

Antioxidant Activity of Endophytic Fungi Extract Isolated from Peel of Pineapple (*Ananas comosus* (L.) Merr. 'Prabumulih')

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ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr. 'Prabumulih') is a tropical fruit that is very popular for its unique aroma and sweet taste. It is also commonly believed that this fruit has potential applications in traditional medicine. However, the peel is not fully harnessed, despite its rich and intricate composition. This study presented findings regarding the endophytic fungi discovered within the peel of *A. comosus* (L.) Merr. 'Prabumulih' and their performance as antioxidants. The endophytic fungal species were identified based on their morphological characteristics. The antioxidant assessment was carried out using the DPPH method. According to the findings of morphological identification, *Trichoderma harzianum*, an endophytic fungus, was found to exhibit the highest potential as an antioxidant source. Antioxidant activity showed a very strong category ($IC_{50} = 18.74 \mu\text{g/mL}$). Through the isolation of its pure constituents and subsequent *in vivo* testing, there is the potential for this extract from the endophytic fungus to evolve into an innovative source of antioxidants.

Keywords: *Ananas comosus*, Antioxidant, Endophytic Fungi, Pineapple Peel

INTRODUCTION

Advancements in technology and science, along with changes in people's lifestyles, have led to adverse effects on health, including reduced physical activity, insufficient rest, and increased smoking habits. Furthermore, pollution, radiation sources, and the presence of harmful chemicals in the environment serve as sources of free radicals (Beutel et al., 2021; Sharifi-Rad et al., 2020). A free radical is an unstable and highly reactive atom or molecule with one or more unpaired electrons, making it transient in nature. Through their disruption of the structural integrity of lipids, proteins, and DNA, they possess the potential to damage biomolecules, leading to elevated oxidative stress and contributing to conditions such as cancer, diabetes, cardiovascular disease, and

neurodegenerative disorders (Nandi et al., 2019; Radi, 2018; Rahaman et al., 2023). Substances possessing antioxidant activity can hinder the oxidation caused by free radicals.

The capacity of free radical molecules or compounds to carry out free radical chain reactions is reduced by antioxidants, which are molecules or compounds that are stable enough to contribute electrons or hydrogen to them (Carr & Maggini, 2017; Nwozo et al., 2023; Shen et al., 2023). While the body employs various enzyme systems to scavenge free radicals, the primary micronutrient antioxidants consist of vitamins such as vitamin E, vitamin C (ascorbic acid), and β -carotene. Additionally, there are numerous secondary metabolic compounds found in

plants, including phenolic compounds, flavonoids, and organic acids, which also serve as antioxidants (AlMousa et al., 2022; Madrid et al., 2014; Palimariciuc et al., 2023; Stuper-Szablewska et al., 2023; Verma et al., 2022). Pineapples (*Ananas comosus*) are among the plants that exhibit antioxidant properties.

Pineapple (*A. comosus*) is a highly favored tropical fruit, largely attributed to its unique scent and delectable taste (Mohd Ali et al., 2020). This fruit is recognized for its flavorful nature due to the presence of volatile compounds. In addition, pineapple contains a lot of healthy vitamins and minerals (Zdrojewicz et al., 2018). Fresh pineapples contain a significant amount of the anti-inflammatory enzyme bromelain. In addition, pineapple contains eight phenolic chemicals, including o-coumaric acid, gallic acid, gentisic acid, syringic acid, vanillin, ferulic acid, sinapic acid, and gentisic acid. This compound exhibits antioxidant activity (Andonova et al., 2023; Chakraborty et al., 2021; Olech et al., 2020; Olszowy-Tomczyk & Wianowska, 2023; A. K. Singh et al., 2022; Skroza et al., 2022; Tang et al., 2021; Wijekoon et al., 2022; Zeng et al., 2023). However, soil conditions and agro-climate greatly affect the quality of enzymes and secondary metabolites in plants (Bustamante et al., 2020; Gfeller et al., 2023; Jurić et al., 2020; Qaderi et al., 2023; Raza et al., 2019; Yang et al., 2018). Therefore, pineapple of the same type will differ in taste and content due to the influence of soil and agro-climate, such as pineapple from Prabumulih.

According to the observations and discussions with farmers and the Prabumulih City Agriculture Service, it has been determined that Prabumulih pineapples are of the queen type and are known to possess the sweetest flavor in Indonesia. The unique sweetness, measured at a Brix level of 13, is a

quality exclusive to Prabumulih pineapples and distinguishes them from pineapples cultivated in other areas. The sweet taste of Prabumulih pineapples contributes significantly to their popularity among the public. Consequently, the peel is not utilized in the same way as the fruit's flesh.

Pineapple peel contains lots of fiber, vitamins, and the enzyme bromelain. More bromelain is found in this part compared to the flesh of the fruit (Agrawal et al., 2022; Varilla et al., 2021). Pineapple peels are frequently underestimated by people, despite the fact that their content and associated benefits are highly valuable. Therefore, it is crucial to utilize pineapple peels to the fullest extent to unlock the potential of their valuable contents, particularly by harnessing the power of endophytic fungi.

Endophytic fungi are types of fungi that live symbiotically in host plant tissue without causing any disturbances or symptoms (Caruso et al., 2020; Oktiansyah, Elfita, Widjajanti, Setiawan, Mardiyanto, et al., 2023; Rai et al., 2021). Endophytic fungi have the capacity to synthesize bioactive compounds that may resemble or differ from those found in their host plants. This ability arises from the fungi's capability to replicate and alter compounds originating from their host plants. (Alam et al., 2021; Sharma et al., 2021; Vigneshwari et al., 2019). Many studies describe that endophytic fungal extracts isolated from plants, especially plants that have medicinal properties, have excellent bioactivity equivalent to or even better than their host plants (Almustafa & Yehia, 2023; Bertoni et al., 2023; Oktiansyah, et al., 2023; Santos et al., 2020; Wen et al., 2022). Therefore, the extraction from endophytic fungi is effective and efficient due to the short cultivation time, enabling the utilization of secondary metabolites as required.

Secondary metabolites produced by endophytic fungi from pineapple peel are believed to exhibit antioxidant properties, potentially serving as a means to counteract free radicals responsible for the onset of neurodegenerative diseases. The technology involving these endophytic fungi requires minimal plant biomass, ensuring the preservation of nature is not compromised. Additionally, it allows for the rapid generation of bioactive components in a short timeframe.

MATERIAL AND METHOD

Preparation of plant samples

The fresh and healthy pineapple peel was used to isolate endophytic fungi collected from Prabumulih City in South Sumatra, Indonesia. The plant samples collected were subsequently identified or analyzed in *Generasi Biologi Indonesia*. (08.161/Genbinesia/I/2023).

Isolation and purification of endophytic fungi

Pineapple peel was washed for \pm 3 minutes using running water. The samples were then soaked in 70% alcohol for \pm 1 minute, rinsed for \pm 1 minute using sterile distilled water, and soaked using NaOCl 10% for \pm 1 minute. After sterilization, the samples were aseptically cut and inoculated into PDA (Potato Dextrose Agar) media in petri dishes. The inoculant was incubated for 3-15 days at room temperature. Colonies that developed near the sample were purified by moving them to a fresh petri plate with PDA medium and culturing them twice for 24 hours (Elfita et al., 2012; Hapida et al., 2021; Oktiansyah, et al., 2023).

Identification of Endophytic Fungi Morphologically

For morphological identification, both microscopic (hyphae (partitioned or

not) and spores observed through culture slides using a microscope up to 1000X magnification) and macroscopic (colonial surface color, colony reverse color, colony texture, looks of exudate dots, radial lines, and concentric circles) characteristics were used. The figures observed during the examination were compared with the relevant references. (Jha et al., 2023; Shen et al., 2023; Walsh et al., 2018; Wang et al., 2023; Watanabe, 2010).

Extraction and Cultivation

Endophytic fungal isolates were cultivated in 15 culture bottles containing 300 ml of PDB (Potato Dextrose Broth) media and incubated for 30 days at room condition. After the incubation period, the biomass was extracted, and an equal part of ethyl acetate solvent was introduced to the medium. A rotary evaporator was used to evaporate the solvent (Syarifah et al., 2021).

Test of Antioxidant Activity

Various concentrations of endophytic fungal extracts in methanol, specifically 1000, 500, 250, 125, 62.5, 31.25, and 15.625 $\mu\text{g/mL}$ were tested for their antioxidant properties using the DPPH method. A mixture of 3.8 ml of a DPPH solution (0.05 mM) was homogenized with 0.2 ml of concentrated extract and then incubated (in the dark) for 30 minutes. The UV-VIS spectrophotometer (Shimadzu UVVIS-UV1900) was used to measure the absorbance at a wavelength of 517 nm. Antioxidant properties was measured based on the IC_{50} value. The linear regression of inhibitor percentation obtained in the form: $y = b + ax$, was used to find the IC_{50} value (50% inhibitor concentration) for each sample, indicating that the x value is the IC_{50} and the y value is 50%. The IC_{50} value represents the concentration of the sample solution required to reduce DPPH

by 50% (Abbas et al., 2021; Elfita et al., 2022).

RESULT AND DISCUSSION

Characteristics of endophytic fungi isolated from pineapple peel

The characteristics found in endophytic fungal colonies isolated from

pineapple peel showed variations, exhibited variations, both at the macroscopic and microscopic levels. (Figure 1). This study identified six different isolates of endophytic fungi. Tables 1 and 2 displayed the results obtained from the examination of the characteristics of the endophytic fungi.

Table 1. Macroscopic characteristics of endophytic fungi from pineapple peel

Code	Surface Colony	Reverse Colony	Structure	Elevation	Pattern	Exudate Drops	Radial line	Concentric circle
DKN1	Black	Black	Cottony	Umbonate	Radiate	-	-	-
DKN2	Green	Green	Cottony	Umbonate	Radiate	-	√	-
DKN3	Green to yellow	Green to yellow	Cottony	Umbonate	Spread	-	-	-
DKN4	Black to White	Black to White	Cottony	Rugose	Zonate	-	-	-
DKN5	Black	Black	Cottony	Umbonate	Radiate	-	-	-
DKN6	Brown to white	Brown to white	Cottony	Umbonate	Zonate	-	√	√
DKN7	Brown to Black	Brown to Black	Cottony	Umbonate	Radiate	-	-	-

Table 2. Microscopic characteristics of endophytic fungi from pineapple peel

Isolate	Spore	Shape	Hyphae	Characteristic	Species of Identification
DKN1	Conidia	Globose	Septate	Conidiophores are simple, round, hyaline, single conidia apically	<i>Nigrospora oryzae</i>
DKN2	Conidia	Globose	Septate	Conidia are septate, hyaline, round, septate hyphae	<i>Trichoderma harzianum</i>
DKN3	Sporangia	Globose	Septate	Columnar, conidia phialosporous, round, echinulate, conidiosporous erect	<i>Aspergillus parasiticus</i>
DKN4	Conidia	Phialides	Septate	Sympodulosporous conidia, pale brown conidiospores, simple branching	<i>Ramichloridium subulatum</i>
DKN5	Conidia	Cylindrical	Septate	Porosporous conidia, dark brown conidiophorous, erect, porous conidia, cylindrical	<i>Curvularia</i> sp
DKN6	Conidia	Globose	Septate	Conidiophosphorus pale brown, tapering from base to apex, ellipsoidal	<i>Gonytrichum chlamydosporium</i>
DKN7	Conidia	Phialides	Septate	Conidiospores erect, rarely branched, hyaline, conidia phialosporous, moon-shaped	<i>Conidaea maire</i>

A total of seven different endophytic fungal species were discovered in this study, and their macroscopic and microscopic characteristics have been detailed in Tables 1 and 2. The characteristics that were observed served as a reference for identifying the

discovered endophytic fungi. The endophytic fungi found were *Nigrospora oryzae*, *Trichoderma harzianum*, *Aspergillus parasiticus*, *Ramichloridium subulatum*, *Curvularia sp.*, *Gonytrichum chlamydosporium*, and *Conidaea maire*.

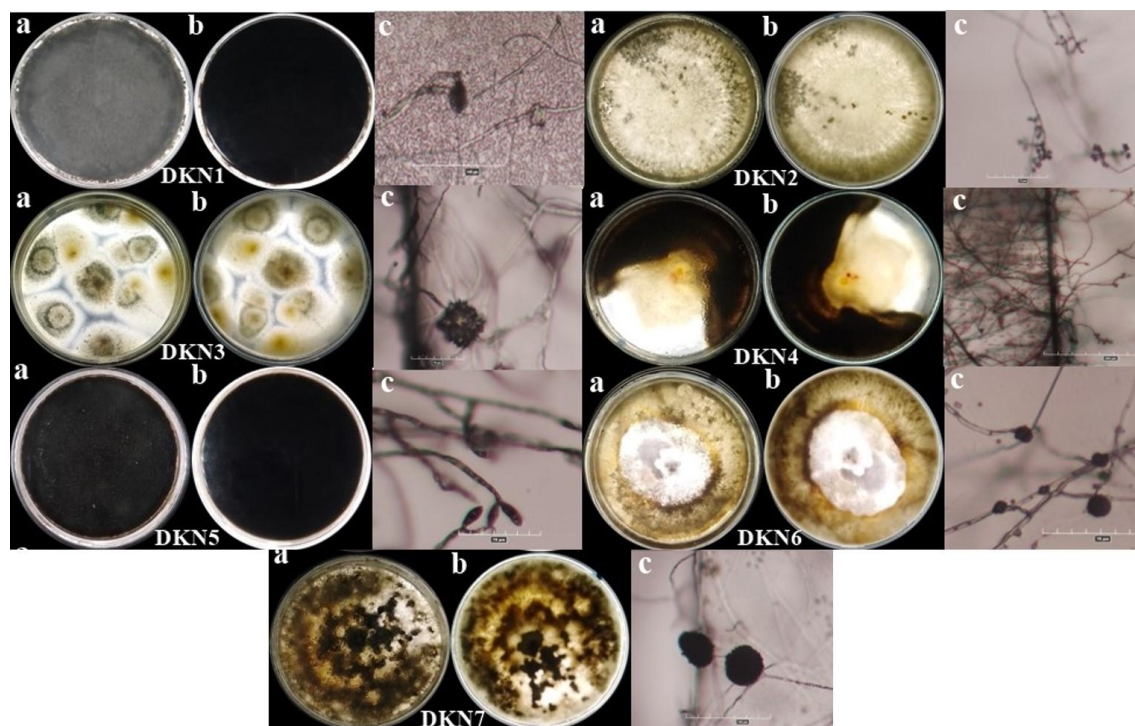


Figure 1. Macroscopic (a: front view; b: reverse view) and microscopic (c) characteristics

Antioxidant Activity of Endophytic Fungi Extract

The ethyl acetate extract derived from endophytic fungi found in pineapple peels exhibited antioxidant properties. All of the extracts exhibited varying levels of

antioxidant activity. However, one of the endophytic fungal extracts exhibited very strong antioxidant properties. The results of antioxidant activity are presented in Tabel 1.

Table 3. Endophytic fungal extract from pineapple peel was tested for antioxidant activity (IC₅₀ value) using ascorbic acid as a positive control

Sample	Extract	Antioxidant Activity IC ₅₀ (µg/ml)
Host Plant	Methanol pineapple peel	19,37 ****
Endophytic Fungi	DKN1	53,35 ***
	DKN2	18,74 ****
	DKN3	29,96 ***
	DKN4	123,61 **
	DKN5	60,48 ***
	DKN6	26,20 ***
	DKN7	121,82 **

Positive Control	Ascorbic ACid	10,08 ****
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Note: antioxidant activity IC₅₀ (µg/mL): ****very strong < 20 µg/mL, ***strong 20 ≤ IC₅₀ < 100 µg/mL; **moderat 100-500 µg/mL; * weak > 500 µg/mL (Elfita et al., 2022; Oktiansyah et al., 2023)

Table 3 reveals that the methanol extract of the host plant (pineapple peel) exhibited remarkably strong antioxidant activity. The ethyl acetate extract of endophytic fungi isolated from the host plant demonstrated different levels of antioxidant activity across various categories. The DKN2 isolates exhibited a highly potent antioxidant effect with IC₅₀ values of 18.74 µg/mL, which were the closest to the results of the positive control. Based on morphological identification, DKN2 was *Trichoderma harzianum*.

The fungus *T. harzianum* is also employed as a fungicide. It is used topically, as a seed treatment, and as a soil treatment to control fungi responsible for various plant diseases. According to the study, *T. harzianum* is easily distributed throughout the plant, and there is no specific information about the particular tissue where it grows. Hence, it can be found in various parts of the plant. (El-Komy et al., 2015; Lahlali et al., 2022). In many cases, endophytic fungi isolated from plants, particularly medicinal plants, show remarkable biological activity. Host plants utilize the secondary metabolites that endophytic fungus can create for defense, and these substances can be exploited as substitute raw materials for medications. Studies on *T. harzianum* from different plants have shown its secondary metabolites, which have anti-inflammatory, antibacterial, and antioxidant effects (Galindo-Solís & Fernández, 2022; Redkar et al., 2022; Sharma et al., 2021; A. Singh et al., 2021).

Strong antioxidant activity was demonstrated by the *T. harzianum* ethyl acetate extract. The high bioactivity of *T. harzianum*'s crude extract is attributed to the metabolites it contains. Research has

indicated that the majority of compounds found in *T. harzianum* are flavonoids and phenolics. (Guo et al., 2022; Khan et al., 2020; Lakhdari et al., 2023). Phenolic compounds are able to reduce oxidative stress. (Adamczak et al., 2020; Burel et al., 2021). According to the literature, the host plant's secondary metabolites are identical to those found in the extract of endophytic fungus. This suggests that endophytic fungi have the capability to replicate the secondary metabolites of their host plants as part of their mutualistic relationships.

CONCLUSION

Seven endophytic fungi were found in this study, namely *Nigrospora oryzae*, *Trichoderma harzianum*, *Aspergillus parasiticus*, *Ramichloridium subulatum*, *Curvularia sp.*, *Gonytrichum chlamydosporium*, and *Conidaea maire*. The fungus *T. harzianum* has antioxidant activity in very strong category. In future studies, the isolation of antioxidant compounds that have not been previously reported will be conducted for advanced research purposes, including in vivo testing.

Acknowledgments

The author is grateful to Universitas Islam Negeri Raden Fatah for providing financial support through the *Penelitian Berbasis Standar Biaya Keluaran dengan Sumber Dana DIPA BLU Tahun Anggaran 2023* (number: 730 Tahun 2023).

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