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Anti-free Radical Activity Test of Endophytic Fungal Fermentate Extract on The Snake Plants (*Sansevieria trifasciata* Hort. Ex Prain) Using the TLC-Autography Method

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Abstract. Degenerative diseases occur due to damage to cells, fat tissue, and the immune system caused by several factors. One theory that causes degenerative diseases is the presence of free radicals. The Snake plant is one of the plants in Indonesia that has long been known as a plant useful as anti-free radicals. The research method used in this study was the isolation of endophytic fungi and to determine the autogram profile of the free anti-radical activity of the Snake plant. The purification results obtained seven isolates, followed by macroscopic examination and fermentation of each isolate. The screening test results showed that two fermentates were active as anti-free radicals, namely AF 1 and AF 3. Fermentate isolates AF 1 and AF 3 were identified by TLC-autography using a mixture of n-hexane: ethyl acetate (7:1). TLC-autography test results by spraying DPPH isolates AF 1 and isolates AF 3 showed yellow spots on a purple background with Rf1 values of 0.90 and Rf2 of 0.2 in AF1 isolates and Rf1 values of 0.83 and Rf2 of 0.16 on AF3 isolates. Potential as anti-free radicals. From the results of this study, it was concluded that there was anti-free radical activity in the Snake plant.

Introduction

Degenerative diseases occur due to damage to cells, fatty tissue, protein, immune system, and DNA caused by several factors, whether they occur naturally due to radiation or carcinogenic chemical substances. One of the theories regarding the causes of degenerative diseases is the free radical reaction theory. According to this theory, the cause of degenerative diseases results from the emergence of hydroxyl radicals in biochemical mechanisms that occur in the body (Wardatun, 2011).

Free radicals are molecules with an unpaired electron in their outer orbital, so they are highly reactive. These radicals tend to hold a chain reaction if they occur in the body and cause ongoing and continuous damage. The human body has an endogenous defense system against free radical attacks, mainly through normal cell metabolism and inflammation. The number of free radicals can increase due to stress factors, radiation, cigarette smoke, and

environmental pollution, causing the body's existing defense system to be inadequate, so the body needs additional antioxidants from the outside that can protect against free radical attacks (Wahdaningsih, Setyowati and Wahyuono, 2011; Bencheikh et al., 2021).

Prevention of free radical formation includes the use of nutrients that can act as anti-free radicals such as vitamin E, carotene, vitamin C, BHT, BHA, and other drugs that can capture free radical molecules. Anti-free radicals function to protect cells from damage caused by free radical molecules (Ayoola et al., 2008).

Indonesia is a country that has a variety of biodiversity, and traditionally Indonesian people use plants as medicinal ingredients (Elfina, Martina and Roza, 2014). The Snake plant is one of the plants in Indonesia that has long been known as an ornamental plant and is rich in benefits. Besides being able to beautify and be healthy because it can absorb toxins and pollution in the air, as well as radiation emitted from electronic devices and communication tools, the Snake plant is also a fiber-producing plant (Idhan, 2015). Besides having these benefits, the Snake plant also has health benefits; namely, it is trusted by the community

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to treat earaches, stomachaches, toothaches, wounds, ulcers, hemorrhoids as an antiseptic and anticancer. Number of studies have demonstrated that the nutritional profile of Snake plant is comparable to those of better known fruits like mango, kiwi and apple, owing to its richness in antioxidants, phenolic, vitamins and minerals (Raslan et al., 2021; Sarjani et al., 2021).

Based on research conducted by Dey (2014) that the methanolic extract of the snake plant contains terpenoids, flavonoids, tannins, triterpenes, polyphenols, steroids, ketones, alcohols, terpenoids, and phenols. The active compounds contained in the snake plant include in the antioxidant group (Utami, 2018). According to research by Nurlaila (2011), it is known that the Snake Plant is an ornamental plant that is also useful as an antibacterial and antioxidant due to the presence of phenolic compounds and flavonoids (Sagita, Aliyah and Safitri, 2019).

In plant tissue, there are living microorganisms called endophytic microbes. Endophytic microbes live in plant tissues such as roots, stems, leaves, flowers, and fruits. Endophytes and their host plants can produce symbiotic mutualism, such as endophytes that get nutrients from the metabolism of plants that have activities to protect their host plants. In contrast, while plants get the nutrients, they need during their lives (Sumampouw, 2014). Endophytic fungi live intracellularly in healthy plant tissues, which induce the host to produce secondary metabolites (Murdiyah, 2017).

Based on the description above, a study was conducted on the test of free radical activity of fermentate extract of endophytic fungi on the Snake Plant using the TLC-autography method.

Experimental

Material and Methods

The tools used in this study were a spirit lamp, petri dish (Normax), Erlenmeyer glass (Iwaki Pyrex), 250 and 500 mL beakers (Iwaki Pyrex), incubator (Memert), Laminar Air Flow (LAF), UV lamp 254 nm and 366 nm TLC plate (E.Merck), Autoclave (SMIC Model YX-280 B), oven (Memert), micropipette, pipe capillaries, shakers, analytical balances (Chyo), and vials.

The materials used in this study were distilled water, Maltose Yast Broth (MYB) medium, Nutrient Agar (NA) medium, PDA+Cloramphenicol (PDAC) medium, Diphenylpicrylhydrazyl (DDPH), ethyl acetate, n-hexane, and 70% ethanol.

Procedures

The leaves of the fresh the Snake plant that had been taken from Makassar, South Sulawesi, were cleaned of dirt, then soaked in 70% ethanol solution for 30 seconds to avoid bacterial contamination. Furthermore, it was cut into small pieces and then stored on Potato Dextrose Agar (PDA) media in a sterile petri dish to which 0.2 g/L chloramphenicol had been added-after then stored at 25oC for 3 x 24 hours to grow fungi (Sumampouw, 2014).

Purification was carried out until a single pure fungal isolate was obtained, and macroscopic analysis was carried out by directly observing the shape and color of the colony (Adriani, 2015). The purified endophytic fungi were grown in Maltose Yeast Broth (MYB) fermentation medium. After that, it was filtered, and fermentate was taken for activity testing (Yunus, 2015). For the screening test of anti-free radicals activity, the fermented endophytic fungi isolate was put into a test tube as much as 5 mL with 1 L of 0.04% DPPH added. The active compound as an antidote to free radicals will give a yellow color change to purplish-brown (Irwanto, 2014).

The production of endophytic fungi free antiradical compounds that have activity in the screening test was fermented with a shake culture by making 50 mL of endophytic fungi inoculum, inoculated into 200 mL of the fermentation medium-then shaken at 200 rpm for 14 days. After the fermentation process ends, filtering is carried out using filter paper. The supernatant obtained was extracted with ethyl acetate solvent, then put into a separating funnel, shaken gently for 5 minutes. After being shaken, the solvent containing dissolved bioactive compounds was poured into a porcelain cup, then evaporated to produce an extract. The fermentate extract obtained was identified by Thin Layer Chromatography (TLC) using a mixture of n-hexane : ethyl acetate eluent in the ratio (7:1).

The resulting chromatograms were observed under UV light at 254 nm, 366 nm, and their Rf values were measured. Free radical activity test by TLC- Autograph, 1.0 mg of fermentate extract was dissolved in 2.0 mL of ethyl acetate, then identified on TLC with the appropriate eluent. After drying, the TLC plate was sprayed with 0.04% DPPH. The active compound as an antidote to free radicals will give yellow spots on a purple background after being stored for 30 minutes (Irwanto, 2014).

Result and Discussion

The results of the isolation of endophytic fungi from the leaves of the Snake plant can be seen in Figure 1.



Figure 1. The results of the isolation of endophytic fungi on the leaves of the Snake plant.

From the isolation of the Snake plant's leaves, seven colonies of fungi were obtained with different characteristics, as shown in Figure 1. Subsequently, the seven colonies of fungi were purified on the new PDA

medium. After that, a single or pure fungal isolate was obtained, which was then continued for macroscopic testing to characterize the type of fungus. These observations include colony shape, elevation, edge, and color, as shown in Table 1 below.

Table 1. Macroscopic tests of endophytic fungal isolates of ginger elephant rhizomes.

isolate Code	Morphological characteristics of fungi			
	Colony Configuration	Evaluation	Margins	Color
AF-1	Round with scalloped margin	Flat	Createriform	White bone
AF-2	Round with scalloped margin	Convex	Smooth	White
AF-3	concentric	Drop-like	Wavy	Brown
AF-4	Round with radating margin	Raised	Smooth	Gray on a black background
AF-5	sircular	Drop-like	Braching	White on a dark brown background
AF-6	concentric	Umbonate	Wooly	White
AF-7	Round with radating margin	Umbonate	Branching	Grey brown

The results of the fermentation were then screened for free radical testing. DPPH was added to the fermentate in

the vial until it turned brown to purple. The test of the results can be seen in Table 2 below.

Table 2. Fermentate free antiradical screening test results of endophytic fungi isolate on the leaves of the Snake plant.

Isolate Code	Isolate Code Endhophytic Fungi	Total of DPPH / μL *)
1	AF 1	2000
2	AF 2	800
3	AF 3	2400
4	AF 4	400
5	AF 5	300
6	AF 6	400
7	AF 7	500

*) DPPH : Diphenylpicrylhydrazyl

From the test results, fermentate from isolates AF1 and AF3 with a few drops of DPPH changed color to purplish brown. Both have anti-free radical activity. The Snake plant can also be used as an antimicrobial. Based on the results of research (Lombogia, Budiarmo and Bodhi, 2016), ethanol extract of the leaves of the Snake plant (*Sansevieria trifasciata*) has antimicrobial activity against the growth of *E. coli* and *Streptococcus sp* bacteria.

In this study, the fermentation medium was MYB because this is a liquid medium containing yeast extract as a protein source, maltose and dextrose as a carbon source, and peptone as a source of amino acids, which are needed for growth, cell synthesis, energy requirements in the metabolism of microorganisms (Winarsih, 2007).

The active isolates selected in this case, namely AF1 and AF3, were continued to the production stage to increase the fermentate obtained for further testing. In producing anti-free radicals, fermentation carried out using a fermentation medium that meets the nutrients of endophytic fungi, namely MYB. The isolate from the active fermentate put into an Erlenmeyer containing 200 mL of MYB medium, which was then incubated and shaken at 200 rpm for 14 x 24 hours.

The fermentate obtained was extracted using ethyl acetate to obtain a dry extract. After obtaining the dry fermentate extract, a screening test of several solvents was carried out based on the level of polarity. The solvent used is ethyl acetate. The screening test carried out by looking at the TLC profile of the solvent, then the fermentate extract AF1 and AF3 were tested with a mixture of n-hexane: ethyl 7:1 eluent. The excellent appearance of the spots seen from the two isolates, namely fermentate extracts AF1 and AF3.

From the results of identification with a TLC profile using an appropriate mobile phase and showing good spots, an anti-free radical activity test carried out by the TLC-Autographic method. This method has used a small number of samples to show its activity and can directly localize compounds that provide anti-radical activity. Free to facilitate the process of isolation or separation of the active compound from other compounds.

Table 3. Autographic TLC test results of ethyl acetate extract of fungi isolates on leaves of the Snake plant.

Isolate Code	Rf 1 Value	Rf 2 Value
AF 1	0.90	0.2
AF 3	0.83	0.16

Based on the TLC-Autograph test performed in table 3, two spots obtained on isolate AF1 with Rf values = 0.90 and

0.2 and two spots on isolate AF3 with Rf values = 0.83 and 0.16. These two isolates have activity as anti-free radicals. It indicated by the colour of yellow spots on a purple background. Research result from Elis Suwarni, et al. (2016) showed the presence of free radical anti-radical activity against the ethanol extract of flower Kecombrang (*Etlingera elatior*) from the results of the TLC- Autograph test, indicated by the presence of two yellow spots on a purple background with Rf values = 0.98 and 0.90 (Suwarni and Cahyadi, 2016).

Conclusion

The research concludes that an endophytic fungal isolate of the Snake plant leaves was obtained with isolate codes AF1 and AF3, which gave the most active activity as anti-free radicals. From the results of the Autographic TLC test, compounds that have anti-free radical activity in the ethyl acetate extract are fermentate extracts of endophytic fungi isolates AF1 and AF 3 with the appearance of yellow spots on a purple background and with Rf1 values on isolates AF1 0.90 and Rf2 0.2 while in AF3 Rf1 is 0.83 and Rf2 is 0.16.

Conflict of Interest

The authors declare that there is no conflict of interest.

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