

Antimicrobial Activity of Endophytic Fungi Isolated from *Physalis angulata* L. Plant

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Abstract: Some endophytic fungi live in plant tissue and does not caused any damage on their host plant. The endophytic fungi could produce antimicrobial secondary metabolites. Some endophytic fungi have been isolated from a medicinal plant *Physalis angulata*, i.e: *Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, *Aspergillus nidulans*, Mycelia sterilia 1, Mycelia sterilia 2, and *Rhizoctonia* sp. The purpose of this research are: 1) to examine the antimicrobial activity of each endophytic fungi species toward *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and, 2) to determine the endophytic fungi species that have highest antimicrobial activity. Each endophytic fungi isolates were inoculated in Potato Dextrose Broth (PDB) medium and shaken in 120 rpm, during 7 x 24 hours, then the broth culture centrifugated in the rate of 3000 rpm for 10 minutes. The supernatants from each endophytic fungi were treat to the bacteria to know the antimicrobial activity by agar diffusion method. Afterwards the antibacterial effect of each endophytic fungi species were measured. Then the highest antimicrobial effect were determined. The research results shows that: 1) each endophytic fungi species have antimicrobial activity towards *B. subtilis*, *E. coli*, *S. aureus*, and; 2) *Penicillium verucossum* secondary metabolites have the highest antimicrobial activity.

Keywords: Endophytic Fungi, Secondary Metabolites, Antimicrobial, *P. Angulata*

1. Introduction

Some research about antimicrobial activity of medicinal plants have been done. People try to find an alternative material from nature for antibiotic substance. It is because some pathogenic bacteria was resistance against synthetic antibiotic. Medicinal plants could produce antibacterial secondary metabolites, i. e: flavonoid, alkaloid, tannin, there for it is potentially to use as antibiotic sources [1]. Some endophytic fungi could produce secondary metabolites that have characters; antifungal, antibacterial, antiinflammatory, antiviral, antitumor; and the compounds belonging to: flavonoids, alkaloids, terpenoids, and steroids [2] [3].

Some endophytic fungi species live in symbiotic mutualism interaction with meditional plants as the host plant. The endophytic fungi found in roots, stem, and leaf tissues of

potato plants [4]. Although the endophytic fungi live in the host plants it does not cause any damage, but the fungi could produce some antimicrobial secondary metabolites. The last research results shows that some endophytic fungi species live in *Hedychium acuminatum* tissue, it was: *Fusarium oxysporum*, *Rhizoctonia* sp., *Fusarium solani*, *Fusarium semitectum*, *Colletotrichum alienum*, *Colletotrichum ti*, *Colletotrichum aotearoa*, *Colletotrichum gloesporoides*, and *Aspergillus parasiticus*. These fungi species have an ability to produce antimicrobial secondary metabolites as well as the host plant produce, i.e: alkaloid, flavonoid, terpenoid, and tannin [5]. It was also proved that the secondary metabolites in liquid culture of each endophytic fungi species isolated from *H. acuminatum* have antibacterial effect towards *Staphylococcus aureus*, and *Bacillus subtilis* [6].

Physalis angulata is another sort of medicinal plant. This plant could produce some secondary metabolities i. e:

alkaloid, flavonoid, saponin, polifenol, terpenoid. People use this plant for infectious disease. Some endophytic fungi live in this plant tissue. In the preliminary research some endophytic fungi species have been isolated from *Physalis angulata* plant tissues, i.e: *Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, *Aspergillus nidulans*, Mycelia sterilia 1, Mycelia sterilia 2, and *Rhizoctonia* sp. The purpose of this research are: 1) examine the antimicrobial activity of each endophytic fungi species towards *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, 2) determine the endophytic fungi species isolated from *P. angulata* plant that have the highest antimicrobial activity.

2. Material and Methods

Materials: endophytic fungi isolates (*Penicillium verrucosum*, *Colletotrichum alienum*, *Fusarium subglutinans*, Mycelia sterilia 1, Mycelia sterilia 2, and *Rhizoctonia* sp.), cork borer, Potato Dextrose Agar (PDA) medium from MERCK, Potato Dextrose Broth (PDB) medium (consist of potato tuber essence, Dextrose from MERCK and sterilized water), Nutrient Broth (NB) medium (consist of meat extract for Microbiology from MERCK), Nutrient Agar (NA) medium from MERCK, chloramphenicol, and 70% alcohol. The method for antimicrobial examination was the Agar Diffusion Method. The procedure is written as follows.

2.1. Preparation for Endophytic Fungi Liquid Cultures

The preparation of endophytic fungus liquid culture was conducted obtain the secondary metabolites produced by each endophytic fungi species [7]. Each endophytic fungus isolates inoculated on PDA plate medium containing chloramphenicol (100 mg/L) and incubated in 26°-27°C for 7-24 hours. Then each endophytic fungus species culture were cutted into five pieces of 1×1 cm each. The endophytic fungus pieces were inoculated in 100 mL PDB medium and shaken at 120 rpm for 7×24 hours in 26°-27°C. Afterwards the liquid were centrifugated at the rate of 3000 rpm for 10 min. The supernatant of each endophytic fungus species of 20 µL each were used for antimicrobial examination in vitro [8].

2.2. Examination of Endophytic Fungi Liquid Cultures Antimicrobial Effect

The examination of each endophytic fungus liquid culture antimicrobial effect againts *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* was done by the agar diffusion methods. Each bacteria species were inoculated on NB medium, standardized with MC Farland 0,5 ($1,5 \times 10^8$ cfu/mL), the result of the incubation hour in 18 hours. The NA medium were prepared and cutted with sterile cork borer to make wells. Then the bacteria culture were inoculated on NB medium and incubated at 37°C for 18 hours. Afterwards

the bacteria liquid cultures were inoculated on NA medium and the wells were filled with 20 µL supernatant of each fungus spesies, then incubated at 37°C for 1 x 24 hour. The positive control used 5 mg/5 mL chloramphenicol and the negative control used PDB medium. The antimicrobial effect of each endophytic species against *S. aureus*, *E. coli* and *B. subtilis* was determined by measuring the growth inhibition zone around the wells on NA medium. The data were analyzed to know whether any differences in the antimicrobial activity of each endophytic fungi species by Anova and continued by Duncan's to determine the endophytic fungi species that have the highest antibacterial activity compared with the other species based on the growth inhibition zone diameter size.

2.3. Detection of Secondary Metabolites Content of Each Endophytic Fungi Species

The flavonoid, alkaloid, tannin, and saponin content in the liquid cultures of each endophytic fungi species were analyzed by spectrophotometry.

3. Result and Discussion

The secondary metabolites of endophytic fungi antimicrobial activity could be examine based on the diameter of inhibition zone around the well contained with the endophytic fungi liquid cultures on the NA medium. The NA medium have been inoculated with *B. subtilis*, *E. coli* and *S. aureus* culture. The research result showed that secondary metabolites of each endophytic fungi species isolated from *P. angulata*, i.e: *Penicillium verrucosum*, *Colletotrichum alieum*, *Fusarium subglutinans*, *Aspergillus nidulans*, Mycelia sterilia 1, Mycelia sterilia 2, and *Rhizoctonia* sp. have an antimicrobial activity toward *E. coli*, *S. aureus*, and *B. subtilis* (Figure 1)

The data were analyzed with ANOVA and continued with Duncan 5% to know which endophytic fungi liquid culture that have the highest antibacterial activity towards *E. coli*, *B. subtilis*, and *S. aureus*. There are differences of growth inhibition zone diameter between each endophytic fungi species toward *B. subtilis*, *E. coli*, and *S. aureus*. *P. verrucosum* liquid culture produce the biggest growth inhibition zone diameter compare with the other species used in this research toward *B. subtilis* and *S. aureus* (Table 1).

The clear zone around the well contains of each endophytic fungi secondary metabolities proved that there are an inhibition activity to the bacteria growth (Figure 1), It is proved that the fungi secondary metabolites in the well diffused to the medium and inhibited the bacterial growth. The reserch results proved that each endophytic fungi species could produced the four antibacterial compounds although the endophytic fungi species have already isolated from *Physalis angulata* as the host plant that could also produce the same secondary metabolites.

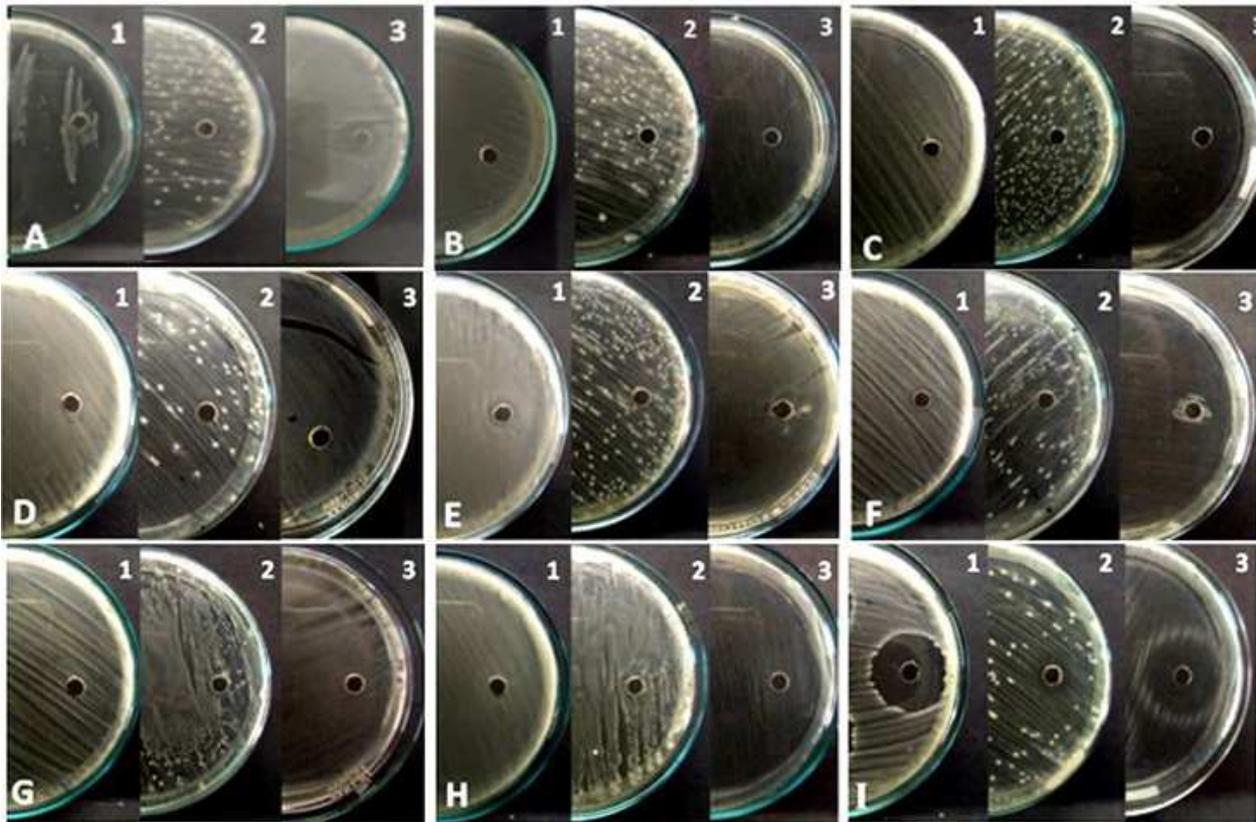


Figure 1. The Growth Inhibition Zone of Each Endophytic Fungi Species Secondary Metabolite towards *E. coli*, *B. subtilis* and *S. aureus*.

(1) *E. coli*, (2) *B. subtilis*, (3) *S. aureus*; (A) *P. verrucosum*, (B) *C. alienum*, (C) *F. subglutinans*, (D) *A. nidulans*, (E) *Mycelia sterilia* 1, (F) *Mycelia sterilia* 2, (G) *Rhizoctonia* sp., (H) negative control, (I) positive control.

Table 1. Duncan 5% of Each Endophytic fungi liquid culture antimicrobial activity toward *B. subtilis*, *E. coli*, and *S. Aureus*.

Treatment		Average	Notation
Negative control	<i>E. coli</i>	7,0700	a
Negative control	<i>S. aureus</i>	7,0700	a
Negative control	<i>B. subtilis</i>	7,0700	a
<i>Aspergillus nidulans</i>	<i>E. coli</i>	7,0700	a
<i>Aspergillus nidulans</i>	<i>S. aureus</i>	7,0700	a
<i>Mycelia sterilia</i> 1	<i>E. coli</i>	7,0700	a
<i>Mycelia sterilia</i> 2	<i>E. coli</i>	7,0700	a
<i>Rhizoctonia</i> sp.	<i>B. subtilis</i>	7,0700	a
<i>Aspergillus nidulans</i>	<i>B. subtilis</i>	7,1750	a
<i>Colletotrichum alieum</i>	<i>B. subtilis</i>	7,3350	a
<i>Colletotrichum alieum</i>	<i>E. coli</i>	7,4150	a
<i>Fusarium subglutinans</i>	<i>B. subtilis</i>	7,4850	a
<i>Fusarium subglutinans</i>	<i>E. coli</i>	7,5200	a
<i>Rhizoctonia</i> sp.	<i>S. aureus</i>	7,6850	a
<i>Colletotrichum alieum</i>	<i>S. aureus</i>	7,8000	a
<i>Penicillium verrucosum</i>	<i>E. coli</i>	7,8000	a
<i>Rhizoctonia</i> sp.	<i>E. coli</i>	7,8150	a
<i>Mycelia sterilia</i> 1	<i>S. aureus</i>	8,1050	a
<i>Mycelia sterilia</i> 1	<i>B. subtilis</i>	8,1800	a
<i>Fusarium subglutinans</i>	<i>S. aureus</i>	8,2650	a
<i>Mycelia sterilia</i> 2	<i>B. subtilis</i>	10,3500	b
Positive control	<i>E. coli</i>	10,9000	b
<i>Penicillium verrucosum</i>	<i>B. subtilis</i>	12,7450	c
<i>Mycelia sterilia</i> 2	<i>S. aureus</i>	13,2350	c
<i>Penicillium verrucosum</i>	<i>S. aureus</i>	14,4650	d
Positive control	<i>S. aureus</i>	14,9350	d
Positive control	<i>B. subtilis</i>	16,1200	e

The secondary metabolites content of each endophytic fungi species isolated from *P. angulata* plant were analyzses (Table 2).

Table 2. The Secondary Metabolite Content of the Endophytic Fungi Species Liquid Cultures Isolated from *Physalis angulata* Plant.

Isolate code	Species	Secondary Metabolite (mg.kg ⁻¹)			
		Flavonoid	Alkaloid	Tannin	Saponin
A	<i>P. verrucosum</i>	635.63	17.76	25.36	6.19
B	<i>C. alienum</i>	493.44	12.41	19.68	4.14
C	<i>F. subglutinans</i>	509.84	13.02	20.33	4.38
D	<i>A. nidulans</i>	504.38	12.82	20.17	4.29
E	<i>Mycelia sterilia</i> 1	556.72	14.79	22.20	5.06
F	<i>Mycelia sterilia</i> 2	597.34	16.32	23.83	5.64
G	<i>Rhizoctonia</i> sp.	567.66	15.20	22.64	5.21

Each endophytic fungi species could produce antimicrobial secondary metabolites that protected *P. angulata* as the host plant from pathogenic bacteria infection. This fact is advantage for *P. angulata* [9] [10].

Each endophytic fungi species isolated from *P. angulata* plant liquid cultures could produce flavonoid, alkaloid, tannin, and saponin as well as in the host plant. There are differences content of the secondary metabolites produced by each endophytic fungi species. *Penicillium verrucosum* is the most potential species to produce its secondary metabolites in the highest content compared with the others species.

The four secondary metabolites produced by endophytic

fungi species take a role in the bacteria colony growth inhibition. Alkaloid could inhibit some enzyme activity, i.e: esterase, RNA polymerase, DNA polymerase, and cellular respiration enzyme [11]. Flavonoid take a role in bacteria cell membrane damaged and inhibit the cellular metabolism enzymes activity [12]. Besides that, OH⁻ ion in phenol compound could bound to H⁺ ion in hydrogen bound at protein cell wall structural protein and cause protein denaturation [13]. Then it cause bacteria cell wall and cell membrane damaged. The bacteria cell membrane semipermeability will be decreased and caused the cell water and enzymes removed from the cell. It caused metabolism and the cell growth inhibition.

Tannin could dissolve the lipid layer on bacteria cell wall and also could denature the structural protein in bacteria cell membrane [14] [15]. This case caused cell wall structure damaged and also decrease the cell membrane semipermeability. Then the nutrients and enzymes removed from the cell and caused cellular metabolims and cell growth inhibition.

This research result proved that the liquid culture of each endophytic fungi species contains antibacterial secondary metabolites have antimicrobial activity towards *B. subtilis*, *E. coli*, and *S. aureus*. *P. verrucosum* liquid culture have the highest antibacterial activity compared with the other endophytic fungi species isolated from *P. angulata* plant. The liquid culture of *P. verrucosum* also contains flavonoid, alkaloid, tannin, saponin in the highest content. Based on this research result people could use this endophytic fungi liquid cultures as an antibiotic source. It is an alternative way besides use the *P. angulata* plant parts extract.

4. Conclusion

The conclusions are: 1) each endophytic fungi species isolated from *P. angulata* plant have antimicrobial activity toward *B. subtilis*, *E. coli*, and *S. aureus*; 2) *Penicillium verrucosum* secondary metabolites in liquid culture have the highest antimicrobial activity. For the suggestion, it is need to make the next research to prove that secondary metabolites produced by the endophytic fungi species isolated from another parts of *P. angulata* plant also have an antibacterial effect to *E. coli*, *S. aureus*, and *B. subtilis*.

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