Research Article

Fusarium sp., an Endophytic Fungi Isolated from Sengon (*Falcataria moluccana*) Gall Rust, Shows Antimicrobial and Antioxidant Potential

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Abstract | Many problems caused by antibiotic resistance encourage the exploration of new antibiotic compounds from natural materials, especially from endophytic fungal cultures. This study aims to evaluate different culture media that can support endophytic fungi to produce bioactive compounds that have antibacterial and antioxidant activity. The method used in this study was to ferment endophytic fungi from gall rust sengon in different media, namely PDB, PDB plus sengon wood powder, and PDB plus yeast, for 9 days, followed by extraction using ethyl acetate solvent and testing of antioxidant and antibacterial activity. The DPPH radical was used to measure antioxidant activity, and *Bacillus subtillis* was used as a representative for Gram-positive and Gram-negative For testing of antimicrobials, *Escherichia coli* and *Pseudomonas aeruginosa* were used. The experiment showed that the addition of yeast and sengon wood to PDB medium increased the growth and production of bioactive compounds extracted from fungal cultures. A fungal endophyte extract in ethyl acetate from all media used produced antioxidant compounds with high activity. Antibacterial activity was obtained from the extract of fungal cultures grown on PDB plus Sengon wood floor on day 6 for *P. aeruginosa* and *E. coli*, and day 3 for *B. subtillis*. In general, the three media used in this study encouraged endophytic fungi to produce antioxidant and antibacterial compounds.

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Introduction

The abundance of synthetic antibiotic resistance in tackling pathogenic bacteria and antifungal agents has forced researchers to explore natural materials to obtain new antimicrobial agents. Research facts show that endophytic microbes, especially endophytic fungi, produce new and interesting bioactive compounds to be utilized in various applications, both in agriculture and pharmaceuticals, which has made many researchers interested in conducting new research studies related to these microbes.

The most commonly encountered endophytes are fungi, and it seems that other forms of microbes must exist in plants as endophytes, but there is little information about their existence and use. So far, medicinal plant's endophytic fungi have been

extensively studied because of their capability to synthesize heterogenous bioactive metabolites (Xia *et al*., 2022). Endophytic microbial secondary metabolites ability to act as host plant secondary metabolites is a very appealing and reliable method of obtaining secondary metabolites from endophytic microbials with similar capacities to the host plant.

Endophytes have a high chance of being exploited because the number of species spread on the earth is 300,000 and each plant has one or many fungal or bacteria-type endophytic microorganisms (Xia *et al*., 2022). Environmental factors that influence the synthesize of fungal secondary metabolites in fungi include the composition of the medium, pH, temperature, agitation, and lighting (Venugopalan and Srivastava, 2015), geographical factors, dehydration, lighting, drought, pH, and the sources produce carbon. As stated by Kinyungu *et al*. (2019), Numerous environmental parameters, including pH, carbon and nitrogen sources, have a significant impact on the synthesis of secondary metabolites such aflatoxins in *Aspergillus*.

The amount of biomass presents during the production phase and incubation period, as well as the culture conditions, influence metabolite biosynthesis in fungi (Magwaza *et al*., 2019). Stress or hazards that require these organisms to defend themselves cause secondary metabolites to be created at a specific growth rate. These substances are produced in modest amounts and have no significant impact on basic metabolic functions. In endophytic fungi, favorable growing circumstances substantially encourage the formation of secondary metabolites.

The growth of fungi was affected by biotic factors as well as abiotic factors, which have a great influence on the growth of microorganisms. Biotic factors that usually appear consist of the form of microorganisms, the nature of microorganisms, such as their response to environmental changes, their ability to adapt to the growing environment, and the presence of other organisms that greatly affect the living environment. Meanwhile, abiotic factors existed in the environment and included the composition and amount of compounds required inside the culture medium or substrate, the physical environment (temperature, humidity, and light), and the presence of other compounds that may be toxic, inhibitory, or promoters, whether derived from the environment or self-generated (Sivakumar *et al*., 2020).

In a previous study, gall rust's endophytic fungi from the sengon plant possess the capacity to create antioxidant and antibacterial substances (Rumidatul *et al*., 2021). Extract of fungal culture possesses the capacity to prevent *B. subtilis,* as well as *E. coli* and *P. aeruginosa* (Rahmawati *et al*., 2018). The experiment result met the consistency of the host plant (*Falcataria moluccana),* which also produces secondary metabolites that have antimicrobial activity (Listiani *et al*., 2021) and antioxidants (Rumidatul *et al*., 2021). This study focused on isolates that produced high levels of antioxidant and antibacterial compounds and their activities in previous studies and were grown in the best media under optimal fermentation conditions to produce secondary metabolites in order to increase bioactive compound produced by endophytic fungal culture.

Optimization of the production of antioxidant and antibacterial compounds is very important to obtain optimum growth conditions for endophytic fungi that can produce secondary metabolites with bioactive compounds that have high antioxidant and antibacterial activity. To achieve this goal, optimum treatment conditions are urgently required, especially media endophytic fungus growth and secondary metabolite production, including anti-inflammatory and antibacterial substances.

Materials and Methods

Materials

Between January and October of 2021, the study was carried out at the ITB Jatinangor's microbiology lab. Samples of gall rust was taken from sengon plant that was lived at ITB campus at Jatinangor, Nutrient Broth (NB), Potato Dextrose Agar (PDA), and Nutrient Agar (NA), *B. subtilis*, *E. coli* and *P. aeruginosa* and organic solvent utilized in this study was ethyl acetate. The radical source for the antioxidant activity tests was DPPH.

Fermentation and extraction of endophytic fungi

On 3 different media, including PDB, PDB with sengon woodmeal, and PDB plus yeast, 4 pure culture agar blocks (in diameter 10 mm) of actively developing fungi were raised in 100 mL Erlenmeyer flasks with a capacity of 250 cc. At a temperature of 27 °C, the flasks were incubated under shaker conditions for 0, 3,

and 9 days. The mycelial mats were removed from the culture by filtering it through paper. In a separating funnel, the liquid broth was collected and vigorously shaken for an hour while using the same amount of ethyl acetate to extract. To determine the mycelium's weight, the cell mass was divided and weighed. The chemical was created after the solvent was evaporated, dried with $MgSO_4$, and concentrated to produce a crude extract (Al-Rashdi *et al.*, 2021).

Measurement of dry weight of mycelium

Endophytic fungal cultures at any sampling time (T0 to T4 or H0 to H9) were filtered using filter paper to separate mycelium and liquid culture. The mycelium was weighed to attain a constant dry weight after being oven-dried at 80 °C (dry weight of mycelium).

Extraction of endophytic fungal culture

Extraction was performed on endophytic fungal cultures using ethyl acetate. A rotary evaporator was used to dry the resulting extract continued by weighing the extract to determine the dry weight for further testing.

The 2,2-Diphenyl-1-pikrilhidrazil (DPPH) is used to determine action as an anti-oxidative

Making use the radical scavenger 2, 2-Diphenyl-1-pikrilhidrazine (Picrylhydrazyl), an antioxidant capacity test was conducted. The test for scavenging hydroxyl radicals was performed according to what Al-Rashidi *et al.* (2021) with a little modification. 250 µl of DPPH (1 mM) and 100 l of extract samples at various concentrations were combined and reacted in 30 minutes; A 517 nm reading of the absorbance was then made. In order to calculate the scavenging of DPPH radicals rate, the following formula was utilized.

Scavenging of DPPH radical(%)= [(A1-A2)/A1] x 100

A2 how much reagent absorbs and A1 whether the control's (complete with all reagents except for the crude extracts) absorption (Al-Rashdi *et al.*, 2021).

Antibacterial bioassay

Individual tests were conducted on extracts from endophytic fungal cultures against panels of pathogenic pathogens, bacterial species both gram-positive and gram-negative. The paper disc method was used to examine the repressive impact influence on microorganisms of fungal isolates extracts (Pelo *et al.*, 2020). By using the paper disc method, the endophytic fungal extract's ability to inhibit bacterial growth was evaluated. 10 mg/mL (W/V) in methanol concentration of endophytic microbial culture extract was applied to disc paper, dried, and then deposited on NA medium for bacterial testing.

The zone of inhibition's diameter in millimetres of fungal isolates culture samples against bacterial infections was measured in comparison to control samples. In the treatment, including as positive control of harmful microorganisms, chloramphenicol was utilized. On each petri plate, four-disc paper replications were employed. Clinical bacterial strains were incubated in petri dishes that were infected with fungi and bacteria and kept at 27 °C for 24 hours. The test organism's clear zone was measured in with a view to evaluating the antibacterial activity. Each test was performed three times for this investigation.

Results and Discussion

The growth of endophytic fungi

The fungal growth was assessed by measuring mycelium expansion. The isolate of endophytic fungus was cultured on PDB, PDB plus sengon wood meal, and PDB plus yeast medium for 9 days at a temperature of 27±2°C under shaking conditions. The sampling was done every 3 days by measuring the weight of mycelium and the weight of the compound extracted by ethyl acetate. Endophytic fungi grew on the mycelium's dry weight. Figure 1 shows the development of an endophytic fungus mycelium during the course of a 9-days fermentation.

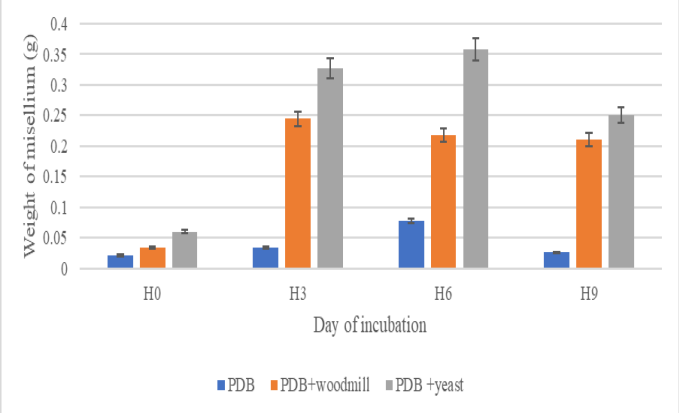

Figure 1: *The effect of different medium and incubation day to the growth of endophytic fungi.*

These findings indicate that the isolated endophytic fungus from gall rust can thrive in all the media used

in this research, so it was expected that it would also produce an optimal level of secondary metabolites. Substrate was the main nutrient source for fungal growth. New nutrients could be exploited after the fungi secreted extracellular enzymes that could break down complex compounds from the substrate into simpler compounds.

Growth curves were measured by monitoring the weight of the mycelium as it ferments. Mycelium weighs very little at the start of the development phase. Mycelium weight started to rise when it got to H3, peaked at H6, and then remained stable until finishing. The growth curves showed that day 3 marks the beginning of exponential growth, which peaks at H6, while the initial stage of growth is still at an adaption level. In H6, static circumstances were found, and in H9, they started to deteriorate.

The development of micellium in different media showed a different rate. The highest rate was shown by endophytic fungi that were grown on PDB plus yeast medium, followed by PDB plus woodmeal, and the lowest was the PDB medium. The addition of yeast extract and woodmeal sengon increased the growth of the fungi. It happened because the addition of nutrition to the medium increased the micellium growth.

Bioactive compound manufacturing

We quantified how much secondary metabolite endophytic fungus produce cultured in different media produce by weighing the extract of the fungal culture that endophytic fungi grown in various mediums produce. Culture media were extracted using the ethyl acetate extraction technique. Given that ethyl acetate has a medium polarity, it can dissolve both polar and nonpolar active chemicals. Solvents containing ethyl acetate were frequently employed to extract endophytic fungal colonies. Its semi-polar nature made it possible for it to contain components from the fungal culture (Nawaz *et al*., 2020).

According to Figure 2, endophytic fungi, the highest second metabolite was produced by *Fusarium* sp. days 3 and 6 when growing in PDB plus woodmeal medium, respectively. In contrast, when growing in PDB plus yeast medium, the highest levels of secondary metabolites were created on day 9. The fungus that was cultivated on PDB plus yeast medium produced the most extract from the culture.

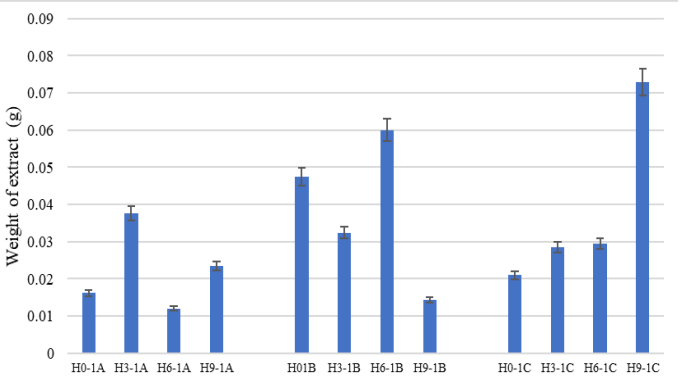

Figure 2: *The influence of different medium and incubation day on the production of secondary metabolite. A: PDB; B: PDB +Woodmill; C: PDB+yeast.*

Daou *et al*. (2021) states that the sporulation process requires favorable environmental conditions, which are also a factor when creating secondary metabolites. The secretion of metabolites may be influenced by variables such the pH, temperature, salt concentration, and variations in the carbon and nitrogen supplies.

Antioxidant activity

It was discovered that the sengon plant's endophytic fungus, *Fusarium* sp. produces bioactive chemicals with antioxidant activity, such as gall rust sengon, which was determined by measuring the inhibitory power of DPPH radicals. The DPPH test is among the most widely used techniques for evaluating free racial radical scavenging. Radical DPPH in methanol solvent is stable and made purple to yellow color shift when it becomes a non-radical form. The hue shift from purple to yellow, which illustrates how the radical has changed DPPH into the stable chemical 2, 2-diphenyl-1-hydrazine complex, was used in this work to test the antioxidant activity. The hydroxyl group of antioxidant compounds derived from natural materials can play a role in this function. Antioxidant substances can neutralize radicals by giving them hydrogen (Munteanu and Apetrei, 2021).

The bioactive substances made by an endophytic fungus isolated from gall rust sengon had a significant level of antioxidant activity. *Fusarium* sp. can grow in any medium and create bioactive substances with antioxidant properties. The extract of the ethyl acetate of the fungal culture had the highest antioxidant activity on the third day, and it started to lose that activity on the sixth day. Despite the fact that all media inhibited DPPH radicals by more than 70%, PDB medium combined with sengon wood flour and PDB medium combined with yeast produced high

antioxidant activity of fungal extracts, whereas PDB medium alone produced slightly lower antioxidant activity.

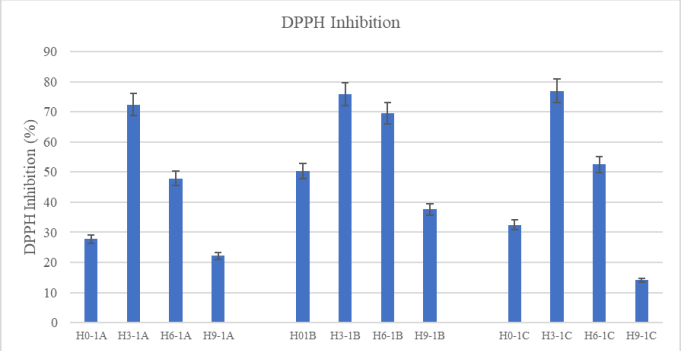

Figure 3: *A graph illustrating the antioxidant property (DPPH free radical inhibition) produced by the development of endophytic fungal cultures in various media. A: PDB; B: PDB +Woodmill; C: PDB+yeast.*

Fusarium sp., which was isolated from gall rust sengon using ethyl acetate as a solvent and has a high level of inhibition against DPPH radicals, has the ability to act as an antioxidant component. In comparison to methanol and n-hexane solvents, ethyl acetate is the best solvent for endophytic fungal culture extraction, producing antioxidant bioactive chemicals (Rahmawati *et al*., 2018). By donating hydrogen, this isolated molecule can scavenge radicals and prevent the formation of DPPH radicals. Additionally, the isolated compound's hydroxyl group might contribute to the inhibitory effect. In the DPPH radical scavenging experiment, antioxidants extracted from fungi cultures provide DPPH radicals with electrons or hydrogen ions to transform them into stable molecules that affect the color of the DPPH (Al-Rashdi *et al*., 2021).

Trigonella foenum-graecum stems from the isolates fungal *Aspergillus* sp. produced the greatest total phenol, which was [(89.9±7.1) mg GAE/g] comparable to gallic acid and demonstrated antioxidant activity with a value of 18.0±0.1 mg/mL for the IC_{50}. One of the world's rarest and most costly spices, saffron (*Crocus sativus* L.), is a traditional medicinal plant. Endophytic fungi *Aspergillus niger* and *Rhizopus oryzae* produced bioactive compounds with antioxidant potential when exposed to DPPH (Chamkhi *et al*., 2018).

Several earlier studies have demonstrated that gall rust and leaf extract from sengon create antioxidant and antibacterial chemicals, which can be used as a reference for an exploratory study on endophytic fungi capable of creating bioactive compounds with antioxidant and antibacterial activities. Research on bioactive compounds from endophytic fungi was conducted because of the properties of endophytic fungi, which may create bioactive chemicals similar to those produced by their plant species. Previous research revealed that the DPPH radical inhibitory activity of extract from endophytic fungal cultures using methanol and ethyl acetate was 71.48% (Rahmawati *et al*., 2018). Endophytic fungi from the stem (Rahmawati *et al*., 2016), twigs, and leaves of *Toona sinensis* that were tested for antioxidant activity yielded a number of isolates that had the potential to create antioxidant chemicals (Rahmawati *et al*., 2018).

Antibacterial activity of fungal culture grown in different media

By quantifying the clear zone created by bacteria on agar medium, endophytic fungal cultures obtained from gall rust sengon have been shown to have antibacterial properties. It was decided to grow on three different media. *B. subtillis*, *P. auregunisa,* and *E. coli* bacteria were both used in this investigation. The antibacterial efficacy of an endophytic fungal culture isolated from the gall rust sengon, *Fusarium* sp., is displayed in Table 1.

Table 1: *Gall rust sengon's antibacterial properties extract from fungal endophytic towards (B. subtilis; P. aregunisa and E. coli).*

	P. aregunisa	***B. subtillis***	***E. coli***
HOA	0.73 ± 0.10	0.41 ± 0.05	0.6 ± 0.09
H3A	0.75 ± 0.06	0.66 ± 0.05	0.61 ± 0.06
H6A	0.95 ± 0.09	0.65 ± 0.1	0.84 ± 0.05
H9A	0.75 ± 0.08	0.56 ± 0.06	0.77 ± 0.09
HOB	0.85 ± 0.08	0.7 ± 0.07	0.64 ± 0.05
H3B	0.73 ± 0.08	1.08 ± 0.07	0.7 ± 0.05
H6B	1.03 ± 0.18	0.69 ± 0.01	0.88 ± 0.06
H9B	0.68 ± 0.11	0.63 ± 0.14	0.62 ± 0.09
HOC	0.56 ± 0.05	0.7 ± 0.05	0.66 ± 0.09
H3C	0.68 ± 0.06	0.92 ± 0.08	0.61 ± 0.08
H6C	0.8 ± 0.05	0.73 ± 0.16	0.86 ± 0.03
H9C	0.55 ± 0.07	0.49 ± 0.11	0.61 ± 0.05
Chlorampenicol	1.0 ± 0.05	1.0 ± 0.05	1.2 ± 0.05

A: PDB medium; B: PDB + woodmeal sengon medium; C: PDB + yeast medium.

On PDB, PDB plus woodmeal, and PDB plus yeast medium, the gall rust sengon endophytic fungus was fermented for 9 days before the crude extract of EtOH from the fungal culture was examined for antibacterial activity. A mixture of *B. subtilis*

and pathogenic bacteria (*P. aerugunisa* and *E. coli*), were subjected to the EtOH extract of 10 mg/mL concentrated fungus fermentation broth. A positive control and antibacterial standard, chlorampenicol (100 mg/L), was employed instead of the methanol solvent as a negative control. The inhibitory zone's diameter, assessed after three repetitions, was used to determine the antimicrobial activity.

It has also been acknowledged that fungi that live asymptomatically inside plant tissues are known as endophytes, are a source of potential second-generation metabolites therapeutic value. When grown aerobically on PDB medium, crude extracts from endophytic fungal culture broths demonstrated significant antibacterial activity against a panel of bacteria. Crude extract of ethyl acetate fungal culture showed the best antibacterial inhibition against bacteria *B. subtilis* and *P. aeruginosa* when compared to methanol extract and n-hexane extract.

In studies with *B. subtillis* bacteria, the crude extract of ethyl acetate fungal culture grown on PDB plus woodmeal, PDB plus yeast, and PDB medium showed the strongest inhibitory activity. The isolates produced on PDB with woodmeal demonstrated the best inhibitory activity in the test with *P. aeruginosa* bacteria, followed by PDB and PDB plus yeast. Endophytic fungus extract in ethyl acetate cultivated on plants showed anti-*E. coli,* has the strongest antibacterial action.

Similar findings showed that endophytic fungi isolated from sengon wood and extracted with ethyl acetate produced antibacterial activity in an earlier investigation. A further study discovered that an endophytic fungus isolated from surian (*Toona sinensis*) twigs and leaves also produced antibacterial activity (Rahmawati *et al*., 2018). Observations from another research shows, the majority of the examined microorganisms were significantly resistant to the antibacterial effects of *Rhizopus oryzae* extract, including *Bacillus* sp. and five gram-negative strains, like *Pseudomonas putida, Luteibacter* sp., *E. coliI, Pantoea* sp., and *Stenotrophomonas* sp. (DH5α) reference strain (Chamkhi *et al*., 2018).

Conclusions and Recommendations

Gall rust sengon endophytic fungus can thrive in three different media: PDB, PDB woodmeal, and PDB yeast, though the greater growth was shown by fungi grown in PDB woodmeal and PDB yeast. The highest production of bioactive compounds was achieved by fungi that were grown in PDB yeast medium after 9 days of fermentation. A fungal culture's extract with ethyl acetate from *Fusarium* sp. isolated from gall rust sengon, inhibited DPPH in an almost identical proportion on day 3 from three different medium. Endophytic fungi with antibacterial activity were grown in three different mediums: PDB, PDB plua woodmeal, and PDB plus yeast. The best activity was achieved by an ethyl acetate extract of fungal cultures from fungi grown in PDB woodmeal medium.

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Novelty Statement

This study provides new information regarding the difference medium that can support the endophytic fungi to produce bioactive compounds.

Author's Contribution

Sopandi Sunarya: Performed experiments and wrote the manuscript.
Alfi Rumidatul: Helped in data analysis and manuscript writing.
Noor Rahmawati: Designed the experiments, the data was analyzed, and the manuscript was edited.

Conflict of interest
The authors have declared no conflicts of interest.

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