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POTENTIAL OF ENDOPHYTIC FUNGI DERIVING FROM ASIATIC PENNYWORTH TO PRODUCE ANTIOXIDANTS

Potensi Jamur Endofit Tanaman Pegagan sebagai Penghasil Antioksidan

Nani Radiastuti[a], Dwi Ningsih Susilowati[b], Nurhasni[a], Lisca Puji Rustanti[a], and Ika Roostika Tambunan[b]

[a]*Department of Biology, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta, Jalan Ir. H. Djuanda No. 90, Ciputat, Banten, Indonesia*
[b]*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development Jalan Tentara Pelajar 3A Bogor, West Java, Indonesia*

** Correspondence author: d_nengsusi@yahoo.com*

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ABSTRACT

Asiatic pennyworth is a medicinal plant that contains triterpenoids, saponin, flavonoids, and tannins which possess antioxidants. Endophytic fungi from the plant could produce a similar compound; therefore, antioxidants could be made in the laboratory if the fungi are isolated. This study aimed to evaluate the potential of endophytic fungi isolated from Asiatic pennyworth to produce antioxidants. The study used 34 endophytic fungal isolates from Asiatic pennyworth accessions of Malaysia (17 isolates) and Bengkulu, Indonesia (17 isolates) collected by the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development. The fungi were propagated in a potato dextrose broth medium, then mycelia mats and filtrates were separated and then freeze-dried. The antioxidant activities were first tested with 1,1-diphenyl-2-picrylhydrazyl (DPPH) using thin layer chromatography (TLC), then UV-Vis spectrophotometry λ517 nm with five variations of concentration. Results showed all 34 fungal isolates have antioxidant activities based on a yellowish-white color change after applying 0.002% DPPH solution of the TLC method and IC_{50} value of the UV-Vis spectrophotometer. The highest antioxidant activity was shown by *Aspergillus austroafricanus* MB 1 (IC_{50} = 12.08 ppm) from Bengkulu accession and *A. oryzae* MM 13 (IC_{50} = 10.29 ppm) from Malaysia accession. *A. austroafricanus* MB 1 produced more antioxidant compounds (seven) than *A. oryzae* MM 13 (six). The antioxidant compounds produced by both endophytic fungi included in the group of flavonoids, fatty acids, and carboxylic acids. The research implies that *A. austroafricanus* MB 1 and *A. oryzae* MM 13 could be further developed as sources of antioxidants.

[Keywords: *Aspergillus austroafricanus, Aspergillus oryzae,* Bengkulu, Malaysia, pennyworth accessions]

ABSTRAK

Pegagan merupakan tanaman obat yang mengandung triterpenoid, saponin, flavonoid, dan tanin yang memiliki potensi sebagai antioksidan. Jamur endofit dari tanaman ini dapat menghasilkan senyawa metabolit sekunder yang sama dengan tanaman inangnya. Oleh karena itu, antioksidan dapat diproduksi di laboratorium jika jamur endofit tersebut dapat diisolasi. Penelitian ini bertujuan untuk mengevaluasi potensi isolat jamur endofit asal tanaman pegagan sebagai penghasil antioksidan. Penelitian menggunakan 34 isolat jamur endofit pegagan aksesi Malaysia (17 isolat) dan Bengkulu, Indonesia (17 isolat) koleksi Balai Besar Penelitian dan Pengembangan Bioteknologi dan Sumberdaya Genetik Pertanian. Jamur ditumbuhkan dalam medium potato dekstrosa cair. Selanjutnya, filtrat dipisahkan dan dikeringbekukan. Aktivitas antioksidan isolat jamur endofit diskrining awal dengan metode 1,1-difenil-2-pikrilhidrazil (DPPH) dengan kromatografi lapis tipis (KLT) dan dilanjutkan dengan uji antioksidan dengan spektrofotometri UV-Vis λ517 nm dengan lima variasi konsentrasi. Sebanyak 34 isolat jamur menunjukkan adanya aktivitas antioksidan yang ditandai dengan perubahan warna menjadi putih kekuningan setelah disemprot larutan DPPH 0,002% di sekitar fraksi senyawa. Aktivitas antioksidan tertinggi ditunjukkan oleh Aspergillus austroafricanus *MB 1 (IC50 = 12,08 ppm) dari aksesi Bengkulu dan* A. oryzae *MM 13 (IC50 = 10,29 ppm) dari aksesi Malaysia.* Aspergillus austroafricanus *MB 1 menghasilkan lebih banyak komponen senyawa aktif (7 komponen) daripada* A. oryzae *MM 13 (6 komponen). Senyawa antioksidan yang dihasilkan kedua jamur endofit tersebut termasuk ke dalam kelompik flavonoid, asam lemak, dan asam karboksilat. Hasil penelitian ini menunjukkan bahwa* A. austroafricanus *MB 1 dan* A. oryzae *MM 13 asal tanaman pegagan aksesi Bengkulu dan Malaysia dapat dikembangkan lebih lanjut sebagai sumber antioksidan.*

[Kata kunci: *Aspergillus austroafricanus, Aspergillus oryzae,* Bengkulu, Malaysia, pegagan]

INTRODUCTION

Asiatic pennyworth (*Centella asiatica* L.) is a medicinal plant producing bioactive compounds, such as asiaticosides, saponins, flavonoids, tannins, phytosterols, and essential oils and volatile oil. This plant's bioactive content has extensively been used for a major stockpile for pharmaceuticals (Shetty et al. 2008; George et al. 2010; Rao et al. 2007). The Asiatic pennyworth has a symbiosis with endophytic fungi that live in the plant tissues without causing harmful effects (Strobel and

Daisy 2003). Endophytes are capable of producing metabolites that may or may not be present in the host plants. Some endophytic fungi have specific relationships with host plants that can significantly affect the production of medicinal compounds from their host (Jia et al. 2016).

Previous researches have identified several bioactivities, such as extracellular enzymes (asparaginase, amylase, cellulase, pectin, protease, glucanase, laccase) producer, antimicrobial, and antioxidant activity produced by endophytic fungi from Asiatic pennyworth (Susilowati et al. 2020). It has been found that the endophytes produce a significant amount of antioxidants, which prevent oxidative damage to cellular components. Antioxidants obtained from endophytic fungi possess different antioxidants comparable or even better in their potency with standard antioxidants. Pestalotiopsis microspora produced 1,3-dihydroisofuran, pestacin, and isopestacin which exhibited antifungal and antioxidant activity 11 times greater than the vitamin E derivative Trolox (Harper et al. 2003). Aspergillus oryzae isolated from Asiatic pennyworth roots in India produced several antioxidants (Nath et al. 2014). It means having microbes, such as endophytic fungi, that could produce antioxidants are essential for the more efficient production (easier fermentation set up and faster production) of antioxidants in the laboratory compared to higher plants and animals.

Endophytic fungi from Asiatic pennyworth have been collected in the Biogen Culture Collection (Biogen CC) of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Indonesian Agency for Agricultural Research and Development, but their potential to produce antioxidants have not yet been studied. The study aimed to evaluate the potential production of antioxidants from endophytic fungi isolated from Asiatic pennyworth accessions of Bengkulu and Malaysia.

MATERIALS AND METHODS

Fungi Source

Thirty-four cultures of already identified endophytic fungi isolated from various parts of Asiatic pennyworth accessions, i.e., leaves, roots, petioles, and stolon, were used in the study; 17 from Bengkulu, Indonesia, and 17 from Malaysia, respectively (Radiastuti et al. 2019). All the fungal isolates are kept in the Biogen Culture Collection of ICABIOGRAD, Bogor, Indonesia.

Cultivation

The pure fungal isolates were inoculated into 200 ml of sterilized potato dextrose broth (PDB) medium in a 500 ml Erlenmeyer flask. The culture was incubated for three weeks under the static condition at 27 ºC in a dark room for secondary metabolite production (Bungihan et al. 2013). After the incubation, the fungal culture was separated into mycelia mat and culture filtrate using Whatman No. 1 filter paper. After that, the mycelia mat and culture filtrate was freeze-dried. The dry crude of mycelia mat and fungal filtrate obtained were analyzed for antioxidant potential (Cui et al. 2015).

Extraction

Fungal extraction was carried out by maceration with methanol (PA grade) two times at a ratio of 1:1, and each immersion was performed for 24 hours. Simultaneously, the maceration stage was carried out by soaking the mixture of filtrate and fungal biomass in solvent for 24 hours. The results were concentrated using a rotary evaporator at 50 °C with a speed of 100 rpm, and then the crude extract was weighed (Radiastuti 2015).

Antioxidant Activity

Testing Using Thin Layer Chromatography

The extract was taken 1 mg, then dissolved with 10 ml of methanol p.a and stirred until it dissolved. Subsequently, 10 µl of filtrate was placed on the thin layer chromatography (TLC) plate of silica $F_{254,}$ and vitamin C solution was used as a positive control. The F_{254} TLC plate was inserted into the chamber containing the mobile phase of chloroform, methanol, and distilled water (6:4:1) (Praptiwi et al. 2020). The TLC plate was sprayed with 0.002% DPPH solution, and the observed spots were used to calculate the retardation factor (Rf) with the following formula:

$$Rf = \frac{\text{Compound mileage}}{\text{Solvent mileage}}$$

The plates sprayed with 0.002% DPPH solution were compared with those eluted with vitamin C by obtaining the Rf value that showed a color change in the compound fraction of endophytic fungal isolates of Bengkulu and Malaysia accessions.

Testing with a UV-Vis Spectrophotometer

The dilution series was made from a stock solution of fungal extract (50 mg fungal extract dissolved with 50 ml

of methanol p.a) at five variations of concentration (20, 40, 60, 80, and 100 ppm). Each sample was taken as much as 6 ml and added with 6 ml of 0.002% DPPH solution, then left for 30 minutes in a dark room. The control was vitamin C solution in methanol p.a with various concentrations (1, 2, 3, 4, and 5 ppm). The absorbance measurement was performed three times using a UV-Vis spectrophotometer λ517 nm (Akar et al. 2017).

Calculation of Inhibitor Concentration Value

The antioxidant activity test results for inhibitory concentration (IC_{50}) were made in a curve using the Microsoft Excel 2016 program. The % inhibition of fungal extract towards DPPH solution was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \text{ x } 100\%$$

The IC_{50} value was calculated based on the % inhibition value of various concentrations (X-axis) against a % inhibition of DPPH reduction activity (Y-axis) and presented in a linear regression curve plotted. Meanwhile, the IC_{50} value was determined from the linear regression equation Y = ax + b, with Y = 50 (Akar et al. 2017). The IC_{50} antioxidant activity values were categorized as very strong (50 ppm), strong (50–100 ppm), moderate (100–150 ppm), weak (150–200 ppm), and very weak (>200 ppm) (Molyneux 2004).

Active Compound Analysis

The fungal extract showing the lowest IC_{50} value for each accession of Asiatic pennyworth from Bengkulu and Malaysia was further analyzed in the gas chromatography-mass spectrometer (GC-MS) SHIMADZU QP 2010. Before use, the GC-MS was first calibrated by injecting 1 µl of test mix. A total of 1000 µl of extract samples were injected into GC with conditions of oven column temperature of 40 ˚C, injector temperature of 210 ˚C, and split ratio of 100, and MS conditions of ion source temperature of 270 ˚C and interface temperature of 230 ˚C. The results showed that the compounds contained in each sample matched with the references of WILEY8.lib and NIST14.lib (Kanjana et al. 2019).

Data Analysis

The TLC antioxidant activity test results and the IC50 calculation values were analyzed descriptively. The data analysis encompased simple and informative summary for each observed parameter.

RESULTS AND DISCUSSION

Antioxidant Activity by Thin Layer Chromatography

All the 34 fungal isolates produced antioxidant compounds based on the spots on the TLC plates after adding a 0.002% DPPH solution. The Rf values of the TLC spots created by the fungal isolates varied from 0.01 to 1.0, whereas the Rf value of vitamin C (the positive control) was 0.62. The number of spots and their Rf values representing the antioxidant activity of the endophytic fungal isolates of Bengkulu accession were shown in Table 1.

The Rf values of the spots that had the same or parallel with standard (vitamin C) indicated that the samples have the same bioactive compound (Akar et al. 2017). Based on the number of spots produced, five endophytic fungal isolates of Bengkulu accessions, namely *A. austroafricanus* MB1, *F. falciforme* 2 MB8, *C. tabaci* 2 MB18, *C. cornigerum* MB20, and *M. gentianae* MB21, produced three spots or antioxidant compounds, while the other 12 isolates produced two spots (Table 1). Since the fungal isolate of Malaysia accessions produced more antioxidant compounds, (four TLC spots), it means that fungal isolates of Malaysia accessions had more antioxidant compounds than that of Bengkulu accessions (Table 2). Our results were comparable with *Aspergillus* isolated from *Mangifera casturi* producing five spots (Nuraini et al. 2019)Based on Table 2, two endophytic fungal isolates, i.e., *F. striatum* MM20 and *C. tabaci* 2 MM23, produced the highest antioxidant activities (four spots). Crude extract of *Coletotrichum karstii* MM2, *C. siamense* MM9, *P. stereoides* MM12, *C. gigasporum* MM14, and *P. capsulatum* MM15 had an reported by (Rahmaniah et al. 2019) antioxidant activity. This study is in line with another study that *Colletotrichum* from jeruju plant (*Acanthus ilicifolius* L.) had an antioxidant activity with Rf value of 0.35 using the eluent of n-hexane and ethyl acetate in a ratio of 5:5 (Rahmaniah et al. 2019). The *C. siamense* MM9 isolated from Malaysia Asiatic pennyworth accession had an Rf value of 0.01–1.00, indicating that the solvent and the eluent used affected the elution process.

Antioxidant Activity Test with a UV-Vis Spectrophotometer

The IC_{50} values of endophytic fungal isolates from Bengkulu and Malaysia accessions varied and differed from the IC_{50} values of the positive control (vitamin C)

Table 1. The Rf value representing the antioxidant bioautography of endophytic fungi isolated from Asiatic pennyworth accessions of Bengkulu using thin layer chromatography.

Species name	Antioxidant activity	Rf value		
		Spot 1	Spot 2	Spot 3
Aspergillus austroafricanus MB1	+	0.50	0.56	0.81
Phanerochaete chrysosporium MB2	+	0.01	1.00	-
Fusarium oxysporum 1 MB3	+	0.01	1.00	-
Acrocalymma vagum MB4	+	0.01	1.00	-
Perenniporia tephropora MB5	+	0.01	1.00	-
Fusarium falciforme 1 MB7	+	0.60	1.00	-
Fusarium falciforme 2 MB8	+	0.01	0.93	0.95
Fusarium oxysporum 2 MB9	+	0.61	0.97	-
Fusarium falciforme 3 MB10	+	0.01	1.00	-
Trichaptum sp. MB11	+	0.01	1.00	-
Fusarium keratoplasticum MB12	+	0.61	1.00	-
Colletotrichum tabaci 1 MB14	+	0.01	1.00	-
Phoma multirostrata MB16	+	0.01	1.00	-
Fusarium oxysporum 3 MB17	+	0.01	1.00	-
Colletotrichum tabaci 2 MB18	+	0.01	0.56	1.00
Ceratobasidium cornigerum MB20	+	0.01	0.53	1.00
Mycochaetophora gentianae MB21	+	0.01	0.58	1.00

Table 2. Rf value of antioxidant bioautography for endophytic fungi isolated from Asiatic pennyworth accessions of Malaysia using thin layer chromatography.

Species name	Antioxidant activity	Rf value			
		Spot 1	Spot 2	Spot 3	Spot 4
Colletotrichum karstii MM2	+		0.01 – 1.00		
Fusarium solani 1 MM3	+	0.01	1.00	-	-
Fusarium falciforme MM4	+	0.37	1.00	-	-
Eutypella sp. MM5	+	0.01	-	-	-
Colletotrichum siamense MM9	+		0.01 – 1.00		
Peroneutypa scoparia MM10	+	0.01	0.43	-	-
Phanerochaete stereoides MM12	+		0.01 – 1.00		
Aspergillus oryzae MM13	+	0.01	0.43	0.91	-
Colletotrichum gigasporum MM14	+		0.01 – 1.00		
Penicillium capsulatum MM15	+		0.01 – 1.00		
Talaromyces pinophilus MM16	+	0.43	1.00	-	-
Fusarium solani 2 MM17	+	0.51	1.00	-	-
Colletotrichum tabaci 1MM18	+	0.01	0.37	1.00	-
Chaetomium globosum MM19	+	0.01	0.37	1.00	-
Fusarium striatum MM20	+	0.01	0.37	0.64	1.00
Perenniporia corticola MM21	+	0.01	0.37	1.00	-
Colletotrichum tabaci 2 MM23	+	0.01	0.32	0.37	1.00

that had 2.68 ppm. The IC_{50} indicated a 50% hindrance of free radicals (Abe et al. 2012). The IC_{50} values representing the antioxidant activity of endophytic fungal isolates of Bengkulu accessions were shown in Figure 1.

Based on the IC_{50} values, the endophytic fungal isolates of Bengkulu accessions had very strong-antioxidant activity, especially the three isolates, i.e., *F. oxsyporum* 1 MB3 (90.65 ppm), *P. tephropora* MB5 (52.70 ppm), and *F. falciforme* 2 MB8 (79.81 ppm). In contrast, the other 14 isolates had very strong activity. *A. austroafricanus* MB1 (12.08 ppm) had the lowest IC_{50} value. The *F. oxysporum* 1 MB3 isolate had a moderate antioxidant activity with the highest IC_{50} value of 90.65 ppm. The higher the

antioxidant activity, the lower the IC_{50} value (Pratiwi et al., 2013). A previous study by Danagoudar et al. (2017) showed that *Aspergillus austroafricanus* of *Zingiber officinale* had an antioxidant activity with an IC_{50} value of 82 ± 0.78 µg mg^{-1}. The IC_{50} of the endophytic isolates of Malaysia accessions were expressed in graphical form, as shown in Figure 2.

Perementypa scoparia MM10 and *T. pinophilus* MM16 showed strong antioxidant activities with IC_{50} values of 50–100 ppm. *F. solani* 1 MM3, *Eutypella* sp. MM5, *F. solani* 2 MM17, and 12 other isolates had very strong antioxidant activity. The *A. oryzae* MM13 isolate had the lowest IC_{50} value of 10.29 ppm than previously reported from Indian Asiatic pennyworth with an IC_{50} value of 75 ppm (Nath et al. 2014). Another study by Nuraini et al. (2019) showed that *A. oryzae* isolated from *Mangifera casturi* plant had an IC_{50} value of 145.01 ppm, which was most lower antioxidant activity than that of *A. oryzae* MM13 in this study.

Analysis of Active Compounds as Antioxidants Using GC-MS

The GC-MS technique wad used to evaluate the fungal extracts having the lowest IC value (*A. austroafricanus* MB1 and *A. oryzae* MM13), and some major compounds were identified (Table 3 and 4). Twelve active compounds were found in *A. austroafricanus* MB1 methanol extract (Table 3), and ten compounds were found in *A. oryzae* MM13 to have potentials as antioxidants (Table 4). The identified antioxidants included flavonoids, carboxylic, and fatty acids. The highest antioxidant compound was 2-decanoic acid (13.54%), and the lowest was hexadecanoic acid, methyl ester (5.26%).

The analysis of active compounds using GC-MS in ethanol extract of Asiatic pennyworth leaves, as Suresh et al. (2010) stated, produced 19 active compounds. Three of the antioxidants had the same composition as *A. austroafricanus* MB1 methanol extract, namely hexadecanoic acid (CAS), palmitic acid (21.77%), and 13-octadecenoic acid, methyl ester (1.27%). The analysis

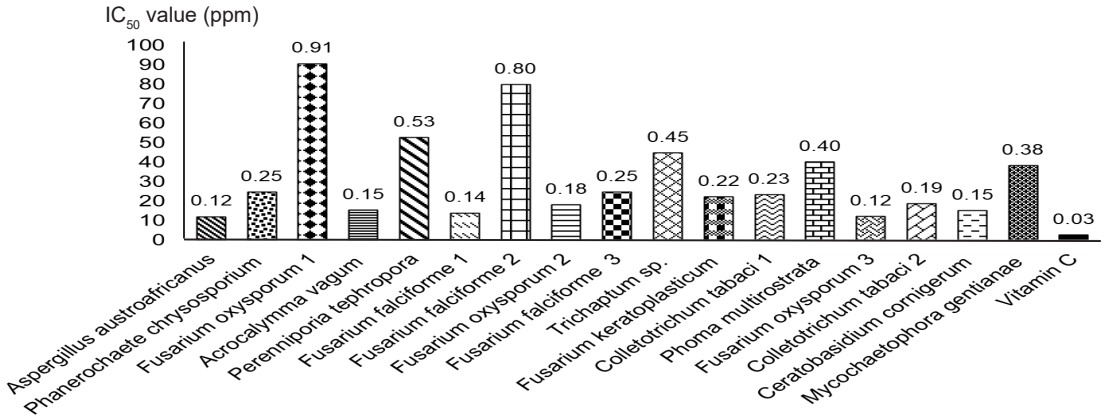

Fig. 1. Antioxidant activity of endophytic fungi isolated from Asiatic pennyworth accessions of Bengkulu based on IC_{50} value of the UV-Vis spectrophotometer.

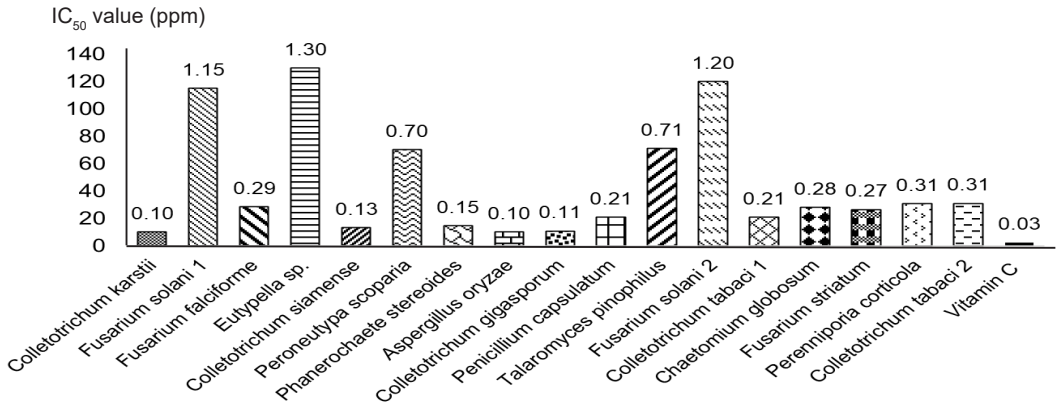

Fig. 2. Antioxidant activity of endophytic fungi isolated from Asiatic pennyworth accessions of Malaysian based on IC_{50} value of the UV-vis spectrophotometer.

Table 3. Components of active compounds in methanol extract of *Aspergillus austroafricanus* MB1.

Peak	% area	Compound name	Molecular formulas	Group	Antioxidant reference
1	12.40	Acetic acid (CAS) Ethylic acid*	$C_2H_4O_2$	Carboxylic acid	
2	2.72	1,4-Dioxin, 2,3-dihydro-5,6-dimethyl-	$C_6H_{10}O_2$	Carboxylic acid	-
3	2.44	Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester	$C_6H_8N_4O_2$	Carboxylic acid	-
4	9.02	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-*	$C_6H_8O_4$	Flavonoids	(Reddy et al. 2017)
5	6.92	1.alpha.,6.beta.,7.beta.-Triacetoxy-9,13-epoxy-8-hydroxy-labd-14-en-11-one	$C_{26}H_{38}O_9$	-	-
6	5.38	1,1,3,3-Tetraphenyl-2,4-di(2,4,4-trimethylpent-1-enylidene)-1,3-disilacyclobutane	$C_{42}H_{48}Si_2$	-	-
7	13.54	2-decenoic acid*	$C_{10}H_{18}O_2$	Fatty acid	(Pavel et al., 2014); Kolayli et al. 2016)
8	5.26	Hexadecanoic acid, methyl ester*	$C_{17}H_{34}O_2$	Fatty acid	(Uma et al., 2011)
9	8.23	Hexadecanoic acid (CAS) Palmitic acid*	$C_{16}H_{32}O_2$	Fatty acid	
10	9.63	13-Octadecenoic acid, methyl ester*	$C_{19}H_{36}O_2$	Fatty acid	(Krishnamoorthy and Subramaniam, 2014)
11	7.40	9-Octadecenoic acid (Z)- (CAS) Oleic acid*	$C_{18}H_{34}O_2$	Fatty acid	(Gideon 2015)
12	17.07	Benzonitrile, 2-(2-pyridinyl)-	$C_{12}H_8N_2$	-	-

*Antioxidant compounds

Table 4. Components of active compounds in methanol extract of *Aspergillus oryzae* MM13

Peak	% Area	Compound name	Molecular formulas	Group	Antioxidant reference
1	2.41	Acetic acid (CAS) Ethylic acid*	$C_2H_4O_2$	Carboxylic acid	
2	9.62	1,2,3-Propanetriol (CAS) Glycerol	$C_3H_8O_3$	Alcohol	-
3	1.74	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one*	$C_6H_8O_4$	Flavonoids	(Reddy et al. 2017)
4	3.11	5-Methyl-7-propyl-1,3-diazaadamantan-6-one	$C_{12}H_{20}N_2O$	-	-
5	3.09	4,7,7-Trimethylbicyclo[2.2.1]heptan-2,3-dione, 2-O-methyloxime	$C_{11}H_{17}NO_2$	-	-
6	16.00	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate*	$C_{17}H_{34}O_2$	Fatty acid	(Reddy et al. 2017)
7	8.43	Hexadecanoic acid (CAS) Palmitic acid*	$C_{16}H_{32}O_2$	Fatty acid	
8	25.45	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate*	$C_{19}H_{34}O_2$	Fatty acid	(Krishna et al. 2012)
9	19.57	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate*	$C_{19}H_{36}O_2$	Fatty acid	(Gideon 2015)
10	10.57	Octadecanoic acid, methyl ester (CAS) Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid	-

*Antioxidant compounds

of active compounds contained in ethyl acetate extract of *Colletotrichum gloeosporioides*, endophytic fungi of Asiatic pennyworth leaves in India, produced 55 active compounds, four of which had the same content as methanol extract of *A. austroafricanus* MB1, namely 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (9.02%), hexadecanoic acid, methyl ester (0.12%), 13-octadecenoic acid, methyl ester (13.37%), and 9-octadecenoic acid (Z) - (CAS) oleic acid (13.37%) (Parmar 2015).

The analysis of active compounds using GC-MS in *A. oryzae* MM13 methanol extract produced ten active compounds, but only six which had an antioxidant activity. Five similar active compounds were identified from the methanol extract of *A. austroafricanus* MB1, namely acetic acid (CAS) ethylic acid; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; fatty acid groups, such as hexadecanoic acid, methyl ester (CAS) methyl palmitate, hexadecanoic acid (CAS) palmitic acid, and 9-octadecenoic acid (Z) methyl ester (CAS) methyl oleate.

The results of active compound analysis of methanol extract of *A. oryzae* MM13 were shown in Table 4. The

active compounds contained in the methanol extract of *A. oryzae* MM13 had six antioxidants. Those with the highest antioxidant activity were 9,12-octadecadienoic acid (Z,Z), methyl ester (CAS), and methyl linoleate (25.45%), while that with the lowest activity was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (1.74%). The active compounds found in the methanol extract of *A. oryzae* MM13 were flavonoids, carboxylic, and fatty acids. Two of the antioxidants in *A. oryzae* MM13, namely hexadecanoic acid (CAS), palmitic acid (21.77%) and 9-octadecenoic acid (Z), methyl ester (CAS) methyl oleate (1.27%), were also found in ethanol extract of Asiatic pennyworth leaves from India (Suresh et al. 2010). Moreover, four active compounds, namely 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (1.26%), hexadecanoic acid (CAS) palmitic acid (6.26%), 9-octadecenoic acid (Z) methyl ester (CAS) methyl oleate (13.37%), and octadecanoic acid, methyl ester (CAS) methyl stearate (13.37%) were also found in ethyl acetate extract of *Colletotrichum gloeosporioides* from Asiatic pennyworth leaves (Parmar 2015).

The present study showed that two endophytic fungal isolates from Asiatic pennyworth, i.e., *A. austroafricanus* MB1 and *A. oryzae* MM13, are promising to be developed further for their antioxidant activity in the laboratory. Hence concerning our result, advance analyses are required to explore other bioactive compounds.

CONCLUSION

Thirty-four endophytic fungi isolated from Bengkulu and Malaysia Asiatic pennyworth showed antioxidant activities. Two of them, i.e., *A. austroafricanus* MB1 and *A. oryzae* MM13, had the highest antioxidant activities, as demonstrated from the IC_{50} value of 12.08 ppm and 10.29 ppm, respectively. The *A. austroafricanus* MB1 produced seven antioxidant compounds and *A. oryzae* MM13 produced six antioxidant compounds. The components of antioxidant compounds consisted of flavonoids, fatty acids, and carboxylic acids.

Aspergillus austroafricanus MB1 and *A. oryzae* MM13 isolates could be further analyzed using metabolomics to explore in detail other bioactive compounds. Moreover, by using advance microbial fermentation processes and genetic engineering, potential of both endophytic fungi can be manipulated to make them more beneficial for mankind.

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