

## Identification and Histological Observation of Endophytic Fungi Isolated from Medicinal Plant, *Physalis angulata* L.

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### ABSTRACT

Some endophytic fungi species live in medicinal plant tissue and does not make any damage, but live in symbiotic mutualism relationship with the host plant. This research was done to: 1) identify the endophytic fungi species isolated from *P. angulata* leaf, twig, and stem bark tissues, 2) determine the endophytic fungi colonization in the *P. angulata* plant tissue by histologic observation. The endophytic fungi was isolated from healthy *P. angulata* plant parts, then inoculated on Potato Dextrose Agar medium and incubated in 27°C for 7-14 days. Each endophytic fungi isolates were identified. The histologic observation was done by microscopic observation to determine the endophytic fungi position in the plant tissue. The conclusion are: 1) seven endophytic fungi species were found: *Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, *Aspergillus nidulans*, *Mycelia sterilia* 1, *Mycelia sterilia* 2, and *Rhizoctonia* sp.; 2) the endophytic fungi micelium was found on the leaf epidermis cell wall, on the twig epidermis cell wall, and parenchyma cell wall, on the stem bark epidermis cell wall. The suggestion of the study: it is need to make the next research about secondary metabolites content produced by endophytic fungi species isolated from *P. angulata* and their antimicrobial activity.

## 1. Introduction

*Physalis angulata* L. Is a kind of medicinal plant. People use this plant to treat infection on gums, skin, intestine. This plant was cultivated for exploit as medicinal treatment purpose. The leaf and the stem parts of this plant contains: flavonoid, alkaloid, tannin, glycoside, and steroid (Nanumala *et al.* 2012). These compounds have antibacterial character, so this plant used in infection treatment.

Some medicinal plant live in symbiotic mutualism relationship with endophytic fungi that live in the plant tissues. The endophytic fungi does not cause any damage to the host plant, but could produce some antimicrobial secondary metabolites. The host plant protected from microbe infection by endophytic fungi antimicrobial secondary metabolites. The endophytic fungi also take some advantages through nutrients, protection from environmental stress (Clay

*et al.* 2002; Stanley *et al.* 2002; Kogel *et al.* 2006). The antimicrobial secondary metabolites produced by endophytic fungi species could be used as an antibiotic source. In the previous research it was found an endophytic fungi: *Penicillium* sp, isolated from *Curcuma longa* produced an antimicrobial compound (Singh *et al.* 2014). Another research proved that some endophytic fungi, i.e: *Fusarium oxysporum*, *Rhizoctonia* sp., *Fusarium solani*, *Colletotrichum alienum*, *Colletotrichum ti*, *Colletotrichum aotearoa*, *Aspergillus parasiticus*, *Hansfordia biophila*, *Fusarium semitectum*, *Colletotrichum coccodes*, and *Colletotrichum gloeosporoides* isolated from *Hedychium acuminatum* could produced antibacterial compounds: alkaloid, flavonoid, terpenoid, and tannin in the secondary metabolites (Hastuti *et al.* 2018). It is also proved that the secondary metabolites produced by those endophytic fungi species isolated from *H. acuminatum* have antimicrobial activity towards *Staphylococcus aureus*, and *Bacillus subtilis* (Hastuti *et al.* 2019b). The endophytic fungi live on the parenchyma cell wall and intercellular space of

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rhizome, epidermis, and parenchyma cell wall of leaf sheath, epidermis cell wall of *H. acuminatum* plant (Hastuti *et al.* 2018). The histologic observation can be used to observe the endophytic fungi colonization in plant tissues. The endophytic fungi could be isolated and identified to determine the species. This research was done to: 1) determine the endophytic fungi position in the *P. angulata* plant parts tissue by histologic observation; 2) identify the endophytic fungi species isolated from *P. angulata* bark, twig, and leaf.

## 2. Materials and Methods

### 2.1. Material

*Physalis angulata* plant parts: bark, twig, and leaf. The plant was taken from Balai Materia Medica, Batu, Malang in May 2019. Potato Dextrose Agar (PDA) medium from MERCK, 70% alcohol, 95% alcohol, 1% NaOCl, chloramphenicol, lactophenol, and lactophenol cotton blue.

### 2.2. Methods

#### 2.2.1. Preparation of *P. angulata* Leaf, Twig, and Bark for Endophytic Fungi Position on the *P. angulata* Plant Tissue for Microscopic Observation

The bark, twig, and leaf of *P. angulata* were rinsed with sterile distilled water, then sliced in paradermal, transversal, and longitudinal sections and made into microscopic slides. Then the endophytic fungi in the *P. angulata* plant tissue position were observed by microscopic histological observation.

#### 2.2.2. Endophytic Fungi Isolation and Identification

*P. angulata* bark, twig, and leaf were washed and soaked in 1% NaOCl during 1 min., then washed with sterilized water. Afterward soaked in 70% alcohol during 1 min. and washed again with sterilized water (Gangadewi *et al.* 2008). The leaf was cut into 1 x 1 cm<sup>2</sup> each, the twig and the bark were cut into the thickness of 0.5 cm each. Afterwards each sample was inoculated on PDA plate medium containing chloramphenicol (100 mg/l) and incubated at 27°C during 7-14 days for endophytic fungi isolation. Each fungi isolate was made into a slide culture for microscopic observation and afterwards the fungi characters were referred to the fungi identification book for each isolate identification need. The identification

books are: Pictorial Atlas of Soil and Seed Fungi-Morphologies of Cultured Fungi and Key to Species by: Tsuneo Watanabe 2002. CRC Press: Florida, Fungi, and Food Spoilage by John I. Pitt and Ailsa D. Hocking, 1985. Academic Press: Tokyo, Illustrated Genera of Imperfect Fungi by: H. L. Barnett and Barry B. Hunter, 1972. Burgess Publishing Company: Minnesota.

## 3. Results

### 3.1. The Histological Observation of Endophytic Fungi on *Physalis angulata* Leaf, Twig, and Bark Colonization

The colonization of endophytic fungi in *P. angulata* tissues was observed microscopically. The endophytic fungi species were found in bark, twig, and leaf of *P. angulata*. The fungi mycelium was found on the bark epidermis cell wall (Figure 1a), on the leaf epidermis cell wall (Figure 1b) and on the leaf parenchyma cell wall (Figure 1c), on the leaf spongy cell wall (Figure 1d).

The endophytic mycelium was found on the epidermis cell wall in bark, twig, and leaf of *P. angulata*, but it is also found on the leaf spongy cell wall. This result shows that the endophytic fungi were not only found on the leaf but also found in another plant's part, i.e.: twig and bark of *P. angulata* plant. In another research the endophytic fungi were found in rhizome, leaf, and leaf sheath of *Hedychium acuminatum* plant. Based on the histological observation it was found that there are no mycelium inserted into cell cytoplasm, so the mycelium does not make any damage to the plant tissue. That is why the host plant is still in healthy condition.

### 3.2. The Identification Result of Endophytic Fungi Isolated from *Physalis angulata*

Seven endophytic fungi species were found in *P. angulata* bark, twig, and leaf. There are: *Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, *Aspergillus nidulans*, *Mycelia sterilia* 1, *Mycelia sterilia* 2, and *Rhizoctonia* sp. Figure 2 shows the seven endophytic fungi colonies.

The characteristic description of each endophytic fungi isolate is described in Table 1. There was variation in fungi colony color, i.e.: green, whitish orange, white, greyish white, brown and black. The colony characters were velvety and the others were cottony. There was variation in conidia shape, i.e.: subspheroid, cylindrical, fusiform, and slightly

curved. *Penicillium verrucosum* had subspheroid conidia shape with smooth walled. *Aspergillus nidulans* had sphaerical conidia shape with

roughened wall. *Colletotrichum alienum* also had conidia as reproduction organ in cylindrical with round edge shape and smooth walled. *C. alienum*

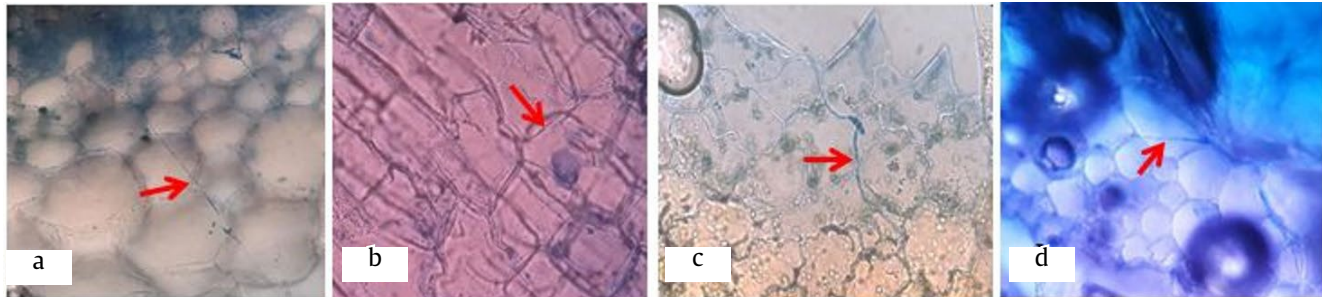


Figure 1. The histological observation of endophytic fungi position on *Pysalis angulata* tissues. (a) On the bark epidermis cell wall (arrow), (b) on the twig epidermis cell wall, (c) on the leaf epidermis cell wall (arrow), (d) on the leaf spongy cell wall

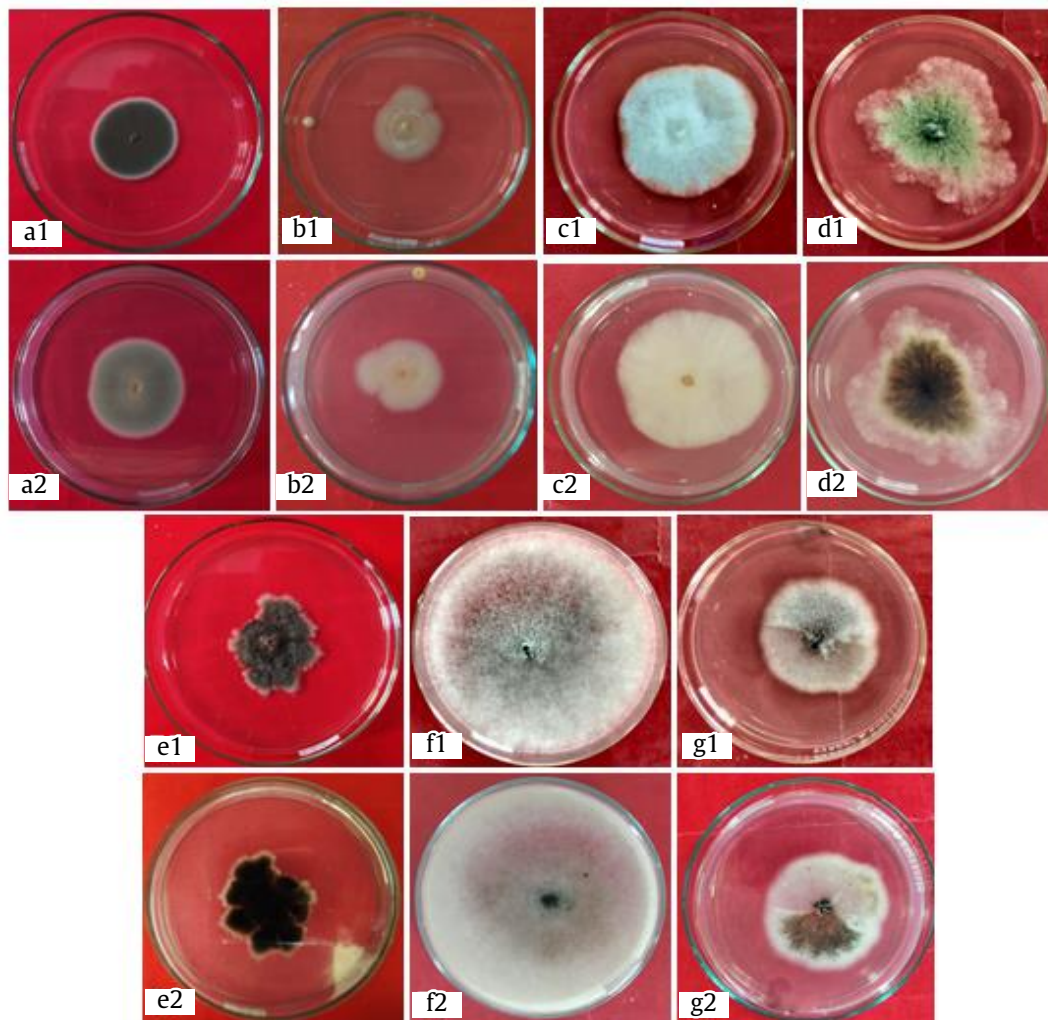


Figure 2. The endophytic fungi colonies on PDA medium after 7 days incubation. 1: upper, 2: reverse (a) *Penicillium verrucosum*, (b) *Colletotrichum alienum*, (c) *Fusarium subglutinans*, (d) *Aspergillus nidulans*, (e) *Mycelia sterilia* 1, (f) *Mycelia sterilia* 2, (g) *Rhizoctonia* sp.

Table 1. The characteristics of each endophytic fungi isolates from *P. angulata* L. plant

Species	Isolate code	The plant part	Colony morphology	Apresorium (shape, size)	Vesicle (shape, size)	Chlamydo-spore (shape, position, size)	Conidia (colour, shape, size, wall character)	Sclerotium (shape, size)
<i>Penicillium verrucosum</i>	A	Leaf, twig, bark	Colour: green, velvety, reverse: yellow	-	-	-	Colour: green, shape: subsphaeroid smooth walled, size: 2-5 µm	-
<i>Colletotrichum alienum</i>	B	Leaf	Colour: whitish orange, reverse: light orange with grey spotted, cottony	Ellipsoidal, irregular shape, size: 17.5 - 22.5 x 10 µm	-	-	Colour: light green, cylindrical with round edge shape, smooth walled, size: 22.5 - 25 x 5 µm	-
<i>Fusarium subglutinans</i>	C	Leaf, bark	Colour: white, reverse: orange, cottony	-	-	-	Microconidia: hyaline, fusiform shape: aseptate smooth walled, size: 6.5 - 13.75 x 2.5 µm, macroconidia: hyalin, slightly curved, smooth walled, 3 - 5 septa	-
<i>Aspergillus nidulans</i>	D	Leaf, bark	Colour: white-violet, reverse: violet-brown, velvety	-	Pyriform shape, size: 7.5 µm, phialide: ampuliform, size: 7.5 x 2.5 µm, metulae: size 8 x 2.5 µm	-	Green, roughened walls, spherical, 2.5 µm	-
<i>Mycelia sterilia</i> 1	E	Leaf, bark	Colour: brown, reverse: blackiest brown, cottony	-	-	Irregular, terminal and intercalar, size: 3.5 x 3 - 7.5 µm	-	Irregular, 37.5 - 40 µm
<i>Mycelia sterilia</i> 2	F	Bark	Colour: brown, reverse: blackiest brown, cottony	-	-	Irregular, terminal and intercalar, size: 3.5 x 3 - 7.5 µm	-	-
<i>Rhizoctonia</i> sp.	G	Leaf	Black, reverse: black, cottony	-	-	Irregular, terminal and intercalar, size: 5 - 13 x 3 - 7.5 µm	-	Irregular, 50 - 60 x 40 - 45 µm

also have appresorium with cylindrical and irregular shape. *Fusarium subglutinans* have fusiform shape microconidia and slightly curved shape macroconidia. *Mycelia sterilia* do not have conidia. This fungi have sclerotium, a compacted mass of mycelium.

#### 4. Discussion

The research result shows that it was found seven endophytic fungi species isolated from *P. angulata* bark, twig, and leaf, i.e: *P. verrucosum*, *C. alienum*, *F. subglutinans*, *A. nidulans*, *Mycelia sterilia* 1, *Mycelia sterilia* 2, and *Rhizoctonia* sp. These endophytic fungi species could be found only in leaf, but certain species also found in leaf, as well as in bark and twig of *P. angulata*. In previous research about endophytic fungi in medicinal plant *Withania somnifera* shows that 20 endophytic fungi species have been isolated from the leaf, stem, and root parts (Khan *et al.* 2010). In another research were proved that 9 endophytic fungi species were found in leaf, twig, and flower petal of *Cananga odorata* plant (Hastuti *et al.* 2019a). These research results proved that endophytic fungi could be found in different or same plant parts.

This research shows that each endophytic fungi species live in *P. angulata* as the host plant in healthy condition. It is proved that there is mutualistic symbiosis relationship between each endophytic fungi and *P. angulata* plant as the host plant. In previous research it was found that each endophytic fungi species could produce some antimicrobial metabolites. This fact showed that the endophytic fungi could protect the host plant from bacterial infection. The endophytic fungi play an important role in the host plant protection from insects, pests, and herbivore (Khan *et al.* 2010). On the other way, the fungi also receive nutrition, and also protected from harmful environment factors, such as: high air temperature, low humidity, etc (Clay *et al.* 2002; Stanley *et al.* 2002; Kogel *et al.* 2006).

The seven endophytic fungi were isolated from healthy *P. angulata* plant parts. In histological observation result, it also proved that there are no mycelium inserted in to cell cytoplasm and the fungi does not make any damage to the plant tissue. This fact shows that endophytic fungi which colonize living, internal tissues of the plant does not cause any harm to their host plant. There are a symbiotic

mutualism interaction between endophytic fungi and *P. angulata* plant.

Some endophytic fungi species isolated from *P. angulata* plant could be found in another plant. *Colletotrichum alienum* also found as one of the endophytic fungi species isolated from *Hedygium acuminatum* (Hastuti *et al.* 2018), from *Cananga odorata* (Hastuti *et al.* 2019a). Another species of *Colletotrichum* have been isolated from *Piper nigrum*, i.e: *Colletotrichum gloeosporoides* (Chitra *et al.* 2014), in tomato fruit, i.e: *Colletotrichum acutatum* (Zivkovic *et al.* 2010). *Fusarium* spp. could found as endophytic fungi in another plant. *Fusarium nivale* and *Fusarium solani* were found in *Dendrobium crumenatum* Sw. (Mangunwardoyo *et al.* 2012). *F. semitectum* were found in *Talinum paniculatum* leaf (Hastuti *et al.* 2015). *Mycelia sterilia* oftenly found in medicinal plant. This endophytic fungi found in Chinese medicinal plant (Huang *et al.* 2008). It is need to make the next research about secondary metabolites content produced by endophytic fungi species isolated from *P. angulata* and their antimicrobial activity.

#### 5. Conclusion

This research result proved that: 1) seven endophytic fungi species were found in *P. angulata* plant, i.e: *Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, *Aspergillus nidulans*, *Mycelia sterilia* 1, *Mycelia sterilia* 2, and *Rhizoctonia* sp. Each fungi species could be found in the leaf, twig, and bark parts of *P. agulata* plant. It shows the diversity of endophytic fungi species isolated from *P. angulata* plant; 2) the seven endophytic fungi were found on the bark epidermis cell wall, on the twig epidermis cell wall, on the leaf epidermis cell wall, and on the spongy cell wall of *P. angulata* plant. It shows that the endophytic fungi could be found in some plant parts. For the suggestion, it is need to make the next research about secondary metabolites content produced by endophytic fungi species isolated from *P. angulata* plant and their antimicrobial activity.

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