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Identification of chemical constituents from fruit of *Antidesma bunius* by GC-MS and HPLC-DAD-ESI-MS

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Abstract

Antidesma bunius is an edible berry fruit with many benefits, such as natural antimicrobials, anticancer, natural dyes, etc. However, data on chemical content in the fruit is still limited. The purpose of this research is to identify volatile compounds of *Antidesma bunius* fruit. We extracted and analyzed the *A. bunius* fruit's chemical content using GC-MS and HPLC–DAD-ESI-MS methods. Forty-nine compounds representing 99.91% of the total chromatogram's relative peak area were detected. *Antidesma bunius* is rich in 5-hydroxymethylfurfural (HMF) and ten other compounds with relative peak area >1%, such as furaldehyde, citric acid and others. We also found 109 compounds tentatively identified through HPLC–DAD-ESI-MS. *Antidesma bunius* contained HMF, several volatile compounds, organic acid, long-chain fatty acid, and photochromic compound.

Keywords: *Antidesma bunius*; berry; bignay, GC-MS; HPLC–DAD-ESI -MS; volatile.

Practical Application: The study results indicate the possible use of the fruit of *A. bunius* for food flavoring, antimicrobial and anticancer agents.

1 Introduction

Antidesma bunius belongs to the Phyllantaceae family. The fruit is included in the type of berries. *Antidesma bunius* comes from east and south Asia. This plant has several other names, such as bignay, bignai, bugnay, Chinese laurel, Queensland cherry, wild cherry, currant tree, salamander-tree. In Indonesia, this fruit is known as the Buni or Huni fruit (Lizardo et al., 2015).

In Indonesia, Buni fruit is usually consumed directly when ripe or processed into local foods such as Rujak. The acidic taste and the striking color become an attraction for consumption. Besides its refreshing taste, Buni fruit is known to have many health benefits and can be applied to other aspects.

Antidesma bunius has many benefits for human health. Almost all parts of *Antidesma bunius* have bioactive compounds that can be used as a source of medicine. Buni fruit contains antioxidant compounds, anticancer, anti-inflammation, anti-diabetic, anti-hyperlipidemia and can also be used as an anti-parasitic agent (Chowtivannakul et al., 2016; Jorjong et al., 2015; Lizardo et al., 2015).

Health benefits and also applications of Buni fruit have been widely studied. However, data regarding the content of chemical compounds in the fruit are still limited. The research that has been carried out only covers the characterization of phytochemical content, polyphenol compounds and anthocyanin, but its chemical constituents remain incompletely investigated. Furthermore, no studies have examined the content of volatile compounds and essential oils in the fruit of *Antidesma bunius*. (Hardinasinta et al., 2020; Jorjong et al., 2015).

Thus, comprehensive profiling of the phytochemicals of *A. bunius* fruit using sensitive tools is necessary. Therefore, this study aims to analyze volatile compounds in the fruit of *Antidesma bunius* by using high-performance liquid chromatography-diode array detector-hyphenated with tandem mass spectrometry (HPLC–DAD–ESI-MS) and gas chromatography with tandem mass spectrometry (GC-MS) as a powerful analytical technique.

2. Methods

2.1 Plant materials

Fresh ripe fruit of *Antidesma bunius* was manually harvested in Sumedang, West Java Province, Indonesia during March 2019. The fruit was maintained at -18 °C immediately after collection until they were used.

2.2 Extraction

The fresh ripe fruit of *Antidesma bunius* (200 g) was crushed and then extracted using 1 L of 80% methanol in the darkroom temperature overnight. The extraction process was repeated three times to ensure exhaustive extraction. The supernatant is then filtered using filter paper, and the resulting filtrate is evaporated using a rotary evaporator at a temperature of 45 °C.

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2.3 Gas Chromatography-Mass Spectrometry

GCMS analyses were performed on a GC-2010 GC coupled with a GCMS-QP2010 Ultra mass spectrometer. The column was a Rxi-5ms fused-silica capillary column (30.0 m Length x 0.25 mm ID x 0.25 µm Thickness) with helium as the carrier gas and was run at a constant pressure of 37.1 psi. The injection was conducted using a split-less mode at an injector temperature of 250 °C. The oven temperature was ramped from 50 to 300 °C (10 min hold) at a rate of 3 °C/min. The oven temperature was held at 300 °C for 6 min following each analysis. The total run time for each sample was approximately 25 min. The GCMS interface temperature was set to 250 °C. MS mode was used during analytical scanning from 40-700 atomic mass units (amu). The ion source temperature was set to 200 °C. The blank was injected first, followed by the sample injection. The chromatograms obtained from the total ion current (TIC) were integrated without any correction for co-eluting peaks, and the results were expressed as total abundance. TIC peaks and chromatograms were analyzed using Agilent MSD ChemStation G1701DA software (version D 02.00, CA, USA). All peaks were identified based on mass spectral matching (≥ 90%) from both the National Institute of Standards and Technology (NIST) and Wiley libraries. Only compounds with 90% or greater spectral matching accuracy were reported.

2.4 HPLC–DAD-ESI-MS analysis

Separation of phenolic compounds from *Antidesma bunius* extract was performed on an Agilent 1200 series Rapid Resolution LC (Agilent Technologies, Santa Clara, CA). This instrument was equipped with an Agilent Zorbax C18 column (4.6 x 150 mm, 5 µm) from Agilent Technologies. Acidified water (0.5% acetic acid, v/v) and acetonitrile were used as mobile phases A and B, respectively. The gradient was programmed as follows: 0 min, 0% B; 20 min, 20% B; 30 min, 30% B; 40 min, 50% B; 50 min, 75% B; 60 min, 100% B; 62 min 0% B, and finally, the initial conditions were held for 8 min as a re-equilibration step. The flow rate was set at 0.80 mL/min throughout the gradient. The flow from the HPLC system into the ESI-MS detector was 0.2 mL/min. The injection volume was 10 µL and the column temperature was maintained at 25 °C.

3 Results and discussion

3.1 Chemical components identified in Antidesma bunius *fruit by GC-MS*

A methanol extract of *Antidesma bunius* has a deep purple color. In the analysis of volatile components present in *Antidesma bunius* extract, a total of fifty compounds were detected (Figure 1). Among these components, 5-hydroxymethylfurfural (5-HMF) was found in the most significant concentrations, representing 47.07% of all volatiles. Other predominant components were furfural, citric acid, furan carboxylic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one, hexanoic acid, 1,6-Anhydro-.beta.-D-glucofuranose, 5,5'-oxy-Dimethylene-bis(2-Furaldehyde), Linolenic acid and gamma-Sitosterol.

The dominant compound detected in *Antidesma bunius* extract was 5-HMF with a relative amount of 47.07% (Table 1). This compound can be found naturally in honey and processed foods such as fruit juice, ketchup, UHT milk, etc. (Saeed et al., 2018). HMF is a compound that can be used as an intermediate for polymers, pharmaceuticals, liquid fuels, and the synthesis of several groups of compounds such as dialdehyde, ethers and other organic compounds. 5-HMF can be produced from hexoses such as fructose through several treatments such as dehydration (Gomes et al., 2015). This study result shows the potential of the *Antidesma bunius* fruit as a natural source of HMF.

This study also detected 5-HMF derivative compounds, namely 5.5'-oxy-Dimethylene-bis (2-Furaldehyde). This compound is the result of the thermal decomposition of 5-HMF. Formation of 5,5'-oxy-Dimethylene-bis (2-Furaldehyde) compounds occur at temperatures of 100-220°C (Nikolov & Yaylayan, 2011).

One of predominant component detected in *Antidesma bunius* extract was furfural. This compound is commonly found in all fruits and vegetables and other plant parts that contain lignin

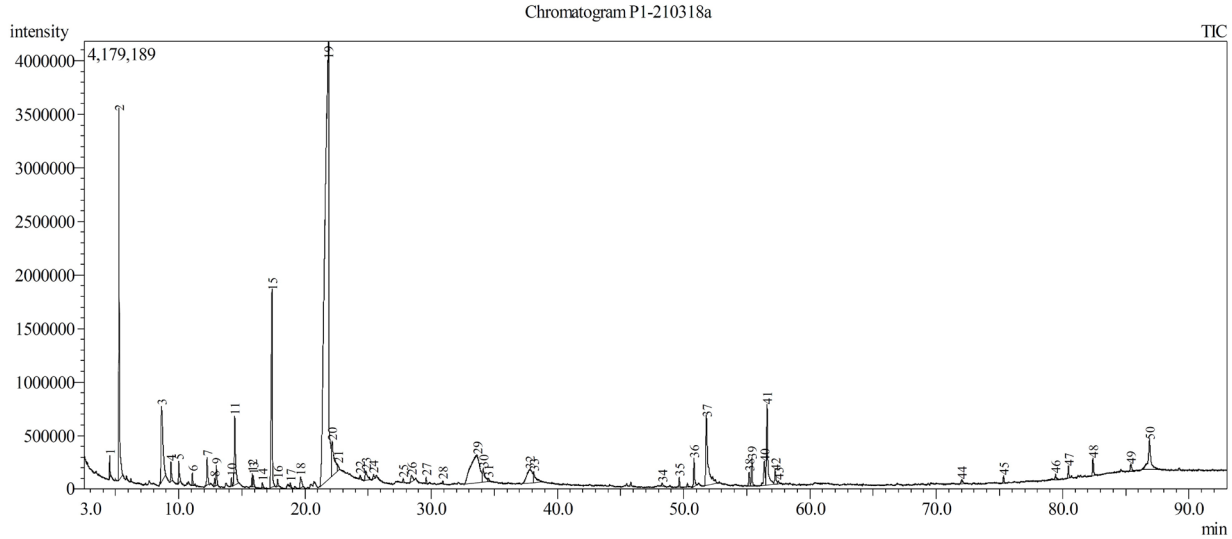

Figure 1. Gas Chromatogram of Volatile Compounds of *Antidesma bunius* extract.

Table 1. Volatile components identified in *Antidesma bunius* fruit by GC-MS.

No	Retention Time (RT) in minutes	Compounds	Molecular Formula*)	Relative Abundance (%)	MS Identification*)
1	4.534	Furazane, 3-amino-4-iodo-	$C_2H_2IN_3O$	0.25	MS
2	5.273	Furfural	$C_5H_4O_2$	5.89	MS
3	8.647	Citric acid	$C_6H_8O_7$	3.99	MS
4	9.389	5-methyl furfural	$C_6H_6O_2$	0.44	MS
5	10.009	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.53	MS
6	11.085	2,2,6,6-Tetramethylpiperidine	$C_9H_{19}N$	0.24	MS
7	12.250	Itaconic anhydride	$C_5H_4O_3$	0.80	MS
8	12.841	2-Methyl-1,3-cyclohexanedione 97%	$C_7H_{10}O_2$	0.16	MS
9	12.987	Pentanoic acid, 4-oxo	$C_5H_8O_3$	0.48	MS
10	14.172	Orcinol	$C_7H_8O_2$	0.18	MS
11	14.447	Methyl 2-furoate	$C_6H_6O_3$	2.38	MS
12	15.835	Levoglucosenone	$C_6H_6O_3$	0.31	MS
13	15.908	Butylacetate	$C_6H_{12}O_2$	0.28	MS
14	16.628	6-Methyltridecane	$C_{14}H_{30}$	0.16	MS
15	17.397	Hydroxydihydromaltol	$C_6H_8O_4$	6.46	MS
16	17.828	Methacrolein dimer	$C_8H_{12}O_2$	0.16	MS
17	18.843	Pyrimidine, 4-chloro-5-ethoxy-2-methyl-	$C_7H_9ClN_2O$	0.10	MS
18	19.653	2-Methylbutyl propionate	$C_8H_{16}O_2$	0.48	MS
19	21.842	5-hydroxymethylfurfural	$C_6H_6O_3$	47.07	MS
20	22.153	2-Cyclohexenone	C_6H_8O	2.55	MS
21	22.622	No hit compound	-	0.23	MS
22	24.375	No hit compound	-	0.09	MS
23	24.813	3-Heptanol	$C_7H_{16}O$	0.53	MS
24	25.437	2-Ethyl-3-nitroso-1,3-oxazinane	$C_6H_{12}N_2O_2$	0.15	MS
25	27.787	Decanoic acid	$C_{10}H_{20}O_2$	0.11	MS
26	28.452	Propanoic acid, 2-methyl-, 2-(hydroxymethyl)-1-propylbutyl ester	$C_{12}H_{24}O_3$	0.34	MS
27	29.607	Dodecanal	$C_{12}H_{24}O$	0.14	MS
28	30.922	Artemisia ketone	$C_{10}H_{16}O$	0.08	MS
29	33.640	Levoglucosan	$C_6H_{10}O_5$	6.76	MS
30	34.128	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	0.80	MS
31	34.528	No hit compound	-	0.14	MS
32	37.793	Scyllo-Inositol	$C_6H_{12}O_6$	2.03	MS
33	38.128	Cyclododecanol	$C_{12}H_{24}O$	0.77	MS
34	43.308	No hit compound	-	0.14	MS
35	49.655	Methyl Hexadecanoate	$C_{17}H_{34}O_2$	0.23	MS
36	50.827	Hexadecanoic acid	$C_{16}H_{32}O_2$	0.79	MS
37	51.798	5,5'-oxy-Dimethylene-bis(2-Furaldehyde)	$C_{12}H_{10}O_5$	4.21	MS
38	55.195	Methyl Linoleate	$C_{19}H_{34}O_2$	0.39	MS
39	55.405	Methyl Linolenate	$C_{19}H_{32}O_2$	0.70	MS
40	56.392	Ambrettolide	$C_{16}H_{28}O_2$	0.86	MS
41	56.612	Linolenic acid	$C_{18}H_{30}O_2$	3.16	MS
42	57.267	Stearic acid	$C_{18}H_{36}O_2$	0.43	MS
43	57.515	No hit compound	-	0.11	MS
44	72.035	No hit compound	-	0.21	MS
45	75.347	Squalene	$C_{30}H_{50}$	0.19	MS
46	79.457	Farnesol	$C_{15}H_{26}O$	0.19	MS
47	80.477	Tetraprenol	$C_{20}H_{34}O$	0.28	MS
48	82.427	Vitamin E	$C_{29}H_{50}O_2$	0.52	MS
49	85.432	Stigmasterol	$C_{29}H_{48}O$	0.29	MS
50	86.903	Rhamnol	$C_{29}H_{50}O$	2.23	MS

*) Based on comparison of MS spectrum with database of WILEY229.LIB.

and cellulose. Furfural is also a multi-purpose compound such as 5-HMF. Furfural can be applied in the petroleum industry, medicine, and also agriculture as insecticides, pesticides, antiseptics, and can also be used as bio-fuel (Wankasi & Tarawou, 2013).

Citric acid was also detected in *Antidesma bunius* extract (3.99%). This compound is generally found in citrus fruits. Citric acid provides an acidic taste in some fruits such as lemon, orange, lime, grapefruit. *Antidesma bunius* fruit also has a strong sour taste. Based on this study results, citric acid may also be a contributor to *Antidesma bunius* fruit sour taste (Chanukya et al., 2017).

Furancarboxylic acid or 2-furoic acid is a heterocyclic carboxylic acid, consisting of a five-membered aromatic ring and a carboxylic acid group. In the industry, this compound is used as a preservative, acting as a bactericide and fungicide. *Antidesma bunius* also own this antimicrobial property. Previous research shows the presence of antibacterial and antifungal activity from *Antidesma bunius* extract and food preservative effects (Lizardo et al., 2015). This study shows that 2-furoic acid compounds can provide antimicrobial effects in *Antidesma bunius*.

Farnesol is a bioactive compound that is also detected in this study. Farnesol is a natural 15-carbon organic compound which is acyclic sesquiterpene alcohol. Farnesol is present in many essential oils and used in perfumery. Farnesol is also known as a natural pesticide for mites and flavoring ingredients. Farnesol has antibacterial activity and is used as a deodorant in cosmetic products. It has been reported to exhibit anticancer and anti-inflammatory effects and alleviate allergic asthma, gliosis, and edema. These findings support the development of *Antidesma bunius* as a potential source of the pharmacological agent (Jung et al., 2018; Kromidas et al., 2006).

Another compound detected in this study is Hydroxydihydromaltol (2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one). It was identified as a novel potent aroma compound in a dairy product, such as Ryazhenka kefir, and found as odorants in roasted chicory (Preininger et al., 2008). This compound is thought to be formed due to the heating process and can affect processed foods' antioxidant activity. For example, increased Hydroxydihydromaltol is accompanied by an increase in antioxidant activity in dry prune products (Čechovská et al., 2011).

Hexanoic acid is an organic acid compound found in *Antidesma bunius*. This compound is also found in other fruit such as noni, pineapple and raspberry (Ferlinahayati et al., 2016; Aprea et al., 2015). Hexanoic acid has anticancer activity and also can improve energy metabolism (Narayanan et al., 2015; Miyamoto et al., 2016). Other studies have shown the anticancer activity of *Antidesma bunius* extract in Hela cells (Puspitasari & Ulfa, 2009). Hexanoic acid can be one of the compounds that provide anticancer activity from *Antidesma bunius*.

Another predominant compound detected in *Antidesma bunius* fruit is 1,6-Anhydro-beta-D-glucofuranose. This compound is anhydrosugar and commonly formed from the results of glucose pyrolysis (Hu et al., 2017). However, this compound is also naturally found in Oak bark, Acacia honey and *Punica granatum*. (Deryabin & Tolmacheva, 2015; Sangeetha & Vijayalakshmi, 2011).

Linolenic acid is an essential fatty acid belonging to the omega-3 fatty acids group. It is highly concentrated in certain plant oils and fruits (Saini & Keum, 2017). Linolenic acid has been reported to inhibit prostaglandin synthesis, resulting in reduced inflammation and the prevention of certain chronic diseases. It also has anti-carcinogenic, lipid metabolism regulation, anti-inflammatory, anti-obese and antioxidant activities (Yuan et al., 2014).

Gamma-Sitosterol is a phytosterol with structural similarity to cholesterol and can be found in plants, animals, and fungi. This compound has many beneficial effects on human health, such as hypolipidemic agents, anticancer, antioxidant, etc. (Balamurugan et al., 2015). In this study, we detected gamma-sitosterol in *Antidesma bunius*. This result suggests that *Antidesma bunius* can be a hypolipidemic agent, as proven in a previous study (Chowtivannakul et al., 2016).

2,4-di-tert-butylphenol is a member of the class of phenols carrying two tert-butyl substituents at positions 2 and 4. It has a role as a bacterial metabolite, an antioxidant, and a marine metabolite. It is an alkylbenzene and a member of phenols. Antioxidants/ Stabilizers Fuels and fuel additives Intermediate Processing aids.

This study also detected several compounds that can act as flavoring agents. 2-cyclohexenone has an organoleptic property such as roasted savory green odor, 2-Methylbutyl propionate, and Methyl 2-furoate taste like sweet caramel brown sugar musty.

3.2 Chemical components identified in Antidesma bunius fruit by HPLC–DAD-ESI-MS

Among those identified compounds, several compounds have not been detected in *Antidesma bunius* fruit before. This study is the first to describe these results. Table 2 shows the list of 109 compounds detected through HPLC–DAD-ESI-MS experiments along with their retention times (tR). Seventy-eight compounds are tentatively identified, and 31 compounds remain unknown (Figure 2). The compounds detected in this work were tentatively characterized by MS data, together with the interpretation of the observed MS/MS spectra compared with those found in the literature. Several public databases were consulted in the identification process, such as ChemSpider (Royal Society of Chemistry, 2020), and PubChem (National Center for Biotechnology Information, 2020).

5-Methoxysalicylic acid is also known as 2-hydroxy-5-methoxybenzoate. It is an extremely weak basic (nearly neutral) compound (based on its pKa). 5-Methoxysalicylic acid has been detected in herbs, spices and tea. (Dieryckx et al., 2015) However, this study results indicate that 5-Methoxysalicylic acid is also found in fruits such as *A. bunius*.

In this research, several fatty acid compounds were identified. (15Z)-9,12,13-Trihydroxy-15-octadecenoic acid is a long-chain fatty acid. This compound can be used for various purposes, such as antifoaming agents, coagulating agents, dispersion agents, emulsifiers, flotation agents, foaming agents, viscosity adjustors, etc. Butanedioic acid, 2-(6-hydroxyhexyl)-3-methylene- is a small acid found in *Aspergillus tubingensis* as its secondary metabolite. This compound has the potential to have bioactivity (Frisvad et al., 2018). However, there was no research to prove this.

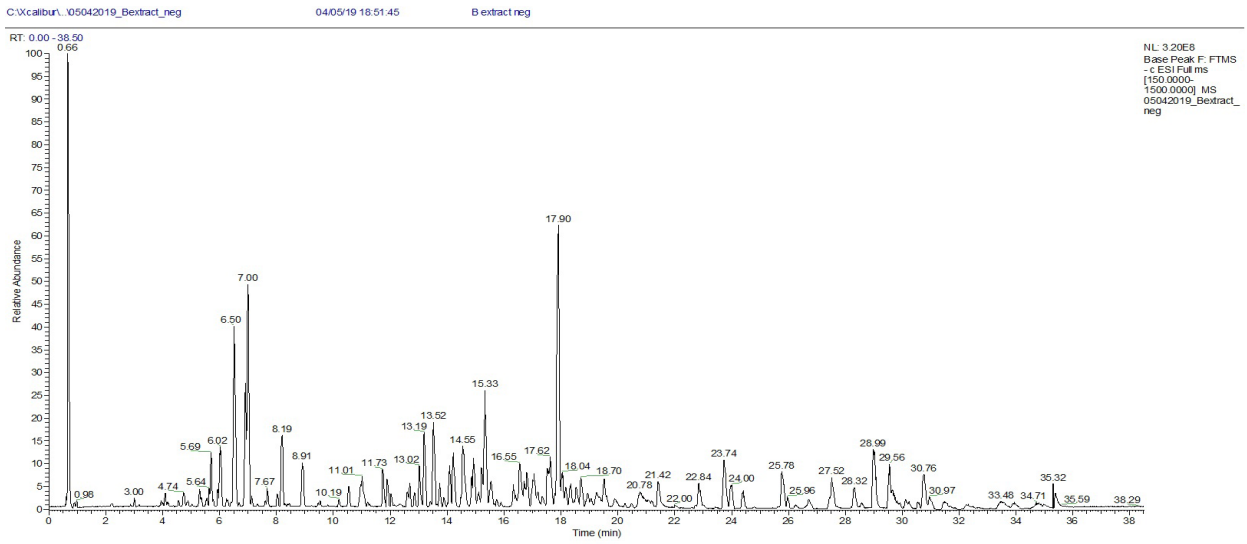

Figure 2. HPLC-DAD Base Peak Chromatogram (BPC) for *Antidesma bunius* extract.

Table 2. Chemical components identified in *Antidesma bunius* fruit by LC-MS/MS.

Peak No	Retention Time (RT) in minutes	Compounds	Molecular Weight**)	Molecular Formula**)	Reference MS Identification**)
1	4.74	5-Methoxy salicylic acid	168.041	$C_8H_8O_4$	MS
2	5.3	Suberic acid	174.088	$C_8H_{14}O_4$	MS
3	5.64	Butanedioic acid, 2-(6-hydroxyhexyl)-3-methylene-	230.115	$C_{11}H_{18}O_5$	MS
4	5.68	Methyl 5-[2-(4-methoxy-2-methyl-4-oxobutyl)-5-methyl-1,3-dioxolan-4-yl]pentanoate	316.188	$C_{16}H_{28}O_6$	MS
5	5.69	Lariciresinol 4-O-glucoside	568.215	$C_{26}H_{34}O_{11}$	MS
6	5.94	Unknown	497.253	$C_{28}H_{31}N_7O_2$	MS
7	6.02	Similar to: 1-(Carboxymethyl)cyclohexanecarboxylic Acid; ΔMass: -208.0708 Da)	394.16	$C_{16}H_{22}N_6O_6$	MS
8	6.03	1- (Carboxymethyl)cyclohexanecarboxylic Acid	186.088	$C_9H_{14}O_4$	MS
9	6.51	Phospocholine conjugate acid	184.073	$C_5H_{15}NO_4P$	MS
10	6.51	Similar to: Disperse red 17	390.129	$C_{17}H_{20}N_4O_4$	MS
11	6.51	Unknown	590.167	$C_{28}H_{27}N_6O_7P$	-
12	6.51	Unknown	796.223	$C_{26}H_{43}N_{10}O_{13}P_3$	-
13	6.51	[Similar to: 2-(3,4-Dihydroxyphenyl)ethyl (1S,4aR,7aR)-1-(β-D-glucopyranosyloxy)-4a-hydroxy-7-methyl-5-oxo-1,4a,5,6,7,7ahexahydrocyclopenta[c]pyran-4-carboxylate; ΔMass: -68.0201 Da]	594.189	$C_{21}H_{37}N_6O_8P_3$	MS
14	6.51	Malonic acid,isobutylidene-,bimol. cyclic ethylene ester	368.147	$C_{18}H_{24}O_8$	MS
15	6.53	[Similar to: 2-(3,4-Dihydroxyphenyl)ethyl (1S,4aR,7aR)-1-(β-D-glucopyranosyloxy)-4a-hydroxy-7-methyl-5-oxo-1,4a,5,6,7,7ahexahydrocyclopenta[c]pyran-4-carboxylate; ΔMass: 274.1081 Da]	252.061	$C_8H_8N_6O_4$	MS
16	6.91	Unknown	596.214	$C_{28}H_{33}N_6O_7P$	MS
17	6.91	[Similar to: 1-(Carboxymethyl)cyclohexanecarboxylic acid; ΔMass: -414.1466 Da]	600.236	$C_{21}H_{43}N_6O_8P_3$	MS
18	6.91	Similar to: 1-(Carboxymethyl)cyclohexanecarboxylic Acid; ΔMass: -208.0708 Da)	394.16	$C_{16}H_{22}N_6O_6$	MS

**) Based on comparison of MS spectrum with database of National Institute of Standards and Technology (NIST)

Table 2. Continued...

Peak No	Retention Time (RT) in minutes	Compounds	Molecular Weight**)	Molecular Formula**)	Reference MS Identification**)
19	6.92	[Similar to: 1-(Carboxymethyl)cyclohexanecarboxylic acid; ΔMass: -67.9870 Da]	254.076	$C_7H_{14}N_2O_8$	MS
20	6.99	1,3,3-trimethylindolino-6'-nitrobenzopyrylospiran	322.131	$C_{19}H_{18}N_2O_3$	MS
21	7.67	1,3-Bis(2,4-dimethoxyphenyl)-1,3-propanedione	344.126	$C_{19}H_{20}O_6$	MS
22	7.68	Rubone	374.136	$C_{20}H_{22}O_7$	MS
23	8.03	[Similar to: [7-Hydroxy-1-(4-hydroxy-3, 5-dimethoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenyl]methyl pentopyranoside; ΔMass: -11.9636 Da]	564.184	$C_{27}H_{36}O_{12}$	MS
24	8.19	[Similar to: 1-(Carboxymethyl)cyclohexanecarboxylic acid; ΔMass: -208.0709 Da]	394.16	$C_{16}H_{22}N_6O_6$	MS
25	8.91	4-Ethyl-3-oxa-5-thia-4-boratricyclo[5.2.1.0~2,6~]decane	182.093	$C_9H_{15}BOS$	MS
26	10.54	Unknown	524.263	$C_{29}H_{32}N_8O_2$	-
27	10.96	Ethyl 5-methoxy-2-[(4-methoxyphenoxy)methyl]-1-benzofuran-3-carboxylate	356.126	$C_{20}H_{20}O_6$	MS
28	10.98	N-[2-(Adamantan-1-yloxy)ethyl]-3,5-Dimethoxybenzamide	359.209	$C_{21}H_{29}NO_4$	MS
29	11	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	330.241	$C_{18}H_{34}O_5$	MS
30	11.74	Ethyl 1,4-dioxaspiro[4.5]decane-6-Carboxylate	214.12	$C_{11}H_{18}O_4$	MS
31	11.74	[Similar to: 11(Z),14(Z),17(Z)-Eicosatrienoic acid; ΔMass: 136.1262 Da]	170.13	-	MS
32	11.9	Isoindole-1,3-dione, 2-[2-(adamantan1-yloxy)ethyl]-5-methyl-3a,4,7,7atetrahydro	343.214	$C_{21}H_{29}NO_3$	MS
33	12.03	1-Allyl 2-dodecyl 1,2-pyrrolidinedicarboxylate	367.272	$C_{21}H_{37}NO_4$	MS
34	12.6	Unknown	508.268	$C_{29}H_{32}N_8O$	MS
35	12.64	Unknown	547.279	$C_{31}H_{33}N_9O$	MS
36	12.68	[Similar to: Cholic acid; ΔMass: -0.9951 Da]	409.283	$C_{24}H_{35}N_5O$	MS
37	12.85	2-Nitro-1,3-bis(octyloxy)benzene	379.272	$C_{22}H_{37}NO_4$	MS
38	13.02	Unknown	427.293	$C_{24}H_{37}N_5O_2$	-
39	13.04	Unknown	367.272	$C_{15}H_{38}N_5O_3P$	-
40	13.09	Unknown	397.282	$C_{23}H_{35}N_5O$	-
41	13.19	(11alpha,13E,15S)-11,15-Dihydroxy-N- (2-hydroxyethyl)-9-oxoprost-13-en-1- amide	397.282	$C_{22}H_{39}NO_5$	MS
42	13.55	[Similar to: 3-[2-(2,3-Dihydroxy-5,6,8atrimethyloctahydro-2Hspiro[naphthalene-1,2'-oxiran]-5-yl)ethyl]-5-hydroxy-2(5H)-furanone; ΔMass: -73.0889 Da]	439.239	$C_{18}H_{42}N_5O_5P$	MS
43	13.74	[Similar to: 20-Hydroxy-(5Z,8Z,11Z,14Z)-eicosatetraenoic acid; ΔMass: -135.0531 Da]	455.288	$C_{25}H_{37}N_5O_3$	MS
44	13.88	(9Z,12Z)-6,8-Dihydroxy-9,12-octadecadienoic acid	312.23	$C_{18}H_{32}O_4$	MS
45	14.09	Unknown	425.278	$C_{24}H_{35}N_5O_2$	MS

**) Based on comparison of MS spectrum with database of National Institute of Standards and Technology (NIST)

Table 2. Continued...

Peak No	Retention Time (RT) in minutes	Compounds	Molecular Weight**)	Molecular Formula**)	Reference MS Identification**)
46	14.21	1-Allyl 2-tridecyl 1,2-Pyrrolidinedicarboxylate	381.288	$C_{22}H_{39}NO_4$	MS
47	14.49	11-Deoxy prostaglandin F2β	338.246	$C_{20}H_{34}O_4$	MS
48	14.55	(11alpha,13E,15S)-11,15-Dihydroxy-N-(2-hydroxyethyl)-9-oxoprost-13-en-1-amide	397.282	$C_{22}H_{39}NO_5$	MS
49	14.56	10-Undecen-1-yl N-(cyclohexylcarbonyl)alaninate	351.277	$C_{21}H_{37}NO_3$	MS
50	14.84	Unknown	455.288	$C_{25}H_{37}N_5O_3$	-
51	14.86	Glycocholic acid	465.309	$C_{26}H_{43}NO_6$	MS
52	14.93	Unknown	439.293	$C_{24}H_{41}NO_6$	-
53	15.03	4-Dodecylbenzenesulfonic acid	326.192	$C_{18}H_{30}O_3S$	MS
54	15.11	Unknown	425.278	$C_{24}H_{35}N_5O_2$	-
55	15.19	(+/-)12(13)-DiHOME /(Z)-12,13-dihydroxyoctadec-9-enoic acid	314.246	$C_{18}H_{34}O_4$	MS
56	15.21	Unknown	435.298	$C_{26}H_{37}N_5O$	-
57	15.23	Unknown	469.304	$C_{29}H_{43}NO_2S$	-
58	15.23	17-(4-Hydroxyphenyl)-16-oxa-1,6,10,23-tetraazatetracyclo[9.8.6.2~12,15~.0~14,18~]heptacosa12,14,26-triene-19,24-dione	492.273	$C_{28}H_{36}N_4O_4$	MS
59	15.33	Similar to: Cholic acid; ΔMass: -0.9947 Da	409.282	$C_{24}H_{35}N_5O$	MS
60	15.33	[Similar to: Cholic acid; ΔMass: - 68.9820 Da]	477.27	$C_{20}H_{40}N_5O_6P$	MS
61	15.54	Unknown	395.267	$C_{23}H_{33}N_5O$	-
62	16.25	Unknown	453.273	$C_{18}H_{40}N_5O_6P$	-
63	16.33	Unknown	409.283	$C_{24}H_{35}N_5O$	-
64	16.34	OCTOXYNOL-2	294.219	$C_{18}H_{30}O_3$	MS
65	16.41	Dodecyl 6-{[(2-methoxyethoxy)carbonyl]amino}Hexanoate	401.314	$C_{22}H_{43}NO_5$	MS
66	16.49	8-Iso-15-keto-prostaglandin-F2β	352.225	$C_{20}H_{32}O_5$	MS
67	16.55	Pentadecyl N-2-furoylalaninate	393.288	$C_{23}H_{39}NO_4$	MS
68	16.62	Dodecyl sulfate	266.155	$C_{12}H_{25}O_4S^-$	MS
69	16.63	Unknown	375.298	$C_{21}H_{37}N_5O$	-
70	16.69	Dodecyl p-toluenesulfonate	340.207	$C_{19}H_{32}O_3S$	MS
71	16.71	2-Nitro-1,3-bis(octyloxy)benzene	379.272	$C_{22}H_{37}NO_4$	MS
72	16.8	2-Nitro-1,3-bis(octyloxy)benzene	379.272	$C_{22}H_{37}NO_4$	MS
73	16.8	N-({(1R,2R)-3-Oxo-2-[(2E)-2-penten-1-yl]cyclopentyl}acetyl)-L-valyl-L-leucine	422.278	$C_{23}H_{38}N_2O_5$	MS
74	16.99	3,5,6-Trihydroxyandrostan-17-one	322.214	$C_{19}H_{30}O_4$	MS
75	17.05	Unknown	551.404	$C_{29}H_{53}N_5O_5$	-
76	17.06	Unknown	535.351	$C_{31}H_{45}N_5O_3$	-
77	17.18	2-Nitro-1,3-bis(octyloxy)benzene	379.272	$C_{22}H_{37}NO_4$	MS
78	17.21	Unknown	425.277	$C_{24}H_{35}N_5O_2$	-
79	17.23	Unknown	437.278	$C_{25}H_{35}N_5O_2$	-
80	17.33	[Similar to: Methyl 3-{1-[3-hydroxy-4-(hydroxymethyl)tetrahydrofuran-2-yl]-2,4-dioxo-1,2,3,4-tetrahydropyrimidin5-yl}acrylate; ΔMass: -143.1715 Da]	455.267		MS
81	17.35	[Similar to: 9-Nitrooleate; ΔMass: -150.0443 Da]	477.285	$C_{25}H_{39}N_3O_6$	MS
82	17.53	Cyclo (isoleucylleucylisoleucylleucylleucyl)	565.419	$C_{30}H_{55}N_5O_5$	MS

**) Based on comparison of MS spectrum with database of National Institute of Standards and Technology (NIST)

Table 2. Continued...

Peak No	Retention Time (RT) in minutes	Compounds	Molecular Weight**)	Molecular Formula**)	Reference MS Identification**)
83	17.6	DPPH	395.086	$C_{18}H_{12}N_5O_6$	MS
84	17.62	Unknown	596.296	$C_{28}H_{45}N_4O_8P$	-
85	17.63	[Similar to: 9-Nitrooleate; ΔMass: -150.0443 Da]	477.285	$C_{25}H_{39}N_3O_6$	MS
86	17.64	[Similar to: 9-Nitrooleate; ΔMass: -178.0757 Da]	505.317	$C_{21}H_{44}N_7O_5P$	MS
88	17.79	Phenyl laurate	276.209	$C_{18}H_{28}O_2$	MS
89	17.81	4-Undecylbenzenesulfonic acid	312.176	$C_{17}H_{28}O_3S$	MS
90	17.9	Unknown	679.412	$C_{32}H_{63}N_3O_8P_2$	-
91	17.91	Unknown	601.397	$C_{30}H_{56}ClN_5O_5$	-
92	18.04	Methyl N-[(benzyloxy)carbonyl] leucylleucylleucinate	505.317	$C_{27}H_{43}N_3O_6$	MS
93	18.17	5-(4-Carboxy-3-methylbutyl)-1,4adimethyl-6-methylenedecahydro-1-naphthalenecarboxylic acid	336.23	$C_{20}H_{32}O_4$	MS
94	18.31	OCTOXYNOL-2	294.219	$C_{18}H_{30}O_3$	MS
95	18.34	13(S)-Hydroperoxylinolenic acid	246.199	$C_{18}H_{30}O_4$	MS
96	18.42	Unknown	345.288	$C_{19}H_{39}NO_4$	-
97	18.53	Unknown	407.267	$C_{17}H_{38}N_5O_4P$	-
98	18.54	[Similar to: Phosphatidylinositol-1,2-dipalmitoyl; ΔMass: 238.2296 Da]	572.296	$C_{27}H_{41}N_8O_4P$	MS
99	18.7	HEXADECYL 4-NITROPHENYL ETHER	363.277	$C_{22}H_{37}NO_3$	MS
100	18.72	17-EPIOXANDROLONE	306.219	$C_{19}H_{30}O_3$	MS
101	18.76	Unknown	533.335	$C_{31}H_{43}N_5O_3$	-
102	18.94	16,17-Dihydroxykauran-18-oic acid	290.225	$C_{20}H_{32}O_4$	MS
103	18.99	[Similar to: Phosphatidylinositol-1,2-dipalmitoyl; ΔMass: 329.2087 Da]	481.317	$C_{24}H_{44}N_5O_3P$	MS
104	19.05	[Similar to: Sorbitan monooleate; ΔMass: -50.9877 Da]	479.302	$C_{18}H_{43}N_9O_2P_2$	MS
105	19.26	4-Dodecylbenzenesulfonic acid	326.192	$C_{18}H_{30}O_3S$	MS
106	19.26	Unknown	598.312	$C_{28}H_{47}N_4O_8P$	-
107	19.38	Unknown	563.346	$C_{32}H_{45}N_5O_4$	-
108	19.51	Laurophenone	260.214	$C_{18}H_{28}O$	MS
109	19.58	1-{[(2-Aminoethoxy)(hydroxy)phosphoryl]oxy} -3-hydroxy-2-propanyl (4Z)-4-icosenoate	507.333	$C_{25}H_{50}NO_7P$	MS

**) Based on comparison of MS spectrum with database of National Institute of Standards and Technology (NIST)

Lariciresinol 4-O-glucoside is a lignan, a type of phenylpropanoids. In food, it is found in sesame seeds, flax and Brassica vegetables (Tchoumtchoua et al., 2019). It is also found in the bark and wood of white fir (*Abies alba*). The lignans are a large group of polyphenols found in plants and one of the major classes of phytoestrogens. It also acts as antioxidants and has potential anti-inflammatory activity (Korkina et al., 2011).

Phosphocholine is the phosphate of choline; and the parent compound of the phosphocholine family. It has a role as an epitope, a hapten, a human metabolite, a mouse metabolite, and an allergen. It is the conjugate acid of a choline phosphate (1-). Phosphocoline was also found in other fruits such as strawberry and Longan (Antunes et al., 2019; Wang et al., 2020).

There is a compound that is identified similar to Disperse red 17. Disperse dyes are a class of water-insoluble dyes that penetrate synthetic fibers and are held in place by physical forces without forming chemical bonds (Al-Etaibi & El-Apasery, 2019). It is used as a colorant, commonly in hair dyeing agents and the textile industry. Disperse red-17 is a red dye that has red powder, soluble in ethanol and acetone. Other than that, 1,3,3-trimethylindolino-6'-nitrobenzopyrylospiran also detected in this research. It is a photochromic compound. It can change their molecular structures by reversible process upon irradiation of lights of two different wavelengths. Several photochromic compounds were known to have the stability of heat (Malinauskiene et al., 2013).

These results indicate the potential of Buni fruit as a source of good quality natural colorant.

Rubone was also detected in this research. This compound was also found in another plant such as *Malva verticillata* and *Myrica nagi* fruit (Bao et al., 2018; Patel & Prashar, 2020) Several studies have shown that it has vigorous anticancer activity. Rubone and paclitaxel (PTX) combination therapy retarded cancer cell growth, migration and cancer stem-like cells (CSC) population growth (Xiao & Chen, 2015).

This study also found that several compounds belong to the steroid group. 11-Deoxy prostaglandin F2β is an analog of PGF2. 8-Iso-15-keto-prostaglandin-F2β is an isomer of PGF2α of non-enzymatic origin. These results indicate the potential of *Antidesma bunius* fruit to be used as a functional food.

4 Conclusions

Based on the study results, it can be concluded that the volatile components present in *Antidesma bunius* extract identified by GC-MS method as many as fifty compounds. Among these components, 5-hydroxymethylfurfural (5-HMF) was the one found in the greatest concentration. Also, there are ten predominant components and several compounds that can be used as flavoring agents. *Antidesma bunius* also contained several organic acids, long-chain fatty acids, and photochromic compounds. The compounds detected in this study may have biological activities which showed the great potential of Buni fruit to be applied in several aspects such as functional food, food processing and medicine.

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