

Research Article

Potential Antioxidant Compound From the Culture of Endophytic Fungi from Surian Trees (*Toona sinensis* Roem)

Noor Rahmawati*, Dea Indriani Astuti and Pingkan Aditiwati

School of Life Science and Technology, Institute Technology Bandung, Jl Ganesha No. 10 Bandung.

Abstract | Due to genetic exchange between host plants and endophytes, endophytic fungi can produce the same secondary metabolites as their host plants. Numerous research on the bioactive elements of secondary metabolites from endophytic microorganisms, particularly endophytic fungi, have been undertaken on plants that are able to produce secondary metabolites with unique properties. When tested using the DPPH radical-scavenging assay, the secondary metabolite *Toona sinensis* Roem, which was extracted using 80% acetone, shown antioxidant activity. To create antioxidant chemicals, the endophytic fungus *T. sinensis* must be isolated and researched. Thin-layer chromatography (TLC) and spectrophotometric methods were used right away to assess the potential of endophytic fungus to create antioxidant chemicals. Additional investigation on the endophytic fungi that produce antioxidant chemicals was carried out using ITS molecular analysis. Thirty strains of endophytic fungus were taken from twigs and leaves. Based on the early TLC examination, a number of endophytic fungus showed the presence of compounds with antioxidant function. Endophytic fungi crude extracts have been shown to be able to inhibit DPPH radicals by up to 86 percent by A26 and A19 and by 80 percent by B3 in spectrophotometric studies on cultures with antioxidant activity. This is the first report on endophytic fungus isolates from branches and leaves of *T. sinensis* that had antioxidant potential. The fungi were identified as *Giberrella* sp. and *Fusarium* sp. Future research on these microbes is highly encouraged because the results of this study indicate that they may be a reliable source of bioactive compounds, notably antioxidants.

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***Correspondence** | Noor Rahmawati, School of Life Science and Technology, Institute Technology Bandung, Jl Ganesha No. 10 Bandung;

Email: noor.rahmawati@itb.ac.id

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Introduction

Since they could grow well in all plant organs, including xylem and phloem, leaves, roots, and stems, endophytic microorganisms, including

endophytic fungi, were detected in practically all plants (Tan and Zou, 2001; Strobel and Deasy, 2003) stated that endophytic microorganisms that colonized plant tissues typically benefited from the host plants nutrients and defences, and in exchange,

they boosted the host plants' resilience by creating a few specific functional metabolites (Tan and Zou, 2001). Endophytic fungi are widely distributed in nature. This is in reference to the research of Strobel and Deasy (2003), who calculated that there were 1-4 species of fungi linked with plants and endophytes that were culturable (could be grown in artificial circumstances). According to Augusta (2009), 99% of all endophytic fungi are unculturable and incapable of being grown artificially. Endophytic populations may be impacted by environmental factors affecting the host plants. The tropics are thought to have more diversified endophytic microbial profiles than temperate areas. Endophytic microorganisms from tropical forests produced natural compounds that were not only more active but also produced in greater amounts than those produced by endophytic microbes from temperate locations, according to Bills *et al.* (2002); (Strobel and Deasy, 2003). The endophytic fungus was able to synthesize bioactive compounds like those produced by host plants thanks to the incorporation of genetic material from the host plant into it. It was predicted that the endophytic fungus isolated from this particular host plant would be able to produce substances with comparable action. *Taxomyces andreanae* was an endophytic fungus isolated by Stirle *et al.* (1993) from *Taxus brevifolia* (Pacific yew) capable of producing paclitaxel (Taxol), an anti-cancer chemical that is produced by the host plant. The insertion of genetic material from plants into endophytic microorganisms may result in the production of secondary metabolites by endophytic microbes that are similar to those of their host plants. Endophytic microorganisms were able to adapt to their host plants through this mechanism, which also served to defend them from viruses, insects, and herbivores (Strobel and Deasy, 2003). Antioxidant chemicals were known to be produced by Surian leaves. Fresh leaf shoots from the Surian (*T. sinensis*) plant had significant antioxidant activity in inhibiting DPPH radicals when extracted with acetone. The leaf extract of *T. sinensis* contained 12 phenolic compounds, including gallic acid (1), methyl gallate (2), trigallic acid (3), 6-O-galloyl-D-glucose (4), 1,2,3-tri-O-galloyl-D-glucopyranose (5), 1, 2, 3, 6-tetra-O-galloyl-D-glucopyranose (6), 1, 2, 3, 4, 6-penta-O-galloyl-D-glucopyranose (7), rutin (10), kaempferol-3-O-B-D-glucopyranoside (astragalin) (11) and kaempferol-3-arabinopyranoside (juglalin) (12). Gallic acid and its derivatives, galotanin, and flavonol glycosides, which were the

primary anti-oxidative components, were significant antioxidants (Cheng *et al.*, 2009; Hseu *et al.*, 2008). *Aspergillus niger* and *Alternaria alternata*, which were isolated from various organs of *Tabebuia argentea*, showed the highest antioxidant capacity of 4, 29–5, 27 $\mu\text{mol/L}$ in an ethanol acetate extract and total phenol of 2.5–2.6 mg/100 mL cultures, which is equivalent to phenolic acid (Sadananda *et al.*, 2011). The antioxidant capacity of endophytic fungal cultures was substantially linked with total phenol, according to an analysis of 292 endophytic fungi isolated from 29 traditional Chinese medicines (Huang *et al.*, 2007). According to numerous studies that looked at the prospect of finding an endophytic fungus with the capacity to manufacture antioxidant compounds similar to those found in host plants, isolation and screening techniques were crucial to the process's success. In this investigation, it was anticipated that some endophytic fungus isolates with the capacity to create antioxidant chemicals would be found. Several things must be considered in the exploration of bioactive compounds from endophytic microbes, including the ability of host plants to produce useful bioactive compounds. Endophytic microbes will produce bioactive compounds similar to those produced by their host plants, so the selection of host is very important. Another thing is the isolation process of endophytic microbes from host plants, as well as the process of extracting bioactive compounds from endophytic microbial cultures.

Materials and Methods

Extraction of twigs and leaves of surian

Extraction of Twigs and leaves of Surian Maceration was a method used to extract Surian's twigs and leaves. The surian tree's twigs and leaves were ground into a powder and dried for over a week before being extracted with three distinct solvents: acetone, ethyl acetate, and chloroform. Based on the degree of polarity, several solvents were selected: acetone was polar, ethyl acetate was semi-polar, and chloroform was non-polar (Harborne, 1987).

Isolation of endophytic fungi from surian twig and leaf

From the twigs and leaves of a 12-years old surian collected in ITB Sumedang, West Java, Indonesia, endophytic fungi were isolated. The approach of Zeng *et al.* (2011) was applied to isolate endophytic fungi from plants with a little modification. Twigs and leaves of surian were cleaned under running

water to remove any contaminants. They were then successively cleaned with 75% ethanol for 1 minute, 5.25% sodium hypochlorite for 15 minutes, and then soaked again in 70% ethanol for 1 minute before being sterilized three times with sterile water. To help the endophytic fungus remove the sterile twigs and leaves, the samples were cut using scissors in an aseptic manner. They were then placed on a PDA (potato dextrose agar) medium.

Screening process of antioxidant compounds from endophytic fungal culture

The pure endophytic fungal isolate was cultivated on PDA media for a week before being used as an inoculum source. A 250-mL Erlenmeyer flask containing 100 mL PDB (potato dextrose broth) medium and four mycelia plugs (0.5 cm in diameter) from each isolated were inoculated, and the mixture was then cultured for two weeks at room temperature with 150 rpm of shaking per minute. To separate the mycelia and the filtrate, the fermented broth was filtered thereafter. Mycelia were homogenized before being extracted with ethyl acetate from the filtrate at an equivalent volume. The ethyl acetate extracts were concentrated in a rotary evaporator at low pressure and 45 °C to provide a crude extract for testing the DPPH radical scavenging ability. qualitative assay of extract endophytic fungus culture. Thin-layer chromatography (TLC) was used to perform a qualitative antioxidant activity assessment. In this procedure, a combination of butanol, glacial acetic acid, and water was employed as the mobile phase and the TLC plate (silica gel) as the stationary phase. The endophytic fungal culture's ethyl acetate extract was put on the TLC plate, eluted with butanol, glacial acetic acid, and water (4: 1: 5), and then dried before being sprayed with 0.2% DPPH solution. The color would shift from purple to pale yellow when extracts containing antioxidant chemicals were added (Harborne, 1987).

Antioxidant activity assay (activity of radical inhibition of DPPH) quantitatively

The 1-diphenyl-2-picrylhydrazil (DPPH) radical was used to gauge the extract of ethyl acetate's ability to inhibit free radicals. With a few modest adjustments, a previous approach was used to conduct the DPPH radical scavenging activity. In brief, 250 µl of a 1 mM DPPH radical solution in methanol was combined with ethyl acetate extracts of various concentrations of endophytic fungal cultures. The control was a

similar solution without the sample. The mixture was vigorously shaken, placed in the dark for 30 minutes, and the absorbance was then measured at 517 nm in comparison to the control. The following equation was used to determine the DPPH radical inhibition capacity: DPPH inhibition effect (%) is equal to $[(A_0 - A_1/A_0) 100]$, where A_0 represents the absorbance of the blank reaction and A_1 represents the absorbance of the solution plus extract (Zeng *et al.*, 2011).

Identification of endophytic fungi molecularly

After being screened for endophytic fungi that produce a high level of antioxidant activity, the identification of endophytic fungi was carried out molecularly. To enhance the 5.8 S and ITS areas, primary ITS1 (5-GTACTTTTGCATAATGGGTCAGC-3') and ITS4 (5-GAAGTAAAAGTCGTAAGG-3') were employed. The PCR samples were amplified at the Korean company Macrogen Sequencing Services. To find the pair of homology sequences, the sequencing data was compared to the GenBank database using NCBI's Basic Local Alignment Search Tool (BLAST). Mega 5 software was used to create a phylogenetic tree from the sequence of the study's isolates and the homologous pair (Zeng *et al.*, 2011).

Results and Discussion

This study examined the antioxidant activity of extracts made from Surian twigs and leaves using acetone, ethyl acetate, and chloroform solvents. It also involved the isolation of endophytic fungi from Surian twigs and leaves, the qualitative and quantitative screening of endophytic fungi from twigs and leaves that produced antioxidant compounds, the study of the antioxidant activity of extracts made from endophytic fungi, and the molecular identification of some isolates endophytic fungi with the highest antioxidant activity. Antioxidant Activity Twigs and Leaf Extract Surian. A preliminary investigation was done to see if the Surian tree's twigs and leaves produced antioxidant chemicals prior to isolating endophytic fungus from them. A non-polar to polar solvent was used to extract the leaves and twigs and got the bioactive compound from them. To symbolize each, chloroform, ethyl acetate, and acetone were chosen. The substance was extracted using a solvent from the twigs and leaves of Surian, and its antioxidant activity was then tested. The outcome is shown in Figure 1. Ethyl acetate extract demonstrated the highest antioxidant activity (82 percent Inhibition),

compared to acetone extract (62 percent Inhibition) and chloroform extract (11 percent Inhibition), when the antioxidant activity of twigs and leaves of Surian were extracted using a variety of solvents. Although it seemed that the link between extract concentration and radical inhibitory level was not always linear, increasing the concentration of the extract would enhance its capacity to inhibit the DPPH radicals. As the extract's concentration increased, a slower rate of DPPH radical inhibition was seen. It was discovered that increasing concentration from 100 μ l to 200 μ l would increase DPPH inhibition by 20%, making the relationship between DPPH inhibition rate and concentration increasing rate of extract 2; when the concentration of extract was increased by three times, the relationship between the two became 18.5 times; and when the concentration of extract was increased by four times, the relationship between the two became 12 times while the concentration increase was five times; At a concentration of 300 μ L, antioxidant activity from leaves extracted with ethyl acetate produced IC 50 (Figure 1). The amount of free radicals that reacted with the antioxidant components in the extract determined the percentage of free radical inhibition. The results of this experiment shown that the rate of DPPH radical inhibition rose up to a point where the extract concentration was increased three times, and then it started to decline after being increased four to five times. The DPPH radical concentrations were insufficient to produce enough radicals for antioxidant chemicals to absorb, which is what triggered the event. Less uniform extracts were the second option. In this experiment, a crude extract was employed, and it contained an additional component that had no antioxidant properties. The ability of antioxidant activity, which was held by the extract of twigs and leaves and was demonstrated by high radical inhibition, was used to determine the efficacy of the ethyl acetate solvent. It was assumed that the antioxidant chemicals present in the twigs and leaves were likewise semi-polar because antioxidant compounds found in twigs and leaves that had been extracted with acetate solvent were semi-polar. Some researchers have studied the antioxidant properties of the twigs and leaves of *T. sinensis*, including extracting antioxidant chemicals from the leaves of Surian (*T. sinensis*) using 80% acetone (Wang *et al.*, 2007) and water methanol extract (Cheng *et al.*, 2009). According to (Wang *et al.*, 2007) and (Cheng *et al.*, 2009) studies, Surian leaf antioxidant was more polar. Because the samples utilized varied, it was possible

to determine the efficacy of the extracting solution in the extraction of the active chemicals found in the twigs and leaves. While samples used in this study were from the tropics, those used in studies by (Wang *et al.*, 2007) and (Cheng *et al.*, 2009) came from the temperate zone. The bioactive chemicals found in Surian twigs and leaves might vary depending on the sampling area.

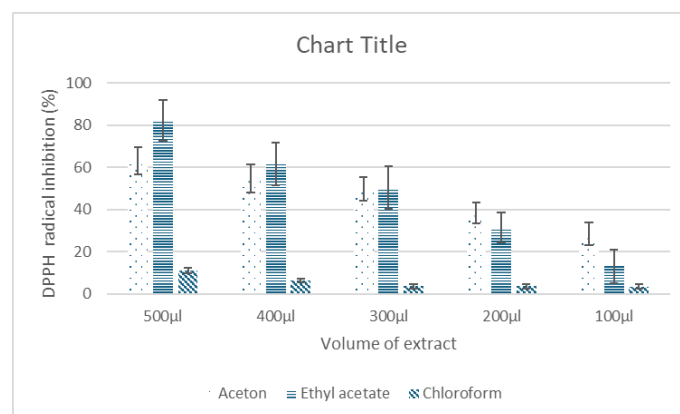


Figure 1: Antioxidant activity of extract twigs and leaves of surian using various organic solvents.

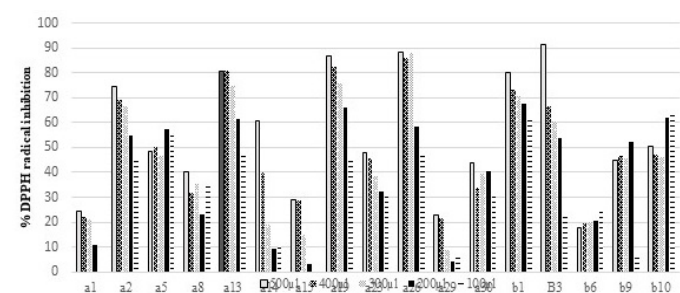


Figure 2: Additional testing of endophytic fungi with the capacity to create antioxidant chemicals that block DPPH radicals.

Isolation endophytic fungi from twigs and leaves of surian

Endophytic fungi were isolated from the twigs and leaves of 12-year-old Surian trees on the campus of ITB. Table 1 and 2 show the outcomes for the isolated organisms. When endophytic fungi were isolated from the twigs and leaves of a Surian tree using PDA media, 35 isolates were obtained from the twigs and 11 isolates were obtained from the leaves. Twelve year old *T. sinensis* twigs and leaves were used to collect the endophytic fungus, which were grown in PDA media and differentiated by morphological variations, spore color, mycelium, and medium discolouration. No investigations had revealed the existence of plant species without endophytes, hence they may be found in any plant. Another hallmark of endophytic microorganisms was high species diversity. The abundance of endophytic fungi isolates

Table 1: Results of qualitative antioxidant test on ethyl acetate extract of endophytic fungi culture by TLC method.

No	Sample	Result	No	Sample	Result	No	Sample	Result
1	A1	Present	16	A17	-	31	A32	-
2	A2	Present	17	A18	-	32	A33	-
3	A3	-	18	A19	Present	33	A34	-
4	A4	-	19	A20	-	34	A35	-
5	A5	Present	20	A21	-	35	A36	-
6	A6	-	21	A22	-	36	B1	Present
7	A7	-	22	A23	Present	37	B2	-
8	A8	Present	23	A24	-	38	B3	Present
9	A9	-	24	A25	-	39	B5	-
10	A10	-	25	A26	Present	40	B6	Present
11	A11	-	26	A27	-	41	B7	-
12	A13	Present	27	A28	-	42	B8	-
13	A14	Present	28	A29	Present	43	B9	Present
14	A15	Present	29	A30	Present	44	B10	Present
15	A16	-	30	A31-	-	45	B11	-
						46	B12	-

Table 2: Superior Isolate of endophytic fungi that produced antioxidant compounds with the high radicals inhibition.

No	Code	Isolates	Similarity
1	NR 01(A19)	Gibberella moniliformis DBT -112	99%
2	NR02 (A26)	Fusarium sp Da167185	99%
3	NR03 (B3)	Fusarium sp NR 2006 M41	100%

from Surian twigs and leaves supported research from (Strobel and Deasy, 2003) studies that suggested all plants were harbours for some type of endophytic fungi. Due to their mutualistic symbiosis with plants, all endophytic fungi serve a variety of distinct purposes for plants. The stem of a Surian included endophytic fungi that had been isolated from it (Rahmawati et al., 2016). The colonization rate of the stem was 82 percent and that of the leaves was 47 percent, indicating that the stem had more nutrients than the leaves (Li et al., 2011). Environmental parameters (temperature and humidity), chemical variation, architecture, and the maturity of the colonized host organ all had a significant impact on the abundance of the endophytic fungus community's composition (Sanchez-Azofeila et al., 2012). According to leaf age (Suryanarayanan and Thennarasan, 2004) and even plant organ type (Sanchez-Azofeila et al., 2012), the community of endophytic fungus may alter significantly (Sanchez-Azofeila et al., 2012). Richnes abundance and endophytic quantities were

higher in plants with older leaves and those that were near to twigs. The colonization pattern revealed that the mature, older leaves provided an environment that was conducive for the growth of the endophytic fungus. Younger leaves often have higher quantities of anti-fungus and anti-herbivore compounds compared to older leaves. Due of *Fusarium* spp. reputation's as a plant disease, the existence of endophytic fungus *Fusarium* spp. seemed particularly intriguing. This finding supported the theory that the endophytic fungus was originally harmful but later evolved into an endophytic fungus that did not cause disease in its host plant (Schulz and Boyle, 2005). According to Sieber (2007), the first stage in which endophytic fungi entered the host plant was by recognition, germination, and penetration. This explanation helped to explain how the endophytic fungus could enter the host plant without creating the disease symptoms. Plant pathogenic fungal infections went through a similar process. Endophytic fungus must first get past the plant's defenses along the way of the process. Fungi's spores frequently identified host plants using chemicals like lectins. After germination, the fungus would enter the plant tissue either by mechanically weakening the cuticle or by softening the epidermal cell wall. The host's defense mechanisms stopped working once the fungus entered the plant tissue and transformed into a dormant condition. The gene for gene (GFG) model was used to explain this phenomenon. In this model, the avirulence genes

(AVR) of the endophytes were encoded into an elicitor and recognized by the product of the resistance gene (R) of the host plant as well as the hypersensitive reaction of the host plant. The serenity then occurs through the signal transduction pathway. Instead, pathogenic fungus lacked the AVR gene; as a result, product R was not generated and disease symptoms would emerge (Sieber, 2007). Endophytic fungi's interactions with host plants led to a compromise between mutualism and hostility that culminated in a successful symbiotic relationship. Endophytes might have their growth restricted by plants, which would allow them to adapt their survival strategies. Endophytes not only created helpful substances and/or supported or promoted the growth of host plants to create a balanced environment, but also described some plant metabolites with ectoenzymes to take essential nutrients and energy to survive (Sieber, 2007). An adequate regulatory balance between the virulence of endophytic fungus and plant defence was what defined interactions between host plants and these organisms. Disease would emerge if this equilibrium was upset by a reduction in plant defence or an increase in the virulence of the fungus. In order to compete with epiphytes and pathogens and remain in the host, endophytes must produce metabolites. Host plant metabolism may also be regulated in a healthy connection between host plants and endophytic fungus (Schulz *et al.*, 2002). A morphological change was also brought on by the interaction of host plants and endophytic fungus. The difference between endophytes and pathogens was often blurred. Endophytes might be latent or opportunistic. Once in the plant tissue, they could remain silent until environmental was changed or decreased in host defences allowed them to become pathogens. Another possibility was a pathogen that has invaded the wrong host and therefore could not cause the disease. Endophytes were also said to be pathogens that had been lost their ability to cause disease or pathogens that have infected non-host species. Some experimental evidence showed that endophytes could be derived from pathogens due to pathogenicity loss. However, many endophytes were still capable of producing phytotoxic secondary metabolites and still capable of causing illness, but the plant's inhabitants were able to limit their growth (Bayman, 2007).

Analysis of antioxidant activity

Two methods were used to analyse the antioxidant

activity of endophytic fungus extract cultures from Surian twigs and leaves. Specifically, a qualitative method utilizing TLC for preliminary screening and a quantitative method employing DPPH radical for secondary screening. A solvent with high antioxidant activity was utilized to extract the fungal culture from the twigs and leaves, according to the Surian extract results. Using TLC, the fermentation broth of endophytic fungus isolated from the twigs and leaves of the Surian tree was extracted using ethyl acetate. The culture extract of endophytic fungi was put to TLC and sprayed with DPPH reagent, which caused the purple hue to turn pale yellow, indicating the presence of possible antioxidant chemicals. Some isolates with potential to create antioxidant chemicals in their culture were found by the screening of antioxidant activity. Table 1 contains a list of isolates that may have created antioxidant chemicals.

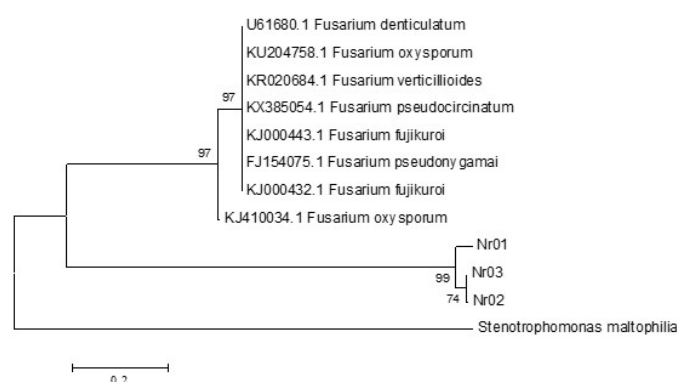


Figure 3: Phylogenetic tree of endophytic fungi potential for producing antioxidant compound.

By using the TLC method, one of 46 ethyl acetate extracts from several endophytic fungal cultures showed the capacity to create antioxidant chemicals. 17 endophytic fungi, out of the 46 fungi tested with the ability to create antioxidant chemicals, were found as a consequence of the screening of antioxidant activity with TLC. The antioxidant activity was assessed by employing DPPH radicals after an endophytic fungal culture extract that may have produced antioxidant chemicals. Figure 2 displays the findings of the additional screening.

The results of a screening for endophytic fungi with high antioxidant activity were displayed in Figure 2. Endophytic fungi isolates A19 and A26 from twigs were able to inhibit the DPPH radical 86 to 88 percent, whereas isolates B1 and B3 from leaves were endophytic fungi isolates and could only inhibit the DPPH radical by 80 to 90 percent. The capacity

of the ethyl acetate solvent to extract the antioxidant component from the endophytic fungus culture resulted in 80–90% DPPH radical inhibition. Because many antioxidant chemicals in endophytic fungal culture were semi-polar, it was thought that the extent of DPPH radical inhibition capability in ethyl acetate extract of endophytic fungus culture may be greater than that of non-polar solvents like n hexane. Several studies, including those by [Zeng *et al.* \(2011\)](#) and [Elfiti *et al.* \(2012\)](#) and based on research by [Estrada *et al.* \(2012\)](#) have employed the solvent ethyl acetate to extract endophytic fungal cultures in order to attract antioxidant chemicals ([Govindappa *et al.*, 2013](#)). The capacity to suppress the DPPH radical was found in the ethyl acetate culture extract of endophytic fungal broth isolated from stem of surian tree ([Rahmawati *et al.*, 2016](#)). Up until now, the quest for new compounds produced by endophytic fungus has mostly focused on the random isolation of isolates. By taking into account (1) synthesized secondary metabolites that may be synthesized according to their respective ecological niche ([Gloer, 1997](#)); and (2) metabolic interactions that could increase the synthesis of secondary metabolites, efforts to optimize the search for secondary metabolites that were bioactive were highly relevant. The screened fungus must therefore come from fungi biotopes that have not before been separated for biochemical purposes and interact metabolically with their surroundings. This is an illustration of intelligent screening and a tactic for utilizing the untapped capacity of fungi to produce secondary metabolites. One option for intelligent screening that meets both of the aforementioned requirements is endophytic fungus. They can develop in hosts plants without displaying any disease symptoms ([Boyle *et al.*, 2001](#)). Endophytic fungus and their host plants constantly interact metabolically to support growth in this ecosystem ([Petrini, 1991](#)).

Fungi with endophytes exist due of its reputation as a plant pathogen, *Fusarium* sp. was particularly intriguing. This outcome supported the hypothesis that the endophytic fungus was a pathogenic fungus that evolved into an endophytic fungus that did not cause disease symptoms in its host plant ([Schulz and Boyle, 2005](#)). Numerous *fusarium* species were recognized as plant diseases. Endophytic fungi were latently pathogenic fungi that would not hurt plants when environmental conditions were good; however, if conditions were unfavorable to fungi, the virulence of the fungus would be minimal, and it would not

harm the plant. This was in line with the findings of [Schulz *et al.* \(2002\)](#) research, which contends that disease will develop from an imbalanced antagonistic relationship between the host and the pathogens as well as the interactions between endophytes and host plants. Instead of fungus, the penetration, colonization, and proliferation of endophytes and pathogens were observed under a microscope. While pathogenic fungi, on the other hand, are able to penetrate straight through the cell wall, endophytic penetration in nuts and barley took occurred through the stomata and along the anticlinal epidermal cells ([Boyle *et al.*, 2001](#)). In the roots of larch and barley, endophytes and pathogens expanded widely, systemically, and on both internal and extracellular surfaces ([Schulz *et al.*, 1999](#)). Some microbes appeared to actively penetrate plant tissue through open or wound attack and utilised hydrolytic enzymes including pectinase and cellulase. Endophytic fungi have slowly adapted to the microenvironment over the course of their long co-evolutionary history through genetic variation, including the insertion of some host plant DNA segments into the endophytic fungal genome and the insertion of endophytic fungal DNA segments into the genome of the host plant. Some particular endophytic fungi developed the capacity to biosynthesize bioactive substances, such as host plants, as a result of this mechanism ([Zhao *et al.*, 2011](#)).

Conclusions and Recommendations

The study's findings indicate that ethyl acetate extract of surian twigs and leaves produced antioxidant compounds with better antioxidant activity compared to acetone and chloroform extracts. Up to 35 different isolates of the endophytic fungus from twigs and up to 11 different isolates from leaves were successfully obtained. By examining the color of the mycelium colony and the color change in the medium brought on by the pigment, each isolate was identified by changes in macroscopic morphology.

From 46 isolates screened for antioxidant activity qualitatively using TLC, 17 were found to have the ability to create antioxidant chemicals. Three isolates were found through additional screening for antioxidant activity utilizing spectroscopy. Endophytic fungi from Surian twigs and leaves were extracted using ethyl acetate, producing compounds with strong antioxidant activity, such as A26 from

twigs (88.06 percent) and B3 from leaves (91.57 percent) to block DPPH radicals.

Based on molecular analysis, possible isolates A19 (NR01), A26 (NR02), and B3 (NR 03) were determined to be *Gibberella moniliformis* DBT -112 and *Fusarium sp* Da167185 and and *Fusarium sp* NR 2006 M41.

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Novelty Statement

The novelty of this research was to find an isolate of endophytic fungi from the twigs and leaves of *Toona sinensis* that can produce antioxidant compounds. We are also finding out which solvent can be used to extract antioxidant compounds from the culture of endophytic fungi.

Author's Contribution

Noor Rahmawati: Performed experiments and wrote the manuscript.

Dea Indriani Astuti: Helped in data analysis and manuscript writing.

Pingkan Adiatiawati: Help the data was analyzed, and writing manuscript and discussion about fungal secondary metabolite especially endophytic fungi.

Conflict of interest

The authors have declared no conflict of interest.

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