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# Isolation of Endophyte Fungus from *Taxus sumatrana* Leaves and Their Potential as the Antimicrobial Producer

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***ABSTRACT***

*Resistant pathogenic microorganisms are a severe global problem today. The occurrence of resistant pathogens makes infections more difficult to treat and leads to increased mortality rates. One alternative that can be used in overcoming antimicrobial resistance is using medicinal plants. Pine Sumatra (Taxus sumatrana) is a medicinal plant with as an anticancer, antibacterial, and antifungal. This medicinal plant contains active compounds taxol, phenolics, flavonoids, and lignans. The purpose of this study was to isolate and test the antimicrobial activity of endophytic fungi from Taxus sumatrana leaves. The method used to isolate the fungus is the Direct Seed Planting technique. The isolated fungi were identified based on their morphology. Endophytic fungi were fermented for 7 days and tested for the antimicrobial activity to obtain secondary metabolite products. This study successfully isolated nine endophytic fungi with different characteristics in terms of morphology. The results of the antimicrobial activity test against S. aureus bacteria obtained a diameter of 14 mm by isolating TD3. In E. coli bacteria, the highest antimicrobial activity was 8.50 mm by isolating K1. Furthermore, in C. albicans, the highest antimicrobial activity was produced by isolate D6 at 9.93 mm. The best antimicrobial activity was produced by isolating TD3 against S. aureus bacteria.*

***Keywords:*** *Antimicrobial, Endophytic fungi, Taxus sumatrana, Leaves*

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## INTRODUCTION

Antimicrobial resistance to pathogens is a serious global problem. The emergence of resistant pathogens complicates the treatment of infectious diseases and leads to increased mortality. Based on data from the Centers for Disease Control (2019), antimicrobial resistance kills nearly 5 million people worldwide. In the United States, more than 2.8 million infections with antimicrobial-resistant pathogens occur each year. Furthermore, data from Indonesia's Antimicrobial Resistance (AMLIN-Study) reports that E. coli is resistant to antibiotics. Research into new drug sources is needed to overcome the problem of antibiotic resistance.

Medicinal plants are an alternative widely used as a source of new antimicrobial active compounds. Many plants are known for their ability to produce antimicrobial compounds.

One of the medicinal plants currently being studied for its potential, especially as an anticancer, antibacterial, and antifungal, is the Sumatran Pine (*Taxus sumatrana*). Research by Iszkuło *et al*. (2013) reported that the compound *taxane diterpenoid* or *paclitaxel* (*taxol*) has the potential as an anticancer. Recent research (Gai *et al*., 2020) reported other active compounds contained in *Taxus sumatrana* are phenolic compounds, lignans, and flavonoids. To obtain compounds from these medicinal plants, it is possible to isolate the endophytic microbes present in the plants. One of the endophytic microbes that can be utilized is endophytic fungi

Endophytic fungi live in plant tissues and do not harm their hosts. The interaction between endophytic fungi and hosts is generally mutualism. Where endophytic fungi obtain nutrients from plants, otherwise host plants can be protected from disease-causing pathogens such as viruses, fungi, and bacteria (Akmalasari *et al*., 2013; Gunawan & Hartanti 2019). The ability of endophytic fungi to protect the host is due to the ability of endophytic fungi to produce the same active compounds as the host plant.

Isolation of endophytic fungi which can produce active antimicrobial compounds has been widely carried out. Research conducted by Handayani *et al*., (2020) succeeded in isolating 7 endophytic fungi from Andalas could to inhibit the growth of *S. aureus* and *E. coli* but not *C. albicans*. Furthermore, (Ryla *et al*., 2022) successfully isolated four endophytic fungi from the leaves and one from the branches of the Mangrove plant (*Rhizophora apiculata* Blume.). Apart from endophytic fungi, other endophytic microbes, namely bacteria, have been isolated by Afifah *et al.* (2018) as many as nine endophytic Andalas bacteria from old stems and 2 young stems.

Information on the endophytic fungus *Taxus sumatrana* producing antimicrobial compounds is limited. Several researchers isolated the endophytic fungus *Taxus sumatrana* and focused on producing anticancer compounds. Several researchers have succeeded in isolating the endophytic fungus *Taxus sumatrana* (Karossi *et al.*, 2009; Artanti *et al.,* 2011; Mustarichie & Udin 2018; Vélëz *et al.*, 2022) which produces active compounds that are effective in treating several types of cancer. Limited data regarding the endophytic fungus *Taxus sumatrana* that produces antimicrobial compounds is a study conducted by Maharani (2019) who tested the antifungal activity of the extract of the endophytic fungus *Taxus sumatrana* on the growth of *C. albicans*. The results showed that the two isolated endophytic fungi had antifungal activity at concentrations of 8 - 10 mg/mL.

According to Maharani (2019), the endophytic fungus *Taxus sumatrana* exhibits low-level antibacterial action. The potential to obtain an isolate of the endophytic fungus *Taxus sumatrana* capable of producing better antimicrobial compounds is a challenge for many researchers. This is based on the content of active compounds in host plants that have the potential as antimicrobial active ingredients.

## METHODS

### Endophytic Fungi Isolation

Endophytic fungi were isolated using the *Direct Seed Planting technique* (Hasiani *et al.*, 2015). Before use, the leaves are separated from the twigs, cut, and washed under running water. The surface sterilization process was carried out by immersing it in 70% alcohol for 1 minute Then, the leaves were washed with sterile *aquadest* and then placed in a hypochlorite solution for 2 minutes. Next, the leaves were again soaked in 70% alcohol for 30 seconds and washed again with sterile *aquadest* (Putri 2018). 3 pieces of leaf pieces were placed on the PDA medium and incubated for 24-72 hours at room temperature. The endophytic fungi were isolated in laminar airflow (LAF)in Laminar Air Flow (LAF).

Endophytic fungi that had grown around the leaves were transferred to a new PDA medium to be purified. This endophytic fungus was purified repeatedly until a completely

pure fungus was obtained without being combined with other fungi. Each pure isolate is stored at room temperature.

**Endophytic Fungus Fermentation**

Fermentation aims to obtain secondary metabolite products from fungi. Fermentation was carried out in PDB medium by taking 3 pieces of the fungus using an ose needle and then inoculating them into 100 mL of PDB medium in a 250 mL *Erlenmeyer.* Furthermore, rocking fermentation was carried out using a *rotary shaker* at a speed of 120 rpm (revolutions/minute) at room temperature for 7 days (Aliyah *et al*., 2021). Samples of the fermented medium were taken at 1 mL, for 7 days of incubation. The sample is centrifuged to separate the supernatant from the pellet. The supernatant was stored at 4°C until use.

**Antimicrobial Activity Test**

The method used for activity testing is the *Diffusion Agar Plate Method*. The microbial pathogens used for the antimicrobial assay were *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The test microbial suspension with turbidity equivalent to *McFarland's* 0.5 was inoculated onto the medium (NA for bacteria and PDA for yeast). The test microbial suspension was inoculated using a sterile *cotton bud* with a review technique (*swab*). Next, as much as 20 µL of the supernatant was dripped onto the disc paper using a micropipette and placed on the medium that had been inoculated with the test microbe. Cultures were incubated for 24-48 hours at room temperature. Antimicrobial activity is expressed by the formation of an inhibition zone. The inhibition zone, in the form of a clear zone around the disc, was measured using a caliper from several sides (Noverita *et al*. 2009). According to (Greenwood *et al*. 1995) the response to microbial growth inhibition has a category of less. The resulting inhibition zone diameter categories can be seen in Table 1.

**Table 1.** Classification of Inhibition Zone Diameter According to (Greenwood *et al*., 1995)

| Diameter | Inhibitory Power |
|---|---|
| >20mm | Very strong |
| 10-20mm | Strong |
| 5-10mm | Moderate/Enough |
| < 5mm | Not enough |

**Data Analysis**

Observational data were processed descriptively. Types of endophytic fungi were observed macroscopically and microscopically, shown in figures and tables. Antimicrobial activity data is displayed in the form of tables and graphs

**RESULT AND DISCUSSION**

Observations during incubation of endophytic fungi growing around *Taxus Sumatrana* leaves tissue on PDA medium. Pictures of the growth of the fungus around the leaf tissue can be seen in Figure 1. Endophytic fungi that have grown around the leaves are then purified to obtain single fungal colonies. The results of the observations obtained 9 types of endophytic fungi isolated from *Taxus* leaves. A single fungus colony from Taxus leaves can be seen in Figure 2. Endophytic fungi isolate that have been obtained, then observed. Macroscopic observations were made by observing the endophytic fungus colonies in terms of colony color, colony shape, colony texture, and colony surface (Table 2).

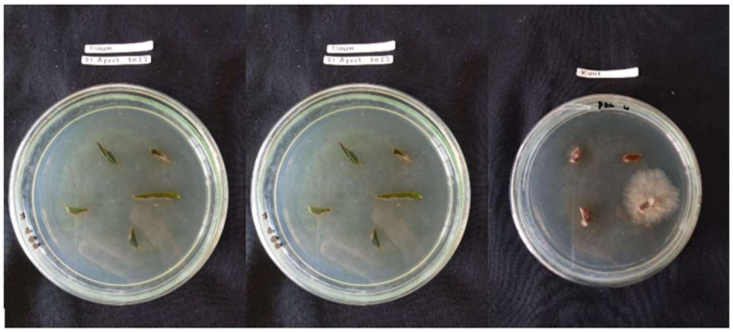

**Figure 1.** Isolation of *Taxus sumatrana* Leaves Endophytic Fungi

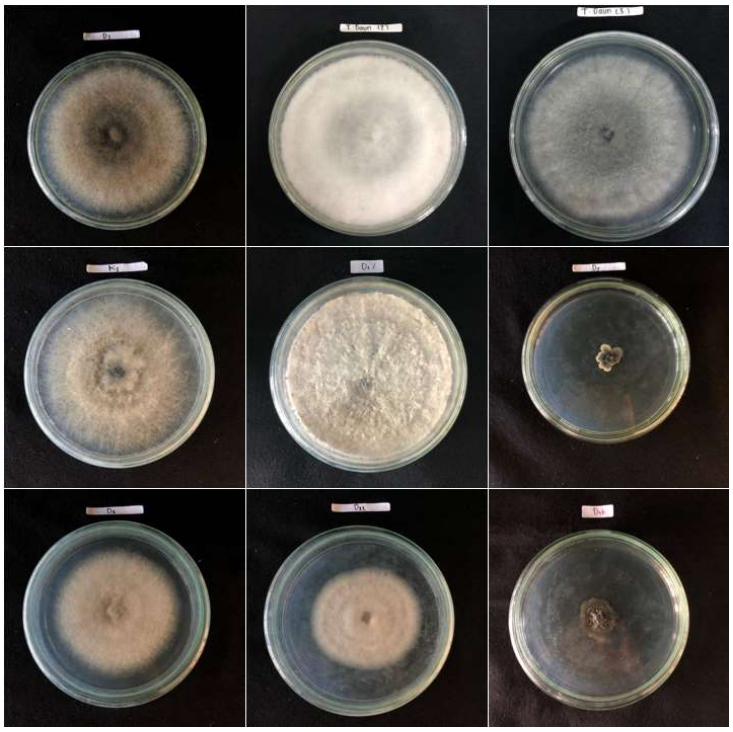

**Figure 2.** Purification Results of *Taxus sumatrana* Endophytic Fungi

Based on the observations that have been made, it can be seen that the endophytic fungi have a variety of colors. Observation of endophytic fungi was carried out for 7 days. The growth of endophytic fungi is not all the same, some quickly fill the petri dish and some grow slowly (isolate D7 and D5b). The morphologically observed endophytic fungi have hyphae that form colonies called mycelium. The mycelium produced from each endophytic fungus is different. According to (Susanti *et al.*, 2021) the color of the mushroom mycelium is usually white at first, but as the fungus gets older, the color of the mycelium will change. Mycelium can contain pigments in red, purple, yellow, brown, gray, and so on (Tournas *et al*., 2001).

**Table 2.** Macroscopic Identification of Endophytic Fungi from *Taxus* Leaves

| Code | Colony Morphology | | | |
|---|---|---|---|---|
| **Isolate** | **Colony color** | **Colony form** | **Colony texture** | **Colony surface** |
| D1 | Chocolate | Round | Cottony | Flat |
| D6 | Chocolate-White | Round | Cottony | Flat |
| TD2 | White | Round | Cattony | Uneven |
| TD3 | Gray | Round | Cottony | Uneven |
| D5b | Green | Irregular | Granular | Uneven |
| K1 | Brownish white | Round | Cottony | Flat |
| D15 | Chocolate | Round | Cottony | Flat |
| D7 | Green | Irregular | Granular | Uneven |
| D2t | Brownish white | Round | Cottony | Flat |

An antimicrobial activity test was carried out on fermented endophytic fungi. Fermentation aims to obtain secondary metabolite products from fungi. The method used is solid agar diffusion (*Diffusion Agar Plate Method*). Based on the results of the research that has been done, the inhibition zone formed against *E. coli* can be seen in Figure 3.

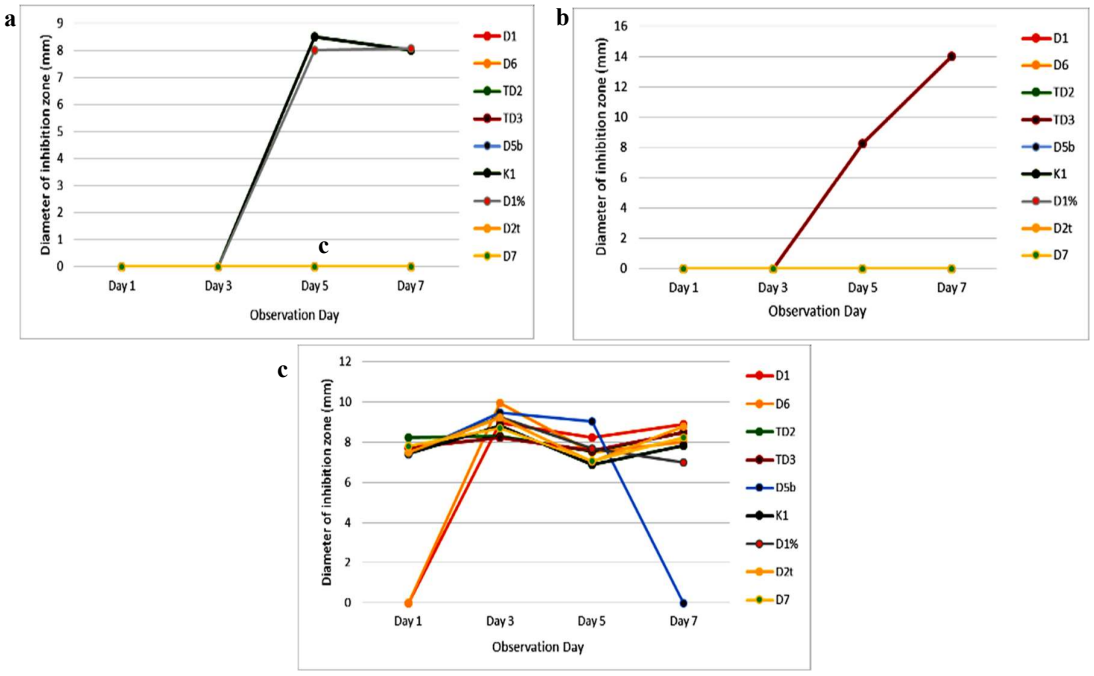


**Figure 3.** Diameter of Inhibition Zone of Fermentation Products of Endophytic Fungi Leaves of *Taxus sumatrana.* (a) Graph of Inhibition Zone Diameter of *E. coli.* (b) Graph of Inhibition Zone Diameter of *S. aureus.* (c) Graph of Inhibition Zone Diameter of *C. albicans*

Based on the results of the activity test that has been carried out, an inhibition zone was obtained for *E. coli* (Figure 3a) on isolates K1 and D1%. The inhibition zone produced by isolate K1 had a diameter of 8.50 mm on the fifth day and decreased to 8.03 mm on the seventh day. The resulting inhibition zones are categorized into moderate/sufficient

inhibition. Furthermore, the activity test results for *S. aureus* (Figure 3b) yielded an inhibition zone of 8.25 mm on the fifth day and 14 mm on the seventh day by TD3 isolate. It can be seen that from the fifth to the seventh day, the inhibition zone produced by TD3 isolates was getting bigger (Figure 3c).

The categories of inhibition strength generated based on Table 1 include strong barriers. This means that the fermented endophytic fungus of Taxus leaves has good antimicrobial potential against *S. aureus* bacteria. Based on the graph (Figure 3), all isolates of the endophytic fungus *Taxus* leaves have activity against the fungus *C. albicans*. The highest inhibition zone was produced by isolate D6 of 9.93 mm on the third day. According to (Jawetz *et al*., 2005) the capacity of each microbe to create antimicrobial active substances varies. A microorganism's antimicrobial activity is influenced by several variables, such as the kind and quantity of an active chemical it contains. The highest inhibition zone diameter of each test microbe can be seen in Table 3.

**Table 3.** Classification of Inhibition Zone Diameter

| Isolate Code | Inhibition Zone Diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** | **Inhibitory Power** | ***E. coli*** | **Inhibitory Power** | ***C. albicans*** | **Inhibitory Power** |
| D1 | 0 | - | 0 | - | 9.18 | Enough |
| D6 | 0 | - | 0 | - | 9.93 | Moderate |
| TD2 | 0 | - | 0 | - | 9.09 | Moderate |
| TD3 | 14 | Strong | 0 | - | 9.20 | Moderate |
| D5b | 0 | - | 0 | - | 9.48 | Moderate |
| K1 | 0 | - | 8.50 | Moderate | 8.81 | Moderate |
| D1% | 0 | - | 8.06 | Moderate | 9.24 | Moderate |
| D2t | 0 | - | 0 | - | 9.20 | Moderate |
| D7 | 0 | - | 0 | - | 8.69 | Moderate |

Based on data in Table 3, good antimicrobial activity was obtained by the TD3 fungus isolate with a diameter of 14 mm against *S. aureus*. In bacteria *E. coli*, the strongest antimicrobial activity was produced by isolate K1 of 8.50 mm, then the antimicrobial activity was the highest against *C. albicans* produced by isolate D6 of 9.93 mm. Research conducted by Nurayni and Handayani (2021) proved that the fermented endophytic fungus Andalas has good antibacterial compounds against *S. aureus* with a diameter of 9.6 mm and *E. coli* 9.4 mm with the optimum fermentation time on the sixth day. Subsequent research, conducted by Nafion *et al.* (2019) stated that fermented endophytic Andalas bacteria were able to inhibit the growth of *S. aureus*. This statement is reinforced by Jawetz *et al*. (2005), the resulting antimicrobial activity was generally greater against Gram-positive bacteria than Gram-negative bacteria. This is because the cell walls of Gram-positive and Gram-negative bacteria are different. The cell wall of Gram-positive bacteria has a more extensive peptidoglycan layer than Gram-negative bacteria (Boleng, 2015). Antimicrobial compounds can inhibit microbial activity (bacteria and fungi). Based on research conducted by Priamsari *et al.* (2016), the Longevity Spinach (*Gynura procumbens*) as a medicinal plant contains active compounds of *triterpenoids*, *polyphenols*, *saponins*, steroids, chlorogenic acid, caffeic acid, vanillic acid, coumaric acid, para hydroxybenzoic acid, flavonoids, and essential oils.

*Taxus sumatrana* contains active compounds *taxol*, phenolics, lignans, and flavonoids which are proven to inhibit the growth of bacteria and fungi (Iszkuło *et al.*, 2013; Gai *et al*., 2020). Activity tests that do not produce inhibition zones, it is possible that the isolates contain different active compounds to inhibit bacterial growth (Harahap & Nurjanah, 2017). The types of antimicrobial metabolites produced by each isolate were different resulting in the diameter of the inhibition zones produced in the three tests microbe varying. According to (Hadioetomo *et al.*, 2016). Several factors affect antimicrobial activity including the concentration of substances, the number of microbes, temperature, active compounds, and Potential of Hydrogen (pH).

## CONCLUSION

This study successfully isolated 9 species of fungi from *Taxus sumatrana* leaves. A total of one isolate can inhibit the growth of *S. aureus* with a strong category, two isolates can inhibit the growth of *E. coli* with a moderate category, and nine isolates can inhibit the growth of *C. albicans* with a moderate category.

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