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Metabolic Profiling of Three Species of *Amorphophallus* (Araceae)

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Abstract

A study was conducted to determine the metabolic contents of corms of three species of *Amorphophallus*, *A. muelleri*, *A. paeoniifolius*, and *A. variabilis* using gas chromatography mass spectrometry (GCMS) with water as polar solvent. The corms were collected from plants cultivated at the Leuwikopo Experimental Station, IPB University, Darmaga, Bogor, Indonesia. Metabolic profiling conducted at the Jakarta Regional Health Laboratory, Indonesian Ministry of Health, demonstrated that the three species of *Amorphophallus* vary in metabolic content, specifically for 12 compounds belonging to antioxidants, vitamins, saturated fatty acids, polyunsaturated fatty acids, phytosterol, alcohol, aldehyde, and alkane. Our study had shown that metabolic profiling is a potentially useful method of differentiating or determining species markers that in turn could be efficient way for genetic conservation and development of future food source.

Keywords: Amorphophallus, functional food, GCMS, metabolic, phytochemicals

Introduction

The plant genus *Amorphophallus* belongs to the family Araceae or Aroid family and is estimated to consist of about 170 species (Hetterscheid and Ittenbach, 1996). *Amorphophallus* is typically lowland plants that grow in primary or secondary forests (Hetterscheid and Ittenbach, 1996). According to Govaerts (1995), *Amorphophallus muelleri* is distributed throughout Southeast Asia, whilst *Amorphophallus paeoniifolius* is distributed throughout tropical and subtropical region of Asia down to Northern Australia, and *Amorphophallus variabilis* is distributed in the island of Java and Philippines. The three *Amorphophallus* species are widely distributed in Indonesia and have long been used as traditional food sources (Sugiyama and Santosa, 2008). *Amorphophallus* has high productivity in wide range of ecology but studies on this crop are still limited (Santosa and Sugiyama, 2016) and it is under-utilized compared to the other staple foods such as rice, maize and wheat. *Amorphophallus* can survive in harsh environment and is an alternative food source when rice is not available (Santosa and Sugiyama, 2016), therefore, it is important to study *Amorphophallus* for the development of more variety food sources in the future.

Species of *Amorphophallus* are considered to be more drought tolerant than cassava. *Amorphophallus muelleri* has been cultivated in several regions of Indonesia, including in East Java (Sugiyama and Santosa, 2008). Harvesting *Amorphophallus* is usually conducted once the plants reached dormant stage, and the first harvest is usually three years after planting (Santosa et. al., 2003). *Amorphophallus paeoniifolius* in Indonesia is known with local name *suweg;* it is commercially cultivated in India and is widely used by local medicine practitioners. *Amorphophallus paeniifolius* is reported to have analgesic, anti-inflammatory, antihelminthic, and hepatoprotective properties (Dey et. al., 2010). *Amorphophallus variabili*s, known as *acung* or *cocoan oray* in Indonesia, has long been used as both food and animal feed (Sugiyama and Santosa, 2008). Datta et al. (2014) reported nutritional values of *A. campanulatus* growing in India; the tubers contain protein, carbohydrate, fat, fibers, ash and vitamin.

Species of *Amorphophallus* have corms that serve as source of carbohydrates and glucomannan (Sugiyama and Santosa, 2008). Glucomannan is a water-soluble, fermentable dietary fiber extracted from the tubers of *Amorphophallus* (Keithley et al., 2013); it is used in pharmaceutical industry for treating various kinds of diseases, including type-2 diabetes and hypertension (Vuksan et. al., 1999), and treatment of obesity through reducing the fat storage in human bodies (Sirotkin, 2021) Studies by Lianah et al. (2018) in mice demonstrated that

consumption of fresh tubers of *Amorphophallus campanulatus* significantly reduced blood sugar. *Amorphophallus* corm contains compounds that have antibacterial, antifungal, and cytotoxic properties, thus has potential as a treatment for cancer (Khan et. al., 2007). The utilization of these three *Amorphophallus* species in Indonesia has declined due to the success of rice cultivation; rice being the preferred choice of staple foods in Indonesia. Our study is aimed to determine the metabolic content of the corms of three *Amorphophallus* species for the possibility of the use of metabolic content as species marker. Information on the metabolic content could be useful for *Amorphophallus* genetic conservation and development of the future food sources.

Materials and Methods

Corms of *A. muelleri*, *A. paeoniifolius*, and *A. variabilis* for this study are from IPB University collection. The corms were planted at Leuwikopo Experimental Field, IPB University, Darmaga, Bogor, West Java, in September 2016 to August 2017. The precipitation from June 2016 to September 2017 ranged from 117-256 mm per month. The air humidity was 82-86%, and temperature was 25-26.55^{o}C. Identification and biochemical content analysis were conducted at the Pusat Pengembangan Pengujian Obat dan Makanan Nasional (PPPOMN), Ministry of Health Regional Laboratory, Rawasari, Jakarta.

Planting and Harvesting

Two weeks prior to planting, land clearing and bed raising were done using tractor, hoe, and garden fork. Ten plant beds that were 5 m long and 1 m wide were set up with a 50 cm-distance between each bed. Planting holes of 50 cm apart with 30 cm diameter and 20 cm depth were set up in the plant bed in a triangular manner. A net with 50% shade was installed above the entire planting area. The plant beds were ameliorated with dolomite with application rate of 10 ton per ha seven days before planting.

Bulbil of *A. muelleri* and corms of *A. paeoniifolius* and *A. variabilis* were used in this study. Bulbils of almost uniform weights were carefully chosen to reduce variability. The corms were cut into pieces and were soaked in a solution of Dithane M-45 (active ingredient: mancozeb 45%) and planted individually.

Planting was conducted by putting one corm at the center of each planting hole. About 200 g of manures were applied in each hole which were then covered with soil to avoid direct contact with the corm.

Plant maintenance include watering three times a week, unless it was raining, and weeding which was conducted manually, when required. Infestation of snail, grasshopper, caterpillar, and termites to the plants were detected, but all can be controlled manually. Harvesting was conducted in July 2017 after all of the plants have reached dormant stage. Dormancy stage was shown by the wilting of the plant leaves and the plant shoots were no longer seen above the ground. The harvested corms were washed, their skin peeled and discarded, then grinded. Extraction was conducted using water.

Data Collection and Analysis

The extracts produced from the plants were injected separately into the GCMS machine using the standard method employed in the Regional Laboratory of PPPOMN, Ministry of Health, Jakarta.
Environmental data, including precipitation rate, ambient humidity, and air temperature, during the study were recorded.

Results and Discussion

Chromatogram Results

GCMS analysis on the extracts of all three corms showed that the highest occurrence of high peaks for all three species are generally at the retention time (RT) of 28.00-34.00 minutes after injection. Those peaks are dominantly fatty acid compounds. Another high occurrence of high peaks of different compounds in *A. variabilis* occurred at RT 03.00-05.00, 07.00-08.00, and 11.00-12.00 minutes after injection (Figure 1).

Compound Grouping

GCMS analysis of the corms of the three species of *Amorphophallus* show compounds from 11 compound groups consisting antioxidants, vitamin, saturated fatty acids, polyunsaturated fatty acids, ketone, alcohol, aldehyde, alkane, alkene, alkyne, and phytosterol (Tables 1-7). Among the 11 compound groups, antioxidant (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one or DDMP) and vitamin (E) were found in all three species of *Amorphophallus* studied. From the total 12 compounds identified, saturated fatty acids, polyunsaturated fatty acids, phytosterol, alcohol, aldehyde, and alkene can be used to differentiate the three species of *Amorphophallus* (Table 7). Many compounds showed different RT results for each species, indicative the existence of different forms or variants of compound within each species. The abundance of compound also varies among species. For instance, *A. variabilis* was found to have higher

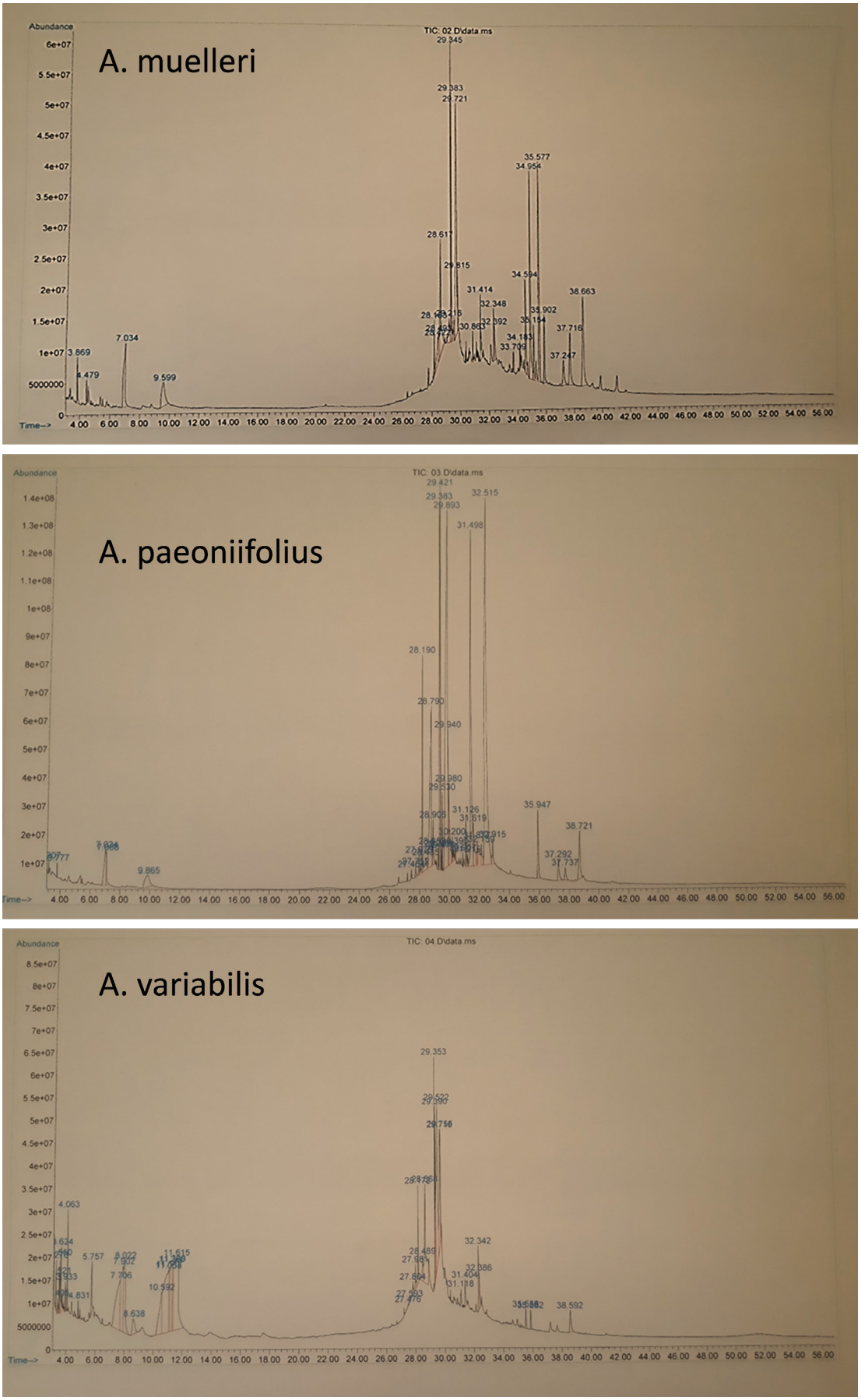

Figure 1. GCMS chromatograms of *A. muelleri*, *A. paeoniifolius*, and *A. variabilis*.

chromatogram area for antioxidant and aldehyde than the other two *Amorphophallus* species. Most of the detected aldehyde compounds are furfurals. On the other hand, chromatogram result of *A. muelleri* shows more area and higher peak compared to the other two species for polyunsaturated fatty acids, phytosterol, and alkene.

Ten compounds of saturated fatty acid group were detected in the three species of *Amorphophallus*. One compound, i.e., hexadecanoic methyl esther, was common to all three species, whereas two compounds (hexadecanoic acid and hexadecanoic 2-hydroxy-1(hydroxymethyl) ethyl esther), were common to both *A. muelleri* and *A. paeoniifolius*. Seven other compounds were unique to each of the three species, with *A. paeoniifolius* containing the highest number of saturated fatty acid compounds compared to the other two species (Table 1).

play important roles in preventing water loss during drought (GERLI, undated). The presence of alkanes or alkenes has also been reported in microorganisms (Ladygina et al., 2006).

A total of five compounds of aldehyde and one alkyne were detected in the species of *Amorphophallus*. None of the compounds from this group was found to be common to the three species, making them good compound identifiers. There were two aldehyde compounds each found in *A. muelleri* and *A. variabilis,* whilst there was one aldehyde compound found in *A. paeoniifolius* only (Table 4).

Six polyunsaturated fatty acid compounds were detected in the *Amorphophallus* samples studied. Of the six poly unsaturated fatty acids, 9,12-octadecedienoic acid (Z,Z) was detected in *A. paeoniifolius* and *A. variabilis*, but not in *A. muelleri*.

Table 1. Saturated fatty acid compounds of the corms of three species of *Amorphophallus*.

No.	Compound name	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
1	(9E)-9-Octadecenoic acid			x
2	9-Hexadecenoic acid, methyl esther (Z)		X	
3	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl esther		X	
4	9-Octadecenoic acid, methyl esther		X	
5	cis-9-Hexadecenoic acid		X	
6	Hexadecanoic acid		X	X
7	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl esther		X	X
8	Hexadecanoic acid, Z-11			X
9	Linoleic acid ethyl esther	X		
10	Hexadecanoic acid, methyl esther	X	X	X

Note: X shows that the compound is detected by GCMS analysis.

Seven compounds of the ketone group were found in the *Amorphophallus* corms. One compound, (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one), was found in all three species. *Amorphophallus muelleri* had the lowest ketone compounds amongst the three species (Table 2).

Four compounds belonging to the alkene group were found in *A. muelleri* and *A. paeoniifolius*, but not in *A. variabilis*. The two species were found to contain two unique alkene compounds, therefore, alkene can be used to identify corm *A. muelleri* and *A. paeoniifolius.* Additionally, there is one alkane compound found in *A. muelleri* only, making it easier to differentiate this species from the other two (Table 3). In plants, alkanes and alkene are part of leaf cuticular waxes and

Amorphophallus muelleri showed three compounds that are not found in the other two species. *Amorphophallus paeoniifolius* and *A. variabilis* showed one unique compound each (Table 5). Fatty acids play important roles in overcoming biotic and abiotic stress in higher plants (He and Ding, 2020).

Four alcohol and three phytosterol compounds were found in *Amorphophallus*. The alcohol compound Stigmast-5-en-3-ol was found in all three species analysed. In addition, Z,Z-10,12-hexadecadien-1-ol acetate was found only in *A. muelleri*, whilst two other compounds, i.e. 9,12-octadecadien-1-ol and 9-methyl-Z,Z-11,12-hexadecadien-ol acetate, were found only in *A. paeoniifolius*. Campesterin and γ-cytosterol from phytosterol group were detected only in *A. muelleri* (Table 6).

Table 2. Ketone compounds of the corms of three species of *Amorphophallus*.

No.	Compound name	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
1	1,13-Tetradecadien-3-one		X	
2	2(3H)-Furanone, dihydro			X
3	2(5H)-Furanone			X
4	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	X	X	X
5	2-Cyclopenten-1-one, 2-hydroxy			X
6	2-Tridecanone		X	
7	Ethanone, 2-bromo-1-(2,4-dimethoxyphenyl)		X	

Note: X shows that the compound is detected by GCMS analysis.

Table 3. Alkene and alkane compounds of the corms of three species of *Amorphophallus*.

No.	Compound name	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
	Alkene			
1	1,3,12-Nonadecatriene	X		
2	4,5,6,7-Tetrahydro-benzo[C]thiophene-1-carboxylic acid (2-chlorophenyl)-amide		X	
3	Cholesta-6,22,24-triene, 4,4-dimethyl	X		
4	E,Z-1,3,12-Nonadecatriene		X	
	Alkane			
1	1-methylcyclobutane	X		

Note: X shows that the compound is detected by GCMS analysis.

Table 4. Aldehyde and alkyne compounds of the corms of three species of *Amorphophallus*.

No.	Compound name	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
	Aldehyde			
1	2,3,7-Trimethyloctanal		X	
2	2-Furancarboxaldehyde, 5-(hydroxymethyl)			X
3	2-Furancarboxaldehyde, 5-methyl			X
4	5,10-Dihexyl-5,10-diihydroindolo [3,2-B] indole-2,7-dicarbaldehyde	X		
5	5-hydroxymethyl-furan-2-carbaldehyde	X		
	Alkyne			
1	7-Pentadecyne			X

Note: X shows that the compound is detected by GCMS analysis.

Table 5. Polyunsaturated fatty acid compounds of the corms of three species of *Amorphophallus*.

No.	Compound name	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
1	9,12,15-Octadecatrienoic acid	X		
2	9,12,15-Octadecatrienoic acid, methyl esther			X
3	9,12-octadecadienoic acid	X		
4	9,12-Octadecadienoic acid (Z,Z)		X	X
5	9,12-Octadecadienoic acid, methyl esther		X	
6	cis-9,cis-12-octadecadienoic acid	X		

Note: X shows that the compound is detected by GCMS analysis.

Table 6. Alcohol and phytosterol compounds of the corms of three species of *Amorphophallus*.

No.	Compound	*A. muelleri*	*A. paeoniifolius*	*A. variabilis*
	Alcohol			
1	9,12-Octadecadien-1-ol		X	
2	9-Methyl-Z,Z-10,12-hexadecadien-ol-acetate		X	
3	Z,Z-10,12-Hexadecadien-1-ol acetat	X		
4	Stigmast-5-en-3-ol	x	X	
	Fitosterol			
1	Ergost-5-en-3-ol		X	
2	Campesterin	X		
3	γ-Cytosterol	X		

Note: X shows that the compound is detected by GCMS analysis.

Table 7. GCMS result for unique compounds of the corms of three species of *Amorphophallus*.

No.	Compounds	RT	Area		
			A. muelleri	*A. paeoniifolius*	*A. variabilis*
Antioxidant and vitamin					
1	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	7.024		2.04	
		7.031	5.83		
		7.086		0.76	
		7.707			7.47
		7.900			5.95
		8.024			5.73
2	Vitamin E	35.881			0.45
		35.902	3.06		
		35.950		1.75	
Unsaturated fatty acids					
1	9,12-Octadecadienoic acid, methyl esther	29.386		7.27	
		29.344	4.83		
		29.386	4.05		
2	9,12-Octadecadienoic acid	29.723	15.42		
		29.813	1.43		
Saturated fatty acids					
1	n-Hexadecanoic acid	28.620	6.93		
2	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	31.406			0.68
		31.495		11.13	
3	Hexadecanoic acid	27.807			0.45
		28.669			5.50
		28.793		8.07	
		28.855		0.25	
		28.903		0.69	
4	(9E)-9-Octadecenoic acid	27.593			0.15
		29.524			9.35
Phytosterol					
1	g-Sitosterol	38.660	7.64		
Alcohol					
1	9,12-Octadecadien-1-ol	32.516		23.20	
Aldehides					
1	2-Furancarboxaldehyde, 5-(hydroxymethyl) (Furfural)	10.589			4.02
		11.058			11.57
		11.120			2.27
		11.285			5.09
		11.327			1.93
		11.616			13.74
		9.865		1.72	
Alkenes					
1	Cholesta-6,22,24-triene, 4,4-dimethyl	34.592	4.08		
		34.957	9.70		
2	Stigmastan-3,5-diene	35.557			0.46
		35.578	10.07		

Further studies related to the biological activity of the compounds detected in each species of *Amorphophallus* can provide information on their benefits as source of food and active compounds for medicinal purposes. It is also important to examine if the cultural practices, such as rate of fertilizer application and time of harvest, could change the quantity and quality of metabolites of the *Amorphophallus* tubers.

Conclusion

Metabolic profiling through the use of gas chromatography mass spectrometry indicates that *A. muelleri*, *A. paeoniifolius*, and *A. variabilis* can be identified using compound markers belonging to antioxidants, vitamins, saturated fatty acids, polyunsaturated fatty acids, phytosterol, alcohol, aldehyde, and alkene. The use of metabolic profiling in identifying *Amorphophallus* has the potential to be used as an efficient method of species marking that in turn could be useful for genetic conservation and development of future food sources.

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