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Antibacterial activity of mycelial extract from a local fungus, *Sclerotium rolfsii*

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ABSTRACT Mycelium-to-sclerotium differentiation in fungi involves not only morphological but also biochemical changes throughout the process, which may contribute to their persistence and be a possible source of bioactive compounds. This study aims to evaluate the antibacterial activity and identify the bioactive compound in the local isolate *Sclerotium rolfsii*. Fungal culture was grown in media containing potato extract (20 g/L), dextrose (20 g/L), and peptone (5 g/L) for 27 days under static conditions at room temperature. Mycelium, sclerotium and filtrate were collected every three days and extracted with methanol, followed by evaporation and antibacterial screening. Significant activity was observed in day three of mycelial extract, which showed morphology of initial sclerotium formation (MIC 0.39 mg/mL) against *B. subtilis* and *E. coli*. An improved extraction method (sequential extraction) was employed for mycelial sample on the third day. N-hexane and ethyl acetate extracts exhibited stronger activities (0.20 mg/mL). Ergosterol was identified after TLC-bioautography, radial chromatography, and NMR elucidation analysis. *S. rolfsii* mycelium (third day-sclerotial initiation) was found to contain ergosterol, demonstrating strong defense against bacteria, and possibly related to sclerotium-differentiation metabolites. These findings may pave the way for more extensive studies of sclerotium differentiation as an interesting phenomenon of fungal development and bioactive compound origins.

KEYWORDS Antibacterial activity; Differentiation; Ergosterol; Mycelium; Sclerotium

1. Introduction

Active compounds from nature have been essential in developing drugs and medical therapies for thousands of years. The diversity of chemical structures in active compounds has the potential to act as therapeutic agents and disease prevention (Dzobo 2022). Researchers continue to explore sources of natural active compounds to obtain a broader and more diverse range of these compounds. One potential yet underexplored source of active compounds comes from organisms that develop survival structures. These structures are presumed to contain bioactive compounds which not only play roles in cell survival but also exhibit bioactive properties for biomedical applications (Gonzalez and Aranda 2023).

Some group of fungi produce a survival strategy called sclerotium which allows the fungi to survive for several years in response to stress conditions (Ajiboye et al. 2025). Aside from abiotic stress, fungi must also withstand biotic challenges, including competition with other microorganisms. Sclerotium has been proposed to also function as a defense strategy against biotic stress, as studies report antifungal (Petersen et al. 2015) and antibacterial (Stanley et al. 2018) properties of sclerotium. These evidences highlight the prospect of sclerotium fungi for antimicrobial discovery.

Sclerotium formation are characterized by three different morphological stages; initiation, development, and maturation phase. This mycelium-sclerotium differentiation involves not only morphological but also biochemical changes throughout the process (Lau and Abdullah 2015). The diversity of metabolites associated with this fungal development may suggest the potential of sclerotium fungi as bioactive compounds reservoirs with various bioactivities through the sclerotium formation stage approach.

Research on sclerotium fungi as potential sources of bioactive compounds remains limited, particularly in Indonesia. Sclerotium fungi are widely recognized as phytopathogens, which has led research to primarily focus on their pathogenic effect and control measures for plants

(Nurhalimah et al. 2022). However, this view neglects their unexplored potential that local sclerotium-forming may serve as bioactive compound origins. In the preliminary research, a locally isolated sclerotium fungus in Central Kalimantan, Indonesia, was identified as *Sclerotium rolfsii*.

Researches regarding *S. rolfsii* as producers of antibacterial bioactive compounds are limited, as it is primarily recognized for their pathogenic effects and can cause devastating loss in agriculture. Though one study by Nyochembeng et al. (2017) also reported antibacterial activity from water mycelial extracts of *S. rolfsii* inhibited the growth of phytopathogen *Erwinia amylovora*. Stanley et al. (2018) showed antimicrobial activities against *Klebsiella* sp., *S. aureus*, *Bacillus* sp., and *Candida albicans* from sclerotial extract of *Pleurotus tuberregium*. Research on isolating and identifying bioactive compounds from sclerotium-forming fungi is still rare, with Petersen et al. (2015) reporting antifungal activity of bioactive compound sclerolizine from sclerotial extract of *Aspergillus sclerotiicarbonarius*. Further studies on the active compounds responsible for the antibacterial activity of this fungal extract are still limited. Nevertheless, their possible applications are of considerable interest. Such compounds may serve as potential leads for the development of new antibacterial drugs but also as adjuvants to enhance the efficacy of existing antibiotics and mitigate resistance.

This study aims to evaluate the antibacterial properties of *S. rolfsii* through mycelium-to-sclerotium differentiation approach, and to isolate and identify the bioactive compound linked to the bioactivity.

2. Materials and Methods

2.1. Materials

Sclerotial bodies were collected from empty oil palm bunches in Central Kalimantan, Indonesia and previously identified by molecular analysis as *Sclerotium rolfsii*. *Bacillus subtilis* and *Escherichia coli* were obtained from the microbial culture collection in the Microbiology Laboratory, SITH, Institut Teknologi Bandung (ITB).

2.2. Fungal cultivation and initial biomass production for antibacterial screening

Fully grown *S. rolfsii* on Potato Dextrose Agar (PDA) was cultivated in liquid media containing 200 g/L of potato extract, 20 g/L dextrose, and 0.5 g/L peptone, then incubated for 27 d at room temperature without agitation. Mycelium, sclerotium, and culture filtrate were collected at 3-day intervals throughout the cultivation period. The biomass (mycelium and sclerotium) was separated from the culture filtrate via vacuum filtration using Whatman No. 1 filter paper. The harvested mycelium and sclerotium were dried using an oven at 50 °C for 24 h. The dried biomass was subsequently weighed and mechanically ground using an electric blender.

2.3. Primary metabolite extraction and antibacterial screening

2.3.1 Extraction

The mycelium and sclerotium powder were extracted by 1:5 (w/v) methanol followed by 10-minutes sonication, and separated via vacuum filtration using Whatman No. 1 filter paper (Graf et al. 2020; Sułkowska-Ziaja et al. 2023). Filtrate extracts were concentrated by lyophilization using freeze-dryer system (Tharavecharak et al. 2023). The lyophilized filtrate was extracted following the same procedure used for the mycelium and sclerotium powder.

2.3.2 Antibacterial assay (agar well diffusion assay)

Antibacterial activity was analyzed using agar well diffusion assay (Balouiri et al. 2016). Bacteria cultures were grown in nutrient broth (NB) media for 24 h with 150 rpm agitation at 37 °C. The cultures were adjusted to a concentration of 1.5×10^8 CFU/mL using a 0.5 McFarland standard as a reference. The cultures were inoculated thoroughly using a sterile cotton bud with the swab method on Mueller-Hinton agar (MHA) plates that had been perforated with a sterile cork borer with a diameter of 5 mm. A total of 20 µL of biomass extract (15 mg/mL), positive control amoxicillin (100 ppm, equivalent to 100 µg/mL) and negative control (methanol) were placed in each test well on MHA plates. All extracts were tested in triplicate. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test at $p < 0.05$.

2.3.3 Antibacterial assay (minimum inhibitory concentration (MIC) assay)

Extracts with significant activity in the agar diffusion assay were subsequently tested for MIC. The extracts were serially prepared to reach the concentration of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 mg/mL. The MIC assay was tested using method described by Shashini Janesha et al. (2020). Amoxicillin (100 ppm, equivalent to 100 µg/mL) as a positive and methanol as negative control. To visualize metabolic activity, resazurin method described by Teh et al. (2017) was followed. All experiments were performed in triplicate. MIC results were classified into weak, moderate, and strong activity according to their concentrations.

2.4. Scanning electron microscopy (SEM) observation

A bacterial culture with a concentration of 1.5×10^8 CFU/mL was contacted with mycelial extract from day 3 (1:1 v/v) for 12 h at 37 °C. The culture mixture was centrifuged at 600 g for 5 min and then washed with saline. The bacterial culture was fixed with 2.5% glutaraldehyde for 90 min and centrifuged at 600 g for 5 min. The bacterial pellet was dried with graded ethanol solutions of 70, 85, and 90% for 20 min for each concentration (García-Salinas et al. 2018).

2.5. Scaled-up biomass production and method optimization

Mycelial production was carried out using described method in Section 2.2, with fungal inoculum (100 mL) cultured in potato dextrose broth (PDB) and cultivated in several flasks, each containing 500 mL medium. The harvested day 3 mycelium was processed as in Section 2.3.1, with the following refinements:

2.5.1 Improved extraction

Mycelial powder was sequentially extracted using three different solvents: n-hexane, ethyl acetate, and methanol in order (Adeniran et al. 2022) with modifications as in Section 2.3.1.

2.5.2 MIC testing of extracts from sequential extraction

N-hexane, ethyl acetate, and methanol extracts were screened for antibacterial activity via MIC assay (as in Section 2.3.3), with adjusted concentration range (6.50, 3.25, 1.60, 0.81, 0.40, 0.20, 0.10, and 0.05 mg/mL).

2.6. Thin-layer chromatography (TLC-Bioautography)

Different solvent mixtures were used as mobile phase; eluent A (n-hexane-ethyl acetate (9:1); eluent B (n-hexane-ethyl acetate (7:3), and eluent C (chloroform-methanol (9:1). Samples of the n-hexane, ethyl acetate, and methanol extracts were spotted at the bottom of the TLC plate. The plate was then placed in a chamber containing one of the solvent systems, developed until the solvent reached the top, and visualized under UV illumination (254/366). The Rf values were determined from the TLC plates. To detect biological activity, the TLC plate was placed onto nutrient agar (NA) plates that had been swabbed with each bacterium (adjusted to 1.5×10^8 CFU/mL) for 30 min at 4 °C to allow any antimicrobial compounds to transfer from the TLC plate to the agar. The TLC plate was then removed, and the agar plates were incubated at 37 °C for 24 h. The appearance of clear zones (inhibition zones) on the agar indicated where antibacterial compounds had transferred. The position of these zones on the agar was then compared to the position of the corresponding Rf spots on the original TLC plate to identify the active compounds (Mawardi et al. 2020).

2.7. Isolation of bioactive compounds and antibacterial assay

The dried fungal biomass was sequentially extracted following the previous step (2.5.1). The n-hexane extract obtained was then purified using radial chromatography.

2.7.1 Radial chromatography

A 1 mm F254 silica was used as the stationary phase, while the mobile phases were solvent with different variations; 100 percent n-hexane, n-hexane-ethyl acetate (9:1), and n-hexane-ethyl acetate (8:2). The n-hexane extract was placed in the center of the silica plate when the plate was rotating and the mobile phases were flowed sequentially and gradually. Compound separation was visualized on silica TLC plate under UV illumination (254/366) and fractions were collected in separate chambers based on spots detected under UV light. To confirm the spot as the target compound, each fraction was subjected to TLC with n-hexane-ethyl acetate (9:1) as the mobile phase. Fraction with spot exhibiting identical Rf value as bioactive Rf in TLC-bioautography, was selected for further analysis.

2.7.2 MIC testing of purified compound

The isolated compound was initially dissolved to achieve a stock concentration of 7 mg/mL and evaluated using the same microdilution assay as in Section 2.3.3, with modified serial dilutions (2.84, 1.42, 0.71, 0.35, 0.18, 0.09, 0.04, 0.02, and 0.01 mg/mL).

2.8. NMR elucidation

The isolated compound was structurally characterized through nuclear magnetic resonance (NMR) spectroscopy analysis: ^{13}C-NMR as well as DEPT-135 to determine the number and type of carbon and ^{1}H-NMR to determine the number, type, and environment of protons contained in the isolated compound.

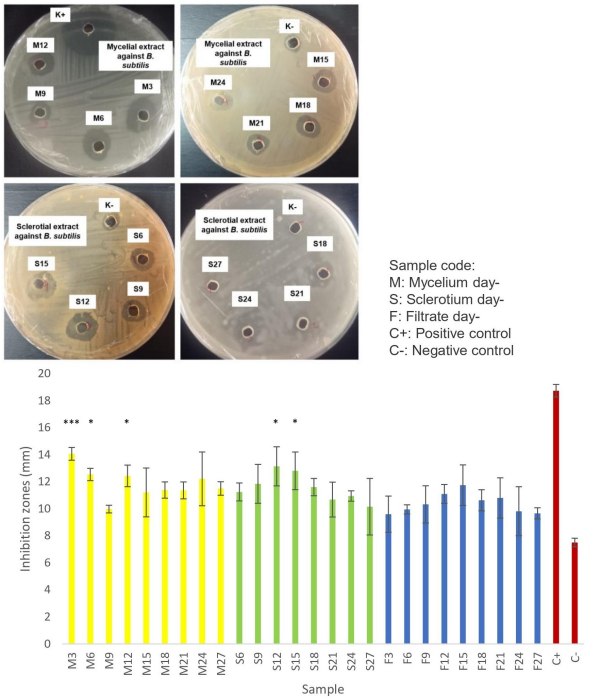
3. Results and Discussion

3.1. Fungal development pattern

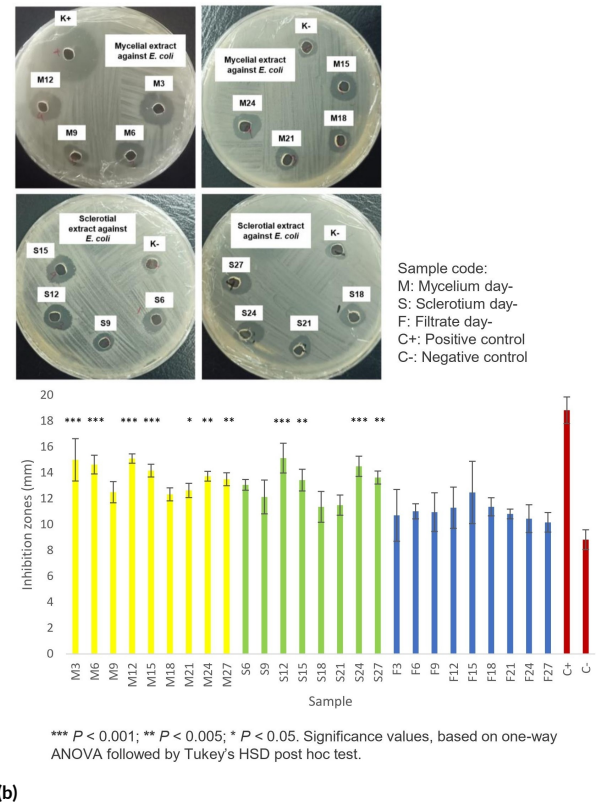
Sclerotium fungi growth can be observed through the development of mycelium and sclerotium. Mycelium was in the log phase on the 3rd to 6th day and entered the stationary phase on the 6th to 21st day, while sclerotium was formed at day 6 (Supplementary Figure 1). Sclerotium fungi begin producing sclerotium when exposed to stress condition (Ajiboye et al. 2025). In this study, the absence of aeration (agitation) triggered mycelium to reach stationary phase, which lead to sclerotia formation. On day 21, a distinct biomass transition was observed, characterized by a decline in mycelial dominance and increasing sclerotial dry weight, presumably due to nutrient exhaustion and other additional stress during late-stage fungal growth.

3.2. Antibacterial inhibitory zone

All extracts were prepared in methanol, thus this solvent became the negative control in this assay. Agar diffusion assay revealed distinct zones of inhibition around certain fungal extracts, indicating antibacterial activity against *B. subtilis* and *E. coli* (Figure 1a and 2b). The significance of the extract's antibacterial activity was compared to the inhibition zone produced by the negative control in this study. Positive control exhibited the strongest significance inhibition (up to 19 mm) from all other groups. Clear zones, ranging from 12,4–14 mm from mycelial extracts (day 3, 6, and 12) and 12,8–13,1 mm from sclerotial extracts (day 12 and 15) against *B. subtilis*, demonstrated



(a)



(b)

FIGURE 1 Antibacterial activity (agar diffusion assay) of *S. rolfsii* biomass extract after 24 h incubation at 37 °C observed on MHA plates and graphic of inhibition zone diameters (mm): (a) against *B. subtilis* and (b) against *E. coli*.

significant antibacterial activity compared to the negative control (Figure 1a). Both mycelial (day 3, 6, 12, 24, 27) and sclerotial (day 12, 15, 24, 27) extracts also exhibited significant antibacterial activity against *E. coli* (13.7–15.1 mm) (Figure 1b). In contrast, filtrate extracts showed not significant antibacterial activity across both tested bacteria (Figure 1a and 1b).

3.3. Antibacterial potency based on MIC

All extracts showing significant activity against *B. subtilis* and *E. coli* in agar diffusion assays were further analyzed using MIC assay, as this method provides a more quantitative result by determining the lowest extract concentration that inhibits bacterial growth. The antibacterial potency of the extracts was classified into three categories based on MIC values, strong (< 0.4 mg/mL), moderate (0.4–0.8 mg/mL), and weak antibacterial activity (> 0.8 mg/mL) (Taguri et al. 2006). Only mycelial extract (day 3) demonstrated strong antibacterial activity, showing the similar MIC values of 0.39 mg/mL against *B. subtilis* and *E. coli*, while the remaining extracts all revealed moderate and weak activities (Table 1). Sclerotium is an aggregated and hardened hyphae which develops melanin layer on its surface. Its protected enclosed structure presumably acts as an effective barrier against bacterial interferences (Ajiboye et al. 2025). Compared to sclerotium, mycelium exists as an exposed form of fungal network, making it more susceptible to microbial disturbance, thus requiring additional protective strategy (Künzler 2018). These findings suggested that mycelium offered better defense against other microorganism through its bioactive compounds.

3.4. Fungal developmental morphology

Although several other sclerotium fungi have reported to exhibit antibacterial activity, no studies have yet reported

TABLE 1 MIC values (mg/mL) of *S. rolfsii* extracts. Values represent the lowest concentration inhibiting visible growth after 24 h.

Microbe	Sample	MIC (mg/mL)	Antimicrobial category
B. subtilis	MT1	0.39	Strong
B. subtilis	MT2	2.08	Weak
B. subtilis	MT4	0.78	Moderate
B. subtilis	ST4	0.65	Moderate
B. subtilis	ST5	50	Weak
B. subtilis	ST8	3.12	Weak
B. subtilis	ST9	3.12	Weak
E. coli	MT1	0.39	Strong
E. coli	MT2	6.25	Weak
E. coli	MT4	0.65	Moderate
E. coli	MT5	5.2	Weak
E. coli	MT8	2.08	Weak
E. coli	ST2	4.16	Weak
E. coli	ST4	1.56	Moderate
E. coli	ST5	50	Weak
E. coli	ST8	3.12	Weak
E. coli	ST9	6.25	Weak

the bioactivity during mycelium-sclerotium differentiation. Under unfavorable conditions, *S. rolfsii* produces sclerotium as a survival structure through three distinct developmental stages: initiation phase or initial sclerotia (hyphae aggregation), development phase (gradual expansion of hyphal structure), and maturation phase (pigmentation of outer surface) (Ordóñez-Valencia et al. 2015). In this study, the presence of white mycelial balls were observed in the morphology of the growing *S. rolfsii* with the highest activity (Supplementary Figure 2), illustrating the initiation phase of sclerotium formation, where the hyphae intertwine and aggregate to form a white mycelial ball structure. The high antibacterial bioactivity during this phase may be linked to fungal development, specifically mycelium-to-sclerotium changes. Other studies also showed that metabolites generated as fungi undergo development, exhibit bioactivities (Calvo and Cary 2015; Petersen et al. 2015). This occurrence may suggest that mycelium-to-sclerotium differentiation involves not only morphological changes, but also alterations in metabolites/compounds production (Lau and Abdullah 2015), which potentially mediate the differentiation process while exhibiting bioactivity.

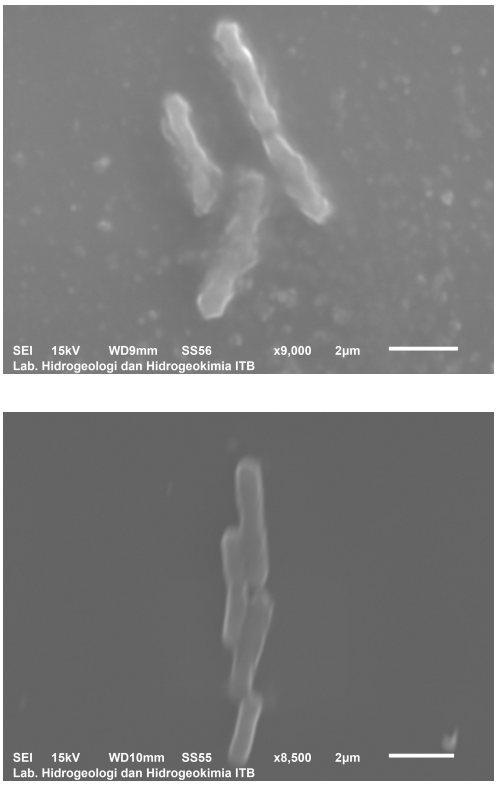


FIGURE 2 Comparison of SEM images of *B. subtilis* cells. (a) treated with M3 (mycelial day-3) extract and (b) treated control (methanol).

3.5. SEM imaging

Control cells exposed to negative control maintained intact basil shape with smooth surface (Figure 2b). In contrast, SEM observation showed an alteration in the morphology of tested bacteria cells contacted with mycelial extract (day 3), displaying irregular and deformation cell shape compared to untreated control (Figure 2a). This result may suggest a disruption of membrane integrity as one of antibacterial actions.

3.6. Optimized mycelial extraction

In the preliminary screening (Figure 1a and 1b), *S. rolfsii* methanol extracts exhibited significant antibacterial activity. Scaled-up production yielded methanol extract that had lost its bioactivity (data not shown). Although methanol can extract a wide range of compounds (Idris and Nadzir 2021), large-scale extraction may result in the formation of precipitates due to the limited solubility of active compounds (Abubakar and Haque 2020). The bulk methanol extract obtained from this large volume contained significant precipitates from less polar compounds. These precipitates can inhibit the bioactivity, since the biological activity of the extract is affected by the solubility and stability of the active compounds (Coltescu et al. 2020). Therefore, sequential extraction was performed to achieve better separation of compounds based on their polarity (n-hexane → ethyl acetate → methanol). The result showed that antibacterial bioactive properties were present in n-hexane and ethyl acetate extracts (Table 2). Although the obtained yields were relatively low, n-hexane and ethyl acetate extracts exhibited bioactivity with lower MIC values (0.20 mg/mL) (Table 2), compared to the previous methanol extracts (0.39 mg/mL) (Table 1), indicating that the bioactive compounds preferentially extracted using less polar solvents.

3.7. TLC-based bioautography

TLC-bioautography is a combination method between thin layer chromatography (TLC) and bioactivity testing, enable detection and localization of bioactive compounds,

TABLE 2 Weight (mg), yield (%), and MIC values (mg/mL) of mycelial extracts obtained by sequential extraction.

Extract	Weight (mg)	Percentage of yield (%)	Bacteria	MIC (mg/mL)	Antibacterial category
N-hexane	76.3	0.42	*B. subtilis*	0.20	Strong
			E. coli	0.20	Strong
Ethyl acetate	117.8	0.65	*B. subtilis*	0.20	Strong
			E. coli	0.20	Strong
Methanol	5491.8	30.55	*B. subtilis*	6.25	Weak
			E. coli	6.25	Weak

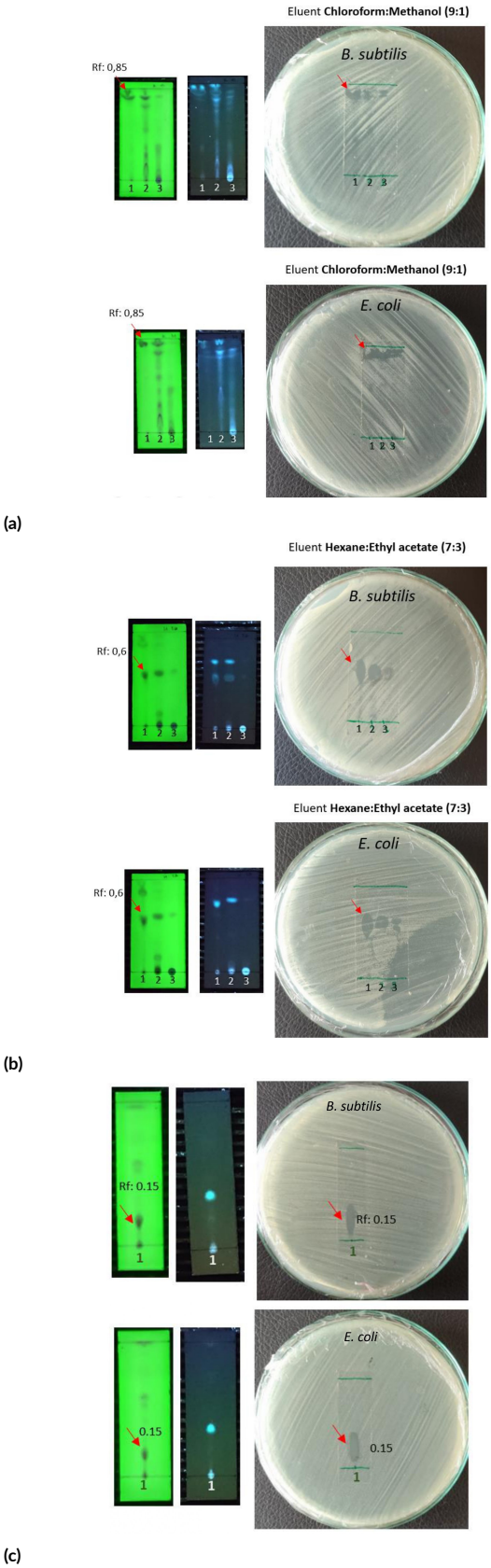


FIGURE 3 Antibacterial screening of fungal extracts analyzed by TLC-bioautography against *B. subtilis* and *E. coli* (sample 1: n-hexane extract, Sample 2: ethyl acetate extract, sample 3: methanol extract): (a) eluent: chloroform-methanol 9:1, (b) eluent: hexane-ethyl acetate 7:3, (c) improvement of TLC-bioautography of n-hexane extract using eluent: n-hexane-ethyl acetate 9:1.

including antibacterial activity (Wang et al. 2021).The clear inhibition zone forms as antibacterial bioactive compounds from the chromatogram spots diffuse into the growth media, suppressing bacterial growth in the affected area (Balouiri et al. 2016; Wang et al. 2021), as observed on MHA plates across all extracts (Figure 3a and 3b). N-hexane, ethyl acetate, and methanol extracts shared the same Rf value (0.85) in TLC-bioautography assay of *B. subtilis* and *E. coli*, similar to their position of clear zones on the NA plate (Figure 3a). The extracts showed a lower Rf value (0.60) in a more non-polar eluent, as well as the inhibition zones position (Figure 3b). However, visual assessment demonstrated larger clear zones in n-hexane and ethyl acetate compared to methanol extract (Figure 3a and 3b), consistent with the MIC data that classified both extracts as strong activities (Table 2). Figure 3a displayed that ethyl acetate extract had numerous spots, whereas n-hexane extract showed only four distinct spots, suggesting that n-hexane extract might contain a simpler chemical profile and selected for further purification process. Additionally, to improve visualization of higher Rf spots in Figure 3b, n-hexane extract was re-analyzed using a different ratio of eluent (Figure 3c). In this eluent system, the target compound exhibited a spot in Rf value of 0.15, and used as a reference for further TLC steps.

3.8. Compound separation by radial chromatography

Radial chromatography resulted in four fractions of n-hexane extract. TLC analysis of fraction 4 showed a spot at Rf 0.15 (Figure 4a), similar to Rf value in Figure 3c, indicating the presence of antibacterial compound (isolate-01). Fraction 4 yielded a yellow oil fraction (dissolved by n-hexane) and a white needle-shaped crystal (dissolved by chloroform) (Figure 4b and 4c). The TLC analysis confirmed the white needle-shaped crystal as the isolated compound, based on a single spot (Rf 0.15) (Figure 4d) matching the previous result (Figure 3c).

The isolated compound also showed a stronger antibacterial activity (0.09 mg/mL) compared to the crude extract (n-hexane extract 0.20 mg/mL) (Table 3). This result indicates that the isolation process not only increases the purity of the compound but also its potential bioactivity.

3.9. Structural elucidation by NMR

The [^{1}H, ^{13}C, and DEPT-135] NMR spectroscopic analysis of the isolated compound (isolate-01) revealed the presence of C28 carbon skeleton and a steroid group. This

TABLE 3 MIC values (mg/mL) comparison of mycelial extract and purified compound.

Sample	MIC (mg/mL)	Bacteria
N-hexane extract (crude)	0.20	*B. subtilis*
	0.20	*E. coli*
Isolate-01	0.09	*B. subtilis*
	0.09	*E. coli*

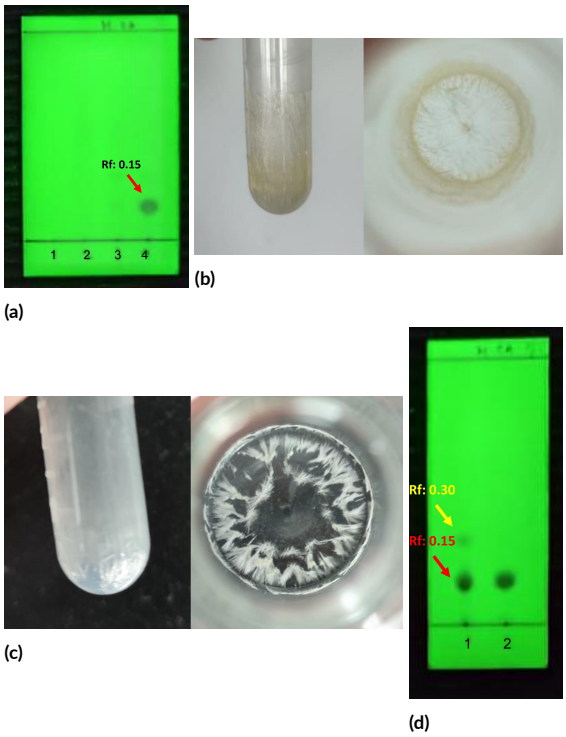


FIGURE 4 Analysis of compound purification: (a) TLC of n-hexane fractions from purification using radial chromatography (sample 1: fraction 1, sample 2: fraction 2, sample 3: fraction 3, sample 4: fraction 4), (b) recrystallization of fraction 4 from radial chromatography resulted in yellow oil and white crystal sediment, (c) separation of white crystal sediment using chloroform, (d) TLC of sample b (1) and c (2) (eluent: hexane-ethyl acetate 9:1) (sample 1: yellow oil and white crystal sediment and sample 2: white crystal sediment) showing single spot at Rf 0.15 (red arrow) for sample 2.

compound has 6 methyl/-CH3 groups, where 2 isolated methyl (singlet peak) (H-18 and H-19) and 4 CH-bound methyl (doublet peaks) (H-21, H-26, H-27, and H-28). Isolate-01 has 1 methine-oxy/-CH-O group at position H-3. In addition, this compound also exhibited the presence of 4 methine-olefin/-CH= protons and 6 olefin carbons derived from 3 double bonds/alkenes, with one conjugated alkene (C-5 to C-8) and one isolated alkene with a trans position (C-22 – C-23). Comparative analysis with literature data (Lini et al. 2020) (Supplementary Table 1), confirmed that isolate-01 is ergosterol (ergosta-5,7,22-trien-3β-ol).

3.10. Antibacterial potential of ergosterol

Ergosterol is a steroid compound (Figure 5) commonly found in fungi, serving essential biological roles in maintaining the integrity, fluidity, and permeability of cell membranes. Ergosterol exhibits wide-spectrum bioactivity, including antioxidant, anticancer, antidiabetic, and antineurogenic activities (Rangsinth et al. 2023). Few investigations have explored the antibacterial activity of ergosterol, with one study reported it inhibited *Helicobacter pylori* with MIC of 0.02 mg/mL (Li et al. 2005). Ergosterol has also been reported to exhibit synergistic effects when combined with commercial antibiotics such as gentamicin, showing enhanced antibacterial activity compared to gentamicin alone (Andrade et al. 2014). These findings indicate the potential of ergosterol for further investigation regarding its antibacterial activity.

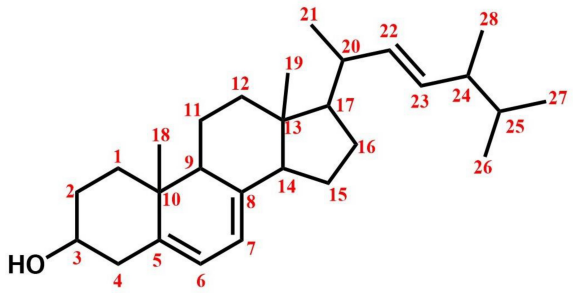


FIGURE 5 Structure of ergosterol ($C_{28}H_{44}O$) from *S. rolfsii*.

The antibacterial mechanism of ergosterol has not been extensively studied, since ergosterol mainly known as a target of antifungal drugs. Ergosterol's mode of action may be analyzed through an approach based on antibacterial activity of non-polar cyclic hydrocarbon compounds. The chemical structure of ergosterol consists of a cyclic hydrocarbon sterol with lipophilic characteristics. This hydrocarbon compound can dissolve in bacterial lipid membranes, accumulate, and expand the membrane, leading to membrane swelling and cell death (Sikkema et al. 1994), this may be in line with alteration in the morphology of treated cells in SEM observations (Figure 2a), suggesting cell membrane damage as antibacterial mechanism. However, further investigation for the exact mode of action of ergosterol remains necessary.

The highest antibacterial activity was observed in the mycelium harvested on the third day, demonstrating the initial formation of sclerotium stage, and ergosterol was subsequently isolated from this mycelial extract. These results may indirectly suggest a connection between ergosterol and sclerotium formation. Several studies support this hypothesis, including: there is an upregulation activity of series of enzymes which played important roles in the final steps of ergosterol biosynthesis in *Polyporus umbellatus* sclerotium (Xing et al. 2022). Impaired ergosterol formation also contributes to premature/abnormal sclerotium growth (Ranjan et al. 2019). In *Verticillium dahlia*, deletion of the ERG2 gene in ergosterol biosynthesis leads to reduced ergosterol content, impaired conidial germination, and a significant decrease in microsclerotia production (Lv et al. 2023). Yaderets et al. (2021) also reported that combination of natural antifungal compounds which both target ergosterol biosynthesis (polyene drug from *Bacillus* and mevastatin from *Penicillium chrysogenum*) inhibits *S. sclerotiorum* growth and suppress sclerotia formation. The modification of ergosterol into aminoacylated form (Erg-Asp and Erg-Gly) plays a critical role in the development switch between conidiation and sclerotia formation in *Aspergillus oryzae*. The absence of these modified ergosterol compounds promotes sclerotia production (Yokokawa et al. 2025). All these findings suggest a po-

tential strong connection between ergosterol biosynthesis and sclerotium differentiation. Ergosterol, which functions to maintain the integrity of fungal cell wall, might facilitate sclerotium formation by supporting the stability of fungal structure during the sclerotium formation. However, further studies must examine detailed mechanism on how ergosterol exactly contributes to sclerotium formation as fungal developmental stage.

4. Conclusions

This research revealed that *S. rolfsii* exhibited potential antibacterial activity. Three-days-old *S. rolfsii* mycelium extracted with n-hexane showed stronger inhibition against *B. subtilis* and *E. coli* (MIC 0.20 mg/mL) compared to other fungal parts (sclerotium and filtrate), indicating that the mycelium represents a key defensive component of the sclerotium-forming fungus against microbial competitors. Ergosterol was purified from this extract with higher antibacterial activity (0.09 mg/mL) against both bacteria, highlighting a new potential activity of this compound. This result also indirectly suggests a link between ergosterol and sclerotium formation, since it was obtained from third day of *S. rolfsii* mycelium, with the morphology of initial stage of sclerotium development. Ergosterol is presumed to play an important role in maintaining fungal membrane stability during sclerotium formation. This finding may further support the prospect of in-depth study of sclerogenesis as fungal development and bioactive compound origins.

While this study identifies ergosterol as a key bioactive compound, its study was limited to a single isolate of sclerotium fungus without full mechanistic characterization. Further works should focus on wider fungal isolation, detailed antibacterial mode of action studies, and time-course analysis correlating ergosterol and sclerotium formation dynamics.

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Authors' contributions

MZ, INPA, S, VSHS designed the study. MZ carried out the laboratory work. MZ, INPA, S, VSHS analyzed the data. MZ, INPA, S, VSHS wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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