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Identification of Secondary Metabolite Compounds in Two Varieties of Young Winged Beans (*Psophocarpus tetragonolobus* L.) at Two Harvest Ages

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Abstract

The winged bean (*Psophocarpus tetragonolobus* L.) is a member of the *Fabaceae* family (beans). Winged bean is developed as a vegetable, and young winged bean pods can be consumed fresh, steamed, fried, or pickled. In Southeast Asia, winged bean pods are generally cooked or consumed as side dishes or salad. Winged bean contains high protein content equivalent to soybean seed. Besides being rich in protein, vitamins, and minerals, the winged bean also has secondary metabolites, including phenolics and flavonoids. This study aimed to identify secondary metabolites in young pods of two varieties, "Fairuz" and "Sandi", at two different harvest ages of 8 and 10 days after anthesis (DAA) and provide the biological activity on each identified compound. The experiment was conducted at the IPB experimental field at Leuwikopo, Bogor, from September 2020 to February 2021. The identification of secondary metabolites of the young green pods "Fairuz", and the young purple pods "Sandi", was performed using the GCMS method at the Regional Health Laboratory (KESDA) DKI Jakarta. The results of the GCMS analysis showed that 1,2-Benzedicarboxylic acid, mono (2-Ethylhexyl) ester was the most abundant compound identified from pods harvested at 8 DAA in both varieties, namely 42.26% in "Fairuz" and 26.66% in "Sandi". Other compounds, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z) (Linoleic acid ester), were found in "Fairuz", whereas (9E,12E)-9,12-Octadecadienoic acid (Linoleic acid) was found in "Sandi"; these compounds are hydroxyl group and phenolic glucoside compounds and are found in pods harvested at 10 DAA.

Keywords: antioxidant, biological activity, days after anthesis, green pods, purple pods

Introduction

Winged bean (*Psophocarpus tetragonolobus* L.) is a member of the *Fabaceae* family (beans). Winged beans thrive in hot and humid tropical regions (Mohanty et al., 2013). India, Thailand, Papua New Guinea, and Indonesia (Khan, 1976; Krisnawati, 2016). Winged bean seed contains high protein content equivalent to soybean plants and can be substituted for soybean needs (Amoo et al., 2006; Handayani, 2013; Alalade et al., 2016). Winged bean has much potential as a protein source because, in addition to stems and roots, almost all parts of the plant, including young leaves, flowers, young pods, dried seeds, and tubers, can be consumed (Tanzi et al., 2019; Mohanty et al., 2020). Young winged pods can be consumed fresh, steamed, fried, or pickled, and in Southeast Asia, winged bean pods are generally cooked or consumed as a side dish or salad (Sriwichai et al., 2021). Therefore, winged bean has great potential to be developed as a useful legume crop (Handayani, 2013).

Young winged bean pods contain 3.99% carbohydrates, 0.16% fat, and 93.50% water content (Pangestu, 2020). Young pods contain around 6.1% albumin, globulin, and glutelin, and have a high lysine content, so young pods contain good quality protein compared to other pods (Tadera et al., 1984). Besides being rich in protein, vitamins, and minerals, winged beans also contain secondary metabolites, including phenolics and flavonoids (Singh et al., 2019), and have high tannin content (Mohanty et al., 2013). Immature winged bean pods contain amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, tryptophan, and valine. The pods also contain 5.0% sucrose, 1.0% raffinose, 2.5% stachyose (NRC, 1981). Traditionally, winged beans can be used to prevent cancer, diabetes, asthma, muscle weakness, overcome migraines, eye diseases, and strengthen the immune system (Singh et al., 2019). The total antioxidant capacity ranges from 1,278 to 1,806 mg AsA/g so winged beans can serve as a good source of antioxidants for humans and animals (Olaiya et al., 2018).

From the many chemical components ingredients in winged bean, studies related to the complete profile of secondary metabolites is not widely known, particularly of the newly developed varieties. Therefore, this study was carried out to investigate the secondary metabolites' profile in young pods of the winged beans. Gas Chromatography-Mass Spectrophotometry (GCMS) was used to analyze the range of secondary metabolites in winged beans. GCMS is one of the best techniques for identifying the constituents of volatiles, long-chain, hydrocarbons, branched-chain, alcohols, acids, and esters (Sermakkani dan Thangapandian, 2012). GCMS has successfully identified the bioactive compounds present in many plant species (Karthikeyan et al., 2019).

There are two new varieties of winged beans, namely "Fairuz" (green pods) and "Sandi" (purple pods). The color difference can indicate the difference in the phytochemical compounds contained in the pods. The phytochemical compounds can also be different from pods harvested at various ages. This study aims to identify phytochemical compounds in young pods of winged bean and compare the results between the two harvest ages 8 and 10 days after anthesis (DAA).

Material and Methods

Experimental Site and Materials

The experiment was conducted at the IPB experimental field Leuwikopo, Bogor, West Java, Indonesia, from September 2020 to February 2021. The analysis of secondary metabolites in winged bean pods was conducted at the Regional Health Laboratory (KESDA), DKI Jakarta.

Two winged bean varieties were used to identify the secondary metabolites in young pods, namely "Fairuz" (green pod) and "Sandi" (purple pod). The young pods were harvested 8 or 10 days after anthesis (DAA).

Sample Preparation

Sample preparation was carried out before injecting test substances into each tool. Samples were dried in an oven at 60°C for approximately 3 hours or completely dry.

Sample Extraction

The dried samples were mashed using a blender and then macerated with methanol for 5x 24hours. Each macerated sample was then put into a 10 ml tube using a pipette and dried for 1 hour at 60°C. After drying, it was dissolved with the remaining 200 µl of the macerated extract, followed by chemical analysis using a Gas Chromatography-Mass Spectrometer (GCMS).

Analysis of Secondary Metabolites using GCMS

The analysis procedure conducted by the Regional Health Laboratory followed the Research Institute for Spices and Medicinal Plants method (Utami et al., 2017). The GCMS was used to analyze the content of secondary metabolites in the Agilent 7890 gas chromatography technology with automatic sampling and 5975 mass selective detectors and data systems. A total of 5 µl samples of young winged pods was injected into the GCMS, which has a capillary column size of HP Ultra 2 with a length of 30 m x 0.20 mm x 0.11 m film thickness. The oven temperature was set at an initial temperature of 80°C, then rose at 3°C/min to 150°C, held for 1 minute, and finally increased 20°C/min to 280°C and held for 26 minutes. The injection temperature was 250°C, the ion source temperature was 230°C, the interface temperature was 280°C, and the quadrupole temperature was 140°C. Helium gas was used as the carrier gas at a constant flow rate of 1.2 mL/min (8:1 split).

Result and Discussion

GCMS had successfully identified the secondary metabolites or phytochemicals of the winged bean pods (Figure 1, 2). Thirteen (13) 13 different compounds were identified in "Fairuz" harvested at 8 and 10 DAA, and 17 and 15 compounds identified in "Sandi" harvested at 8 and 10 DAA, respectively.

Identification of the secondary metabolites was confirmed based on the peak area, retention time (RT), and molecular formula. The retention time depends on the molecular weight of the compounds; our samples had the retention times of 20-30 minutes. In "Fairuz" harvested at 8 DAA, the first compound with a lower retention time was Hexadecanoic Acid, Ethyl Ester (27.831 minutes). In contrast, the last phytochemical compound identified that required the longest retention time was gamma-Sitosterol (37.084 minutes). Pods harvested at 10 DAA had the lowest retention time was tetradecanoic acid (27.162

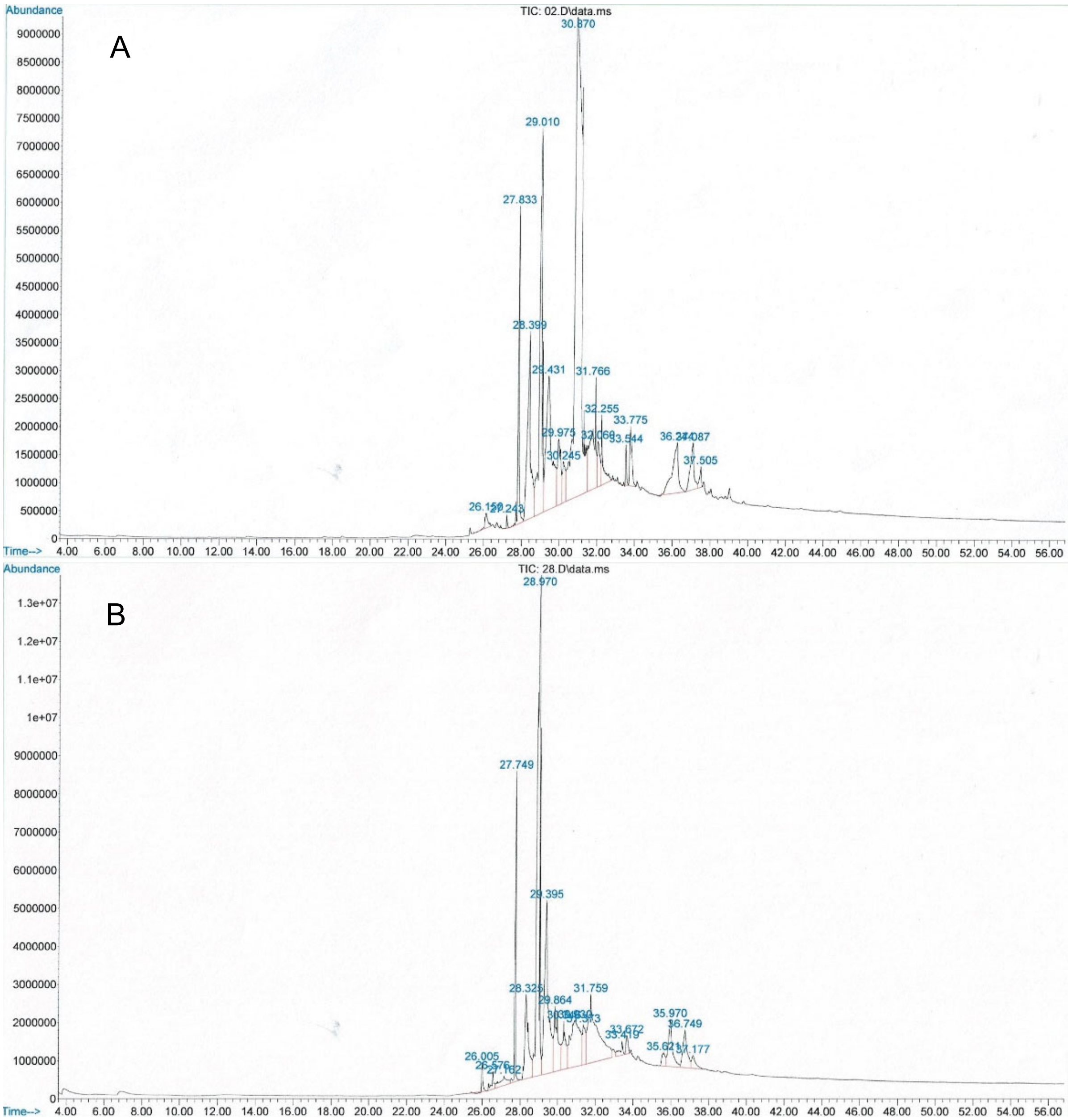

Figure 1. Chromatography of GCMS on young green pods ("Fairuz") harvested at 8 days (A) and 10 days (B) after anthesis

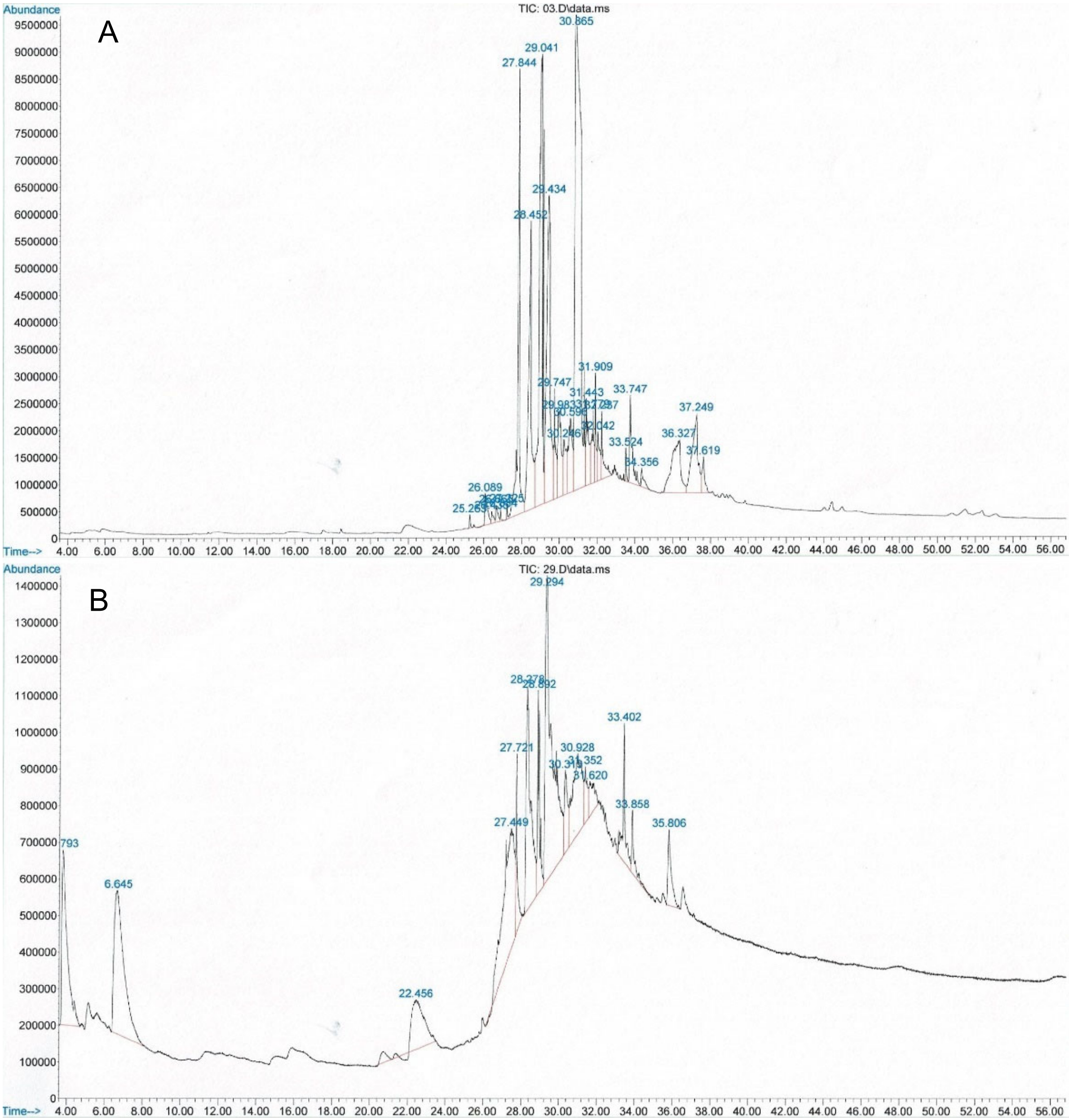

Figure 2. Chromatography of GCMS on young purple pods ("Sandi") harvested at 8 days (A) and 10 days (B) after anthesis.

minutes), while the phytochemical compound that required the longest retention time was Pyrene, hexadecahydro- (37.174 minutes) (Table 1).

In "Sandi" harvested at 8 DAA, the secondary metabolite compound identified with the shortest retention time was Hexadecanoic Acid, Ethyl Ester (27.845 minutes). In contrast, the phytochemical compound identified with the most prolonged retention was gamma-sitosterol (37.250 minutes). In "Sandi" pods harvested at 10 DAA, 2,5-Furandione (3.794 minutes) had the lowest retention time, while stigmasterol (35.809 minutes) was the longest retention time (Table 2).

The phytochemical compounds identified in "Fairuz" and "Sandi" from two harvest dates have biological activity. Therefore, "Fairuz" and "Sandi" are rich sources of secondary metabolites with biological activities (Konovalova et al., 2013).

Two phytochemical compounds were identified in "Fairuz" harvested at 8 DAA with the highest content of >10% (Table 3). The first was 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester (42.26%), a phthalate group chemical. This compound has antifungal, anticancer, immunomodulatory, and antimicrobial activity (Ezhilan dan Neelamegam, 2012; Save et al., 2015; Prawanayoni dan Sudirga, 2020). The

Table 1. Secondary metabolites identified in "Fairuz" harvested at 8 and 10 days after anthesis (DAA) with an abundance >1%

No	Compound	Molecular formula	Molecular weight ($g.mol^{-1}$)	8 DAA*		10 DAA*	
				Retention time	Peak area %	Retention time	Peak area %
1	TETRADECANOIC ACID	$C_{14}H_{28}O_2$	228.37	-	-	27.162	1.33
2	HEXADECANOIC ACID, ETHYL ESTER	$C_{18}H_{36}O_2$	284.5	27.831	3.97	27.748	5.13
3	HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256.42	-	-	28.327	6.05
4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	28.396	7.46	-	-
5	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	$C_{20}H_{34}O_2$	306.5	-	-	28.968	27.53
6	ETHYL (9Z,12Z,15Z)-9,12,15 OCTADECATRIENOATE	$C_{20}H_{34}O_2$	306.5	29.010	13.69	-	-
7	(9E,12E)-9,12-OCTADECADIENOIC ACID	$C_{18}H_{32}O_2$	280.4	29.430	9.78	29.396	14.30
8	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	-	-	29.865	5.38
9	14-METHYL-8-HEXADECYL-1-OL	$C_{17}H_{32}O$	252.4	29.975	2.87	-	-
10	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	254.5	30.244	1.58	-	-
11	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE	$C_{18}H_{31}CLO$	298.9	-	-	30.348	3.35
12	1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester	$C_{16}H_{22}O_4$	278.35	30.872	42.26	-	-
13	[(DIMETHYLSILYL)METHYL](DIMETHYL) SILANE	$C_5H_{14}Si_2$	130.33	-	-	30.927	9.27
14	2-CHLOROETHYL (9Z,12Z)-9,12-OCTADECADIENOATE	$C_{20}H_{35}ClO_2$	342.9	-	-	31.375	2.49
15	Geranylgeraniol	$C_{20}H_{34}O$	290.5	-	-	31.761	12.98
16	i-Propyl 9,12-octadecenadienoate	$C_{21}H_{38}O_2$	322.5	31.768	5.39	-	-
17	Z,Z-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	2804	32.058	1.52	-	-
18	PENTACOSANE	$C_{25}H_{52}$	352.7	32.258	1.63	-	-
19	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.7	33.775	1.83	-	-
20	Stigmasterol	$C_{29}H_{48}O$	412.7	36.243	3.99	35.967	3.99
21	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	37.084	2.36	36.746	3.18
22	Pyrene, hexadecahydro-	$C_{16}H_{26}$	218.38	-	-	37.174	1.10

Note: *DAA= days after anthesis

Table 2. Secondary metabolites identified in winged bean "Sandi" harvested at 8 and 10 days after anthesis (DAA) with an abundance >1%

No	Compound	Molecular formula	Molecular weight ($g.mol^{-1}$)	8 DAA*		10 DAA*	
				Retention time	Peak area %	Retention time	Peak area %
1	2,5-FURANDIONE	-	-	-	-	3.794	9.60
2	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE	$C_6H_8O_4$	144.12	-	-	6.642	13.97
3	1H-Imidazole-4-carboxamide, 5-amino- $$ Imidazole-4-carboxamide, 5-amino-	$C_4H_6N_4O$	126.12	-	-	22.459	6.50
4	.alpha.-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194.18	-	-	27.452	13.77
5	HEXADECANOIC ACID, ETHYL ESTER	$C_{18}H_{36}O_2$	284.5	27.845	7.82	27.721	2.22
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	-	-	28.279	9.92
7	HEXADECANOIC ACID	$C_{16}H_{32}O_2$	256.42	28.451	7.91	-	-
8	ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE	$C_{20}H_{34}O_2$	306.5	-	-	28.893	3.92
9	Linoleic acid ethyl ester	$C_{18}H_{32}O_2$	280.4	29.038	15.30	-	-
10	(9E,12E)-9,12-OCTADECADIENOIC ACID	$C_{18}H_{32}O_2$	280.4	29.437	11.57	29.293	20.02
11	1,2-ETHYLENEDIAMINE, N,N-DIMETHYL-N'-2-PYRIDINYL-N'-(2 PHENYLMETHYL)-	$C_{14}H_{20}ClN_3S$	297.8	29.748	1.83	-	-
12	(9E,12E)-9,12-OCTADECADIENOIC ACID	$C_{18}H_{32}O_2$	280.4	29.982	2.94	-	-
13	E,E-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	280.4	30.244	1.66	-	-
14	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	266.38	-	-	30.313	2.57
15	10-Undecen-1-al, 2-methyl-	$C_{12}H_{22}O$	182.30	30.596	2.81	-	-
16	1,2 Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester	$C_{16}H_{22}O_4$	278.35	30.865	26.66	-	-
17	4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE	$C_{19}H_{34}$	262.5	-	-	30.927	7.32
18	4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE	$C_{19}H_{34}$	262.5	-	-	31.354	1.51
19	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	31.444	2.02	-	-
20	14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE	-	-	-	-	31.623	1.80
21	Ethyl tetracosanoate	$C_{26}H_{52}O_2$	396.7	31.782	1.55	-	-
22	Squalene	$C_{30}H_{50}$	410.7	31.906	1.10	-	-
23	1-CINNAMYL-3-METHYLINDOLE-2-CARBALDEHYDE	$C_{10}H_9NO$	159.18	32.044	1.19	-	-
24	ICOSANE	$C_{20}H_{42}$	282.5	32.237	1.19	-	-
25	Stigmasterol tosylate	$C_{36}H_{54}O_3S$	566.9	-	-	33.402	3.05
26	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.7	33.747	2.12	-	-
27	STIGMAST-5-EN-3-OL	$C_{29}H_{50}O$	414.7	-	-	33.875	1.41
28	Stigmasterol	$C_{29}H_{48}O$	412.7	36.326	4.72	35.809	2.43
29	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	37.250	4.56	-	-

Note: *DAA= days after anthesis

second compound is Ethyl (9Z, 12Z, 15Z)-9,12,15 Octadecatrienoate (13.69%), which belongs to the linolenic acid group. This compound has biological activity as anti-inflammatory, hypocholesterolemic, cancer preventive, antihistaminic, antiacne, antiarthritic, and anticoronary products (Kumar et al., 2010; Starlin et al., 2019).

There were three significant compounds in “Fairuz” pods harvested at 10 DAA with abundance > 10% (Table 4). The predominant one was 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z) (27.53%). These compounds belong to the Linoleic acid ester compound group and have hypocholesterolemic, antirheumatic, antiandrogenic, anticoronary, and anticancer activity. They can also be used as a nematicide and insect repellent (Mamani dan Alhaji, 2019). The compound (9E,12E)-9,12-Octadecadienoic acid (14.30%), belongs to the Linoleic acid group, has the biological activity as anti-inflammatory, hypocholesterolemic, antihistaminic, antiarthritic, and antiacne product (Sermakkani dan Thangapandian 2012). The third compound is Geranygeraniol (12.98%), which belongs to the chloroform group of compounds, has anticancer and antimycobacterial activity (Vik et al., 2007; Sianipar dan Purnamaningsih, 2018).

Similar phytochemical compounds to “Fairuz” variety harvested at 8 DAA are also found in “Sandi” variety harvested at 8 DAA above 10% (Table 5). The phytochemical compound with the highest content was 1,2-Benzenedicarboxylic acid mono(2-Ethylhexyl) ester (26.66%). The second most important product was Linoleic acid ethyl ester (15.30%), which has anti-inflammatory, hypocholesterolemic, antihistamine, antirheumatic, antiacne, and antieczemic activities, antiandrogenic, and anticoronary products (Sermakkani dan Thangapandian, 2012; Sudha et al. 2013). Furthermore (9E,12E)-9,12-Octadecadienoic acid (11.57), which belongs to the linoleic acid group, his activity as an anti-inflammatory, hypocholesterolemic, antihistaminic, antiarthritic, and antiacne products (Sermakkani dan Thangapandian, 2012).

Secondary metabolite compounds were abundant in the “Sandi” harvested at 10 DAA, i.e. > 10% (Table 6). The phytochemical compound with the highest content was (9E,12E)-9,12-Octadecadienoic acid (20.02), as found in “Sandi” variety with pods harvested at 8 DAA. The next important phytochemical compound was 2,3-dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One (13.97%), which belongs to the hydroxyl group, with activity as an antioxidant (Li et al., 2019). Furthermore, alpha.-D-Glucopyranoside, methyl (13.77%), which belongs to the phenolic glucoside group, has apoptotic activity, anticancer, antituberculosis, antioxidant, alpha-amylase inhibitory activity, and as an anticonvulsant (Lyantagaye, 2013; Uchegbu et al., 2017).

The abundance distribution of identified secondary metabolites from these two varieties of winged bean at two harvest ages is shown in the heatmap. The heatmap was based on the distribution of phytochemical with an abundance > 1% (Figure 3). The highest content of secondary metabolite compound from pods harvested at 8 DAA from these two varieties was 1,2-Benzenedicarboxylic acid mono (2-Ethylhexyl) ester. In contrast, at 10 DAA harvest age, the highest abundance compound was 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) in the “Fairuz” and (9E,12E)-9,12-Octadecadienoic acid in the “Sandi”.

Discussion

From the results of the GCMS analysis, it has been shown that the identified compounds in the two wingead bean varieties and at harvest ages are very diverse. The compounds with the highest content found in both varieties at each harvest were derived from the linoleic acid compound group of fatty acids. These compounds have biological activity as anti-inflammatory, hypocholesterolemic, antihistaminic, antiarthritic, antiacne products, as well as nematicidal and insecticidal properties (Sermakkani dan Thangapandian, 2012).

It has been shown that the dominant secondary metabolites, with abundance > 10%, was 1,2-Benzenedicarboxylicacid, mono(2-Ethylhexyl) ester, Ethyl (9Z,12Z,15Z)-9,12,15-Octadecatrienoate, Linoleic acid ethyl ester, (9E,12E)-9,12-Octadecadienoic acid, Geranylgeraniol, 9,12,15-Octadecatrienoic acid, ethyl ester (Z, Z,Z), 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One,.alpha.-D-Glucopyranoside, methyl. Therefore, these compounds can be used as specific compounds in winged beans, especially in young pods.

There are compounds in the “Sandi” harvested at 10 DAA that were not found in those harvested at 8 DAA, or in the “Fairuz” harvested at 8 and 10 DAA. These compounds are from the hydroxyl group and phenolic glucoside, with one of their activities being as antioxidants (Uchegbu et al., 2017; Li et al., 2019). “Sandi” has a purple pod, and the color is darker when harvested at 10 DAA than it is at 8 DAA. The darker color can indicate higher content of metabolite compounds. Previous studies reported that purple, red or blue fruits, and vegetables generally contain anthocyanin pigments (Juansah et al., 2013; Reswari

Table 3. Secondary metabolites and biological activity of winged bean “Fairuz” harvested at 8 days after anthesis (DAA)

No	Retention time	Peak area %	Compound	Compound group	Activity	Reference
1	30.872	42.26	1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester	Phthalate	Antifungal, Anticancer, Immunomodulatory activities, Antimicrobial	(Ezhilan dan Neelamegam 2012; Save et al., 2015; Prawanayoni dan Sudirga 2020)
2	29.010	13.69	ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE	Linolenic acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Nematicide, Insectifuge, Antihistaminic, Antiacne, Antiarthritic, Anticoronary	(Kumar et al., 2010; Starlin et al., 2019)
3	29.430	9.78	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
4	28.396	7.46	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory	(Aparna et al., 2012; Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019)
5	31.768	5.39	i-Propyl 9,12-octadecenadienoate	-	-	-
6	36.243	3.99	Stigmasterol	Steroid	Antimicrobial, Anticancer, Antiarthitic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant	(Sudha et al., 2013; Mary and Giri, 2016)
7	27.831	3.97	HEXADECANOIC ACID, ETHYL ESTER	Ester Compound	Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic	(Sermakkani and Thangapandian, 2012)
8	29.975	2.87	14-METHYL-8-HEXADECYN-1-OL	-	Sclerosant, Spasmolytic, Catechol-O-Methyl-Transferase-Inhibitor, 5-Alpha-Reductase-Inhibitor, Free-Radical Scavenging	(Karthikeyan et al., 2019; Payum, 2020)
9	37.084	2.36	.gamma.-Sitosterol	Steroid	Antidiabetic, Anti-aging, Anticancer, Antimicrobial, Anti-inflammatory, Antiviral	(Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha and Rao, 2018)
10	33.775	1.83	.gamma.-Tocopherol	-	Antioxidant	(Falk and Munné-Bosch, 2010)
11	32.258	1.63	PENTACOSANE	-	-	-
12	30.244	1.58	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	-	-	-
13	32.058	1.52	Z,Z-10,12-Hexadecadien-1-ol acetate	-	Hypoglycemic, Hycholesterolemic, Antioxidant, Anticancerous	(Jain and Rijhwani, 2018)

Table 4. Secondary metabolites and biological activity of winged bean “Fairuz” harvested at 10 days after anthesis (DAA)

No	Retention time	Peak area %	Compound	Compound group	Activity	Reference
1	28.968	27.53	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	Linoleic acid ester	Hypocholesterolemic, Nematicide Antiarthritic, Antiandrogenic, Anticoronary, Insectifuge, Antieczemic Anticancer	(Mamani dan Alhaji, 2019)
2	29.396	14.30	(9E,12E)-9,12-OCTADECADIENOIC ACID	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani dan Thangapandian, 2012)
3	31.761	12.98	Geranylgeraniol	Chloroform	Anticancer, Antimycobacterial	(Vik et al., 2007; Sianipar and Purnamaningsih, 2018)
4	30.927	9.27	[(DIMETHYLSILYL) METHYL](DIMETHYL) SILANE	-	-	-
5	28.327	6.05	HEXADECANOIC ACID	Palmitic acid	Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory	(Aparna et al., 2012; Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019)
6	29.865	5.38	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
7	27.748	5.13	HEXADECANOIC ACID, ETHYL ESTER	Ester Compound	Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic	(Sermakkani and Thangapandian, 2012)
8	35.967	3.99	Stigmasterol	Steroid	Antimicrobial, Anticancer, Antiarthitic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant	(Sudha et al., 2013; Mary and Giri, 2016)
9	30.348	3.35	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE	Chlorinated aliphatic ketone	Anti-inflammatory, Antiarthritic, Antidiabetic, hypolipidemic, Cytotoxic activities	(Sreeja et al., 2018)
10	36.746	3.18	.gamma.-Sitosterol	Steroid	Antidiabetic, Anti-aging, Anticancer, Antimicrobial, Anti-inflammatory, Antiviral	(Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha dan Rao, 2018)
11	31.375	2.49	2-CHLOROETHYL (9Z,12Z)-9,12-OCTADECADIENOATE	Fatty acid	Anticancer, Antioxidant	(Berwal et al., 2019)
12	27.162	1.33	TETRADECANOIC ACID	Myristic acid	Antioxidant, Cancer preventive, Nematicide, Hypocholesterolemic	(Rajeswari et al., 2013)
13	37.174	1.10	Pyrene, hexadecahydro-	-	Dyes and plasticisers precursor, Biodegradation of organic compounds	(Wei et al., 2015; Salim 2018)

Table 5. Secondary metabolites and biological activity of winged bean "Sandi" harvested at 8 days after anthesis (DAA)

No	Retention time	Peak area %	Compound	Compound group	Activity	Reference
1	30.865	26.66	1,2 Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester	Phthalate	Antifungal, Anticancer, Immunomodulatory activities, Antimicrobial	(Ezhilan and Neelamegam, 2012; Save et al., 2015; Prawanayoni and Sudirga, 2020)
2	29.038	15.30	Linoleic acid ethyl ester	Linoleic acid ethyl ester	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne, Antieczemic, Antiandrogenic, , Anticoronary, Insectifuge	(Sermakkani and Thangapandian 2012; Sudha et al., 2013)
3	29.437	11.57	(9E,12E)-9,12-OCTADECADIENOIC ACID	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
4	28.451	7.91	HEXADECANOIC ACID	Palmitic acid	Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory	(Aparna et al., 2012; Sermakkani and Thangapandian, 2012; Korbecki and Bajdak-Rusinek, 2019)
5	27.845	7.82	HEXADECANOIC ACID, ETHYL ESTER	Ester Compound	Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic	(Sermakkani dan Thangapandian 2012)
6	36.326	4.72	Stigmasterol	Steroid	Antimicrobial, Anticancer, Antiarthitic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant	(Sudha et al. 2013; Mary and Giri ,2016)
7	37.250	4.56	.gamma.-Sitosterol	Steroid	Antidiabetic, Anti-aging, Anticancer, Antimicrobial, Anti-inflammatory, Antiviral	(Tripathi et al., 2013a; Tripathi et al., 2013b; Nisha dan Rao, 2018)
8	29.982	2.94	(9E,12E)-9,12-OCTADECADIENOIC ACID	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
9	30.596	2.81	10-Undecen-1-al, 2-methyl-	-	Antimicrobial and Anti-inflammatory	(Hameed et al., 2016)
10	33.747	2.12	.gamma.-Tocopherol	-	Antioxidant	(Falk and Munné-Bosch, 2010)
11	31.444	2.02	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
12	29.748	1.83	1,2-ETHANEDIAMINE, N,N-DIMETHYL-N'-2-PYRIDINYL-N'-(2 PHENYLMETHYL)-	-	-	-
13	30.244	1.66	E,E-10,12-Hexadecadien-1-ol acetate	-	Hypoglycemic, Hycholesterolemic, Antioxidant, Anticancerous, Thyroid inhibiting properties	(Jain and Rijhwani, 2018)
14	31.782	1.55	Ethyl tetracosanoate	Ester	Antibacterial	(Igwe and Okwu, 2013)
15	32.044	1.19	1-CINNAMYL-3-METHYLINDOLE-2-CARBALDEHYDE	-	-	-
16	32.237	1.19	ICOSANE	-	Antifungal, Anticancer	(Ahsan et al., 2017; Sianipar and Purnamaningsih, 2018)
17	31.906	1.10	Squalene	Triterpene	Anticancer, Antimicrobial, Antioxidant, Chemopreventive, Pesticide, Anti-tumor, Antibacterial, Immunostimulant	(Ezhilan and Neelamegam, 2012; Sermakkani and Thangapandian, 2012)

Table 6. Secondary metabolites and biological activity of winged bean "Sandi" and the harvest age of 10 days after anthesis (DAA)

No	Retention time	Peak area %	Compound	Compound group	Activity	Reference
1	29.293	20.02	(9E,12E)-9,12-OCTADECADIENOIC ACID	Linoleic acid	Anti-Inflammatory, Hypocholesterolemic, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Antiacne	(Sermakkani and Thangapandian, 2012)
2	6.642	13.97	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE	Hydroxyl group	Antioxidant	(Li et al., 2019)
3	27.452	13.77	.alpha.-D-Glucopyranoside, methyl	Phenolic glucoside	Apoptotic activity, Anticancer, Antituberculosis, Antioxidant, Alpha-amylase inhibitory activity, Anticonvulsant	(Lyantagaye 2013; Uchegbu et al., 2017)
4	28.279	9.92	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Nematicide, Pesticide, Antiandrogenic, Flavor, Anti-inflammatory	(Aparna et al. 2012; Sermakkani and Thangapandian 2012; Korbecki dan Bajdak-Rusinek, 2019)
5	3.794	9.60	2,5-FURANDIONE	-	-	-
6	30.927	7.32	4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE	-	-	-
7	22.459	6.50	1H-Imidazole-4-carboxamide, 5-amino- $$ Imidazole-4-carboxamide, 5-amino-	-	-	-
8	28.893	3.92	ETHYL (9Z,12Z,15Z)-9,12,15-OCTADECATRIENOATE	Linolenic acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Nematicide, Insectifuge, Antihistaminic, Antiacne, Antiarthritic, Anticoronary	(Kumar et al., 2010; Starlin et al., 2019)
9	33.402	3.05	Stigmasterol tosylate	-	-	-
10	30.313	2.57	2-Dodecen-1-yl(-) succinic anhydride	Ethyl acetate	Antineoplastic agents, Antioxidants, Antimicrobial	(Rawal and Sonawani 2016; Saravanan et al., 2018)
11	35.809	2.43	Stigmasterol	Steroid	Antimicrobial, Anticancer, Antiarthitic, Antiasthma, Diuretic, Anti-inflammatory, Antioxidant	(Sudha et al., 2013; Mary and Giri, 2016)
12	27.721	2.22	HEXADECANOIC ACID, ETHYL ESTER	Ester Compound	Antioxidant, Flavor, Nematicide, Pesticide, Antiandrogenic	(Sermakkani and Thangapandian. 2012)
13	31.623	1.80	14-HYDROXY-20-OXOPREGNAN-3-YL ACETATE	-	-	-
14	31.354	1.51	4-(4-ETHYLCYCLOHEXYL)-1-PENTYL-1-CYCLOHEXENE	-	-	-
15	33.857	1.41	STIGMAST-5-EN-3-OL	Phytosterols	Anticancer, Reduce blood level of glucose, hypercholesterolemia	(Sianipar et al., 2016; Yamuna et al., 2017)

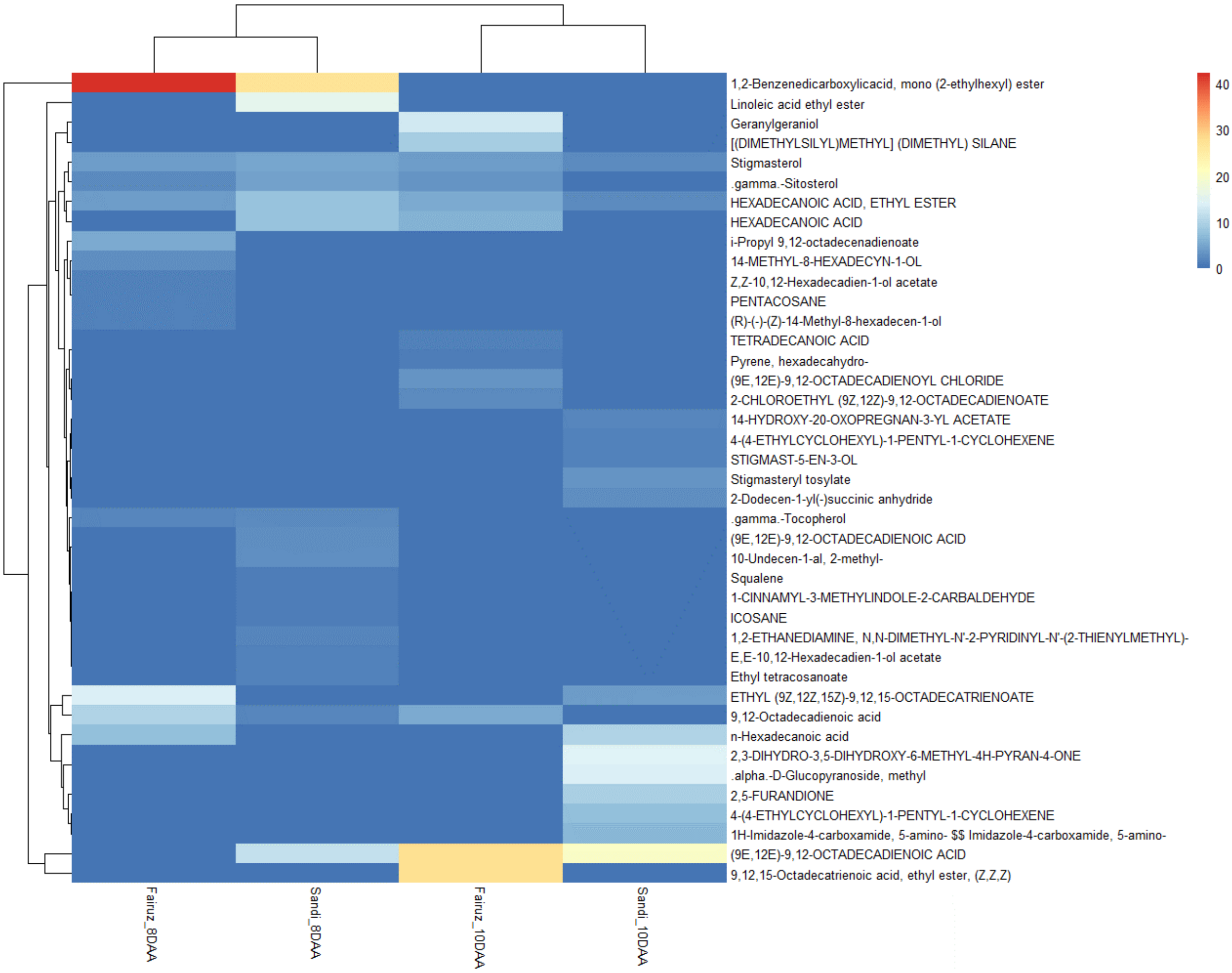

Figure 3. Heatmap of the abundance of secondary metabolites of two varieties harvested at 8 and 10 DAA. The intensity of the color indicates abundance from low (blue) to high (red).

et al., 2019). The anthocyanin pigments are a group of flavonoids whose activity is an antioxidant (Nomer et al., 2019), which in this study was demonstrated in pods harvested at 10 DAA. Many phytochemical compounds with biological activities make young winged pods have good health benefits, and they can be consumed as functional vegetables.

Conclusion

The secondary metabolite compounds mostly from the linoleic acid group were identified from the young green pods "Fairuz" harvested at 8 and 10 DAA. Similar compounds were found in the purple pods "Sandi". The purple pods harvested at 10 DAA have additional compounds with antioxidant activity other than those from the linoleic acid group. 1,2-Benzenedicarboxylic acid, mono(2-Ethylhexyl) ester was the most abundant compound identified from pods harvested at 8 DAA in both varieties. Therefore, young winged bean pods have benefits when consumed as vegetables.

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