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Original Research Article

Molecular Characterization of Endophytic Fungi from the Leaves of Beruwas Laut (*Scaevola taccada* (Gaertn.) Roxb.) as Antibacterial Producer

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ABSTRACT

Endophytic fungi live and develop in plant tissues, are symbiotic, and are host-plant friendly. These fungi generally produce secondary metabolites with anticancer, antiviral, or antibacterial properties. This study aims to identify endophytic fungi isolated from Beruwas laut leaves. The endophytic fungi were isolated from Potato Dextrose Agar (PDA), while the secondary metabolites were produced by fermentation on Maltose Yeast Broth medium, agitated for 18 days at 150 rpm. The chemical composition of the fungi was identified using TLC. The antibacterial activity was assessed against some pathogenic bacteria strains through agar diffusion and TLC-Bioautography methods, while molecular identification was conducted using PCR. The results revealed 16 chemical isolates from the endophytic fungi, among which IFDBL-15 exhibited the most potent antibacterial activity. Inhibitory zones of the isolates against *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25923), and *Salmonella typhi* (NCTC 786) were 15.47, 14.52, 14.64, 14.10, 15.92, and 15.52 mm, respectively. The fermentation extract of the IFDBL-15 isolate contained flavonoids, alkaloids, anthraquinones, and saponins. TLC-Bioautography activity revealed four active spots. The spot that inhibited all test bacteria was identified as a flavonoid (Rf = 0.45). Molecular identification using ITS1 and ITS4 gene regions via Polymerase Chain Reaction showed that the IFDBL-15 isolate exhibited the closest relationship to the species *Parengyodontium album* with an identity match of 99.83%. IFDBL-15 endophytic fungi isolate has shown the potential to generate antibacterial metabolites and could be further explored as a drug lead.

***Keywords*:** endophytic fungi, Beruwas laut, antibacterial, *Scaevola taccada*, *Parengyodontium album*

Introduction

Worldwide, infectious diseases originating from bacteria continue to be prevalent. These diseases are commonly treated with antibiotics. However, antibiotic resistance poses a great threat to disease therapy, possibly due to improper antibiotic usage.[1,2] It has been demonstrated that natural chemicals from a wide range of sources possess antibacterial qualities, particularly against bacteria that have developed antibiotic resistance.[2]

Endophytic fungi are a group of fungi that partially or completely live in living plant tissues. They often do not pose a threat to their hosts and instead generate beneficial secondary metabolites with antibacterial, antiviral, or anticancer properties.[3–5]

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Due to genetic transfer between the endophytic fungi and the host plant, the endophytic fungi may produce bioactive chemicals and secondary metabolites that are either identical to, distinct from, or even superior to those of their hosts, as such endophytic fungi have the propensity to produce useful and unique substances.[5,6] Endophytic fungi also have a short life cycle and can produce large amounts of bioactive compounds, making them a large reservoir of natural, economical, and environmentally friendly sources of medicinal materials.[7] Endophytic fungi can be utilised for timely and large-scale production of antibacterial agents without causing ecological damage.[8]

Endophytes represent a microbial community that lives within plants without displaying any noticeable symptoms, offering the potential for generating a wide array of bioactive compounds and new metabolites. These substances have practical applications in medicine, agriculture, and various industrial settings. Strobel and Daisy,[9–11] suggested that endophytes represent a valuable source of secondary metabolites. They compared *Pestalotiopsis sp*. to "the *E. coli* of the rain forests" and labeled *P. microspora* as a "microbial factory" producing bioactive secondary metabolites. Their research has led to the identification of several chemical structures, including those of subglutinols A and B, taxol, ambuic acid, cryptocandin, and torreyanic acid. Various chemical classes, including alkaloids, cytochalasines, flavonoids, furandiones, phenylpropanoids, lignans, peptides, phenols, phenolic acids, steroids, terpenoids, quinones, aliphatic acids, and chlorinated compounds, are represented among the endophyte-derived metabolites.

A variety of chemical classes, such as steroids, xanthones, phenols, isocoumarins, perylene derivatives, quinines, furandiones, terpenoids, depsipeptides, and cytochalasins, are among the secondary metabolites that are extracted from endophytes. Numerous biological activities, including antibacterial, antiviral, anticancer, antioxidant, insecticidal, antidiabetic, and immunosuppressive qualities, have been established by these substances. Additionally, endophytes contribute significantly to the physiological functions of host plants, strengthening their defenses against nematodes, stress, insects, and diseases.[12–15]

One of the traditional medicinal plants in Indonesia is beruwas laut (*Scaevola taccada* (Gaertn.) Roxb.), known to be effective in treating diabetes, headaches, eye infections, foot swelling, pain, coughs, and influenza. Besides, this plant is commonly used in ethnomedicine, particularly for wound healing.[16,17]

Kosman and Tappang reported the presence of flavonoids in the diethyl ether fraction of sea beruwas leaves.[18] Hasan,[19] on the other hand, reported the presence of terpenes, phenols, tannins and flavonoids in the ethanol extracts of beruwas laut leaves demonstrating activity against *Vibrio cholerae, Pseudomonas aeruginosa, Streptococcus mutans, Salmonella typhi, Escherichia coli, Bacillus subtilis, Propionibacterium acnes,* and *Shigella dysenteries* in TLC-Bioautography tests.

This current study aims to conduct molecular characterization of the active isolates of endophytic fungi from beruwas laut (*Scaevola taccada* (Gaertn.) Roxb.) leaves as a source of antibacterial agents.

Materials and Methods

Pathogens of interest

Staphylococcus aureus (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhi (NCTC 786), *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25923).

Sterilization of tools and preparation of agar media

Materials used for this assay were heated in an oven (180 °C) for 2 hours, while the non-heating tools were sterilized in an autoclave (121 °C) at 1 atm pressure for 15 minutes.[3,20]

Plant Collection and Identification

Fresh and healthy leaves of *Beruwas laut* were collected on March 18, 2023 at Suppa district, Pinrang regency, South Sulawesi, Indonesia, with coordinates 3°56’34.5 “S 119°33’59.8 "E. The samples were kept in a sterile sample bag and washed in running water to remove soil and other extraneous particles adhering to the leaf surface. Leaves of beruwas laut from the same location have been identified with registration number 1363/11.CO2.2/PL/2018 at the Bandung Institute of Technology, registration by researcher Ermalyanti Fiskia (Department of Pharmacy, Faculty of Medicine, Khairun University, Ternate, Indonesia). Herbarium samples are also stored at the Pharmacy Microbiology Laboratory, Universitas Muslim Indonesia, the site used for this study.

Preparation of leaf samples

The surfaces of the leaves were sterilized by placing them in a 250-mL Erlenmeyer flask, submerged in 70% ethanol, and mildly shaken for 3 minutes. Following the removal of the ethanol, 5.25% NaOCl was added and allowed to sit for two minutes. Next, the leaves were washed three times, each for a minute, with sterile distilled water. Aseptic methods were used for sterilization. The leaves were then placed on sterile Petri dishes, as reported by Rante *et al.*[3,21]

Isolation and Purification of Endophytic Fungi

After being sterilized, the leaves were cut into 1 cm pieces using a sterile knife. They were then placed in sterile Potato Dextrose Agar (PDA) supplemented with 0.05% chloramphenicol and left to incubate for five to seven days at room temperature (25°C). It was noted that there was fungal growth surrounding the plant leaves, a sign of endophytic fungal development.[3,21]

Following a 5-day incubation period at 25°C, the fungi that had grown around the explants were sub-cultured on PDA supplemented with 0.05% chloramphenicol. Following this, observations were conducted about the colonies' color formed on the media. Until a pure colony was achieved, every colony with variations in shape and color was recolored on PDA media.[3]

Morphological Identification of Endophytic Fungi

Macroscopic identification of the fungi isolates was carried out to determine the colony morphology by directly examining the colonies’ appearance, focusing on the shape as well as the colours of the colony surface and edge of the petri dish.[3]

Fungal isolates were taken using a needle and transferred to a sterile preparation glass. Lactophenol Cotton Blue solution was added and then observed microscopically under a light microscope with a magnification of 100-400.[20]

Antagonist test

Cultures of endophytic fungus were placed in a petri dish with pathogenic bacteria and a nutrient agar medium. The dishes were incubated for 24 hours at 37°C.[21]

Molecular identification of IFDBL-15 isolates by PCR Method

DNA Extraction, Amplification

DNA extraction, sequencing, and BLAST (Basic Local Alignment Search Tool) were performed in compliance with the protocol. DNA extraction was carried out using the Quick-DNA Magbead Plus Kit. Additionally, the internal transcribed spacer (ITS) region of the rRNA gene was used to characterize fungi by PCR amplification. The ITS universal primer set, which flanked the ITS 1 and ITS 4 regions, was also used. The nucleotide sequence of the ITS 1 primer was 5'-TCCGTAGGTGAACCTGCGG-3', and that of the ITS 4 primer was 3'-TCCTCCGCTTATTGATATGC-5'. PCR was carried out in a PCR System Thermal Cycler (Takara, Japan) with a program consisting of initial denaturation in 1 cycle for 3 minutes at 95°C involving 35 cycles (denaturation for 15 seconds at 95°C, annealing for 30 seconds at 52°C, and extension for 45 seconds at 72°C). The final extension was done in 3 minutes at 72°C, and the cooling was at 4°C for a while. Overall, the PCR process was performed in 38 cycles.

Electrophoresis

One gram of 2% agarose gel was weighed and dissolved in 100 mL of TAE Buffer into an Erlenmeyer flask, heated in the microwave for 2 minutes to boil, then 8 µL of ethidium bromide was added. The gel liquid was poured into an agarose printer until it solidified. Inserted 8 µL of PCR products mixed with 2 µL of loading dye solution, each amplified sample into the wells on agarose submerged in a tank containing TAE Buffer. The electrophoresis process was run at 100 volts for 50 minutes. The electrophoresis gel layer was observed under UV-transluminator light.

Sequencing and Species Identification using BLAST

The sequencing process was carried out by 1st Base through PT Genetika Indonesia. Sequencing was performed by "Single Pass DNA Sequencing" using the same primers as gene amplification in the PCR process. The sequencing results were processed using the Bioedit application. Analysis of the BLAST results provides information and verifies what the organisms or bacteria have in common with the sample DNA sequence so that it can be used for bacterial identification. Information from the BLAST results is in the form of Query Coverage and Maximum identity. Query coverage is the percentage of nucleotide length that is aligned with the database contained in BLAST. Max identity is the highest value of the percentage of identity or match between the query sequence and the aligned database sequence.

Phylogenic Analysis

The data was then processed for phylogenetic analysis using the MEGA 11 application in Windows 11.

Secondary metabolite production

The pure culture of the fungi was rejuvenated on a PDA slant agar medium, followed by incubation at 25 °C for 3–12 days. The inoculum was taken from the slant agar medium, homogenized in sterile distilled water, put into Maltose Yeast Broth (MYB) medium supplemented with yeast extract, and then incubated at 25°C for 3 days. The culture was

transferred into a 1000-mL Erlenmeyer flask containing 800 mL of the same medium. The fermentation was carried out at 25 ℃ under controlled conditions for 18 days, as described by Burhamzah *et al.*[4] After fermentation, the culture was filtered, and the mycelia and culture liquid were separated. Subsequently, ethyl acetate (1:1 v/v) was added to the culture liquid in a separating funnel for 20 minutes. The extracted ethyl acetate solvent was then evaporated using a rotary evaporator to yield a thick extract. The extract was stored in a dryer for use in the next stage of the experiment.[22]

Phytochemical analysis

Aliquots of the extracts were spotted on (TLC) plates (Merck, silica gel 60 F254) to identify the chemical components. The plates were eluted with Hexane and ethyl acetate (6:1) under saturated conditions, and the eluates were visualized under UV light at 254 and 366 nm. The chromatograms were sprayed with 10% H_2SO_4, then incubated at 110 ℃ to achieve the best color development.[21]

Antimicrobial Activity Test

Agar diffusion method

The suspension (100 µL) of test microorganisms, containing 10^8 colony-forming units (CFU)/mL of bacteria cells spread on nutrient agar (NA), was made into wells (6 mm in diameter). 20 µL of extract (250 µg/mL, 500 µg/mL, 1000 µg/mL, 2000 µg/mL, and 4000 µg/mL) was transferred into the wells previously inoculated with the test microorganisms. Wells without samples were used as a negative control, and the leaf extract (1000 µg/mL) was used as a positive reference. The Petri dishes were kept at 4℃ for 2 hours and incubated at 37℃ for 24 hours. The test organisms' zones of growth inhibition in millimeters (including wells diameter of 6 mm) were measured and contrasted with the controls to determine the antibacterial activity of the extracts. Inhibition zone measurements were made for three sample replications, and the results were expressed as the mean of the three duplicate measurements.[23,24]

Bioautographic TLC method

The best chromatogram results were followed by a TLC-bioautography test on a Petri dish. Approximately 10 mL of the nutrient agar was added to a suspension of test bacteria (about 20 µL) and then homogenized. TLC plates that had been eluted were placed on the surface of the agar medium that had been inoculated with test bacteria and then left for 60 minutes. After that, the plate was lifted and removed, then incubated for 24 hours at 37°C until the inhibition zones were formed.[20]

Results and Discussion

Isolation and purification of endophytic fungi from leaves of beruwas laut.

This study reported the isolation of endophytic fungi from the leaves of beruwas laut (*Scaevola taccada* (Gaertn.) Roxb.) using Potato Dextrose Agar medium supplemented with chloramphenicol (PDAC) to prevent bacterial growth on the medium, allowing only the growth of fungi[25] (Figure 1). Appropriate purification processes resulted in the isolation of 16 fungal isolates with noticeable differences identified through macroscopic and microscopic tests (Figure 2). The inhibitory test was carried out by the agar diffusion method using MHA (Muller Hinton Agar) medium against six bacteria strains: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhi, Staphylococcus aureus,* and *Staphylococcus epidermidis*. The antibacterial activity of each isolate was indicated by the inhibition zone formed after incubation. Screening tests showed that the isolate with the best activity was IFDBL-15 (Table 1).

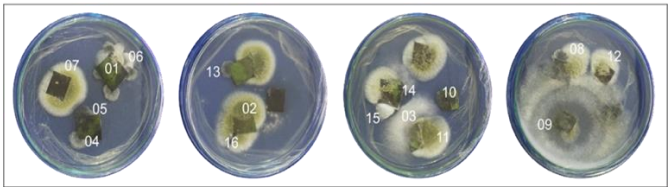

Figure 1: Fungi on the edges of the pieces of leaf

Table 1: Antagonist Test

No	Kode Isolat	Diameters of Inhibition zone (mm)					
		S. aureus	***E. coli***	***B. subtilis***	***P. aeruginosa***	***S.* Typhi**	***S. epidermidis***
1	IFDBL 01	7.2	7.3	7.7	7	7	7
2	IFDBL 02	-	-	-	-	-	-
3	IFDBL 03	-	-	-	-	-	-
4	IFDBL 04	7.6	--	7.5	-	7	-
5	IFDBL 05	-	-	-	-	-	-
6	IFDBL 06	-	-	-	-	+	-
7	IFDBL 07	-	-	-	-	-	-
8	IFDBL 08	7.4	7	7.6	-	7.2	-
9	IFDBL 09	-	-	-	-	7.4	-
10	IFDBL 10	7.3	8.5	-	7.2	7.1	7
11	IFDBL 11	-	-	-	-	-	-
12	IFDBL 12	-	-	-	-	7	7
13	IFDBL 13	-	-	-	7.6	-	-
14	IFDBL 14	8.3	8.2	7.4	7.8	7.5	8.5
15	IFDBL 15	9.2	9.7	9.7	7.8	9.8	9.6
16	IFDBL 16	8.4	8.5	7.8	8.3	8.9	8.6

(Description: *S. aureus= Staphylococcus aureus, S. epidermidis=Staphylococcus epidermidis, P. aeruginosa=Pseudomonas aeruginosa, S.* Typhi=*Salmonella* Typhi, *B. subtilis =Bacillus subtilis, E. coli=Escherichia coli*)

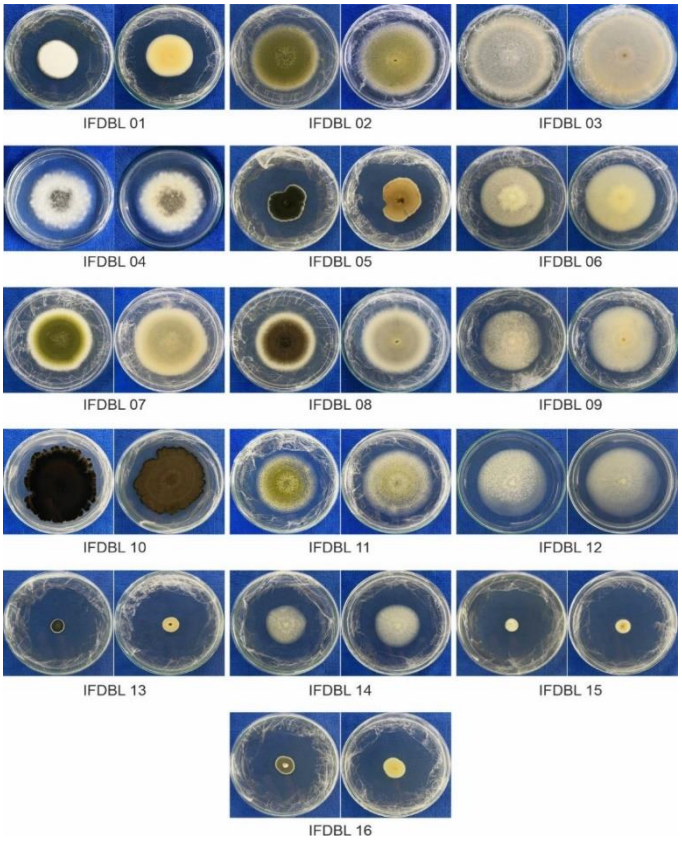

Figure 2: Endophytic fungal isolates

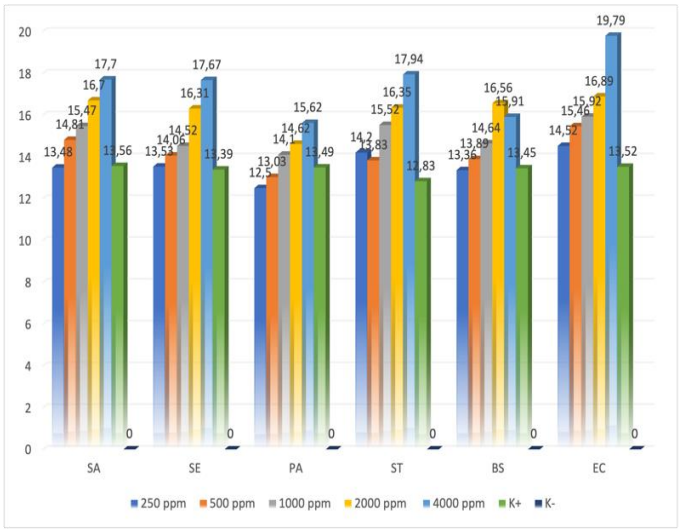

Figure 3: Antimicrobial activity extract of IFDBL-15 against clinical pathogens
(Description: SA=Staphylococcus aureus, SE= Staphylococcus epidermidis, PA=Pseudomonas aeruginosa, ST=Salmonella Typhi, BS=Bacillus subtilis, EC=Escherichia coli, 1=250 µg/mL, 2=500 µg/mL, 3=1000 µg/mL, 4=2000 µg/mL, 5=4000 µg/mL, 6=K+)

Molecular identification of IFDBL-15

The molecular identity of IFDBL-15 was carried out with the PCR method. First, the fungal DNA was isolated and then amplified with PCR, followed by electrophoresis to obtain the band appearance (Figure 7). The band formed from the isolated IFDBL-15 DNA appeared homogeneous but less thick, suggesting that the total extracted DNA had an excessive amount of contamination.[26] The DNA sequencing of the sample yielded 600 base pairs (base pairs bp). The difference in the number of base pairs might be due to differences in nucleotide sequence or the genetic structure of the fungus.[27] Based on the BLAST results, IFDBL-15 exhibited high conformity with the DNA sequence of *Parengyodontium album* species, with an identity level of 99.83%, as recorded in the database. The results of phylogenetic analysis using the MEGA 11 application in Windows 11 (Figure 8) confirmed likewise.

In addition to molecular identification, IFDBL-15 was fermented in Maltose Yeast Broth media for 18 days, followed by the extraction of the fermentation product. Subsequent testing was conducted using 400 mg extract, antimicrobial activity screening, TLC-Bioautography, and the determination of the chromatogram profile.

Antimicrobial activity screening

The results of antibacterial activity testing of the ethyl acetate extract of IFDBL-15 yielded the highest activity against *S. aureus* with an inhibitory zone diameter of 17.70 mm, followed by *S. epidermidis* at 17.67 mm, *P. aeruginosa* at 15.62 mm, *Salmonella* at 17.94 mm, *B. subtilis* at 15.91 mm, and *E. coli* at 19.79 mm (Figure 3). Compared to the ethanol extract leaves of beruwas laut used as a positive control, these results indicated higher values. Antibacterial strength was divided into 4 groups: 20 mm or more was classified as very strong, 10-20 mm was considered strong, 5-10 mm was in the medium category, and 5 mm or less was categorized as weak. The results of this activity test indicated that the endophytic fungi present in leaves of beruwas laut produced antibacterial compounds in a strong ability category.[28,29]

Antimicrobial activity test by TLC-Bioautographic method

The antibacterial activity testing of the ethyl acetate extract of IFDBL-15 fermentation isolate by TLC-Bioautography was done to determine the presence of chemical components with antibacterial activity. The presence of a clear zone visible on the medium characterizes the activity formed. The results of the TLC-Bioautography test on the ethyl acetate extract revealed that the spot with an R_f value of 0.06 inhibited *E. coli*, the spot with an R_f value of 0.45 inhibited all test bacteria, the spot with an R_f value of 0.55 inhibited *S. aureus*, *B. subtilis*, *E. coli*, and *S. epidermidis*, and the spot with an R_f value of 0.96 inhibited *Bacillus subtilis* bacteria (Figure 6).

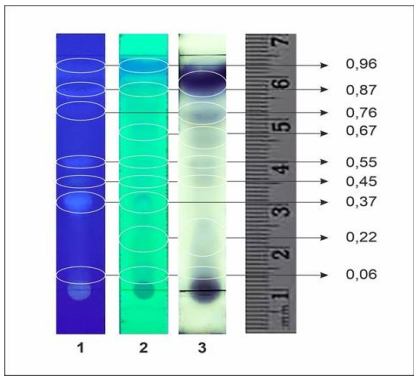

Figure 4: Chromatogram profile of ethyl extract of IFDBL-15 (Description: 1. UV 366, 2. UV 254, 3. H_2SO_4)

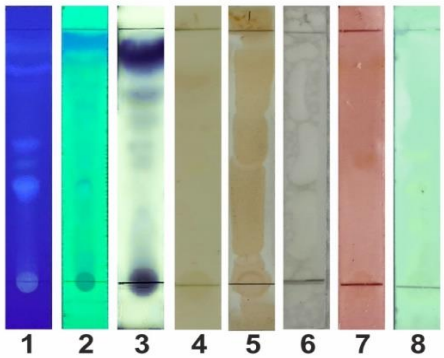

Figure 5: Chemical compound component identification results (Description: 1=UV 366, 2=UV 254, 3=H_2SO_4, 4=$AlCl_3$, 5=Dragendorf, 6=$FeCl_3$, 7= Lieberman Bouchardat, 8=KOH)

Phytochemical analysis

Thin-layer chromatography (TLC) identification is one of the methods used to separate compounds on the basis of differences in the partition coefficient. The extract was eluted with n-hexane: ethyl (6:1), and the spots were visualized at UV 254 nm and UV 366 nm and then sprayed with an H_2SO_4 reagent. The TLC profile of the ethyl acetate extract of IFDBL-15 showed nine spots with varying colors and R_f values, indicating the presence of several chemical components with different polarity values (Figure 4). Differences in polarity indicate different compounds from each spot. The class of chemical components contained in the ethyl acetate extract of IFDBL-15 fermentation isolate was determined by spraying the TLC plates with several specific reagents to reveal the spots. IFDBL-15 spot appeared brownish yellow at R_f values of 0.22 and 0.45 when sprayed with $AlCl_3$ spray reagent, indicating the presence of flavonoids, brown or orange at R_f value of 0.55 with Dragendorf reagent for alkaloids, yellow for anthraquinones with KOH reagent, at R_f =0.06; 0.22; 0.37; and 0.45), and brownish yellow for saponins with Liberman Bouchard spray reagent, at R_f 0.45 and 0.87) (Figure 5).

Conclusion

The isolated endophytic fungi IFDBL-15 demonstrates high conformity with the DNA sequence of the *Parengyodontium album* species, showing an identity level of 99.83%. This isolate contained phytochemical compounds such as flavonoids, alkaloids, anthraquinones, and saponins, which may have contributed to the antibacterial activity of IFDBL-15, which also exhibited inhibitory activity against the bacteria strains (*B. subtilis*, *E. coli*, *P. aeruginosa*, *S. Typhi*, *S. aureus*, and *S. epidermidis*) used in this study. IFDBL-15 could be further explored for potential antibacterial drug lead.

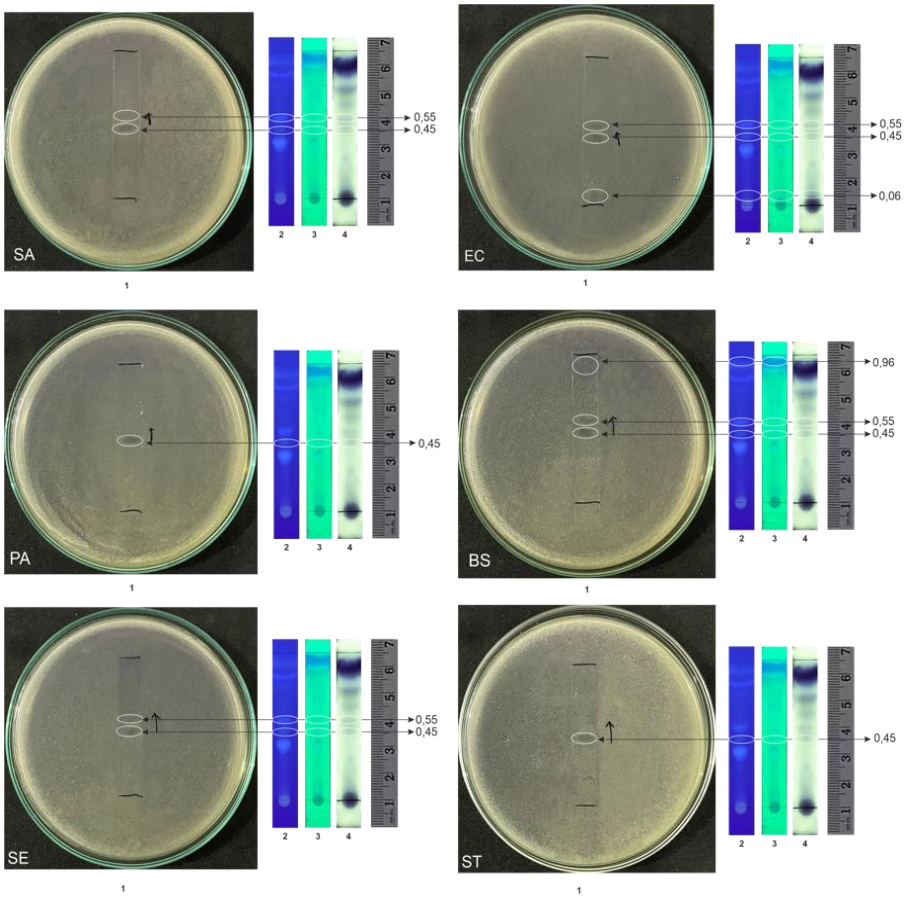

Figure 6: TLC-Bioautography activity test
(Description: SA=Staphylococcus aureus, SE= Staphylococcus epidermidis, PA=Pseudomonas aeruginosa, ST=Salmonella Typhi, BS=Bacillus subtilis, EC=Escherichia coli, 1=UV 366, 2=UV 254, 3=H_2SO_4)

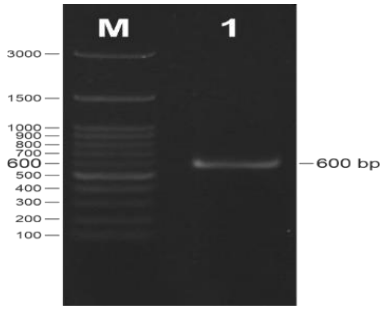

Figure 7: DNA electrophoresis of IFDBL-15 isolate (Description: M=DNA ladder 100 bp, 1=IFDBL-15 Isolate)

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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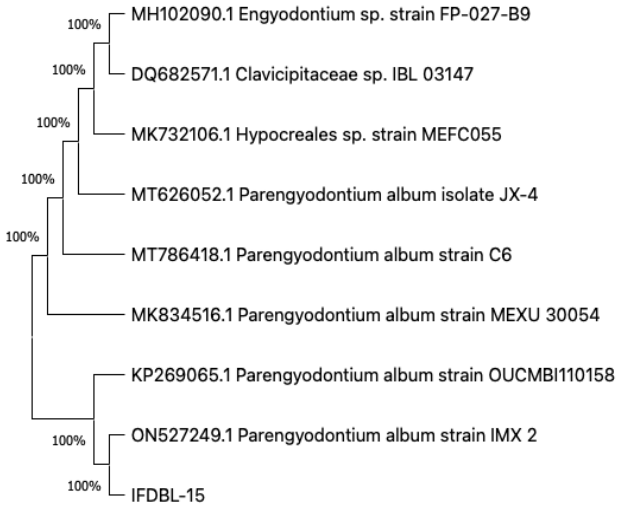

Figure 8: Phylogenetic tree of isolate IFDBL-15

References

1. Mao Z, Zhang W, Wu C. et al. Diversity and antibacterial activity of fungal endophytes from *Eucalyptus exserta*. BMC Microbiol, 2021; 21: 155 https://doi.org/10.1186/s12866-021-02229-8
2. Álvarez-Martínez FJ, Barrajón-Catalán E & Micol V. Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. Biomedicines. 2020; 8(10): 405 https://doi.org/10.3390/biomedicines8100405
3. Rante H, Halim Umar A & Mau DP. Isolation of Endophytic Fungi from Tamarind Leaves (*Tamarindus indica* l.) as a Producer of Antibacterial Compounds. Majalah Farmasi Dan Farmakologi. 2021; 25(2): 66–68.
4. Burhamzah R, Alam G, Rante H. Characterization of Antibacterial-Producing Endophytic Fungi of Syzygiumpolyanthum Leaves. Infect Disord Drug Targets. 2020; 20(4): 448-454 https://doi.org/10.2174/1871526519666181226123541
5. Fitriana F & Nurshitya E. Antibacterial Activity of Extracts of Endophytic Fungal Isolates from Mangrove Roots (*Rhizophora apiculata* Blume) by TLC-Bioautography. Jurnal Ilmiah As-Syifaa. 2017; 09(01): 27-36 https://doi.org/10.56711/jifa.v9i1.234
6. Deponda RA, Fitriana F, Nuryanti S & Herwin H. Isolation of Red Fruit Peel Endophytic Fungi (*Pandanus conoideus* Lam.) which has potential as Antibacterial by TLC - Bioautography Method. Jurnal Ilmiah As-Syifaa. 2019; 11(02): 147-153 https://doi.org/10.56711/jifa.v11i2.583
7. Hasiani VV, Ahmad I & Rijai L. Isolation of Endophytic Fungi and Production of Antioxidant Secondary Metabolites from Girlfriend Leaves (*Lawsonia inermis* L.). Jurnal Sains dan Kesehatan. 2015; 01(04): 146–153.
8. Murdiyah S. Endophytic Fungi in Various Medicinal Plants in the Evergreen Forest Area of Baluran National Park and the Potential for Development as a Mycology Course Manual. Jurnal Pendidikan Biologi Indonesia. 2017; 03(01): 64-71 https://doi.org/10.22219/jpbi.v3i1.3977
9. Strobel GA. Endophytes as sources of bioactive products. Microbes and Infection. Preprint. 2003; 5(6):535-44 https://doi.org/10.1016/S1286-4579(03)00073-X
10. Strobel G & Daisy B. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiology and Molecular Biology Reviews. 2003; 67(4): 491-502 https://doi.org/10.1128%2FMMBR.67.4.491-502.2003
11. Radji M. The Role of Biotechnology and Endophytic Microbes in Herbal Medicine Development. Majalah Ilmu Kefarmasian. 2005; 02(03): 113–126 https://doi.org/10.7454/psr.v2i3.3388
12. Deshmukh SK, Prakash V & Ranjan N. Recent advances in the discovery of bioactive metabolites from Pestalotiopsis. Phytochemist Rev. 2017. 16: 883–920 https://doi.org/10.1007/s11101-017-9495-3
13. Deshmukh SK, Gupta MK, Prakash V & Saxena S. Endophytic fungi: A source of potential antifungal compounds. Journal of Fungi vol. 4 Preprint. 2018; 04(03): 77 https://doi.org/10.3390/jof4030077
14. Gao H, Li G & Lou HX. Structural diversity and biological activities of novel secondary metabolites from endophytes. Molecules 2018; 23(03): 646 https://doi.org/10.3390/molecules23030646
15. Gupta A, Meshram V, Gupta M, Goyal S, Qureshi KA, Jaremko M, Shukla KK. Fungal Endophytes: Microfactories of Novel Bioactive Compounds with Therapeutic Interventions; A Comprehensive Review on the Biotechnological Developments in the Field of Fungal Endophytic Biology over the Last Decade. Biomolecules. 2023; 13(07): 1038 https://doi.org/10.3390/biom13071038
16. Apriandi A. Vitamin and Mineral Content Analysis of Fruit Beruwas Laut (*Scaevola taccada*). Marinade. 2019; 24(03): 325-329 https://doi.org/10.17844/jphpi.v24i3.36874
17. Sari M, Apriandi A & Suhandana M. Toxicity Test of Leaf Extract of Beruwas Laut (*Scaevola taccada*) with Brine Shrimp Lethality Test (BSLT) Method. Marinade. 2020; 03(01): 37-46 https://doi.org/10.31629/marinade.v3i01.2724
18. Kosman R & Tappang K. Isolation and Identification of Chemical Compound Groups of Diethyl Ether Fraction of Leaves Beruwas Laut (*Scaevola taccada* (Gaertn.) Roxb.) from Pinrang Regency (South Sulawesi). Jurnal Ilmiah As-Syifaa. 2012: 04(02): 219-227 https://doi.org/10.56711/jifa.v4i2.87
19. Hasan NI. Antibacterial Activity of Ethanol Leaf Extract of Beruwas Laut (*Scaevola taccada* (Gaertn.) Roxb.) against Pathogenic Bacteria using TLC-Bioautography. Makassar. Universitas Muslim Indonesia. 2022.
20. Amirullah S & Nainu F. Endophytic Fungi from Secang (*Caesalpinia sappan* L) as Producer of Antioxidant Compounds. J Farm Galenika. 2019; 05(01): 26–32 https://dx.doi.org/10.22487/j24428744.2019.v5.i1.12013
21. Rante H, Yulianty R, Evary Y & Hardiana E. Isolation and Antibacterial Activity of Endophytic Fungi from *Melochia umbellata* (Houtt). J Pure Appl Microbiol. 2017; 11(03): 1313–1318. http://dx.doi.org/10.22207/JPAM.11.3.11
22. Peterson SW, Vega FE, Posada F & Nagai C. Penicillium coffeae, a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum, P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. Mycologia. 2005; 97(3): 659–666 https://doi.org/10.3852/mycologia.97.3.659
23. Eltawaty SIA, Suliman MB & El-Hddad S. Chemical Composition, and Antibacterial and Antifungal Activities of Crude Extracts from *Pistacia lentiscus* L. Fruit. Trop J Nat Prod Res. 2023; 07(09): 4049–4054 http://www.doi.org/10.26538/tjnpr/v7i9.30
24. Hanh DT, Ngu TN, Bao PH, Nguyen NP, Trong PV, Loan LT, Lam DT, Nguyen PH, Khuyen PT, Truong PC, Tran MH. Chemical Composition and Biological Activities of Essential Oil from Ocimum basilicum L. Collected in Dak Lak, Vietnam. Trop J Nat Prod Res. 2023; 7(9): 4032–4037 http://www.doi.org/10.26538/tjnpr/v7i9.27
25. Suleman AW, Arna AN, Safaruddin. Isolation of taro tuber endophytic fungi (Colocasia esculenta (l.) Schott) as Antibacterial against *Escherichia coli* and *Staphylococcus aureus* Bacteria by TLC-Bioautography. Medical Sains: Jurnal Ilmiah Kefarmasian. 2022; 07(01): 39-48
26. Mawardi A, Maury HK, Maladan Y. Comparative Analysis of PMSA2 Gene Amplicon Product Quality Between Dried

and Venous Blood Spot Specimens. Jurnal Biologi Papua. 2020; 12(01): 10-18 https://doi.org/10.31957/jbp.949
27. Sardi A. Bioinformatics: Challenges in Integrating Biological Information. Jurnal Biologi Tropis. 2022; 22(04): 1297–1301 https://doi.org/10.29303/jbt.v22i4.4346
28. Davis WW and Stout TR. Disc Plate Method of Microbiological Antibiotic Assay. Appl Microbiol. 1971; 22(4):659-65 https://doi.org/10.1128%2Fam.22.4.659-665.1971

Rundengan CH. Inhibition Test of Ethanol Extract of Yaki Areca Nut Seeds (*Areca vestiaria*) Against Bacteria *Staphylococcus Aureus*, *Escherichia Coli*, *Pseudomonas aeruginosa*. Pharmacon. 2017; 06(01): 37-46 https://doi.org/10.35799/pha.6.2017.15003