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Phytochemical Screening and Biological Activities of Fungal *Phyllosticta capitalensis* Derived from *Andrographis paniculata*

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Abstract: The research aims to evaluate the phytochemical constituents and biological activity (antibacterial and antioxidant activity) of the extract of endophytic fungi isolated from the seeds of *Andrographis paniculata*. Isolation of endophytic fungus followed the dilution method with Potato Dextrose Agar (PDA) media. Three single strains of fungi have been isolated from seeds of *A. panicalata*. They were cultivated on the white, black, and red rice media for three weeks, respectively. Extraction of the cultivated fungi with ethyl acetate followed by evaporation yielded the crude extract. The antibacterial and antioxidant activity were evaluated for nine crude extracts of fungi. Data of their biological activities showed that the ethyl acetate extract from fungus SAP-1 on the black rice media was the rich of active compounds. Molecular identification showing the fungus SAP-1 was *Phyllosticta capitalensis*. Further research of isolation and structure elucidation of bioactive compounds from fungal *P. capitalensis* obtained from the seeds of *A. paniculata* will be performed in the future. Interestingly, phytochemical and biological study of extract of endophytic fungus *P. capitalensis* from the seeds of *A. paniculata* is firstly reported in this study.

Keywords: Andrographis paniculata, Antibacterial, Antioxidant, Endophytic fungus, Phyllosticta capitalensis

1. Introduction

Endophytic fungi are microbes that colonize with internal tissues of plants. They do not cause negative effects to their host plants (Jia *et al.*, 2016, Mangayi and Ateba, 2020, Anshar *et al.*, 2021). Chemically, endophytic fungi produced many secondary metabolites where some of them are novel compounds (Riga *et al.*, 2020, Rustamova *et al.*, 2020, Riga *et al.*, 2021). The isolated compounds from endophytic fungi exhibited various biological activities, including anticancer, antibacterial,

antifungal, and antioxidant (Chen *et al.*, 2018, Riga *et al.*, 2019, Rustamova *et al.*, 2020). Previous research indicated that the medicinal plants are the potential source for the host of endophytic fungi. One of the reported medicinal plants is *Andrographis panicualata.*

A. paniculata is known as Sambiloto in Indonesia and widely used as traditional medicine, including diabetes, fever, diarrhea, skin diseases, flatulence, colic, and influenza (Nyeem *et al.*, 2017, Utaminingrum *et al.*, 2020). This plant belongs to Acanthaceae family. *A. paniculata* reported to yield various secondary metabolites with diverse structures and biological activities (Reddy *et al.*, 2003, Sukesh *et al.*, 2011). Some of the reported bioactivities were antibacterial, antifungal, antioxidant, and anticancer (Rajagopal *et al.*, 2003; Rao *et al.*, 2004; Chao *et al.*, 2010; Akbar, 2011; El Ouadi *et al.*, 2017; Mussard *et al.*, 2020). This data indicated that *A. panicaluta* is the potential host for the growth of endophytic fungi.

Previous study indicated that an endophytic fungus, *Xylaria* sp., obtained from the flowers of *A. paniculata* had antibacterial activity against some pathogenic bacteria (Suryelita *et al.*, 2021). Ethyl acetate extract of *Xylaria* sp. inhibited the growth of three tested bacteria. Furthermore, the MIC values of this extract were 25 μg/mL against *E. coli*, 25 μg/mL against *S. pyogenes*, and 12.5 μg/ml against *S. aureus*. The result showing fungal *Xylaria* sp. derived from the flowers of *A. paniculata* is the potential source for producing antibacterial compounds. The other study reported that some endophytic fungi isolated from the roots, leaves, and stems of *A. paniculata* showed antibacterial activity (Shang, 2016). A new compound (benzochromen derivative) from a fungus obtained from the leaves of *A. paniculata* inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, and *S. typhi* (Munawar *et al.*, 2015). Interestingly, phytochemical, and biological studies of endophytic fungi colonizing with the seeds of *A. paniculata* have not been reported. The study aims to evaluate the chemical composition of endophytic fungus from the seeds of *A. paniculata*. We are also evaluation the antibacterial and antioxidants activities of the extract from the fungal *P. capitalensis*.

2. Materials and Methods

2.1 Sample Preparation

In the current study, we used the seeds of *A. paniculata* collecting from Padang, West Sumatera, Indonesia on March 2022. An hour after collection of the seeds, isolation of endophytic fungi was carried out in laboratory of Chemistry Department, Universitas Negeri Padang.

2.2 Isolation of Endophytic Fungi

Procedure of isolation of endophytic fungi from the seeds of *A. paniculata* followed the previous study (Suryelita *et al.*, 2021). Ethanol 70% (45 s), NaClO 3.5% (30 s), and sterile aquadest (30 s) were used for sterilization of fresh seed of *A. paniculata*. As a negative control, the seed was placed on the PDA media for 5 s. Then, it was cut into 0.2x0.2 cm. The internal part of the seed was inoculated on the sterile PDA media followed by incubation at 28 ^{0}C. Each isolate of endophytic fungi from the seed of *A. paniculata* was sub-cultured to a new PDA medium to obtain single strain of fungus.

2.3 Cultivation and Extraction of Fungus

Each isolate of endophytic fungus was cut into 2x2 cm and cultivated on 250 mL Erlenmeyer flask (25 g rice/30 mL aquadest) following the reported study (Suryelita *et al.*, 2021). Cultivation of

endophytic fungus was fermented on three media (white, red, and black rice media) for every three days. Then, the cultivated endophytic fungus was extracted three times with ethyl acetate. All crude extract of each endophytic fungus were investigated for their phytochemical constituents. The biological activities including antibacterial and antioxidant activities of all extract were also investigated.

2.4. Evaluation of Antibacterial Activity

2.4.1 Inhibition Zone of Fungal Extract

Inhibition zone of three crude extracts were evaluated following the previous procedure (the disc diffusion method) (Anshar *et al.*, 2021). The crude extracts were dissolved in DMSO. The tested bacteria in this study were *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*. The positive control was amoxicillin. MHA media (15 mL) were poured into Petri dishes. Then, the MHA media were inoculated with tested bacteria. 6 mm sterile paper disc were placed on the MHA media. 20 μL of the serial concentrations (1%, 3%, and 5%) of each crude extract were dropped on the paper disc. After incubation for 24 h, the zone of inhibition where there is no growth of bacteria was measured and recorded. The inhibition zone of each crude extract was performed three times. Statically, the zone of inhibition was calculated and presented as mean ± standard deviation.

2.4.2 Minimum Inhibitory Concentration (MIC) of Fungal Extract

Crude extract was assayed for their MIC value following reported method (Suryelita *et al.*, 2021). Tested bacteria were cultured for 24 h and then placed on the wells. Serial concentrations (400 to 3.125 μg/mL) of each extract were added and incubated for 24 h at 37 ^{0}C. The lowest concentration which inhibited any growth visible to the naked eye after incubation was called as the minimum inhibitory concentration (MIC).

2.5. Antioxidant Activity

The free radical scavenging activity of crude extract was determined following 1.1-diphenyl-2-picryl-hydrazil (DPPH) method (Pandey and Negi, 2018). 0.5 mL DPPH solution in ethanol (0.2 mM) was mixed with extract (2.5 mL). Then, the mixture was allowed to stand for 30 min. The absorbance of mixture and blank samples was read at 517 nm. Higher free radical scavenging activity was indicated with lower absorbance of the reaction mixture. The results of DPPH method were expressed as IC_{50} defined as the quantity of antioxidant needed to lower the radical to 50%.

2.6. Phytochemical Screening

The chemical constituents of all crude extract (*n*-hexane, ethyl acetate, and methanol) were tested based on the previous study (Nounah *et al.*, 2019, Suryelita *et al.*, 2021). This step aims to identify alkaloids, phenolic compounds, terpenoids, and steroids in all crude extract. The concentration of tested crude extract was 2%. The presence of them was confirmed by the formation of precipitate or change of color.

2.6.1. Test of Steroids and Terpenoids

Each extract of endophytic fungus was dissolved and shaken in ammonia-chloroform and 2N H_2SO_4. This step formed two layers. The bottom layer was evaporated and mixed with anhydrous

acetic acid and H_2SO_4 p.a. Steroids give green-blue coloring with this reaction, while terpenoids give red coloring.

2.6.2. Test of Alkaloids

The alkaloids were evaluated by three reagents (Dragendorff, Mayer, and Wagner reagent). The top layer in steroids test was added with each reagent in every test tube. Alkaloids give a brown precipitate, a white precipitate, and an orange precipitate, respectively.

2.6.3. Test of Phenolic Compounds

2 mL each extract and 1 mL $FeCl_3$ 1% were added. The color changes to pink in the presence of phenolic compounds.

2.7. Determination of Total Alkaloids

Dried extract was dissolved 2N HCl followed by shaking and filtering. The filtrate was washed by chloroform. The aqueous layer was added ammonia to produce a solution to free alkaloid bases. Then, alkaloids were extracted with chloroform. The combined chloroform extract was filtered using anhydrous sodium sulfate and evaporated under vacuum. The percentage of alkaloid was calculated as w/w of mycelia of fungi.

2.8. Determination of Total Phenolics

The total polyphenol content (TPC) was calculated using gallic acid as standard with spectrophotometer, according to the previous method (Pandey and Negi, 2018). Crude extract (1 mL) in test tube was added with 2 mL of 20% sodium carbonate solution and 1 mL of diluted Folin-Ciocalteau reagent. After 40 minutes, absorbance was measured against reagent blank in 760 nm using UV/Vis spectrophotometer. The gallic acid standard curve was prepared (10-80 µg). Total concentration phenolic was defined as mg GAE/g of extract calculated from standard curve.

2.9. Molecular Identification of Fungus

Molecular identification of fungus was carried by three steps i.e., DNA extraction, DNA amplification and sequencing, according to the previous method (Riga *et al.*, 2019). DNA extraction was carried out by using 5.8S rDNA. The DNA was extracted from sample by using i-genomic BYF DNA Extraction Mini Kit. The DNA amplification was conducted by using go-Taq Master Mix with eucaryotes primer which wa 5.8S rDNA. This primer consists of ITS 4: 5'-TCC TCC GCT TAT TGA TAT GC-3' and ITS 5: 5'-GGA AGT AAA AGT CGT AAC AAG G-3'. Furthermore, the PCR product was sequenced with an automated DNA sequencer. The sequencing data were trimmed and assembled with the BioEdit program and then blasted at National Center for Biotechnology Information (NCBI). Constructing the phylogenetic tree following neighbor-joining method with a bootstrap value of 500 replication used MEGA X software.

3. Results and Discussion

Previous chemical study *of A. paniculata* showed that many secondary metabolites (terpenoids, steroids, alkaloids, and flavonoids) have been isolated. Some of them were the new compounds. The isolated compounds exhibited bioactivities, including antibacterial and antioxidant indicating *A. panicaluta* is the source of bioactive compounds (Sukesh *et al.*, 2011, Utaminingrum *et al.*, 2020).

Another source to produce the bioactive compounds from *A. paniculata* is their endophytic fungi. An endophytic fungus identified as *Xylaria* sp. has been reported from the twigs of *A. paniculata*. The crude extract of *Xylaria* sp. having broad antibacterial activity with the MIC values of 12.5 and 25 μg/mL (Suryelita *et al.*, 2021). To continue our work on bioactive compounds from endophytic fungi associated with *A. paniculata*, we have investigated the chemical and biological properties of fungus from the seeds of *A. paniculata*.

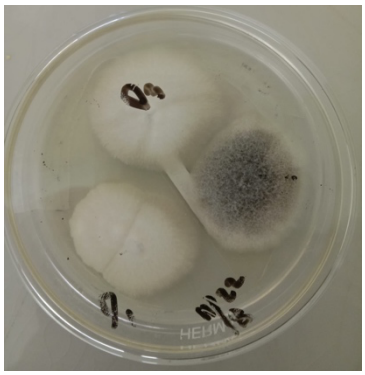

Figure 1. Fungal SAP-1, SAP-2, and SAP-3 (c) from the seeds of *A. paniculate*

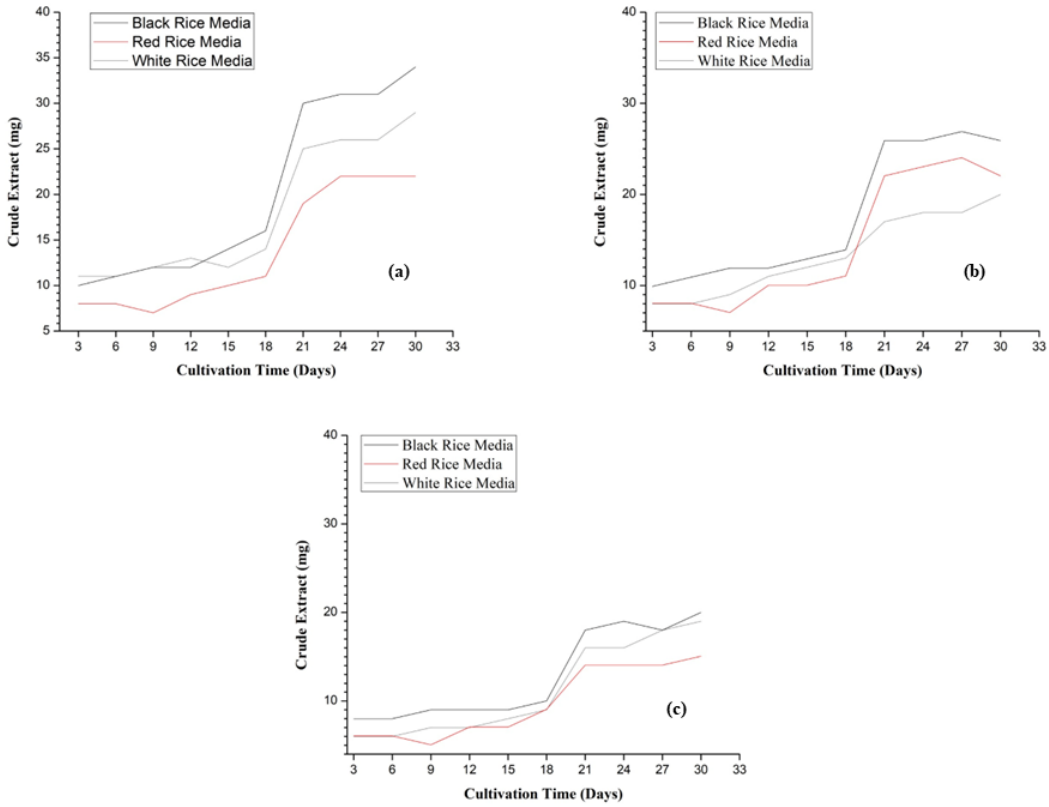

Figure 2. Optimum cultivation time of fungus of SAP-1 (a), SAP-2 (b), and SAP-3 (c) on three rice media

Three isolates of fungi labeled as SAP-1, SAP-2, and SAP-3 (Figure 1) were obtained from the seeds of *A. paniculata*. The time of optimum cultivation of them were identified by analyzing the weight of crude extract in three media (white, red, and black rice media). Figure 2 showed that the

optimum time of all isolated fungi to produce the secondary metabolites was 21 days (three weeks). These results revealing the third week was the stationary period of growing fungi. The stationary period was defined as the period where the size of a population of fungi remains constant and biomass of the fungi does not remain unchanged (Kjer *et al.*, 2010, Nouna *et al.*, 2019). In this period, fungi produce secondary metabolites significantly due to the accumulation of enzymes responsible for producing compounds (Li *et al.*, 2016). Furthermore, endophytic fungi yielded the highest mass of crude extract on black rice media.

Table 1. Inhibition zones of fungus SAP-1

Media	Concentration	*S. aureus*	*S. pygoenes*	*E. coli*
White rice media	1%	3.43 ± 0.06	-	6.13 ± 0.21
	3%	7.20 ± 0.17	6.40 ± 0.26	9.87 ± 0.25
	5%	8.87 ± 0.06	9.37 ± 0.12	12.47 ± 0.21
Red rice media	1%	-	-	-
	3%	4.47 ± 0.21	3.93 ± 0.15	5.60 ± 0.26
	5%	6.23 ± 0.06	7.33 ± 0.15	8.40 ± 0.53
Black rice media	1%	4.80 ± 0.26	5.30 ± 0.20	6.70 ± 0.17
	3%	8.57 ± 0.25	9.20 ± 0.30	10.30 ± 0.17
	5%	10.03 ± 0.21	11.10 ± 0.26	12.97 ± 0.12
Positive Control		12.97 ± 0.21	10.3 ± 0.21	15.2 ± 0.36
Negative Control		-	-	-

*All the values are mean ± SD of three parallel measurements.

Table 2. Inhibition zones of fungus SAP-2

Media	Concentration	*S. aureus*	*S. pygoenes*	*E. coli*
White rice media	1%	3.23 ± 0.15	-	4.03 ± 0.32
	3%	5.40 ± 0.26	5.83 ± 0.31	7.20 ± 0.10
	5%	7.43 ± 0.46	8.37 ± 0.21	10.37 ± 0.25
Red rice media	1%	-	-	-
	3%	-	2.07 ± 0.23	4.47 ± 0.21
	5%	4.10 ± 0.26	6.23 ± 0.06	8.03 ± 0.21
Black rice media	1%	3.87 ± 0.21	3.50 ± 0.26	5.17 ± 0.42
	3%	6.30 ± 0.36	6.93 ± 0.15	9.27 ± 0.47
	5%	8.47 ± 0.35	9.43 ± 0.25	11.27 ± 0.40
Positive Control		12.97 ± 0.21	10.3 ± 0.21	15.2 ± 0.36
Negative Control		-	-	-

*All the values are mean ± SD of three parallel measurements.

The nine-crude extracts from three fungi were evaluated for their antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli* with amoxicillin as a positive control and DMSO as a negative control. The antibacterial activity was presented by zones of inhibition around the disks. All of them were active against tested bacteria on several concentrations. The results of the inhibition zones of the crude extract are presented in Table 1, 2, and 3. Data of the zones of inhibition in Table 1, 2, and 3 indicated that the higher the concentration, the larger the zone of inhibition. The increase of antibacterial activity in higher concentration of extract was caused by the large number of active compounds in the extract (Raja *et al.*, 2011, Khan *et al.*, 2013). The crude extract of fungus SAP-1 on black rice media exhibited the greatest growth inhibition at a concentration of 5% against all tested bacteria (inhibition zones of

10.03 ± 0.21 mm against *S. aureus*, 11.10 ± 0.26 mm against *S. pygoenes*, and 12.97 ± 01.2 against *E. coli*).

Table 3. Inhibition zones of fungus SAP-3

Media	Concentration	*S. aureus*	*S. pygoenes*	*E. coli*
White rice media	1%	-	-	3.13 ± 0.12
	3%	-	4.47 ± 0.21	5.70 ± 0.20
	5%	7.47 ± 0.32	7.90 ± 0.44	7.87 ± 0.21
Red rice media	1%	-	-	-
	3%	-	-	-
	5%	5.23 ± 0.15	4.97 ± 0.21	6.50 ± 0.10
Black rice media	1%	-	-	3.90 ± 0.17
	3%	6.43 ± 0.21	5.83 ± 0.31	6.13 ± 0.42
	5%	8.47 ± 0.21	7.87 ± 0.12	8.23 ± 0.15
Positive Control		12.97 ± 0.21	10.3 ± 0.21	15.2 ± 0.36
Negative Control		-	-	-

*All the values are mean ± SD of three parallel measurements.

Minimum inhibitory concentration (MIC) of the crude extract of fungus SAP-2 on black rice media was calculated using the agar dilution method with amoxicillin as a positive control. The MIC data of fungus SAP-2 are summarized on Table 4. Furthermore, the crude extract of fungal SAP-2 cultivated on black rice media displayed MIC values of 25 μg/ml against *S. aureus*, 50 μg/mL against *S. pyogenes*, and 12.5 μg/mL against *E. coli*. They were only 2 and 4 times higher than a positive control (amoxicillin).

Table 4. MIC values of fungal SAP-1 on black rice media

Bacteria	MIC (μg/mL)	
	Crude Extract	Positive Control
S. aureus	25	12.5
S. pygones	50	12.5
E. coli	12.5	6.25

In addition, the antioxidant activity of crude extracts of isolated fungi (SAP-1, SAP-2, and SAP-3) on various rice media was also evaluated following DPPH method. Antioxidant activity of the extracts was expressed as IC_{50} defined as the concentration of extract to decrease the DPPH radical by 50% (Oussaid *et al.*, 2020). At first the color of DPPH is violet, then radical reduction causes discoloration from violet (DPPH•) to yellow (DPPH-H). The values of IC_{50} of crude extracts were determined through a linear regression equation. Data of them are presented in Table 5. The crude extract of fungal SAP-1 on black rice media showed DPPH radical scavenging activity with the IC_{50} value of 45.73 μg/mL as compared to ascorbic acid (IC_{50} value of 37.92 μg/mL). Data of inhibition zones, MIC as well as IC_{50} values of extract of fungus SAP-1 exhibited strong antibacterial and antioxidant activity so that it has the potential to be a good candidate for the source of bioactive compounds. The biological activities of extract from all fungi, especially fungal SAP-1, indicated that the fungi are rich of secondary metabolites. Results of the chemical screening of fungal extracts revealing the existence of alkaloids, terpenoids, steroids, and phenolic compounds are shown in Table 6.

Table 5. Free radical-scavenging capacities (IC_{50}) of fungal extract

Fungi	Media	IC_{50} (μg/mL)
SAP-1	White rice media	62.89
	Red rice media	109.45
	Black rice media	45.73
SAP-2	White rice media	123.48
	Red rice media	145.94
	Black rice media	95.67
SAP-3	White rice media	>200
	Red rice media	>200
	Black rice media	>200
Reference	Ascorbic acid	37.92

Table 6. Phytochemical screening of fungal extract

Fungi	Media	Steroids	Terpenoids	Alkaloids	Phenolic Compounds
SAP-1	White rice media	+	+	+	+
	Red rice media	+	+	+	+
	Black rice media	+	++	++	++
SAP-2	White rice media	+	+	+	+
	Red rice media	+	+	+	+
	Black rice media	++	+	+	+
SAP-3	White rice media	+	+	-	-
	Red rice media	+	+	-	-
	Black rice media	+	+	-	-

(+) = present; (++) = abundant; (-) = absent

Quantitative analysis of fungal extracts was displayed in Table 7. The data in Table 7 showed that the highest total phenolics and alkaloid was fungal SAP-1 on black rice media (311.99 ± 1.17 mg GAE/g of extract and 0.65 ± 0.11 g %, respectively) followed by fungal SAP-1 on red rice media (125.52 ± 1.06 mg GAE/g of extract and 0.43 ± 0.09 g %, respectively).

The antibacterial activity of the crude extract of endophytic fungi isolated from the seeds of *A. paniculata* may be attributed to the presence terpenoids, steroids, alkaloid, and phenolic compounds therein. Terpenoids disrupted the membrane formation to inhibit the tested bacteria. The mechanism of alkaloids as antibacterial inhibiting is disrupting the peptidoglycan in bacterial cells that will make the membrane will not be perfectly formed (Othman *et al.*, 2019). Furthermore, phenolic compounds inhibited the growth of bacteria through the inhibition of the reverse transcription enzymes, and DNA topoisomerase (Bouarab-Chibane *et al.*, 2019). In addition, the antioxidant properties of their crude extracts due to the presence of phenolics compounds (Sharma *et al.*, 2018). The highest DPPH radical scavenging activity was the fungal SAP-1 on black rice media where the phytochemical screening (Table 6 and 7) showed the abundance of phenolic compounds compared to other fungi and other rice media.

Phylogenetic tree of fungal isolate of SAP-1 (Figure 3) showed that SAP-1 was *P. capitalensis* (Figure 4) with precent homology of 100 %. This phylogenetic tree was constructed by neighbore-joining method with bootsrap value of 500. SAP-1 was clustered with *P. capitalensis* MT649668.1 from Southest Florida (Urbina *et al.*, 2021). The analysis of genetic difference between SAP-1 and *P. capitalensis* MT649668.1 can be observed on Table 8. Based on Kimura2-parameter model,

there was no difference between them or percent similarity of 100 %. Meanwhile, the genetic difference among the other fungi of 1.7%.

Table 7. Quantitative analysis of fungal extracts

Fungi	Media	Alkaloids g % (w/w)	Phenolics (mg GAE/g extract)
SAP-1	White rice media	0.19 ± 0.05	63.98 ± 1.31
	Red rice media	0.43 ± 0.09	125.52 ± 1.06
	Black rice media	0.65 ± 0.11	311.99 ± 1.17
SAP-2	White rice media	0.23 ± 0.10	5.84 ± 0.38
	Red rice media	0.02 ± 0.02	39.98 ± 1.44
	Black rice media	0.26 ± 0.07	77.32 ± 2.11
SAP-3	White rice media	-	-
	Red rice media	-	-
	Black rice media	-	-

*All the values are mean ± SD of three parallel measurements.

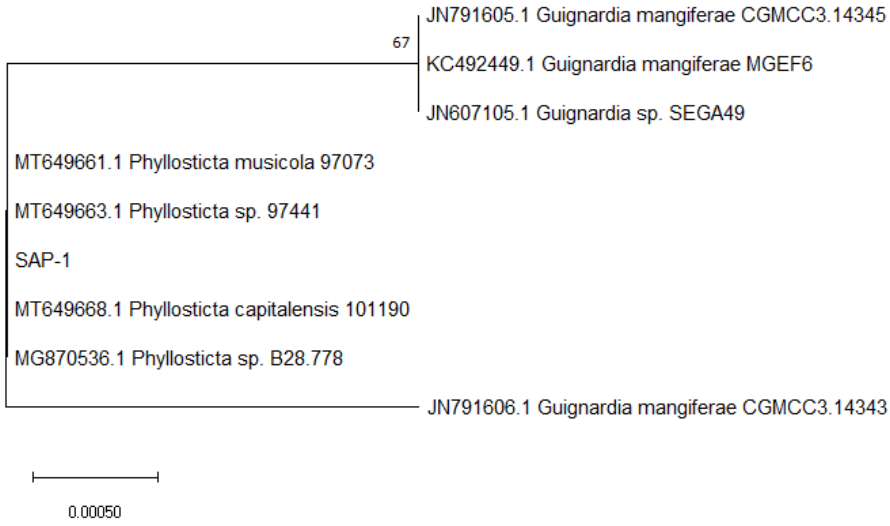

Figure 3. The phylogenetic tree of SAP-1 derived from *A. paniculata* by using neighbor-joining method with the bootsrap value of 500

Table 8. Estimation of evolutionary divergence between sequences using the Kimura 2-parameter model

	Accession Number	1	2	3	4	5	6	7	8	9
1	SAP-1									
2	MT649668.1	0.000								
3	JN791606.1	0.00169	0.00169							
4	JN607105.1	0.00169	0.00169	0.000						
5	JN791605.1	0.00169	0.00169	0.000	0.000					
6	KC492449.1	0.00169	0.00169	0.00169	0.00169	0.00169				
7	MT649663.1	0.00169	0.000	0.00169	0.00169	0.00169	0.000			
8	MG870536.1	0.00169	0.000	0.00169	0.00169	0.00169	0.000	0.000		
9	MT649661.1	0.00169	0.000	0.00169	0.00169	0.00169	0.000	0.000	0.000	

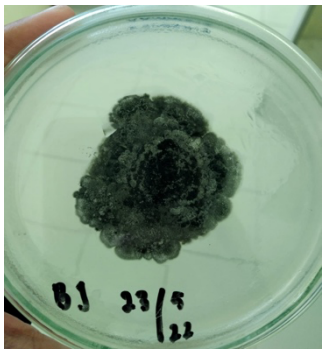

Figure 4. Fungal SAP-1 (*P. capitalensis*) isolated from the seeds of *A. paniculata*

Previous study indicated that fungal genus *Phyllosticta* was the source of bioactive compounds. 49 isolates of fungal *Phyllosticta* were isolated from several host inhibiting at least one of the testing bacteria (Chukeatirote *et al.*, 2015). Another study showed that *P. capitalensis*, *P. citriasiana*, and *P. cordylinophila* could inhibit the growth of *E. coli*, *Bacillus cereus*, and *Pseudomonas aeruginosa* with the inhibition zones of 7 to 11.5 mm (Taylor *et al.*, 2013). Furthermore, A meroterpene, guignardone A, yielded from *P. capitalensis* derived from Chinese mangrove *Bruguiera sexangula* showed the antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with MIC values of 25 μg/mL (Xu *et al.*, 2021). A new compound (phenguignardic acid butyl ester) as well as a known compound (peniisocoumarin G) from the citrus phytopathogen *P. citricarpa* displayed antibacterial activity against S. aureus (MIC values of 111 and 333 μg/mL, respectively) (Savi *et al.*, 2019).

The crude extract of fungal genus *Phyllosticta* was also reported for their antioxidant activity. The ethyl acetate extract of *Phyllosticta* sp associated with the leaves of *Hippobroma longiflora* (L.) G showed antioxidant activity with IC_{50} value of 28.50 μg/mL (Widjajanti *et al.*, 2022). Endophytic fungus identified as *P. capitalensis* isolated from the leaves of *Berberis aristata* DC exhibited 83% DPPH radical scavenging at concentration of 1000 μg/mL (Sharma *et al.*, 2018). Current research and previous data of the antibacterial and antioxidant activities of crude extract as well as isolated compounds from fungus *Phyllosticta* are the references in further studies in the isolation, structure elucidation and bioactivities of pure compounds from *P. capitalensis* obtained from the seeds of *A. paniculata*.

Conclusion

Endophytic fungus *P. capitalensis* has been isolated from the seeds of *A. paniculata*. Fungal *P. capitalensis* cultivated on the black rice media inhibited the growth of three tested bacteria with the MIC values of 12.5 to 50 μg/mL. It also inhibited 50% of the DPPH radical at a concentration of 12.05 μg/mL. This research indicated that fungal *P. capitalensis* from the seeds of *A. paniculata* displayed antioxidant and antimicrobial activity. These results will be useful as the preliminary study of drug discovery in pharmaceutical industry.

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Disclosure statement:

Conflict of Interest: The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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