fragments were subsequently analyzed through bidirectional Sanger DNA sequencing employing capillary

electrophoresis. The acquired DNA sequences were juxtaposed with sequence data in the National Center for Biotechnology Information (NCBI) database utilizing the Basic Local Alignment Search Tool program. A phylogenetic analysis of the sequences was performed, reconstructing a phylogenetic tree via the neighbor-joining method utilizing a substitution model based on *p*-distance, validated by 1000 bootstrap replications, using MEGA11 (Molecular Evolutionary Genetics Analysis) software [17,18].

Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis of endophytic fungi extracts

The LC-MS/MS analysis was conducted using high-performance liquid chromatography coupled with mass spectrometry. The LC-MS/MS analysis utilized an ultra-performance liquid chromatography system with a C18 column (1.8 μ m; 2.1 \times 100 mm) and an XEVO G2-S Q-TOF mass spectrometer. The mobile phase comprised solvent A (water with 5-mM ammonium formate) and solvent B (acetonitrile with 0.05% formic acid), running under a gradient elution system at a 0.2-ml/minute flow rate for 23 minutes. A 10-mg extract sample was dissolved in 10 ml of absolute methanol. A volume of 5 μ l of this solution was introduced into the column maintained at 50°C. Mass spectrometric analysis was performed utilizing electrospray ionization in positive mode, with mass spectra acquired within 50–1,200 *m/z*. Data analysis was conducted utilizing MassLynx software version 4.1. Compounds were identified by analyzing the intensity of ions corresponding to the mass-to-charge ratio (*m/z*) of the molecular ion ($[M+H]^+$) in comparison to the PubChem, MassBank, and ChemSpider databases. The precision of compound identification was validated by the congruence of MS/MS fragment patterns, with a tolerance of under 5 parts per million (ppm).

RESULTS AND DISCUSSION

Fungal endophytes are present in many plant species and are essential for the health of their hosts. The diversity and biological activities of these fungi are mainly unexplored. *Jatropha multifida*, a well-known ornamental and medicinal plant species, is found in West Sumatra, Indonesia. This investigation aimed to detect endophytic fungi that produce antibacterial metabolites in *J. multifida* by collecting samples of its leaves and stems. Endophytic fungi found in marine sponges, mangroves, and medicinal plants in West Sumatra, Indonesia, are the subject of an ongoing research project to identify potential new drugs [7–10,12,15,17,19,20].

Fourteen fungal isolates were successfully obtained from the roots, stems, leaves, and fruits of the *J. multifida* plant (Fig. 1). The endophytic fungi obtained from the roots were labeled with the code JMA, consisting of four distinct isolates. The isolates obtained from the stems were designated JMB and comprised four isolates. The isolates obtained from the leaves were labeled as JMD, amounting to five isolates. Finally, the isolate derived from the fruit was designated as JMBB, indicating a singular isolate (Fig. 2). Fungal isolates were cultivated on rice-based media. After achieving maximum growth, the fungi's secondary metabolites were extracted with EtOAc. The extracts were then concentrated under reduced

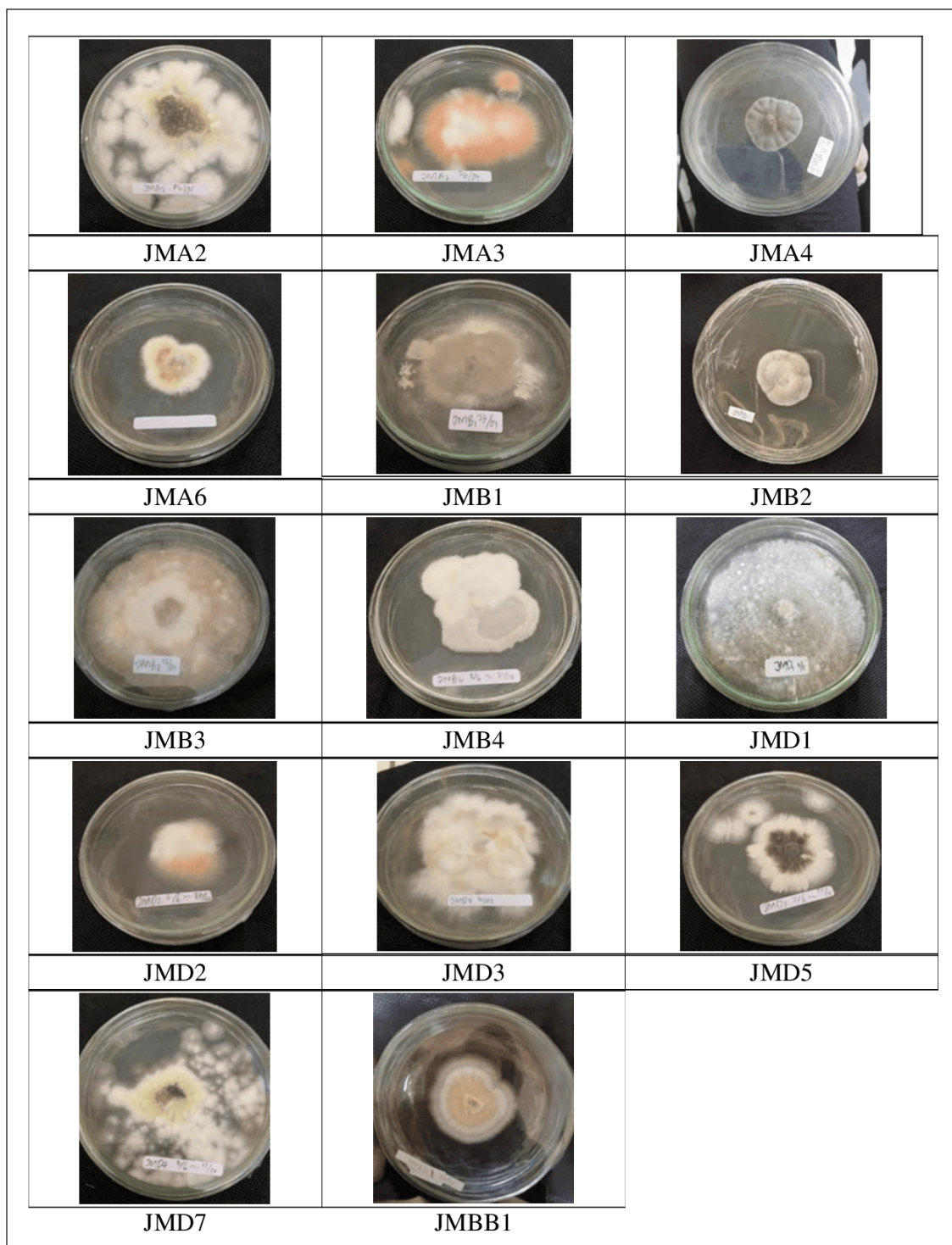


Figure 2. The pictures of isolated fungal endophytes from *J. multifida* L. cultivated on SDA.

pressure until a viscous consistency was attained. The ethyl acetate extract was subsequently evaluated for antimicrobial activity.

Three bacterial strains, specifically *S. aureus*, MRSA, and *E. coli*, were chosen to evaluate the antibacterial activity of fungi extracts. These strains were utilized for preliminary screening due to their availability and established testing

protocols. This can be considered an initial step to identify promising fungal isolates before broadening the testing to a larger spectrum of bacteria. The selection was based on their representation of Gram-positive and Gram-negative bacteria, offering initial insight into the spectrum of activity. These strains are clinically significant and commonly associated with community-acquired and hospital-acquired infections.

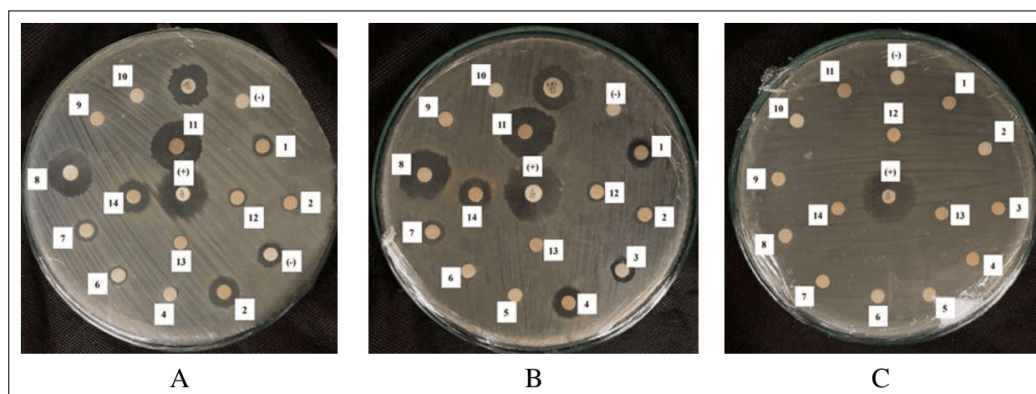


Figure 3. The agar plate pictures of the inhibition zone of fungal isolate extract against the growth of *S. aureus* (A), MRSA (B), and *E. coli* (C).

Table 1. Antibacterial activity screening of endophytic fungi from *J. multifida* L. plant against pathogenic bacteria.

No.	Fungal code	Organ	Average of Inhibition Zone (mm)		
			<i>S. aureus</i>	MRSA	<i>E. coli</i>
1	JMA2	Root	10.15	11.56	—
2	JMA3	Root	7.73	7.31	—
3	JMA4	Root	10.91	10.68	—
4	JMA6	Root	12.69	14.09	7.99
5	JMB1	Stem	7.2	—	—
6	JMB2	Stem	7.78	7.63	—
7	JMB3	Stem	10.30	10.53	7.87
8	JMB4	Stem	22.61	20.42	—
9	JMD1	Leaf	—	—	—
10	JMD2	Leaf	—	—	—
11	JMD3	Leaf	23.28	22.19	—
12	JMD5	Leaf	8.558	8.07	—
13	JMD7	Leaf	—	—	—
14	JMBB1		13.77	13.93	—
	Positive control		25.53	22.56	24.33

Note: Pos. control (Chloramphenicol 30 µg/disk).

MRSA and *E. coli* are acknowledged for their multidrug resistance and are major contributors to severe infections globally.

The antibacterial efficacy of the endophytic fungi extracts was evaluated at a concentration of 5% in DMSO. Antibacterial agents on the disk diffuse into the agar, suppressing bacterial proliferation, as evidenced by a clear zone surrounding the disk. The results of the antibacterial activity screening are shown in Table 1 and Figure 3 and indicate that several extracts demonstrated differing sensitivity levels toward the test bacteria. If there is a clear zone around the disc, this suggests that the presence of secondary metabolite compounds is preventing the growth of fungi and bacteria. Seventy-nine percent of the endophytic fungi collected could inhibit the growth of *S. aureus*, MRSA, and *E. coli* strains in the experiment. This variability is due to genetic differences

Table 2. Antibacterial activity of selected fungal endophyte.

Fungal code	Zone of Inhibition ± Standard Deviation (SD) in mm		
	<i>S. aureus</i>	MRSA	<i>E. coli</i>
JMA2	10.89 ± 0.77***	13.2 ± 0.45***	—
JMA4	10.71 ± 0.38***	10.89 ± 0.61***	—
JMA6	11.87 ± 0.36***	12.79 ± 0.55***	7.76 ± 0.26***
JMB3	10.67 ± 0.27***	10.93 ± 0.24***	7.64 ± 0.23***
JMB4	21.79 ± 0.31*	22.55 ± 0.71*	—
JMD3	23.19 ± 0.58 ^a	23.37 ± 0.77 ^a	—
JMBB1	15.68 ± 0.49***	15.83 ± 0.48***	—

The value is expressed as the mean ± standard deviation; n = 3. ****p* < 0.001, **p* < 0.05, ^a = no difference between sample and control.

among fungal isolates that encode for the biosynthesis of secondary metabolites, producing various compounds with distinct antibacterial activities [21].

At a 5% concentration, seven fungal isolates exhibited significant inhibition of *S. aureus* and MRSA, with inhibition zones surpassing 10 mm. None of the isolates exhibited activity against *E. coli* (Table 2). Furthermore, the antibacterial assays indicated that the endophytic fungi isolates from *J. multifida* exhibit more significant potential to inhibit the growth of Gram-positive bacteria (*S. aureus* and MRSA) compared to Gram-negative bacteria. This phenomenon can be elucidated by the structural disparities in the cell walls of the examined bacteria, wherein Gram-positive bacteria exhibit a more simplistic cell wall composition than their Gram-negative counterparts [22]. Among all tested fungal extracts, isolates JMB4 and JMD3 demonstrated the most significant antibacterial efficacy. The JMB4 isolate exhibited an average inhibition zone of 21.79 ± 0.31 mm against *S. aureus* and 22.55 ± 0.71 mm against MRSA. The JMD3 isolate exhibited an average inhibition zone of 23.19 ± 0.58 mm against *S. aureus* and 23.37 ± 0.77 mm against MRSA (Table 2). The antibacterial efficacy of JMD3 against *S. aureus* and MRSA is not statistically different from that of chloramphenicol; conversely, JMB4 exhibits a significant difference from chloramphenicol for both bacteria at *p* < 0.05.

The extract of JMB4 and JMD3 was subsequently analyzed for their secondary metabolite composition utilizing chemical methodologies (Table 3). Prior research indicates that extracts from the stems of *J. multifida* L. exhibit antibacterial properties and encompass secondary metabolites categorized as alkaloids, flavonoids, steroids, phenolics, and saponins [23,24]. A comparable secondary metabolite profile was observed in the ethyl acetate extract of the endophytic fungus JMB4. Moreover, antibacterial activity has been documented in extracts from *J.*

multifida leaves, which encompass secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, and quinones [4,24]. The results align with the findings of the ethyl acetate extract from the endophytic fungal isolate JMD3, which demonstrated notable antibacterial activity and comprised secondary metabolites from the alkaloid, terpenoid, and steroid categories.

The macroscopic examination of isolate JMB4 revealed a smooth, spherical morphology characterized by a white-cream hue and a distinctly undulating surface (Fig. 4). Conversely, isolate JMD3 exhibited clustered mycelia with a pale orange and pink hue, distinguished by wavy and tapering margins. Microscopic observations (Fig. 5) indicate that fungal isolate JMB4 possesses curved and tapered macroconidia featuring five septa. The microconidia isolate JMB4 is disk-shaped and possesses one septum. The fungal isolate JMD3 possesses curved macroconidia and oval and round microconidia resembling a false head with short monophilia.

Table 3. Phytochemical screening of EtOAc extract of GCA3 from *J. multifida* L.

Fungal code	Chemical constituents				
	Alkaloid	Flavonoid	Terpenoid	Steroid	Phenolic
JMB4	+	–	+	+	+
JMD3	+	–	+	+	–

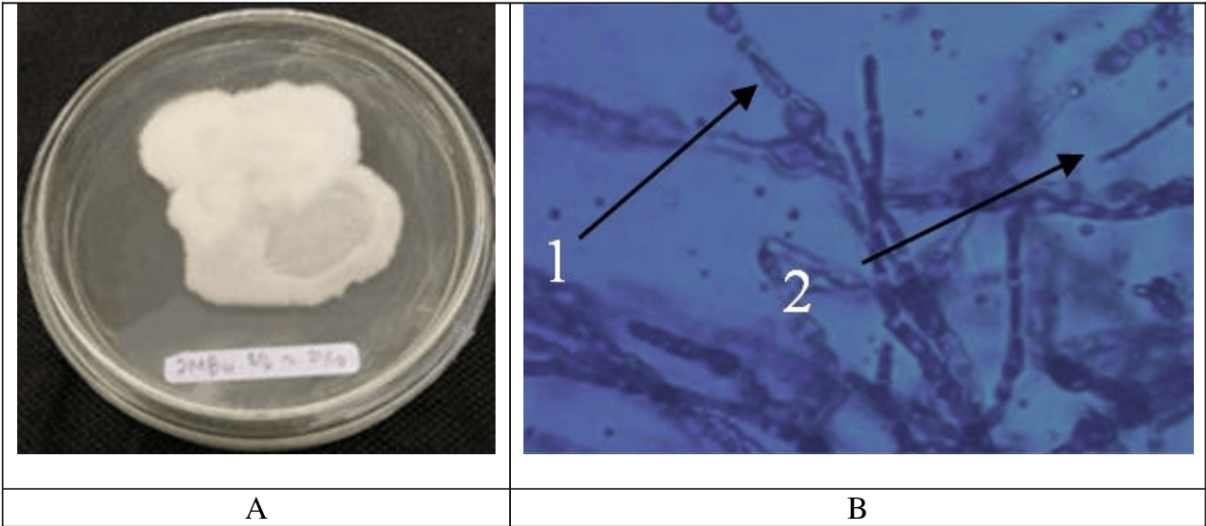


Figure 4. Macroscopic (A) and microscopic (B) pictures of fungal isolate JMB4. (1) Macroconidia. (2) Microconidia.

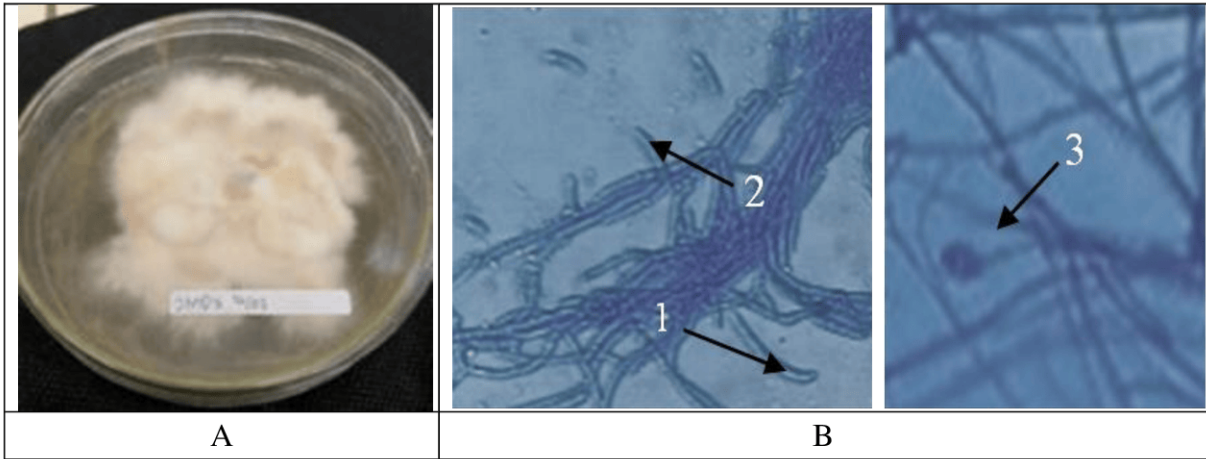


Figure 5. Macroscopic (A) and microscopic (B) pictures of fungal isolate JMD3. (1) Macroconidia. (2,3) Microconidia.

The macroscopic characteristics of both isolates were closely aligned with the descriptions of *Fusarium incarnatum* and *Fusarium oxysporum* in the literature [25].

Molecular identification of fungal isolates JMB4 and JMD3 was performed utilizing PCR techniques. The DNA sequence was acquired in the ITS gene region (Fig. 6). The DNA sequences obtained were aligned with reference sequences from the NCBI database. The results were analyzed and aligned with a phylogenetic tree to validate the identification of the fungal isolates. The neighbor-joining method was utilized to reconstruct the phylogenetic tree, employing the *p*-distance substitution model and a bootstrap value of 1,000 replicates, analyzed with MEGA11 software (Fig. 7). Phylogenetic analysis indicates a close genetic relationship between isolate JMB4 and *F. incarnatum* strain JL5-2 (MT563420.1), exhibiting a percent identity of 99.81% and a query cover of 100%. Additionally,

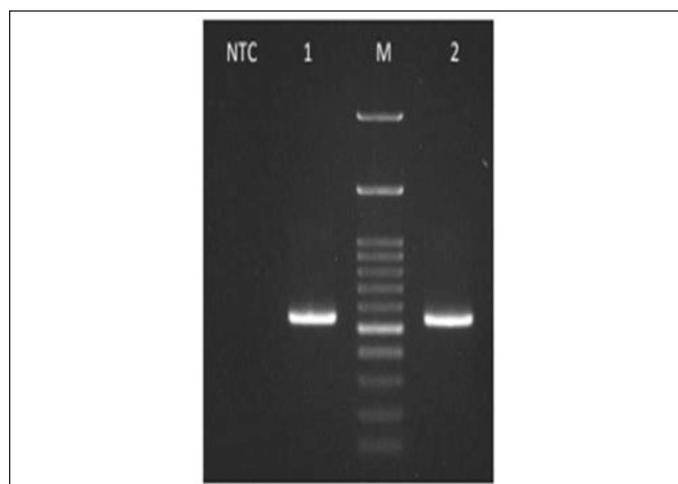


Figure 6. Amplified isolate JMB4 (1) and isolate JMD3 (2) visualized on gel agarose 1% electrophoresis. M. 100 bp DNA ladder. NTC: Negative control amplified.

isolate JMD3 shows a close relationship with *F. oxysporum* isolate FoA10 (PP565979.1), with a percent identity of 99.09% and a query cover of 99% (Fig. 7).

The content of secondary metabolites in both extracts was analyzed using LC-MS/MS. A quadrupole mass analyzer detected small molecules within the mass range of 1–5,000 *m/z*, followed by time-of-flight analysis, which is suitable for both small and large molecules, thereby applicable to all molecular sizes. The detected masses were transformed into mass spectra, illustrating ions associated with *m/z* values of different compounds. The resulting MS fragments were analyzed against LC-MS/MS mass libraries from PubChem, Massbank, and prior studies.

Total ion chromatograms (TICs) for JMB4 and JMD3 extracts are shown in Figures 8 and 9. The TIC of the JMB4 extract displays several peaks, with a prominent peak observed at a retention time of 15.95 minutes. The peak exhibits a distinct adduct ion signal at *m/z* 994.6431 [*M*+*H*]⁺, correlating with the molecular formula C₄₆H₈₃N₁₃O₁₁. In analytical chemistry, specifically, mass spectrometry, adduct ions denote ions generated when a molecule interacts with another ion (typically a small ion, such as a proton, metal ion, or anion) to form a complex while preserving the fundamental structure of the molecule [26].

However, limitations in the literature precluded further identification of this compound. Conversely, the TIC of the JMD3 extract displayed multiple peaks, with the principal peak occurring at a retention time of 14.41 minutes. The peak exhibits a distinct adduct ion signal at *m/z* 374.2330 [*M*+*H*]⁺, correlating with the molecular formula C₂₂H₃₁NO₄. The fragmentation of this ion yields several notable fragment ions, such as *m/z* 356.2228 [*M*+*H*]⁺, which aligns with the molecular formula C₂₂H₃₀NO₃⁺, *m/z* 346.2387 [*M*+*H*]⁺, corresponding to C₂₁H₃₂NO₃⁺, *m/z* 200.0928 [*M*+*H*]⁺, which relates to C₉H₁₄NO₄⁺, and *m/z* 175.1490 [*M*+*H*]⁺, associated with C₁₃H₁₉⁺ (MassBank, 2021). Table 4 presents the *m/z*

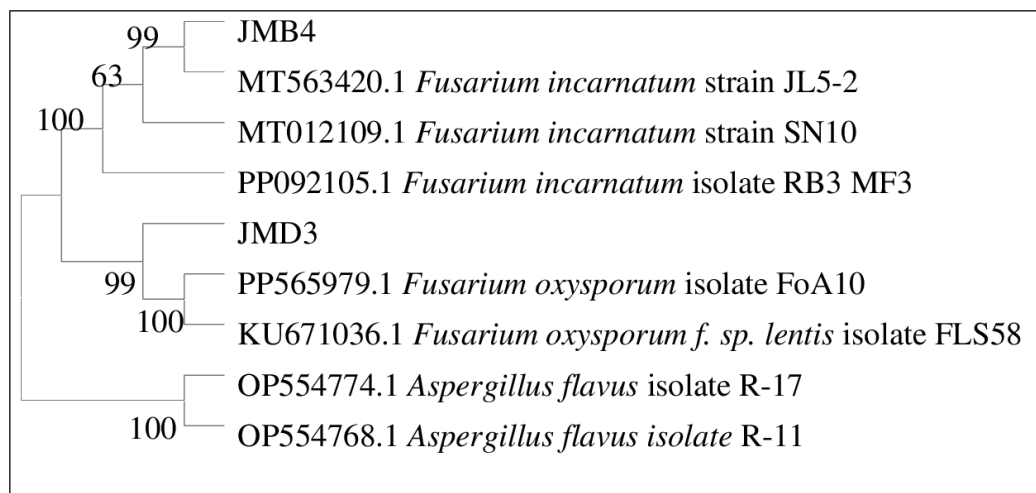


Figure 7. Using the neighbor-joining algorithm and 1,000 bootstraps, concatenated phylogenetic tree of isolate JMB4 and JMD3 with other *Fusarium* species based on internal transcribed spacer (ITS) and large subunit (LSU) rDNA region sequences.

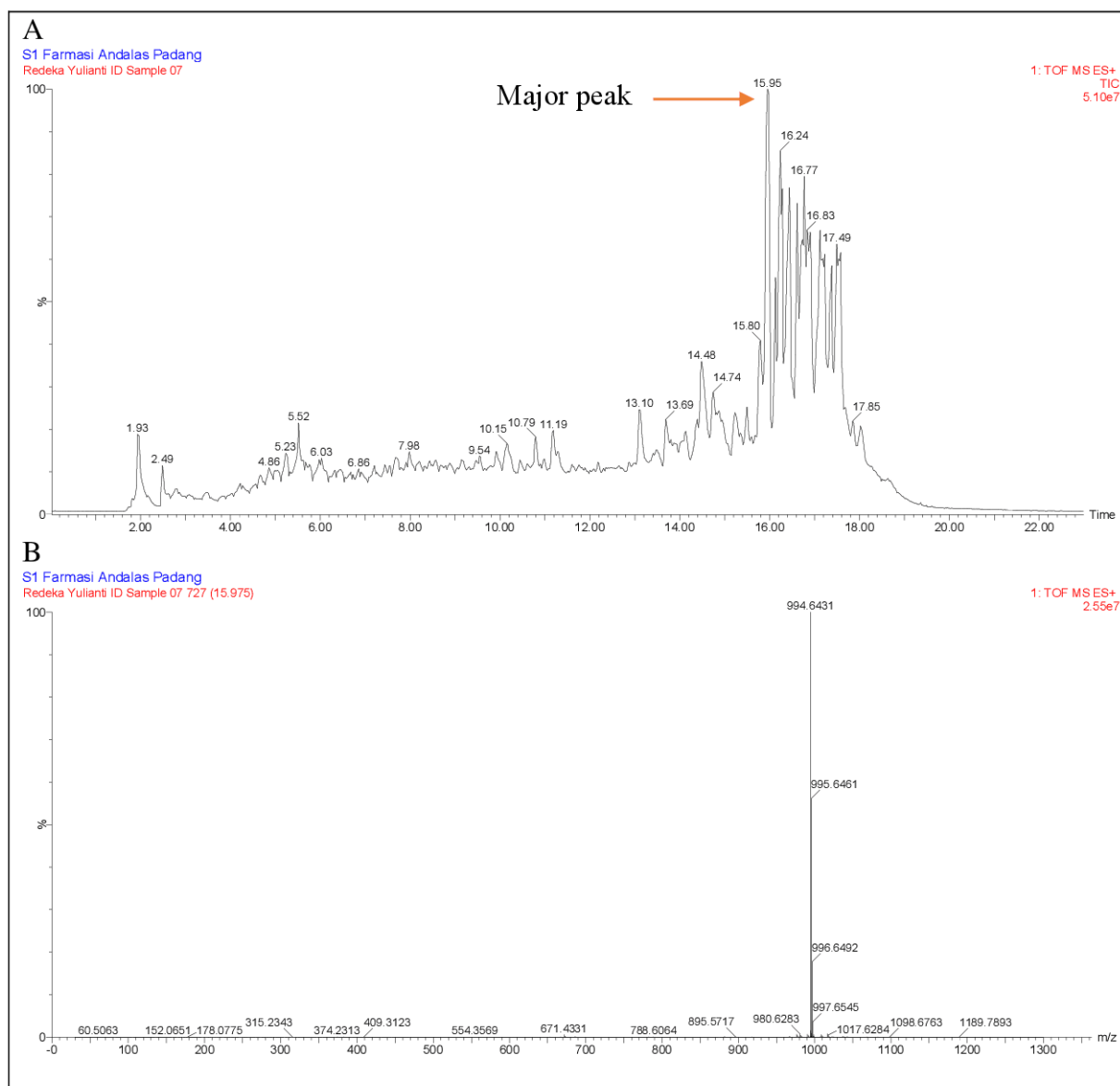


Figure 8. LC-MS/MS data from JMB4 extract (A) and the major peak were observed at retention times of 15.95 minutes using electrospray ionization (ESI) in positive mode (B).

values of the parent ion and daughter ion from the major peak of the JMD3 extract, compared with the literature values for Equisetin. Based on PubChem, ChemSpider, and MassBank data comparisons, this principal peak was tentatively identified as equisetin. Initially separated from the deep-sea fungus *Fusarium equiseti* NRRL 5337, equisetin is an acyl tetramic acid derived from N-methylserine. Although it is not usually connected with any one strain of *Fusarium*, including *F. equiseti*, it is not widely found among a broad spectrum of fungi. The environmental conditions, the particular strain of the fungus, and the substrate on which the fungus is grown will all significantly affect the frequency of equisetin synthesis [27–29]. It demonstrates significant antibacterial activity against multidrug-resistant bacteria, particularly Gram-positive pathogens, through a novel mechanism that involves allosteric binding to biotin carboxylase [30].

Moreover, equisetin inhibits HIV-I integration and quorum sensing in *P. aeruginosa*. This compound was also reported to employ a distinctive dual mechanism to regulate the host-pathogen interaction in eliminating intracellular bacteria [31]. Biosynthetic researchers aim to produce equisetin because of its high activity and rarity. Research into the heritable and environmental variables that promote its growth is continuing in the hopes of improving its cultivated yield.

Although this study has offered some preliminary insights into the antibacterial properties of equisetin from JMD3, it is essential to point out that the chemical analysis techniques utilized have significant limitations. The interpretation of the findings and the generalization of the findings are both hampered by this factor. Continuous research using enhanced isolation methods will be carried out to strengthen the findings and discover additional antibacterial compounds in JMD3 and

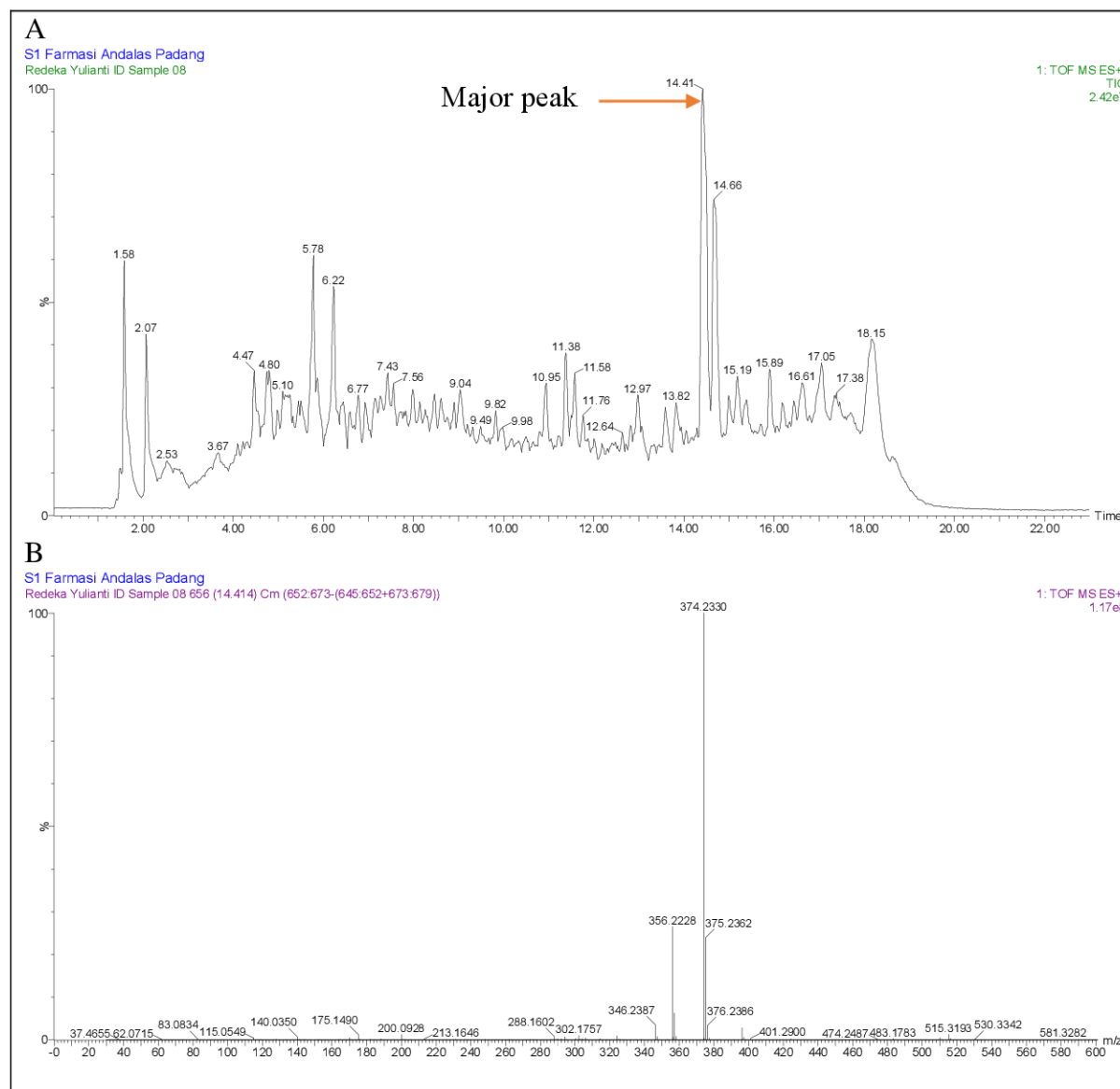


Figure 9. LC-MS/MS data from JMD3 extract (A) and the major peak were observed at retention times of 14.41 minutes using electrospray ionization (ESI) in positive mode (B).

Table 4. m/z parent ion and daughter ion from major peak JMD3 extract, which compared with the literature m/z equisetin.

MS major peak of JMD3		MS equisetin (Mass bank)	
m/z [M+H] ⁺	Intensity	m/z [M+H] ⁺	Intensity
374.2,330	100	374.2,324	999
356.2,228	31.01	356.2,217	127
346.2,387	4	346.2,376	25
200.0,928	1.33	200.0,917	28
175.1,490	1.53	175.1,481	152

JMB4 extracts. This will be done to address the limitations that have been identified.

CONCLUSION

Endophytic fungi from *J. multifida* represent a promising source for producing secondary metabolites exhibiting antibacterial properties. Fourteen endophytic fungal isolates from this plant displayed antibacterial properties against *S. aureus* and MRSA, with two isolates showing notable effectiveness. JMD3 demonstrates enhanced antibacterial efficacy against *S. aureus* and MRSA compared to JMB4. The isolate JMB4 significantly suppressed the proliferation of *S. aureus* and MRSA, generating inhibition zones of 21.79 ± 0.31 mm and 22.5 ± 0.71 mm, respectively. The isolate JMD3 exhibited inhibition zones of 23.19 ± 0.58 mm against *S. aureus* and 23.37 ± 0.77 mm against MRSA. Phylogenetic analysis indicated that isolate JMB4 is 100% identical to *F. incarnatum*, whereas isolate JMD3 exhibits a 99.09% similarity to *F. oxysporum*. Moreover, LC-MS/MS analysis of the JMD3

extract revealed that the predominant peak corresponded to the molecular formula $C_{22}H_{31}NO_4$ and was tentatively identified as equisetin. The results of this study indicate that extracts from these fungi may provide a promising basis for creating new, effective treatments for nosocomial and skin infections, which are currently major global health issues.

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AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

CONFLICTS ON INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All of the collected and analyzed data are in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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