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RESEARCH ARTICLE

Endophytic Fungi from *Piper retrofractum* VAHL: Isolation, Phytochemical Analysis, Antibacterial and Antioxidant Activities

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Abstract

Objective: *Piper retrofractum* Vahl. is an Indonesian plant that has several activities such as antibacterial, antifungal, antioxidant, etc. Endophytic fungi are fungal microbe which can produce secondary metabolites that associated with medical plants can be exploited for curing many diseases. This is the first study of endophytic fungi from *P. retrofractum* which can be an alternative source of natural product besides its host, *P. retrofractum*. Seven endophytic fungi were isolated from the fruits and leaves of *P. Retrofractum* and investigated for their antibacterial and antioxidant activities. Methods: All the isolated fungi were tested for their antibacterial activity using disc diffusion method with several series of concentrations and antioxidant capacity using the DPPH method. Result: The highest activity both antibacterial and antioxidant was demonstrated by DPR-1 extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This extract contained alkaloids, steroids, and flavonoid compounds. Conclusion: This study reported that endophytic fungi from *P. retrofractum* indicate as a potential source of bioactive and chemically novel compounds.

Keywords: *Piper retrofractum, Endophytic fungi, Antibacterial activity, Antioxidant activity.*

Introduction

Piper retrofractum Vahl. is an Indonesian plant that is closely related to pepper plants. Empirically, *P. retrofractum* fruit has been used to treat abdominal pain, as carminative, expectorant, laxative, antiseptic and antibacterial [1], while *P. retrofractum* leaves are used to cure abdominal pain, as a medicine gargle and treat bacterial infections [2]. The *P. retrofractum* leaf extract is reported to have antimicrobial activity against *Candida albicans* [3], *Staphylococcus aureus*, *Bacillus subtilis, Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Escheria coli* [4, 5].

The extract of *P. retrofractum* leaves is also shown in their activity against bacterial growth [6]. The use of *P. retrofractum* extract in drug discovery especially as antibacterial has several disadvantages, for example, many plants are needed to produce extracts. It will cause the plant to become extinct if it continues to be exploited without conservation. So an alternative is needed to overcome this problem, such as using endophytic fungi. Endophytic fungi are fungal microbes that live within plant tissues without causing pathogenic effects and symptoms of the disease on its hosts [7,8]. Endophytic fungi can produce secondary metabolites which are interesting topics for drug discovery researchers. They offer potential sources of novel natural products for exploitation in medicine, agriculture and the pharmaceutical industry [9].

Some studies proved secondary metabolites of endophytic fungi potential against bacterial growth [10-11] [12,13]. Secondary metabolites such as alkaloids, terpenoids, flavonoids, polyphenols, etc., isolated from endophytic fungi, are reported to have various activities such as antifungal [14], antiviral [15], anti-inflammatory & anti-cancer [16], anti-malarial [17], and antibacterial [18, 19-20, 21, 22].

Many endophytic fungi have been identified from different plants belonging to *Piperaceae* which have reported for their antimicrobial and antioxidant activity. Endophytic fungi of *Piper betle, Piper longum, Piper nigrum* has shown antimicrobial such as antifungal and antibacterial [23, 24], while *Piper longum, Piper nigrum* gave strong antioxidant activity [24]. But the activity of endophytic fungi from *P. retrofractum* hasn't explored before. Based on these problems, this study is aimed to isolate endophytic fungi from *P. retrofractum*, phytochemical screening in the fermented extract of *P. retrofractum* endophytic fungi and determine for their antibacterial and antioxidant activities.

Materials and Methods

Collection Plant

P. retrofractum was collected from Jember Regency, East Java, Indonesia. Fresh samples were brought to the laboratory in a sterile bag and processed several hours after sampling. Fruits and leaves from *P. retrofractum* were used in this research.

Isolation and Characterization of Endophytic Fungi

The fruits and leaves were washed in running tap water and surface sterilized using 70% ethanol (1 minute), 1% NaOCl (3 minutes), 70% ethanol (1 minute) and rinsed three times in sterile distilled [25]. All the steps were carried out under LAF and each step change, the sample was dried using sterile paper. After the surface sterilization, the samples were cut into 1-2 cm placed on solid potato dextrose agar (PDA) medium and incubated at 25^{o}C for 5-7 days until the endophytic fungi were grown.

Growing of the endophytic fungi colonies fungi were put on new PDA medium to get single and pure colonies. Characterization of endophytic fungi from *P. retrofractum* was analyzed by observing the morphology of endophytic fungi both macroscopically and microscopically. Macroscopically, the surface color, pigmentation color, and type of fungal growth were observed. Microscopic observations were carried out with septa in hyphae, conidia, and conidiophores.

Screening the Antibacterial Activity of Endophytic Fungi

The screening of antibacterial activity was determined by direct contact between the endophytic fungi's isolate from *P. retrofractum* and *Mueller Hinton Agar* (MHA) medium, that contains testing bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa* and incubated at 27^{o}C for 20 hours. Antimicrobial activity was determined by the presence of an inhibition zone around the endophytic fungi's plug and was measured in millimeter (mm).

Fermentation and Extraction of Endophytic Fungi

Endophytic fungi isolated from *P. retrofractum* were fermented by inoculating in 150 ml of Potato Dextrose Broth (PDB) medium for 14 days at 25^{o}C. This fermentation is placed in a rotary shaker at 100 rpm. The Fermented endophytic fungi supernatant was extracted with ethyl acetate (1:1), 2 times partition.

Phytochemical Analysis of Endophytic Fungi Fermented Extract

Phytochemical screening was analyzed by using the TLC method to determine the chemical content such as alkaloids, flavonoids, and terpenoids [26]. The stain appearance used to detect the presence of alkaloids is dragendorf reagent. Terpenoids determined by using anisaldehyde sulfuric acid reagent, flavonoids determined by using ammonia reagent [27].

Antibacterial Activity Assay

Antibacterial activity testing of the extract was using the disc diffusion method with 10 µg gentamicin disc as a positive control and 10% DMSO as a negative control. The concentration of extract was 1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 6.0 mg/ml and 8.0 mg/ml. Tested bacterium that used in this study was *S. aureus* (ATCC 27853) and *P. aeruginosa* (ATCC 25923). The experiments were repeated in triplicate were triplicate and the result expressed as mean $\pm$ sandard deviation.

Antioxidant Activity Testing

Antioxidant activity was tested by determined the antioxidant capacity of endophytic fungi using the DPPH method. 0.1 mg/ml and 0.25 mg/ml concentration of endophytic fungi were measured with a spectrophotometer at 517 nm after 30 minutes incubation. Absorbation was inserted into the regression equation of trolox's standard until received the value of antioxidant capacity, which is expressed as Trolox Equivalence Antioxidant Capacity (TEAC) [28].

Result and Discussion

Isolation and Characterization of Endophytic Fungi from *P. retrofractum*

Seven endophytic fungi were isolated from *P. retrofractum* which 4 endophytic fungi were isolated from fruits that coded BPR-1, BPR-2, BPR-3, and BPR-4, while 3 endophytic fungi were isolated from the leaves that coded DPR-1, DPR-2, and DPR-3. All the isolated fungi were characterized by macroscopically and microscopically. BPR-1 isolate has radial type mycelium growth, brownish surface color, and brown pigmentation color.

Microscopically, BPR-1 has septa in hyphae, non-branched conidiophores, yellowish conidia that extends and attached to conidiophores (Fig. 1A). BPR-2 isolate has radial type mycelium growth, gray surface, and white pigmentation. This fungus has septa in hyphae, with non-branched conidiophores, black and round conidia (Fig. 1B). BPR-3 isolate has radial type mycelium growth, the surface is dark brown with white spots above it and black pigmentation. BPR-3 has septa in hyphae, with branched conidiophores. Its conidia are dark yellow, elliptical and attached to the conidiophores (Fig. 1C). BPR-4 isolate has radial type mycelium growth, black surface and gray pigmentation. BPR-3 has septa in hyphae, branched conidiophore, white and oval conidia (Fig. 1D). Macroscopically DPR-1 Isolate has concentric type mycelium growth, yellow surface and light yellow pigmentation. DPR-1 has septa in hyphae, with branched conidiophores, gray and oval conidia (Fig. 2A). DPR-2 isolate has radial type mycelium growth, white and smooth surface, white pigmentation.

DPR-2 has septa in hyphae, with branched conidiophores, white and oval conidia which attached to conidiophores (Fig. 2B). DPR-3 isolate has radial type mycelium growth, brown surfaces and black pigmentation. DPR-3 has septa in hyphae, with branched conidiophores, white and round conidia (Fig. 2C). Based on macroscopic and microscopic analysis, 7 endophytic fungi isolated from fruit and *P. retrofractum* leaves had different characteristics. These results suggest that isolated fungi have different species, but to determine its species phylogenetic analysis is needed.

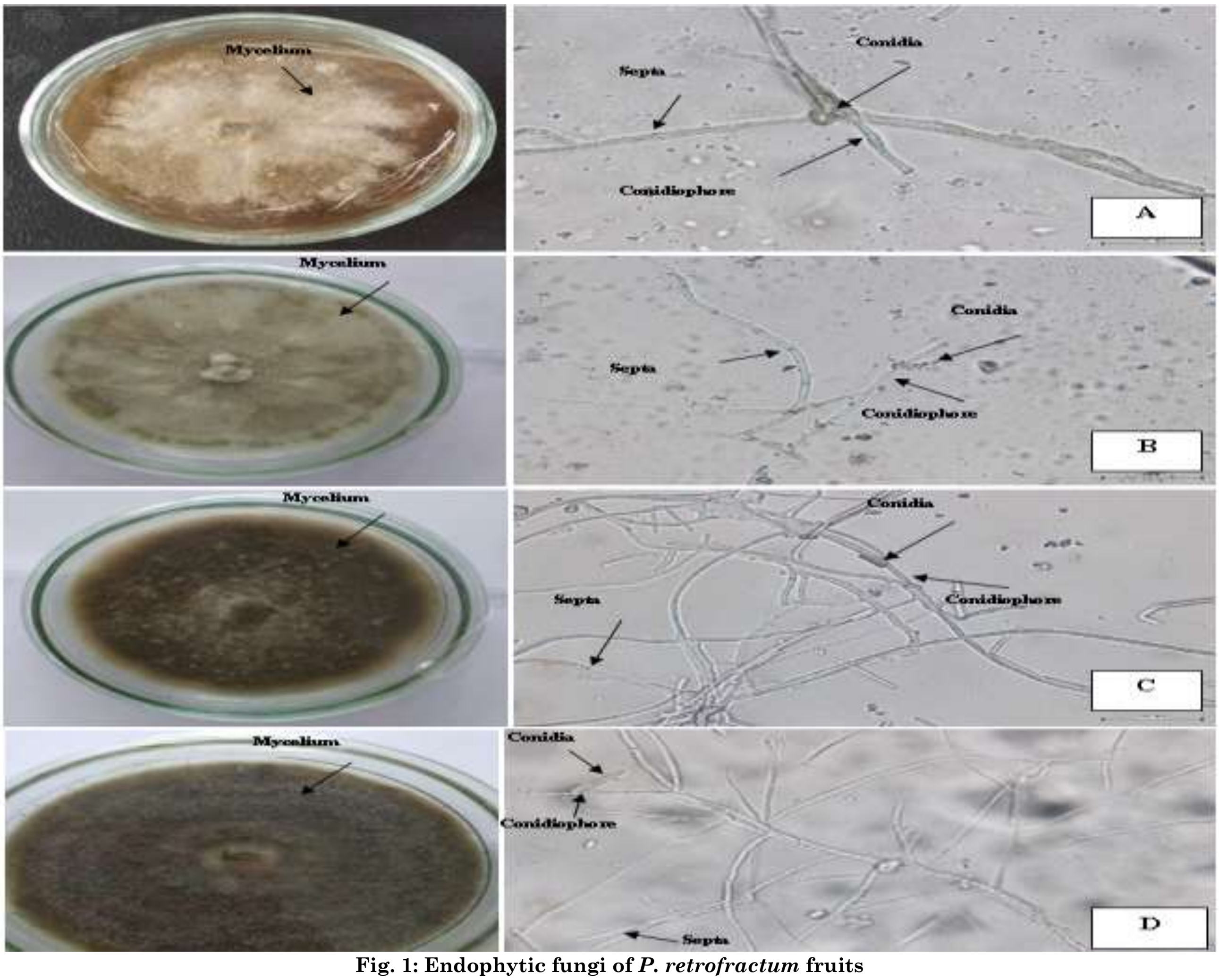

Fig. 1: Endophytic fungi of *P. retrofractum* fruits

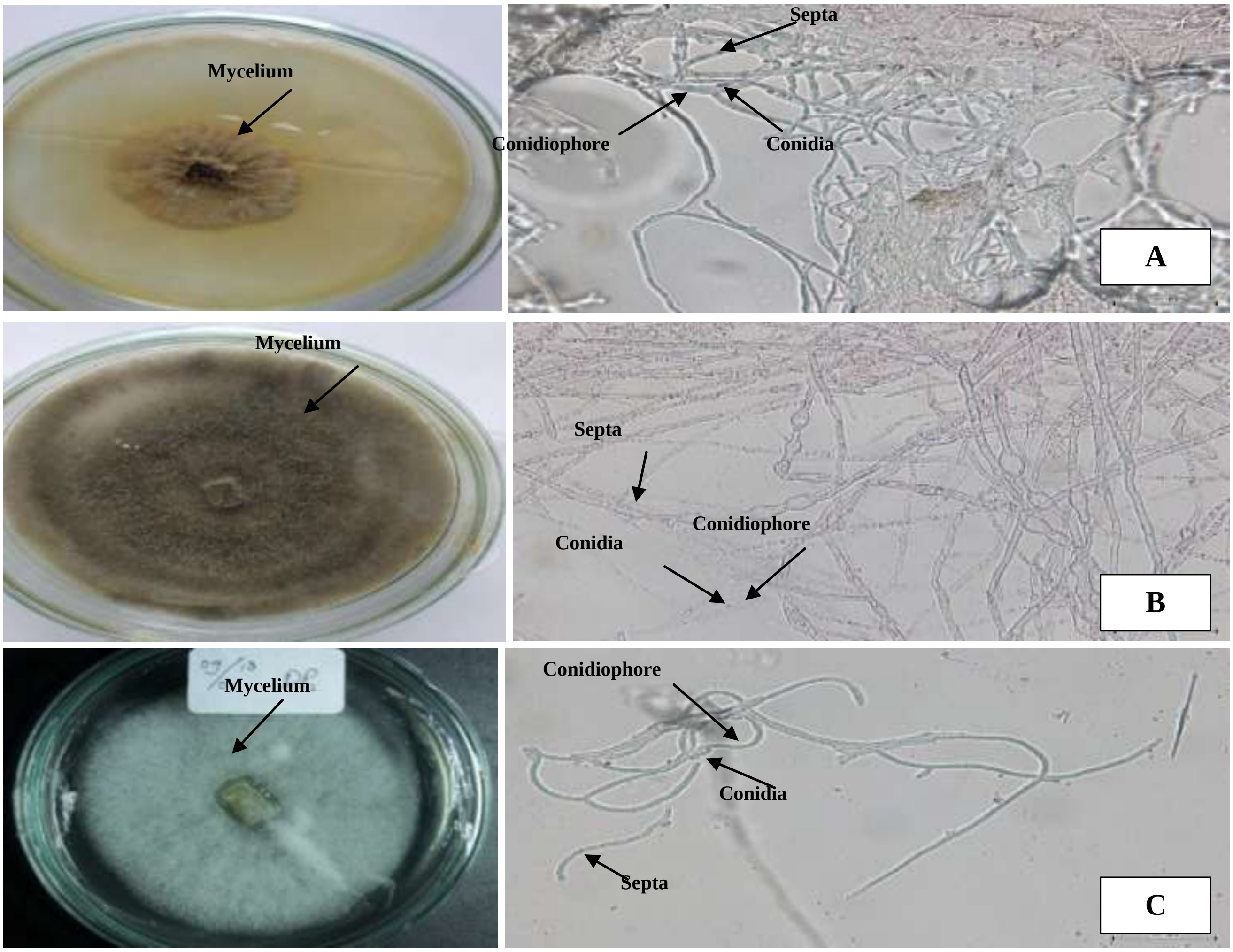

Fig. 2: Endophytic fungi of *P. retrofractum* leaves

Screening the Antibacterial Activity of Endophytic Fungi

The results of screening antibacterial activity of endophytic fungi showed that all the isolated fungi didn't inhibit *S. aureus* and *P. aeruginosa* bacteria (Table 1). There was no inhibition of the endophytic fungi because the tested fungi isolates were thought to haven't produced secondary metabolites or the number of secondary metabolites produced was not enough to inhibit bacterial growth. To ensure the endophytic fungi produce secondary metabolites that have antibacterial activity, fermentation and extraction processes were needed in all isolated fungi.

Table 1: Antibacterial screening activity of *P. retrofractum* endophytic fungi

No	Isolate	Inhibition zone	
		S. aureus	***P. aeruginosa***
1.	BPR-1	ND	ND
2.	BPR-2	ND	ND
3.	BPR-3	ND	ND
4.	BPR-4	ND	ND
5.	DPR-1	ND	ND
6.	DPR2	ND	ND
7.	DPR-3	ND	ND

*ND: Not Detected

Phytochemical Analysis

Phytochemical analysis aims to determine the secondary metabolites that produced by endophytic fungi which are considered to provide antibacterial activity. Their presence is an indicator that they can be exploited as precursors in the development and advancement of synthetic drugs. In the current study, the phytochemical analysis of extracts showed the presence of alkaloids, terpenoids, and flavonoids (Table 2).

Table 2: Phytochemical assay of *P. retrofractum* endophytic fungi

Isolate	Assay		
	Alkaloids	Terpenoids	Flavonoids
BPR-1	-	+	-
BPR-2	+	+	+
BPR-3	+	+	+
BPR-4	-	+	+
DPR-1	+	+	+
DPR-2	+	+	+
DPR-3	-	+	+

* (+): presence, (-): absent

Alkaloids are contained in BPR-2, BPR-3, DPR-1 and DPR-2 extracts, while terpenoids were contained in all the fermented fungus extracts and also flavonoids were absent in BPR-1 extracts. This result is in accordance some research wherein the endophytes have shown the presence of different phytochemicals such as alkaloids, terpenoids, flavonoids, and phenolic compounds are known to possess strong antimicrobial and antioxidant activities [10, 29, 30].

Antibacterial Activity Assay

Based on the antibacterial activity against *S. aureus* (Table 3), BPR-1, BPR-2, BPR-4 and DPR-1 extracts initially inhibited at the lowest concentrations that used. While BPR-3 and DPR-3 extracts initially inhibited at 2.0 mg/mL concentration, then DPR-2 inhibited the bacteria at 6.0 mg/ml concentration. The highest antibacterial activity against *S. aureus* was shown in the DPR-1 extract which exhibited the largest inhibition zone among the other fungi at the lowest concentration. While at another concentration this extract proved a relatively large inhibition zone, compared the others. Endophytic fungi extracts of BPR-2, BPR-3, DPR-1, and DPR-3 reported antibacterial activity against *P. aeruginosa* at the lowest concentrations. While BPR-1, DPR-1, and DPR-2 extract initially inhibited at 4.0 mg/ml concentration. DPR-3 extract gave the highest antibacterial activity against *P. aeruginosa* (Table 4).

Table 3: Diameter of *S. aureus* Inhibition Zone

Isolate	Diameter of Inhibition Zone (mm)				
	1.0 mg/ml	2.0 mg/ml	4.0 mg/ml	6.0 mg/ml	8.0 mg/ml
BPR-1	7.00 ± 0.15	7.68 ± 0.12	7.90 ± 0.03	8.70 ± 0.05	10.18 ± 0.27
BPR-2	7.20 ± 0.03	8.93 ± 0.04	10.38 ± 0.04	11.24 ± 0.01	13.28 ± 0.18
BPR-3	ND	8.25 ± 0.29	9.43 ± 0.18	10.62 ± 0.07	11.91 ± 0.13
BPR-4	7.15 ± 0.06	8.88 ± 0.12	10.35 ± 0.09	11.73 ± 0.51	12.6 ± 0.06
DPR-1	8.20 ± 0.07	10.39 ± 0.08	12.93 ± 0.07	13.51 ± 0.05	14.72 ± 0.14
DPR-2	ND	ND	ND	6.60 ± 0.17	13.64 ± 0.06
DPR-3	ND	8.35 ± 0.09	12.77 ± 0.05	12.99 ± 0.04	13.74 ± 0.01

*ND: Not Detected

* Diameter is expressed in mean ± sandard deviation

Table 4: Diameter of P. aeruginosa Inhibition Zone

Isolate	Diameter of Inhibition Zone (mm)				
	1.0 mg/ml	2.0 mg/ml	4.0 mg/ml	6.0 mg/ml	8.0 mg/ml
BPR-1	ND	ND	7.47 ± 0.22	9.08 ± 0.13	10.38 ± 0.17
BPR-2	7.17 ± 0.14	8.38 ± 0.08	10.39 ± 0.11	11.73 ± 0.03	13.36 ± 0.09
BPR-3	6.89 ± 0.75	7.88 ± 0.09	8.43 ± 0.12	9.37 ± 0.02	11.27 ± 0.40
BPR-4	ND	ND	7.95 ± 0.35	9.23 ± 0.10	10.69 ± 0.16
DPR-1	7.05 ± 0.03	7.94 ± 0.05	9.35 ± 0.14	11.61 ± 0.02	13.52 ± 0.07
DPR-2	ND	ND	7.14 ± 0.10	8.31 ± 0.11	9.42 ± 0.26
DPR-3	8.48 ± 0.07	9.18 ± 0.08	11.28 ± 0.10	12.46 ± 0.07	13.66 ± 0.09

*ND: Not Detected

* Diameter is expressed in mean ± sandard deviation

Based on the results, fermented extracts of endophytic fungus BPR-2 and DPR-1 exhibited good antibacterial activity against *S. aureus* and *P. aeruginosa*. These extracts gave the larger inhibition zone than others at the lowest concentrations, and at other concentrations, these extracts also showed a large inhibition zone. DPR-1 extract reported higher activity than BPR-2. These occur by the differential of chemical content and concentrations of chemical content contained in the fungi. The chemical contents of both extracts are same. They contain alkaloids, terpenoids, and flavonoids compounds. So we suggest, concentrations of chemical content make them have different activity. Antibacterial activity against the tested bacteria showed that endophytic fungi ethyl acetate extract was more active in inhibiting the growth of *S. aureus* than *P. aeruginosa*.

This can be seen from several extract test concentrations which give a greater inhibition diameter value for *S. aureus*. Besides that gentamicin as a positive control also gave a greater inhibition diameter of *S. aureus* than *P. aeruginosa*. This activity was higher for *S. aureus* than *P. aeruginosa* according to the cell wall of the bacteria. The structure of Gram-negative bacterial cell walls is more complex than Gram-positive bacteria [31]. Gram-negative bacteria have cell walls consisting of 3 layers, namely, the outer layer, the middle layer, and the inner layer, while Gram-positive bacteria only have a single layer on the cell wall. The more complex structure of cell walls in Gram-negative bacteria make it harder for antibacterial compounds to enter cells and find targets for the work [32]. Nevertheless, it appears that some fungi have higher activity against *P. aeruginosa*.

Antioxidant Activity Testing

Based on TEAC's value which is identified in 0.1 mg/ml and 0.25 mg/ml concentrations (Table 5), it showed that the antioxidant capacity of endophytic fungi from highest to lowest is DPR-1 > DPR-3 > BPR-2 > DPR-2 > BPR-4 > BPR-3 > BPR-1. In other word, the higher the TEAC's value, it means the antioxidant capacity will be greater.

Table 5: Antioxidant Capacity of Endophytic Fungi

Isolate	TEAC (μmol/g)	
	0.1 mg/ml	0.25 mg/ml
BPR-1	543.79	284.79
BPR-2	824.09	499.33
BPR-3	618.03	314.48
BPR-4	649.85	328.42
DPR-1	1175.61	935.09
DPR-2	696.82	493.27
DPR-3	971.06	514.48

Determined of antioxidant activity related to polyphenols contents such as tannin and flavonoid. This result correlated to phytochemical screening's result of endophytic fungi. Flavonoids were presented in six fungi, except BPR-1 isolate. Antioxidant activity can be influenced by flavonoid contents [33,34]. Flavonoid is oxidized by radicals, being radical is more stable and less-reactive. Flavonoids react with radical reactive compound to stabilize the reactive oxygen species. It explains the high reactivity of the hydroxyl group of the flavonoids, radicals is made inactive [35].

In other hand, strong antioxidant capacity also showed due to the presence of flavonoid [34, 36]. Flavonoid contents are higher, antioxidant activity will be better [28]. Moreover, antioxidant activity is caused by alkaloid and terpenoid as well. These have a role to inhibit some radicals [33, 37, 38]. Endophytic fungi present some biological activities such as antibacterial and antioxidant. It's all affected by their compounds contribution that commonly called secondary metabolite. Those secondary metabolites are alkaloids, flavonoids and terpenoids. Based on our research DPR-1 isolate has the highest antibacterial and antioxidant activities. It may be caused by their secondary metabolite that contains all three compounds we had been screened which are alkaloids, flavonoids and terpenoids. Alkaloids had been explained to have strong antibacterial activity by transcription process and toxin production inhibition [39, 40].

It also affects cell division & virulence genes, respiratory & enzyme inhibition in bacteria and disrupts bacterial membrane [41]. Some alkaloids like Pyrrocidine A & B which isolate from *Acremonium zeae* show antibacterial activity [42]. Beside having antibacterial activity, alkaloids have antioxidant activity as well. Alkaloids contain NH functional group that showed antioxidant activity, donating their hydrogen to DPPH [43]. Antioxidant activity is not only influenced by alkaloid, but also flavonoids and terpenoids. Some researchers prove flavonoids exhibited potential antioxidant activity [44]. Pestacin and isopestacin were obtained from *Penicillium microspore* reported having strong antioxidant activity. Even the antioxidant activity of pestacin isolated from *Pestalotiopsis microspora* shows its ability 11 times higher than Trolox and derivate of vitamin E [45]. Flavonoids also demonstrated antibacterial activity. It inhibited and interfered enzymes in metabolism [46].

Endophytic fungi flavonoid compounds such as pestacin and isopestacin showed antibacterial activity too [30]. *Fusarium tricinctum* endophytic fungi from *Salicornia bigelovii* were isolated and they contain some terpenoid compounds which is fusartricin, fusarielin B and enniatin B. These compounds is known to be able to inhibit bacteria. It means that terpenoids contribute to antibacterial activity [47]. Another side terpenoids show antioxidant activity as evidenced by *Acremonium sp*. compounds. They reported that those compounds exhibited strong antioxidant activity with an IC_{50} value equivalent to ascorbic acid [48]. All the secondary metabolite contributes to both of antibacterial and antioxidant activities with their own mechanism. The highest activity of DPR-1 isolate is also affected by its compounds. We suggest an amount of compounds concentrations among the fungi cause they are at different level of activity. In other words, the higher its concentrations, the greater its activity. Furthermore, these differences are affected by synergistic effect of their compounds. One compound attributes to other phytoconstituents for generating antibacterial and antioxidant activity.

Conclusion

The present study suggests that endophytic fungi of *P. retrofractum* respectively, are effective alternative sources of antibacterial and antioxidant agents. Especially for DPR-1 isolate which presents the highest activity both antibacterial and antioxidant. In the future this study is needed for species identification of the fungi.

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References

1. P Warrier, V Nambiar, K Raman(1995) Piper longum Linn.: Indian medicinal plants. India.: Orient Longman Ltd. Madrs,.
2. IPG Krisnawan, PA Sandhi, AS Duniaji (2017) “Daya hambat ekstrak daun cabe jawa (Piper retrofactum Vahl.) terhadap pertumbuhan Staphylococcus aureus,” 6 (2):P 1-10.
3. ER Sari, ER Nugraheni (2013) “Antifungal activity test of Piper retrofractum leaf ethanol extract on Candida albicans growth,” Biofarmasi, 11 (2): 36-42.
4. Y Jamal, P Irawati, A Fathoni, A Agusta (2013) “Chemical constituents and antibacterial effect of essential oil of Javanese pepper leaves (Piper retrofractum Vahl.),” Indones. Inst. Sci. Res. Cent. Biol. LIPI, Bogor, 23: 65-72.
5. NJ Reddy, DN Vali, M Rani, SS Rani (2014) “Evaluation of antioxidant , antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit,” 34: 115-122.
6. M Khan, M Siddiqui (2007) “Antimicrobial activity of piper fruits,” Nat. Prod. Rad., 6: 111-113.
7. A A L Gunatilaka (2006) “Natural products from plant-associated microorganism: Distribution, structural diversity, bioactivity and implications of their occurrence,” J. Nat. Prod., 69: 509-526.
8. JS Bhore, G Sathisha (2010) “Screening of endophytic colonizing bacteria for cytokinin-like compounds: Crude cell-free broth of endophytic colonizing bacteria is unsuitable in cucumber cotyledon bioassay,” World J. Agric. Sci., 6 (4): 345-352.
9. G Strobel, B Daisy (2003) “Bioprospecting for microbial endophytes and their natural products,” Microb. Mol. Biol. Rev., 67 (4): 491-502.
10. BD Rekha, MB Shivanna (2014) “Diversity, antimicrobial and antioxidant activities of fungal endophytes in Cynodon dactylon (L.) Pers. and Dactyloctenium

aegyptium (L.) P.," Int. J. Curr. Microbiol. Appl. Sci., 3: 573-591.

11. M Stinson, D Ezra, W Hess, J Sears, G Strobel (2003) "An endophytic Gliocladium sp. of Eucryphia cordifolia producing selective volatile antimicrobial compounds," Plant Sci., 165: 913-922.
12. M Corrado, K Rodrigues (2004) "Antimicrobial evaluation of fungal extracts produced by endophytic strains of Phomopsis sp.," J. Basic Microbiol., 44: 157-160.
13. D Ezra, W Hess, G Strobel (2004) "New endophytic isolates of Muscodor albus, a volatile-antibiotic-producing fungus.," Microbiology, 150: 4023-4031.
14. Y Huang, J Wang, G Li, Z Zheng, W Su (2001) "Antitumor and antifungal activities in endophyticfungi isolated from pharmaceutical plants," Immunol. Med. Microbiol., 31: 163-167.
15. K Selim, E-B AA, A-R TM, El-Diwany (2012) "Biology of endophytic fungi," Curr. Res. Environ. Appl. Mycol., 2 (1): 31-82.
16. S Wiyakrutta, N Sriubolmas, W Panphut, N Thongon, KD Kanjana, N Ruangrungsi (2004) "Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants," World J. Microbiol. Biotechnol., 20: 265-272.
17. SJ Higginbotham, AE Arnold, A Ibanez, C Spadafora, PD Coley, TA Kursar (2013) "Bioactivity of fungal endophytes as a function of endophyte taxonomy and the taxonomy and distribution of their host plants," PLoS One, 8: 73192.
18. S Kim, D-S Shin, T Lee, K-B Oh (2004) "Periconicins, two new fusicoccane diterpenes produced by an endophytic fungus Periconia sp. with antibacterial activity," J. Nat. Prod., 67: 448-450.
19. Y Li, Y Song, J Liu, Y Ma, R Tan (2005) "Anti-Helicobacter pylori substances from endophytic fungal cultures," World J. Microbiol. Biotechnol., 21: 553-558.
20. J Liu et al (2004) "Aspergillus fumigatus CY018, an endophytic fungus in Cynodon dactylon as a versatile producer of new and bioactive metabolites," J. Biotechnol., 114: 279-287.
21. P Chomchoen, S Wiyakrutta, N Sriubolmas, N Ngamrojanavanich, D Isarangkul, P Kittakoop (2005) "3-Nitropropionic acid (3-NPA), a potent antimycobacterial agent from endophytic fungi: is 3-NPA in some plants produced by endophytes?," J. Nat. Prod., 68: 1103-1105.
22. I Atmosukarto, U Castillo, W Hess, J Sears, G Strobel (2005) "Isolation and characterization of Muscodor albus I-41.3 s, a volatile antibiotic producing fungus," Plant Sci., 169: 854-861.
23. U Purwanto, F Pasaribu, M Bintang (2014) "Isolasi bakteri endofit dari tanaman sirih hijau (Piper betle L.) dan potensinya sebagai penghasil senyawa antibakteri," Curr. Biochem., 1(1): 51-57.
24. F Uzma, S Chowdappa (2017) "Antimicrobial and antioxidant potential of endophytic fungi isolated from ethnomedicinal plants of Western Ghats, Karnataka," J. Pure Appl. Microbiol., 11: 2.
25. S Feitosa et al (2018) "Antimicrobial activity of crude extracts of endophytic fungi from Oryctanthus alveolatus (Kunth) Kuijt (Mistletoe)," African J. Microbiol. Res., 12 (11): 263-268.
26. AS Nugraha et al (2019) "Antibacterial and anticancer activities of nine lichens of Indonesian Java island," J. Biol. Act. Prod. from Nat., 9 (1): 39-46.
27. H Wagner, S Bladt (1996) Plant Drug Analysis: A thin layer chromatography atlas, 2nd ed. Berlin: Springer-Verlag,.
28. DK Pratoko, FA Wardhani, N Kristiningrum, FA Fajrin, DA Pangaribowo (2018) "Kadar fenolat dan flavonoid total serta kapasitas antioksidan ekstrak etanol dan fraksi jahe merah (Zingiber officinale var. Rubrum)," Al-Kimia, 6(2): 166-177.
29. HY Lai, YY Yau, KH Kim (2010) "Blechnum orientale Linn-a fern with potential as antioxidant, anticancer and antibacterial agent," BMC Complem. Altern. M., 10: 15.
30. RH Patil, MP Patil, VL Maheshwari (2016) Bioactive secondary metabolites from endophytic fungi: A review of biotechnological production and their potential applications, 1st ed.
31. L Panawala (2017) "Difference between gram positive and gram negative bacteria

difference between gram positive and gram negative bacteria stunning images of cells discover how scientists use main difference-gram positive vs gram negative bacteria," Biology (Basel)., 1-13.

32. D Daniels, B Biswas, K Rogers, F Mclaughlin, D Daniels, A Yadav (2015) "Antimicrobial activities of leaf extracts of guava (Psidium guajava L .) on two gram-negative and gram-positive bacteria A," Int. J. Microbiol., 1-8.
33. L Hermawan, Purwanti, UA Dasuki (2017) "Identifikasi senyawa flavonoid dari daun pakis sayur [Diplazium esculentum (retz.) swartz]," 642-650.
34. X Liu, M Dong, X Chen, M Jiang, X Lv, G Yan (2007) "Antioxidant activity and phenolics of an endophytic Xylaria sp. from Ginkgo biloba," Food Chem., 105: 548-554.
35. AN Panche, AD Diwan, SR Chandra (2016) "Flavonoids: an overview," J. Nutr. Sci., 5 (47): 1-15.
36. IY Ningsih, S Zulaikhah, MA Hidayat, B Kuswandi (2016) "Antioxidant activity of various kenitu (Chrysophyllum Cainito L.) leaves extracts from Jember, Indonesia," Agric. Agric. Sci. Procedia, 9: 378-385.
37. M Antony, D Menon, J James, L Dev, K Arun, V Thankamani (2011) "Phytochemical analysis and antioxidant activity of alstonia scholaris," Pharmacogn. J., 3 (26): 13-18.
38. N Al-Jaber, A Awaad, J Moses (2011) "Review on some antioxidant plants growing in Arab world," J. Saudi Chem. Soc., 4 (15): 293-307.
39. TT Cushnie, B Cushnie, AJ Lamb (2014) "Alkaloids: An overview of their antibacterial, antibiotic-enhanching and antivirulence activities," Int. J. Antimicrob. Agents, 44 (5):377-386.
40. D Mabhiza, T Chitemerere, S Mukanganyama (2016) "Antibacterial properties of alkaloid extracts from Callistemon adoensis against Staphylococcus aureus and Pseudomonas aeruginosa," Int. J. Med. Chem.
41. L Othman, A Sleiman, RM Abdel-massih, M Sciences (2019) "Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants," Front. Microbiol.
42. DT Wicklow, S Roth, ST Deyrup, JB Gloer (2005) "A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to Aspergillus flavus and Fusarium verticilioides," Mycol. Res., 109(5): 610-618.
43. A Dalimunthe, APZ Hasibuan, J Silalahi, SF Sinaga, D Satria (2018) "Antioxidant activity of alkaloid compounds from Litsea cubeba Lour.," Orient. J. Chem., 32 (2): 1149-1152.
44. S Sivakrishnan, M Swamivelmanickam (2019) "Journal of Global Pharma Technology Antioxidant Potential , Total Phenolic and flavonoids content of aerial parts of ethanolic extract of Cordia obliqua," J. Glob. Pharma Technol., 11 (8): 5-7.
45. JK Harper, AM Arif, EJ Ford, GA Strobel, JA Porco, DP Tomer (2003) "Pestacin: a 1, 3-dihydro isobenzofuran from Pestalotiopsis microspora possessing antioxidant and antimycotic activities," Tetrahedron, 59: 2471-2476.
46. TT Cushnie, AJ Lamb (2015) "Antimicrobial activity of flavonoids," Int. J. Antimicrob. Agents, 26 (5): 343-356.
47. J Zhang, D Liu, H Wang, T Liu, Z Xin (2015) "Fusartricin, a sesquiterpenoid ether produced by an endophytic fungus Fusarium tricinctum Salicorn 19," Eur. Food Res. Technol., 240: 805-814.
48. E Elfita, M Muharni, MM Rizki (2012) "Isolation of antioxidant compound from endophytic fungi Acremonium sp. from the twigs of Kandis Gajah, Makara," J. Sci., 16: 46-50.

