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Endophytic Fungi as A Symbion in *Polianthes tuberosa* L.: Identification, Microscopic Observation, and Secondary Metabolite Analysis

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Abstract

Background: *Polianthes tuberosa* L. *was known as an ornamental plants. People use this plant flower for ritual purpose because of the fragrant aroma. This plant rarely attacked by microorganism and usually stay healthy. Some endophytic fungi species live with P. tuberosa* L. *plant in mutualistic symbiosis interaction. This study was conducted to determine where the hyphae of endophytic fungi are located within the tissue of P. tuberosa* L. *plant petals and leaves, using findings from microscopic observations; Identification species of endophytic fungi residing in the flower petals and leaves of P. tuberosa L.; and Ascertain the various secondary metabolites produced by each species of endophytic fungi.* **Methodology:** *P. tuberosa plant were obtained from flower market at Malang city. The leaves and flower petal parts were prepared for microscopic observation and identification. Cultures of each endophytic fungal species were grown on PDA plate medium, then cut into pieces measuring 5 x 1 cm and inoculated in PDB medium. These were shaken at a speed of 120 rpm for 7 days. After that, the liquid culture was centrifuged at 3000 rpm for 10 minutes. The contents of several secondary metabolites uses the supernatant of the liquid culture. The phytochemical analysis used spectrophotometry method.* **Findings:** *The study showed the endophytic fungi hyphae are located in stomata neighbor cells, the walls of xylem cells, epidermal cell walls, sponsa cell walls, and also within the petal epidermis cell walls of the flower; Eight endophytic fungi species have been identified which each species of endophytic fungi generates flavonoids, alkaloids, tannins, saponins, and steroids, each with varying content levels.* **Contribution:** *Based on this research describe the endophytic fungi culture can be utilized as natural antibiotic source for better synthetic substitution. Besides that endophytic fungi also can be applied as antagonistic fungi to inhibite pathogenic fungi growth that attack some cultivated plant through appropriate research.*

Keywords: *Endophytic Fungi; Mutualistic Symbiosis Interaction; Polianthes tuberosa* L.*; Secondary Metabolites*

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INTRODUCTION

Although *Polianthes tuberosa* L. is a widely utilized plant, research on it remains limited, particularly regarding the symbiotic mutualism between the plant and endophytic fungi. Unlike most fungi that can cause damage, these specific fungi reside harmlessly within the plant's tissue (Alam et al., 2021). Usually the host plant remains healthy and rarely attacked by microorganisms. *Polianthes tuberosa* L. is sort of ornamental plant Indonesia society usually use this plant for ritual purpose e.g.: visiting graves and scattering the flowers (Sundar & Arunachalam, 2024). This flower has a fragrant aroma (Maiti & Mitra, 2017). This plant distribution especially at Pasuruan East Java and at Tabanan district, Bali island (Rohibni et al., 2023; Putri et al, 2020). The tuberose plant (*Polianthes tuberosa* L.) is known for its resilience to microbial attacks, a trait believed to be a result of the symbiotic relationship it shares with endophytic fungi living within its tissues.

This assumption is supported by the discovery of similar fungi in other plants, such as *Mycelia champaca*, a sort of ornamental and medicinal plant have been isolated; the fungi species are: *Colletotrichum alienum*, *Colletotrichum kahawae*, *Geotrichum candidum*, *Aspergillus ochraceus*, *Rhizoctonia* sp., *Mycelia sterilia*, *Papulospora* sp., *Curvularia lunata*, and *Aspergillus parasiticus* (Hastuti et al., 2024). Another endophytic fungi species have been found in *Jasminum sambac* plant, an ornamental plant that have flowers with fragrant aroma (Hastuti et al., 2023). These plants usually rarely attached by microorganism especially pathogenic microorganism. The plants stay healthy and the leaves is green in colour. The ramie plant is rarely attacked by microorganism. If ramie cultivation attacked by pathogenic microorganism, the quality and quantity of the fiber will decrease (Wang et al., 2011).

P. tuberosa plant is also potentially as medicinal plant and have been used for research (Rahmatullah et al., 2019). In the other side the endophytic fungi are useful for the plant health (Triastuti, 2020). Based on the phytochemical analysis result in another research have been proved that the flower, stem, and tuber of this plant can produce some secondary metabolite compound, i.e: alkaloid, flavonoid, polyphenol, tannin, monoterpene, sesquiterpene, steroid, quinon, and saponin (Setiani et al., 2020). Another research also proved that flower, stem, and tuber parts of *P. tuberosa* plant have potentially inhibit *Staphylococcus epidermidis* and *Propionibacterium acnes* that cause acne (Ariani et al., 2024; Setiani et al., 2020; Paryati et al., 2022).

Fungi residing endophytically within the *P. tuberosa* plant can be extracted and identified. By examining these under a microscope, one can observe the positioning of the endophytic fungi's hyphae within the plant's tissues. Additionally, the secondary metabolites of these fungi can be examined by analyzing the liquid culture of each endophytic fungi. This suggests that antibacterial secondary metabolites can be produced by each specific species of endophytic fungi. The liquid culture from endophytic fungi may serve as a natural antibiotic, potentially replacing synthetic antibiotics (Sundar & Arunachalam, 2024).

This study aimed to locate the hyphae of endophytic fungi within the tissues of the *P. tuberosa* plant, utilizing the results from microscopic observations. Additionally, it sought to identify the endophytic fungi isolated from *P. tuberosa* leaves and flower petals and to assess the presence of alkaloids, steroids, tannins, flavonoids, and saponins in the liquid cultures of these fungi.

This study's findings indicated that hyphae do not penetrate the cells of *P. tuberosa* plants. This demonstrates that the endophytic fungi do not harm their host plant. Based on the analysis of secondary metabolites content, each fungal liquid culture has the potential to serve as a natural antibiotic alternative, replacing synthetic antibiotics.

METHOD

Materials

The plant parts used in this research were the flower petal and leaf of *Polianthes tuberosa* L. from flower market Malang city (7°58'40.1"S 112°37'52.4"E) this research was done in June 2025, Potato Dextrose Broth (PDB), Potato Dextrose Agar (PDA), chloramphenicol 100 mg/L, alcohol 70 %, Lactophenol solution, NaOCl 1 %, and Lactophenol cotton blue solution.

Preparation for Microscopic Observation

To create microscope slides, the flower petals and leaves of *P. tuberosa* were sliced both paradermally and transversely. An Olympus CX 21 FS 1 microscope, with magnifications of 40×10 and 100×10, was utilized to observe the location of endophytic fungi within the tissue of *P. tuberosa*.

Isolation and Identification of Endophytic Fungi

The flower petals and leaves of *P. tuberosa* were cut 0.5 x 0.5 cm in size. The plant parts underwent washing and then were immersed in a 1% NaOCl solution for one minute, after which they were rinsed with distilled water that had been sterilized. Afterwards these samples soaked into 70 % ethanol, a minute and rinsed in distilled water (Gangadewi & Muthumary, 2008). Each sample, consisting of five replicates, was aseptically placed on PDA medium supplemented with 100 mg/L chloramphenicol and incubated at 25–27 °C for 7 days (Sofiya et al., 2021; Arpitha et al., 2022). Each source of endophytic fungi could be identified. Then, each fungal species was inoculated onto PDA medium using slide culture methods and incubated at 25–27 °C for 5 days to prepare slides for microscopic observation (Rosana et al., 2014).

Each endophytic fungal isolate was inoculated onto a 1 x 1 cm² piece of PDA medium placed on a sterile object glass, then put on a sterile cover glass. Afterwards drip distilled water on a piece of filter paper under the sterile object glass, put the Petri dish cover and then incubated in 25 - 27°C for 3 x 24 hours. Observation were done whether the hyphae have been grow on the edge of PDA medium in the Petri dish. If the hyphae in the conidia have been grow take the cover glass where the fungi attacked. Afterwards drip alcohol 95 % on the cover glass. The lactophenol

cottonblue solution were dripped on the object glass. Then, the cover glass were lie face down ward on the object glass. The microscopic structure of the endophytic structure could be described for identification purpose. The identification of fungi based on macroscopic observations includes: the colony diameters, the colony characters, the colony color, and the color of the colony base. The microscopic observations include: the appresorium, metula, phialida, conidia, and sclerotium morphology.

Observations of morphology and microscopic characters of each endophytic fungi using some books about fungi identification, consists of: "Illustrated Genera of Imperfect Fungi" by Barnett & Hunter (1972), "Introduction to Food Borne Fungi" by Samson et al., (1984), and "Fungi and Food Spoilage" by Pitt & Hocking (1985).

Preparation of Endophytic Fungi Liquid Culture

Each colony of endophytic fungi isolated from *P. tuberosa* L. tissues on Potato Dextrose Agar (PDA) medium was sectioned into fragments measuring approximately 5 × 1 cm². These fungal fragments were subsequently transferred into Potato Dextrose Broth (PDB) medium, where five inocula were introduced into every 100 mL of PDB. The cultivation was performed in quintuplicate to ensure experimental reliability. Following inoculation, the cultures were incubated under agitation at 120 rpm and maintained at a temperature range of 25–27 °C for seven days. After incubation, the cultures were centrifuged at 3000 rpm for 10 minutes to separate the supernatant, which was then employed for qualitative phytochemical screening to detect the presence of alkaloids, tannins, saponins, flavonoids, and steroids.

Detection of Secondary Metabolites

The content of alkaloid, tannin, steroid, flavonoid, and saponin in each endophytic fungi liquid cultures were analyze uses spectrophotometry method. The production of these secondary metabolites was found to vary across the different endophytic fungi.

The Quantitative of Flavonoid Determination

The procedure refers to Alara et al., (2018), the preparation of quercetin standard solution (100 mg/L) was carried out by dissolving 100 mg of quercetin in distilled water to obtain a primary stock solution. This stock was subsequently utilized to generate a range of working standards with concentrations of 0.0, 0.5, 1, 10, 25, and 50 mg/L. For the sample preparation process, one gram of the solid material was ground and diluted in 10 mL of methanol. The resulting suspension was then homogenized for 30 minutes to ensure complete dissolution. The homogenate was filtered and subjected to centrifugation at 3000 rpm for 10 minutes, and the supernatant obtained was collected for further analysis. Thereafter, an aliquot of 1 mL from the liquid extract was mixed with 5 mL of methanol, homogenized for 5 minutes, and subsequently filtered through a vacuum filtration system.

For the analytical procedure, a volume of 0.1 mL from either the sample or the standard solution was mixed with an equal volume (0.1 mL) of 2% Al_2Cl_3 reagent.

The resulting mixture was subsequently subjected to homogenization at a speed of 3000 rpm for 60 minutes. The development of a reddish coloration served as an indicator of flavonoid presence. The absorbance of the solution was then recorded at a wavelength of 420 nm, and the flavonoid concentration was calculated based on the standard regression equation.

The Quantitative of Alkaloid Determination

The procedure refers to Tambe et al., (2014), A 0.1 g portion of the sample was initially pulverized and subsequently dissolved in 10 mL of dimethyl sulfoxide (DMSO). To this solution, 1 mL of bromocresol green reagent, 5 mL of 2 N hydrochloric acid (HCl), and 5 mL of phosphate buffer were added, followed by homogenization for 60 minutes to ensure complete mixing. The prepared mixture was afterward transferred into a separatory funnel, where it was treated with 10 mL of chloroform and agitated until two distinct layers were visibly formed.

For the preparation of the standard solution, 5 mL of atropine solution were blended with 5 mL of bromocresol green reagent, 1 mL of 2 N HCl, and 5 mL of phosphate buffer, and the mixture was subsequently homogenized. From this standard solution, 11 mL of the upper phase were carefully collected. The development of a yellowish-orange coloration was observed as a qualitative indication of the presence of alkaloids. The absorbance of the resulting solution was recorded at a wavelength of 470 nm, and the alkaloid concentration was quantified using the calibration curve derived from the standard regression equation.

The Quantitative of Tannin Determination

The procedure refers to Tambe et al., (2014) A tannic acid standard solution with a concentration of 50 mg/L was formulated by dissolving 5 mg of tannic acid into 10 mL of 20% ethanol. This stock solution subsequently served as the basis for the preparation of a series of working standards with concentrations of 0, 1, 5, 10, 25, and 50 mg/L. For the sample preparation, 0.1 g of the material was finely ground and solubilized in 10 mL of methanol, followed by homogenization for 30 minutes to ensure complete dissolution. The homogenate was then subjected to filtration and centrifugation at 3000 rpm for 10 minutes, after which the clear supernatant was collected for subsequent analysis.

In the analytical procedure, a 5 mL aliquot of the standard solution was mixed with 0.5 mL of 0.0008 M potassium ferricyanide ($K_3Fe(CN)_6$) and 0.5 mL of 0.1 M ferric chloride ($FeCl_3$). The resulting mixture was thoroughly homogenized for 30 minutes and then diluted with distilled water to reach a final volume of 10 mL. Absorbance measurements were performed at a wavelength of 620 nm (λ = 620 nm), and the tannin concentration was quantified using the calibration curve generated from the standard regression equation.

The Quantitative of Saponin Determination

The procedure refers to Ezeabara et al., (2014), A standard solution of saponin with a concentration of 100 mg/L was prepared by dissolving 10 mg of saponin in 100 mL of 20% ethanol. This stock solution was subsequently utilized to produce a

range of working standards with concentrations of 0, 1, 5, 10, 25, and 50 mg/L. For the sample preparation, 0.1 g of the sample was finely crushed and dissolved in 10 mL of 90% ethanol. The mixture was subjected to heating in a water bath at 55 °C for 90 minutes, followed by homogenization to ensure uniform extraction. Afterward, the solution was filtered and re-extracted with an additional 10 mL of 90% ethanol. Both extracts were concentrated by heating at 90 °C until their volumes were reduced by half. The concentrated extract was then transferred into a separating funnel, to which 40 mL of diethyl ether was added. The mixture was vigorously shaken and then allowed to stand until two distinct layers were formed.

The resulting filtrate was subsequently dried at 60 °C to obtain a dry saponin residue, which was then re-dissolved in 20% ethanol for further analysis. For the determination process, 5 mL of the standard solution was combined with 0.5 mL of 0.008 M potassium ferricyanide ($K_3Fe(CN)_6$) and 0.5 mL of ferric chloride ($FeCl_3$), followed by homogenization for 30 minutes. The development of a blue coloration indicated the presence of saponins. The reaction mixture was then diluted with chloroform to a final volume of 10 mL, and the absorbance was measured at a wavelength of 470 nm (λ = 470 nm). The saponin concentration was subsequently quantified using the standard regression calibration curve.

The Quantitative of Steroid Determination

The procedure refers to Pharmaguideline (2011), The lanosterol standard solution with a concentration of 100 mg/L was obtained by dissolving 10 mg of lanosterol in 100 mL of chloroform. From this stock solution, a series of working standards were subsequently prepared at concentrations of 0, 0.1, 0.5, 1, 2.5, and 5 mg/L.For the sample preparation, 0.5 g of the sample was finely ground and dissolved in 10 mL of analytical-grade hexane (p.a.), followed by a standing period of 30 minutes to ensure complete dissolution. The resulting suspension was then subjected to either filtration or centrifugation at 3000 rpm for 10 minutes, after which the supernatant was collected and used for further analysis.

In the analytical phase, a 10 mL aliquot of the sample extract was transferred into a volumetric flask, to which 0.01 mL of 10% blue tetrazolium hydroxide and 2 mL of ethanol were added. The mixture was subsequently incubated in the dark for 90 minutes. Following incubation, 0.05 mL of glacial acetic acid was introduced, and the solution was thoroughly homogenized before being adjusted to a final volume of 10 mL using ethanol. The prepared solution was then transferred into a cuvette for spectrophotometric measurement at a wavelength of 525 µm. A blank solution composed of ethanol and identical reagents was used as a reference. The steroid content was determined from the absorbance values using the corresponding standard regression calibration curve.

RESULT AND DISCUSSION

The Endophytic Fungi Hyphae in *P. tuberosa* L. Plant Tissue Position

The positioning of the endophytic fungi hyphae within the leaf and flower petal tissues of *P. tuberosa* L. is detailed in Table 1. The positioning of endophytic fungi

hyphae within *P. tuberosa* L. tissue is depicted in Fig. 1. Upon microscopic examination, it was observed that the hyphae are located on the sponsa cell wall, the wall of neighboring cells of stomata, and the xylem cell wall of the leaf, as well as on the epidermal cell wall of the flower petal. The hyphae do not penetrate into the plant cells. This indicates that the fungi do not intrude into the cells of the *P. tuberosa* L. and consequently do not cause any harm to the plant cells of the host.

Table 1. The Distribution of Endophytic Fungi Hyphae on Tissues of *P. tuberosa* Plant Parts

No.	The Plant Parts	Slice Section	Position
1.	Leaf	Transversal	Sponsa cell, stomata neighbour cell, and xylem cell wall
		Paradermal	Epidermis cell wall
2.	Flower petal	Paradermal	Epidermis cell wall

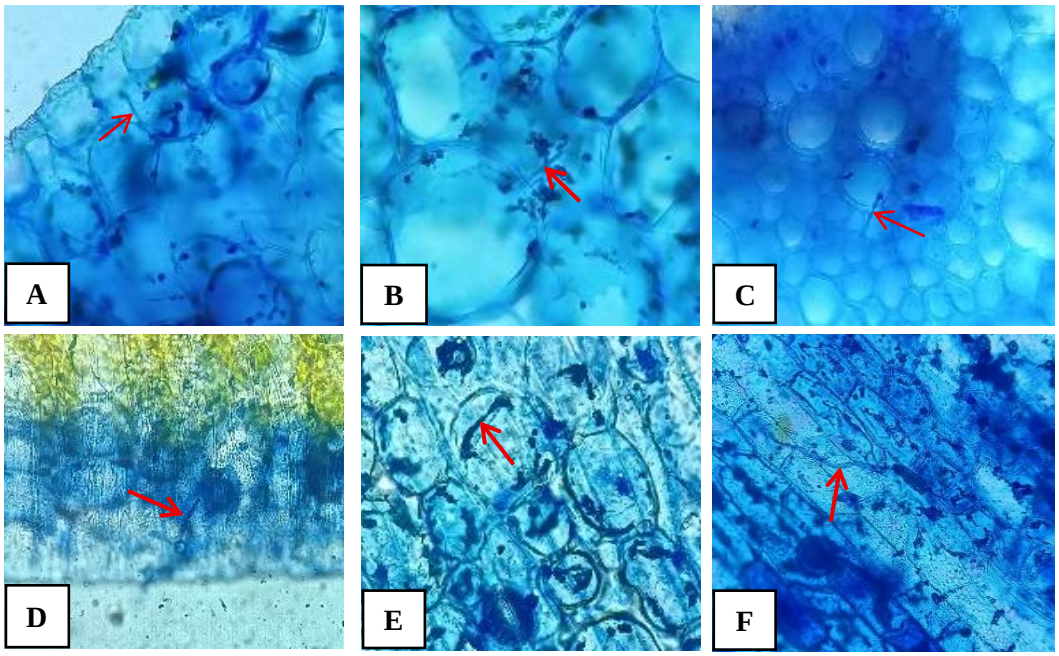

Figure 1. The Microscopic Observation Result of The Endophytic Fungi Position on *P. tuberosa* L. Plant Parts Tissues. A) on the leaf epidermis cell wall (on transversal slices); B) on the leaf spongy cell wall (on transversal slices); C) on the leaf xylem trachea cell wall (on transversal slices); D) on the leaf stomata neighbor cell wall (on transversal slices); E) on the leaf epidermis cell wall (on paradermal slices); F) on the flower petal epidermis cell wall (on paradermal slices). (400x). The red arrow show the fungi hyphae position.

Figure 2. show the characters of each endophytic colonies i.e the colony colour on the above site and bellow site and the colony character.

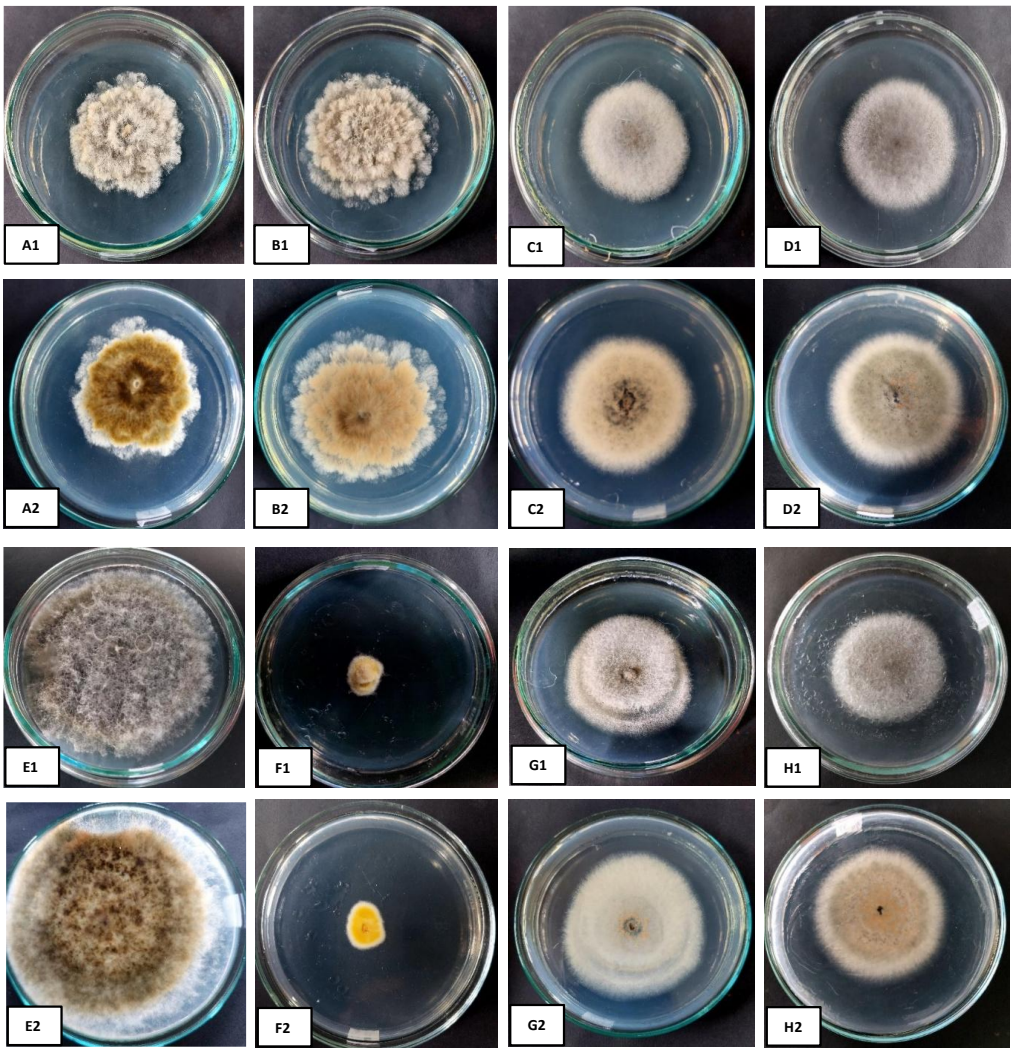

Figure 2. Photomicrograph of Each Endophytic Fungi Colony on PDA plate Medium (7 days). A) *Mycelia sterilia*, B) *Gibellula* sp., C) *Colletotrichum kahawae* B. Weir & P. R Jhonst, D) *Colletotrichum psidii* Curzi, E) *Rhizoctonia* sp., F) *Penicillium citrinum* Thom, G) *Colletotrichum alatae* B. Weir & P. R Jhonst, H) *Colletotrichum alienum* B. Weir & P. R Jhonst, 1 = above side 2 = bellow side. The Petri dish diameter size is 9 cm.

The characteristics that describe each fungi are used for identification purposes. The microscopic features of the fungi extracted from the leaf and flower of *P. tuberosa* are depicted in Figure 3.

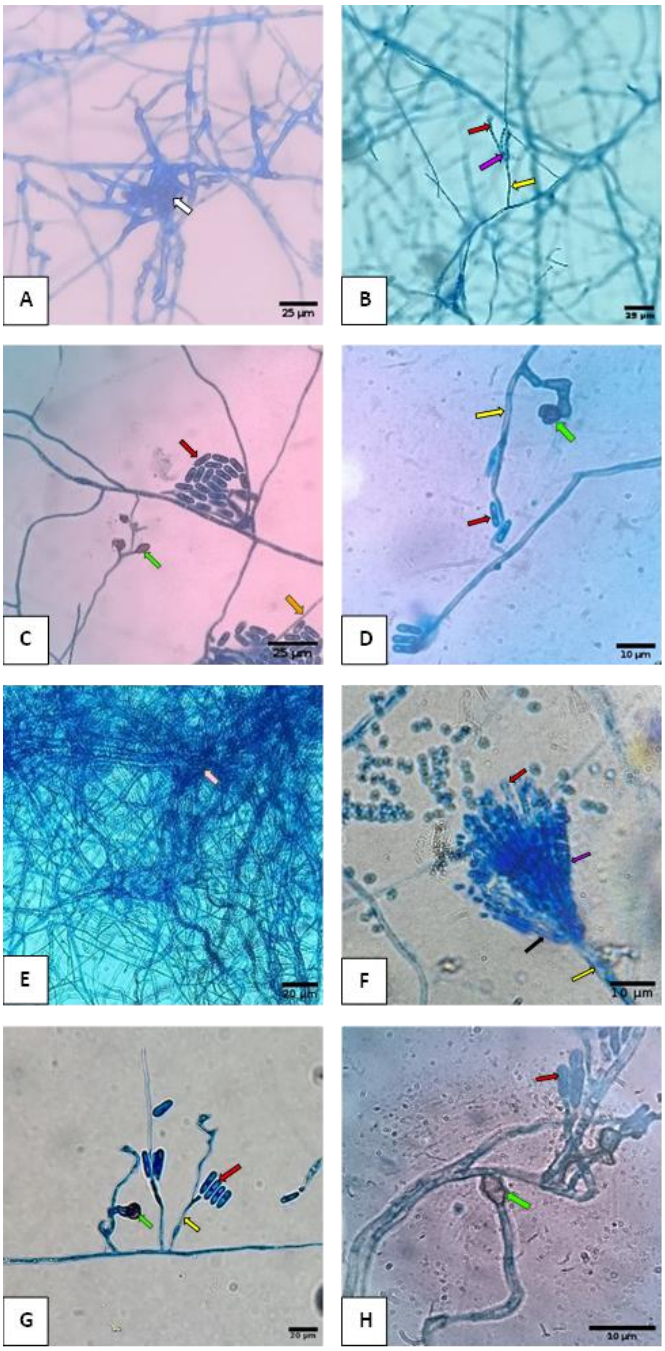

Figure 3. Each Endophytic Fungi Species Isolated From *P. tuberosa* leaf and Flower Petal Photomicrograph. Note: **A**) *Mycelia sterilia*, **B**) *Gibellula* sp., **C**) *Colletotrichum kahawae* B. Weir & P. R Jhonst, **D**) *Colletotrichum psidii* Curzi, **E**) *Rhizoctonia* sp., **F**) *Penicillium citrinum* Thom, **G**) *Colletotrichum alatae* B. Weir & P. R Jhonst, **H**) *Colletotrichum alienum* B. Weir & P. R Jhonst, **White arrow** = mycelium web, **Red arrow** = Conidia, **Purple arrow** = Phialide, **Yellow arrow** = Conidiophore, **Green arrow** = Appresorium, **Orange arrow** = Hyphae, **Pink arrow** = Sclerotium, **Black arrow** = Metula (A, B, C, E & G: 400x; D, F & H: 1000x).

Table 2. The Microscopic Characteristic of Endhophytic Fungi Species Isolated from *Polianthes tuberosa* L. Plant

Species	Isolate Code	Part of Plant	Colony Morphology	Appresorium (Shape, Size)	Metula (Shape, Size)	Phialids (Shape, Size)	Conidia (Colour, Shape, Size, Wall characteristic)	Selerotium (Colour, Size)
Mycelia sterilia	A	Flower petal	• Colour: Brownish white • Reserve: Brownish green, velvety • Diameter size: 6 cm					• Colour: hyaline • Size: 28,75 µm x 18,15 µm
Gibellula sp.	B	Flower petal	• Colour: Brownish white • Reserve: Light brown, velvety • Diameter size: 6,27 cm			• Shape: broadly wedge-shape head	• Shape: ellipsoidal, in short chains	
Colletotrichum kahawae B. Weir and P. R Johnst	C	Leaf	• Colour: White with black and orange spotted • Reserve: Greenish white with black and orange spotted, cottony • Diameter size: 5,1 cm	• Shape: Cylindrical, fusoid with some lobus • Size: 7,5 – 10 µm x 5 – 7,5 µm			• Colour: Greenish • Shape: Cylindrical • Size: 12,5 – 15 µm x 5 µm Smooth wall	
Colletotrichum psidii Curzi	D	Leaf	• Colour: Greenish – yellow white, cottony • Diameter size: 5,25 cm	• Shape: irregular with some lobus • Size: 7 x 5 µm			• Colour: Greenish • Shape: Cylindrical with round edge • Size: 11 – 20 µm x 1-3 µm, smooth wall	

Rhizoctonia sp.	E	Flower petal	• Colour: Greenish white, • Reverse: Brownish green, cottony • Diameter size: 9 cm				• Colour: Black • Size: 62,5 µm
Penicillium citrinum Thom.	F	Flower petal	• Colour: Yellow • Reverse: Yellow, Velvety • Diameter size: 2,2 cm		• Shape: Cylindrical • Colour: Hyaline • Size: 12,5µm x 2,5 µm	• Shape: Ampulliform • Colour: Hyaline • Size: 7,5µm x 1,25 µm	• Colour: Hyaline • Shape: ellipsoidal • Size: 2 –3 µm x 1 – 2 µm Smooth wall
Colletotrichum alatae B. Weir and P. R Johnst	G	Leaf	• Colour: White • Revers: White with orange spotted, Cottony • Diameter size: 5, 97cm	• Shape: elips, fusoid, irregular with some lobus • Size: 10- 17µm x 5 – 7,5µm			• Colour: Hyaline • Shape: Cylindrical • Size: 10 – 22,5 µm x 2,5 - 5 µm
Colletotrichum alienum B. Weir and P. R Johnst	H	Leaf	• Colour: Greenish white with orange spotted • Revers: Greenish white with orange spotted, Cottony • Diameter size: 5, 23cm	• Shape: cylindrical irregular with some lobus • Size: 7,5 – 12,5µm x 5 – 7,5µm			• Colour: Greenish • Shape: Cylindrical • Size: 7,5 – 17,5 µm x 2,5 - 5 µm

Endophytic Fungi Species Found in *P. tuberosa* Plant

It have proved in this research that in *P. tuberosa* plant tissue founded eight endophytic fungi species; four species belongs to genus *Colletotrichum,* one species is belongs to genus *Penicillium,* and the other are *Gibellula* sp., *Rhizoctonia* sp, and *Mycelia sterilia*. The same fungi species also live in another host plant. *Colletotrichum kahawae* as also found in *Cananga odorata* plant (Hastuti et. al., 2019), *Switenia mahogany* plant (Hastuti et. al., 2022), *Jasminum sambac* plant (Hastuti et. al., 2023). *C. alatae* also found in *Cananga odorata* plant (Hastuti et. al., 2019) and *Lycopodium clavatum* plant (Santra & Banerjee, 2022). *C. alienum* also found in *Hedychium acuminatum* plant (Hastuti et. al., 2018), *Cananga odorata* plant (Hastuti et. al., 2019), *Physalis angulata* plant (Hastuti et. al., 2021), *Cosmos caudatus* plant (Hastuti et. al., 2024) and *Mycelia champaca* plant (Hastuti et. al., 2024). *Penicillium citrinum* also found in Ramie *(Boehmeria nivea)* plant (Sari et. al., 2023). *P. citrinum* have an ability to increase the plant nutrients absorption, it means that this fungi play a role in the plant growth (Hakim & Yuwati, 2020).

From microscopic observation, it was observed that the hyphae of the endophytic fungi within the leaf and flower petal tissues were positioned along the cell walls of the leaf epidermis, as well as on the neighboring stomatal cells, spongy mesophyll cells, xylem cells, and the cell walls of the flower petals. No hyphal penetration into the internal tissues of *P. tuberosa*, particularly within the leaf and petal cells, was detected. This structural arrangement indicates a mutualistic symbiotic association between the endophytic fungi and *P. tuberosa* as the host plant (Clay & Schardl, 2002; Kogel et al., 2006). Moreover, such an interaction provides protection to the fungi against adverse environmental factors, including fluctuations in air temperature and low humidity, as the fungal structures reside within the host's tissues (Latz et al., 2018). The endophytic fungi do not extract nutrients directly from the plant cells; instead, they utilize nutrients present in the intercellular spaces that are not exploited by the host plant.

There is a difference fact in pathogenic fungi activity that caused damage at the host plant cells. The fungi hyphae insert into the host plant cell and caused damages in some plant cell. The fungi hyphae tannin was proved insert into the host plant cell and caused damages in some cell. The selulase enzyme produced by phathogenic fungi is a biocatalisator in selulose degradation process, while chitinase enzyme is a biocatalisator in chitin degradation process in plant cell (Leon & Montesano, 2013). Afterwords the fungi hyphae insert into the plant cells and absorb the plant nutrition for the fungi growth, this activity can cause damage on the plant cell that was attacked by the fungi (Gao et al., 2010) This damages can inhibit celluler metabolism and ATP will decrease. It cause wilted to the plant and the leaf become yellowish. The fungi hyphae absorb nutrition from the plant cell and afterwards the metabolism obstructed and plant will death.

Secondary Metabolites Produce by Each Endophytic Fungi Species Isolated from *P. tuberosa* Plant

The eight endophytic fungi species isolated from leaves and flower petals of *P. tuberosa* have potencially produce secondary metabolites, i. e: Alkaloids, flavonoids, tannin, saponin, and steroid (Table 3). The analysis result proved that each of eight endophytic fungi species have potentially produce each sort of secondary metabolites with different contents. This facts proved that eight species of endophytic fungi potentially to be used as antibiotics substance. *Penicillium citrinum* produce secondary metabolites with the highest content compare with the other fungi species in this research.

Table 3. The Analysis Outcome of Various Secondary Metabolite Compounds Produced by Each Endophytic Fungi Species from *Polianthes tuberosa*

Code	Endophytic Fungi Species	Secondary Metabolite (mg/L)				
		Flavonoid	Alkaloid	Tannin	Saponin	Steroid
A	*Mycelia sterilia*	4107.81	2599.41	2089.26	1006.53	891.59
B	*Gibellula* sp.	5256.25	3122.94	2457.55	1174.32	1033.94
C	*Colletotrichum kahawae* B. Weir & P. R Jhonst	6580.47	4008.24	3119.23	1506.53	1356.33
D	*Colletotrichum psidii* Curzi	5521.88	3472.94	2788.39	1338.74	1185.71
E	*Rhizoctonia* sp.	4225.00	2552.35	1976.90	943.47	847.29
F	*Penicillium citrinum* Thom	6646.88	4034.71	3169.16	1565.09	1376.13
G	*Colletotrichum alatae* B. Weir & P. R Jhonst	6252.34	3840.59	3019.35	1496.40	1285.63
H	*Colletotrichum alienum* B. Weir & P. R Jhonst	4350.00	2672.94	2164.17	1088.74	923.64

Note: *Among the endophytic fungi species, Penicillium citrinum was able to produce the highest amounts of different secondary metabolites.*

Some another research about endophytic fungi isolated from some plant proved that each endophytic fungi potencially could produce some antibacterial secondary metabolite with different content each other. Each other, *Alternaria tenuis,* as endophytic fungi isolated from *Jasminum sambac* have potentially produce alkaloid, saponnin, flavonoid, tannin, and steroid with the highest content compared with the other fungi species (Hastuti et. al., 2023). *Colletotrichum alatae* is the endophytic fungi species in *Cananga odorata* (Lam Hook. F & thomson) that have the highest ability to produce tannin, alkaloid, saponin, terpenoid, and flavonoid with the highest content compared with the other fungi species (Hastuti et. al., 2019). While *Colletotrichum alienum,* an endophytic fungi in *Cosmos caudatus* and *Cosmos sulphureus* have

potentially produced alkaloid, tannin, phenolat, flavonoid, and saponin with the highest content compared with the other fungi in this research (Hastuti et. al., 2024).

This secondary metabolites are antimicrobial metabolites. If *P. tuberosa* as the host plant attacked by a pathogenic bacteria, it means the fungi can protect the plant by produced the secondary metabolites. The host plant stay healthy and saved from pathogenic bacteria (Gao et al., 2010; Fuchs & Krauss, 2019). In the other side the endophytic fungi also prevented from the environmental stress, such as the drought, very high air temperature, very low humidity (Morales-Vargas et al., 2024; Javed et al., 2022). The nutrition for endophytic fungi was take from the intercelluler cell space that does not take by the host plant, so it does not cause damage or inhibition to the plant growth. Moreover, the endophytic hyphae do not penetrate the cell's protoplasm; consequently, the water provided by these hyphae passes directly into the epidermal cell wall and through the mesophyll and vascular sheath cells, where it supports the synthesis of photosynthetic products (Wang et al., 2024). On the positive side, endophytic fungi can promote the absorption of vital nutrients like nitrogen and phosphorus from the soil. This greater nutrient availability supports better plant growth, which subsequently boosts the plant's fitness by enhancing its reproductive capacity (Bhardwaj et al., 2023). This fact means that there is a symbiotic mutualism interaction between the *P. tuberosa* plant and the endophytic fungi as symbion.

In another next research, is important to proved the antimicrobial effect of each endophytic fungi liquid cultures to pathogenic bacteria on people or plant growth inhibition. If there is antimicrobial effect of endophytic fungi liquid culture, it proved that the liquid culture can be used as natural antibiotic substance. This natural antibiotics are useful as substitution for synthetic antibiotic. The endophytic fungi also can play a role as an antagonist fungi in the pathogenic fungi growth inhibition, that can examined by research.

CONCLUSION

The findings of this study reveal that the hyphae of endophytic fungi are located in the cells neighboring the stomata, the xylem cell walls, epidermis cell walls, and spongy cell walls of leaves, as well as the epidermis cell walls of the flower petals. The study identified eight species of endophytic fungi: *Mycelia sterilia, Gibellula* sp., *Colletotrichum kahawae* B. Weir & P. R. Jhonst, *Colletotrichum psidii* Curzi, *Rhizoctonia* sp., *Penicillium citrinum* Thom, *Colletotrichum alatae* B. Weir & P. R. Jhonst, and *Colletotrichum alienum* B. Weir & P. R. Jhonst. Each species produced a variety of secondary metabolites, including flavonoids, alkaloids, tannins, steroids, and saponins, each in different amounts. Among them, *Penicillium citrinum* showed the potential to produce these metabolites at the highest levels compared to the other species studied.

The hyphae of the endophytic fungi do not harm *P. tuberosa* plant tissue, the host plant, as there is no penetration of the plant cells. Each species of endophytic fungi demonstrated the potential to produce antibacterial secondary metabolites, suggesting that their liquid culture could be employed as a natural antibiotic source,

offering an alternative to synthetic antibiotics. The research conducted was in vitro, emphasizing the necessity of in vivo studies to further validate these endophytic fungi as a source of natural antibiotics

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