

The metabolomic fingerprinting of four *duku* (*Lansium domesticum*) cultivars from Central Java, Indonesia based on unique metabolites and prospects for future breeding

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Manuscript received: 6 August 2024. Revision accepted: 24 October 2024.

Abstract. Murrinie ED, Fairuzia F, Arni N, Alpandari H, Maharini I. 2024. The metabolomic fingerprinting of four *duku* (*Lansium domesticum*) cultivars from Central Java, Indonesia based on unique metabolites and prospects for future breeding. *Biodiversitas* 25: 3816-3839. This research aims to map the genetic identity of Central Java (Indonesia) *duku* cultivars, Sumber, Woro, Matesih, and Kalikajar, through a metabolomic analysis approach using the Gas Chromatography-Mass Spectrophotometer (GC-MS) method from *duku* fruit peel and flesh. Heatmap and principal component analysis (PCA) of volatile organic compound (VOC) metabolites identified from *duku* fruit peel grouped Sumber, Woro, and Matesih as one group, with Sumber and Woro being more similar, while Kalikajar was in a different group. The identified metabolites of *duku* fruit flesh show that Woro and Matesih cultivars are in the same cluster, followed by Kalikajar, which is closer to the Woro and Matesih metabolites. Sumber mostly has different metabolites. VOC metabolites successfully identified primary and secondary metabolites defined as common metabolites in four cultivars, which have a myriad of functions for plant growth and development, nutritional value, plant defense against biotic stress and environmental stress tolerance, and physiological plant phenotypes. The successful identification of these metabolites not only enhances our understanding of the *duku* cultivars but also reassures the accuracy and reliability of future breeding for superior *duku* plants that are resistant to biotic and abiotic stress based on the VOC metabolites in the Sumber, Woro, Matesih, and Kalikajar cultivars, particularly the unique metabolites that distinguish these cultivars.

Keywords: GC-MS, Kalikajar cultivar, Matesih cultivar, Sumber cultivar, Woro cultivar

INTRODUCTION

Duku fruit (*Lansium domesticum* Corrêa) has high economic value and myriad advantages for human health, such as antioxidant, anti-inflammatory, analgesic, anticancer, antimicrobial, anti-aging, antimalarial, cytotoxic, genotoxicity, and anti-mutagenic (Alfonso et al. 2017; Apridamayanti et al. 2018; Abdallah et al. 2022; Maretha et al. 2022). Moreover, it also holds an advantage in agriculture as bio-plant protection against biotic attack (Salim et al. 2017). Indonesia has several *duku* cultivars from several islands, such as Sumatra, Java, and Maluku (Hanum and Kasiamdari 2012). *Duku* cultivars from Sumatra Island, the *duku komering*, have been popular *duku* fruits consumed by Indonesians (Komala et al. 2007; Susilawati et al. 2017). Besides the *duku komering*, some *duku* cultivars, considered as the favorite *duku* fruit for their unique sweetness and fresh taste, are Sumber, Woro, Matesih, and Kalikajar cultivars from Central Java (Sinaga et al. 2021).

Despite its potential, *duku* production meets several challenges, such as land shifting, degradation, and deforestation (MoEF 2015; Rudy et al. 2021; Hein et al. 2022), pollution caused by agriculture mismanagement (Basarab et al. 2013; Šimanský et al. 2021), household, transportation, and industry (Nordahl et al. 2020; Hailemariam et al. 2023). This contributes to the climate change increment,

which degrades the agriculture condition and suitability of plant habitation, leading to abiotic and biotic stress. Moreover, it has also significantly impacted *duku* production in Indonesia. The decline from 2018 to 2022 was around -93.45% or 237,000 to 89,000 tons per year, a drastic reduction that threatens the economic and health benefits of *duku*. It also affects the decrement of *duku* consumption by around 39.98%, from 5,475 kg per year to 0,366 kg per year (Ministry of Agriculture 2022). Therefore, the new *duku* cultivar harbors tolerance traits to abiotic stress and is resistant to biotic stress to maintain its production.

To obtain the *duku* cultivar with superior cultivar traits, strategic breeding should be acquired. Genetic information, such as morphological characteristics and metabolomic information, is the key to achieving that. *Duku* trait has been identified through morphological and simple molecular methods such as RAPD. The diverse information on *duku* plant characteristics has been collected through morphological characterization in *duku komering* from South Sumatra, Indonesia (Susilawati et al. 2017; Rupiah et al. 2018). The identification of *duku* genetic diversity based on DNA using RAPD markers had been employed in 28 accessions of *duku langsung*, *duku trengganu*, and *duku johor* in Malaysia (Song et al. 2000). The 29 *duku* and *langsar* from

the Bogor Botanical Garden collection in Indonesia were also included (Hanum and Kasiandari 2012).

The other powerful method to identify the organism's genetic diversity is the metabolomic compound. The methods for metabolite identification, as a fingerprinting of plant genetic diversity, are gas chromatography-mass spectrophotometry (GC-MS) (Hill and Roessner 2013; Jumhawan et al. 2015), GC/MS-TOF, C nuclear magnetic resonance (C-NMR), and H-NMR or H-nuclear magnetic resonance (Scott et al. 2010; Canellas et al. 2019; Shirahata et al. 2021), headspace gas chromatography-mass spectrometry (Schmeda-Hirschmann et al. 2020; Ch et al. 2021), liquid chromatography mass spectrophotometry (LC-MS), and rapid method fourier transform infrared (FT-IR) (Rai et al. 2023).

Metabolite compounds from *duku* plant parts have been successfully identified using GC-MS, and each component was subjected to phytochemical qualitative and quantitative analysis to determine its phytochemistry, nutritional value, and biological activity (Taganas et al. 2016; Alfonso et al. 2017; Salim et al. 2017; Abdallah et al. 2022; Maretha et al. 2022; Mayanti et al. 2022; Apridamayanti and Sari 2023). However, metabolite data for genetic diversity characterization purposes in the *duku* plant has never been utilized. Therefore, this research aims to reveal the metabolic fingerprinting in four cultivars of the *duku* plant in Central Java, Indonesia, namely Sumber, Woro, Matesih, and Kalikajar cultivars, through gas chromatography-mass spectrophotometry. The potential impact of this research is to provide valuable insights into the metabolite diversity of *duku* plants, which can be used to develop more resilient and productive cultivars, thereby contributing to the sustainability and profitability of *duku* production.

MATERIALS AND METHODS

Study area

From August 2023 to March 2024, a significant study was conducted. *Duku* fruit samples, a crucial component of the study, were meticulously collected during the harvest season from four distinct regions in Central Java, Indonesia, i.e., Kudus District (Sumber region), Rembang District (Woro region), Karanganyar District (Matesih region), and Purbalingga District (Kalikajar region). These samples were then named the Sumber, Woro, Matesih, and Kalikajar cultivars. The precise region coordinates of the research sample location are presented in Table 1, underscoring the meticulousness of the data collection process.

Procedures

Duku fruit flesh and peel filtrate sample preparation

The sample extraction was based on the sample preparation of previous research (Elsherif et al. 2024) with the modification of ethanol, employment was replaced with methanol. The *duku* fruit flesh and peel from Sumber, Woro, Matesih, and Kalikajar cultivars were meticulously separated and ground using a mortar. The ground *duku* fruit flesh and fruit peel were then carefully macerated with methanol (MeOH) 98% (Merck, German) in separate erlenmeyer flasks for three days in a dark place at room temperature. The liquid extraction then filtrates separated with Whatman 1442-125 Grade 42 Circles, 125 mm, 100/pk pore size 2.5 µm. The methanol filtrate of each *duku* cultivar fruit flesh and peel was then stored in the freezer before further analysis, ensuring the highest quality of the sample preparation.

GC-MS analysis

The *duku* fruit flesh and peel methanol extract was then sent to the Regional Health Laboratory, Jakarta, to analyze volatile metabolite components using a gas chromatography-mass spectrophotometer (GC-MS). The GC-MS analysis was coupled to Agilent Technologies (Agilent Technologies, USA) 7890 instruments. Gas Chromatograph with autosampler and 5975 Mass Selective Detector and ChemStation data system. The GC-MS analysis procedure was using the method from BALITTRO or Research Institute for Spices and Medicinal Plants, Indonesia, with the machine setting was ionization mode (electron impact), electron energy with column (HP Ultra 2. Capillary column length (m) 30 X 0.20 (mm) I.D X 0.11 (µm) film thickness), oven temperature (the initial temperature at 80°C hold for 0 minutes, rising at 3°C/min to 150°C hold for 1 minute and finally rising 20°C/min to 280°C hold for 26 minutes), injection port temperature (250°C), ion source temperature (230°C), interface temperature (280°C), quadrupole temperature (140°C), carrier gas (helium), column mode (constant flow), the flow rate of the carrier gas (helium) was flow column (1.2 mL/minute), injection volume (5 µL), and split (8: 1).

The GC was coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, USA). The injection volume was one µL, and the flow rate of the carrier gas (helium) was 1 mL/min. The oven temperature increased from 60°C to 250°C with a temperature rate of 5°C/min, and 10 min remained at the final temperature. The ionization was taken in an EI mode at 70 eV, followed by scanning the fragment ions in the octupole-quadrupole analyzer with 0.5 s/scan; the split ratio of fragment ions was 1: 10 (Asadollahi et al. 2019).

Table 1. The high meters above sea level (m asl) and the research sample location coordinates

Region/District	Meter above sea level (m asl)	Coordinate
Sumber/Kudus	12	LS-6.801281720531151, 110.89396368481788
Woro/Rembang	500	LS -6.677297164469234, 111.54086120106182
Matesih/Karanganyar	450	LS -7.638056499738324, 111.04546434244462
Kalikajar/Purbalingga	42	LS -7.377200418573956, 109.3812865209861

Notes: Source from BPS (2016, 2019)

Data analysis

The interpretation of the molecular formula and nature of identified compounds was confirmed using PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), Spectra Base (<https://spectrabase.com/>), and mass spectra from commercial databases (NIST) and literature (Klein-Júnior et al. 2016). To match the GC chromatogram, retention time, elution order, and peak area. The metabolites detected using GC-MS Agilent were analyzed using the open software program R 4.4.1 (www.rcran.studio.com). A cluster heatmap data was created to understand the metabolite conserved in the *duku* plant and the specific metabolites that can be differentiated between the four *duku* cultivars, Sumber cultivar, Woro cultivar, Matesih cultivar, and Kalikajar cultivar as their genetic identification. The identified metabolites were then analyzed using the chemical compounds deposited in PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), a National Library of Medicine (NLM) in the US National Institutes of Health (NIH) (<https://www.nlm.nih.gov/>) and literature study in an accredited national and international journal. The multivariate analysis used XL-Stat 2019 (<https://www.xlstat.com/en/>) to obtain the Principal Component Analysis (PCA) to visualize the metabolites discrimination dataset in each *duku* cultivar (Rai et al. 2023; Zhong et al. 2023).

RESULTS AND DISCUSSION

The untargeted GC-MS analysis identifies the volatile organic compounds (VOCs) of Sumber, Woro, Matesih,

and Kalikajar *duku* cultivars fruit peel and flesh. The VOCs were used to determine the metabolite compound diversity of four *duku* cultivars based on the physiological characteristics of the metabolites produced by each cultivar. The unique and similar compounds were analyzed separately from *duku* fruit peel and flesh. These compounds play a significant role in understanding the metabolite compound diversity of *duku* cultivars.

The fruit peel metabolite fingerprinting of four local favorites, *duku* in Central Java

Duku fruit skin metabolites identified from four superior local cultivars, namely the Sumber cultivar, the Kalikajar cultivar, the Matesih cultivar, and the Woro cultivar (Figure 1, Table 2), detected the presence of common metabolites such as 1 1-[-]-4-hydroxy-1-methylproline, Germacrene D, Sphatulenol, and 5 (1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8,-dimethyl-4-(1-methyl ethylidene)-, (8S-cis).

Of the common metabolites identified, 1-[-]-4-hydroxy-1-methylproline was the main component metabolite of *duku* fruit peel, with 1-[-]-4-hydroxy-1-methylproline of Matesih detected the highest among the four cultivars, followed by cultivars Matesih, Woro and Sumber, respectively (Table 1, Figure 1). The metabolite 1-[-]-4-hydroxy-1-methylproline was detected highest in the Matesih cultivar, followed by the Woro cultivar and the Sumber cultivar; the metabolite is the major metabolite in the *duku* fruit peel.

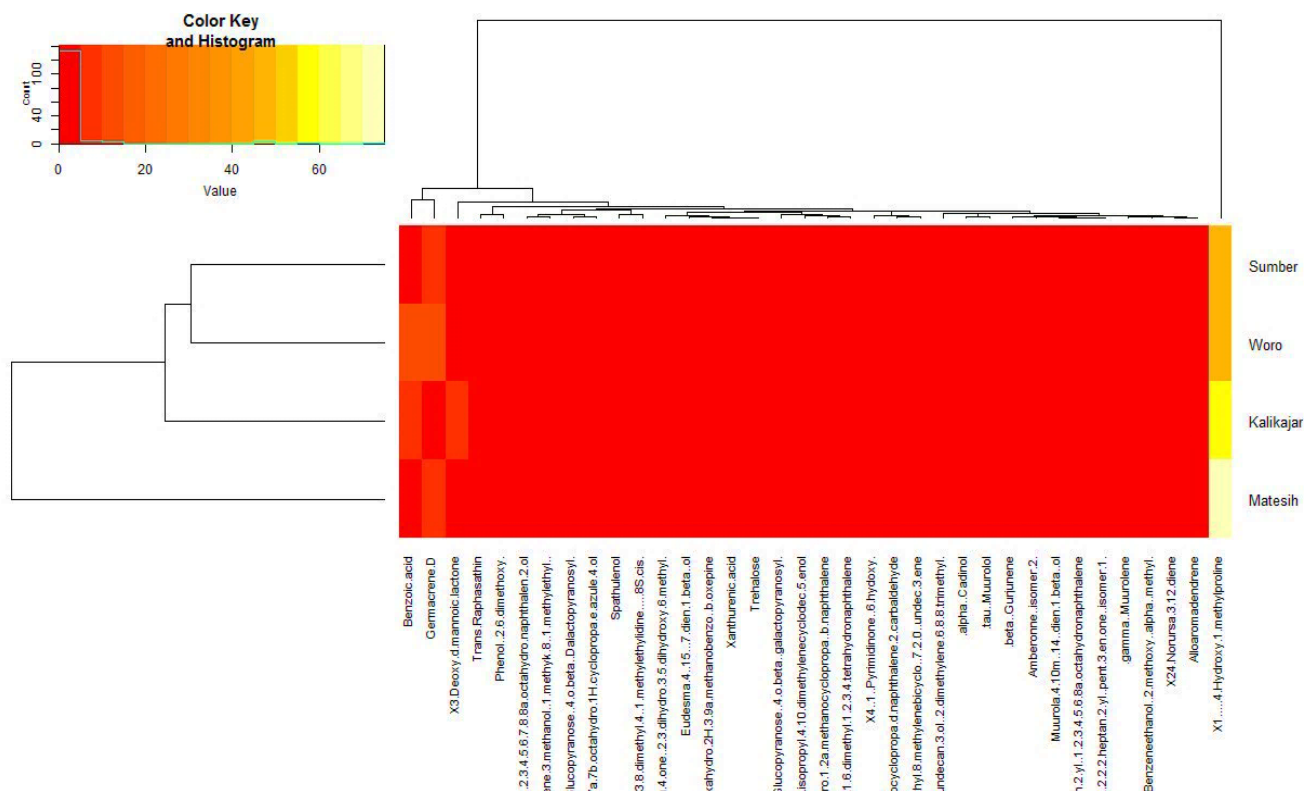


Figure 1. The cluster heat map of *duku* fruit peel of Sumber, Woro, Matesih, and Kalikajar cultivars in Central Java

Table 2. Chemical composition of *duku* fruit peel from Sumber, Mateseh, Woro, and Kalikajar cultivar of Central Java, Indonesia

Compound	Molecule	Compound in nature	Composition (%) / cultivar			
			Sumber	Woro	Matesih	Kalikajar
Phenol, 2,6-dimethoxy-	C ₈ H ₁₀ O ₃	Phenolic	1.32	4.07	-	4.01
Germacrene D	C ₁₅ H ₂₄	Monocyclic sesquiterpene	9.13	12.57	5.79	3.19
Sphatulenol	C ₁₅ H ₂₄ O	Sesquiterpene	2.81	2.90	1.52	1.91
Tricyclo [5.2.2.0 (1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-.tau.-Muurolol	C ₁₅ H ₂₄ O	Sesquiterpene	2.06	1.83	1.42	-
	C ₁₅ H ₂₆ O	Cadinane sesquiterpenoid	1.31	1.98	-	1.09
Alloaromadendrene	C ₁₅ H ₂₄	Sesquiterpene	2.10	-	-	-
6-Isopropenyl-4,8,1-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-2-ol .alpha.-Cadinol	C ₁₅ H ₂₄ O	Sesquiterpene	2.66	-	-	-
	C ₁₅ H ₂₆ O	Cadinane sesquiterpenoid	1.31	1.60	-	1.42
(3R,4aR,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	C ₁₅ H ₂₂	Sesquiterpenoid	1.37	-	-	-
Tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol, 1-methyl-8-(1-methylethyl)-	C ₁₅ H ₂₄	Sesquiterpene	2.89	-	-	-
Muurolo-4,10m(14)-dien-1.beta.-ol	C ₁₅ H ₂₄ O	Sesquiterpene	1.44	-	-	-
Benzeneethanol,2-methoxy-.alpha.-methyl-	C ₁₀ H ₁₄ O ₂	Benzene	1.87	-	-	-
Amberonne (isomer 2)	C ₆ H ₁₂ O	Methyl ketone	1.31	-	-	-
5-(3,3-Dimethylbicyclo[2.2.2] heptan-2-yl) pent-3-en-2-one (isomer 1)	C ₁₅ H ₂₂ O	Sesquiterpene	1.39	-	-	-
(1aR,4S,7R,7aS,7bR)-1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azule-4-ol	C ₁₅ H ₂₄	Sesquiterpene	4.61	-	-	-
1-[-]-4-hydroxy-1-methylproline	C ₆ H ₁₁ NO ₃	Proline	48.58	48.11	75.01	55.40
.alpha.-D-Glucopyranose,4-o.beta.-D-galactopyranosyl-	C ₁₂ H ₂₂ O ₁₁	Trisaccharides	3.82	-	2.70	-
.beta.-Gurjunene	C ₁₅ H ₂₄	Aromadendrane sesquiterpenoids	1.10	-	-	-
.gamma.-Muurolene	C ₁₅ H ₂₄	Sesquiterpene	1.67	-	-	-
5 (1H)-Azulennone,2,4,6,7,8,8a-hexahydro-3,8,-dimethyl-4-(1-methylethylidene)-,(8S-cis)-	C ₁₅ H ₂₂ O	Terpenoid	3.68	1.83	1.51	1.72
24-Norursa-3,12-diene	C ₂₉ H ₄₆	Triterpenoid	2.05	-	-	-
Benzoic acid	C ₇ H ₆ O ₂ /C ₆ H ₅ COOH	Benzene/aromatic carboxylic acid	-	10.86	3.63	7.43
4 (1H)-Pyrimidinone-6-hydroxy-	C ₄ H ₄ N ₂ O ₂	Ketone	-	1.46	-	-
(1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d] naphthalene-2-carbaldehyde	C ₁₅ H ₂₂ O	Sesquiterpene	-	2.31	-	-
1R,3Z,9S-4,11,11-Trimethyl-8-methylenebicyclo [7,20] undec-3-ene	C ₁₅ H ₂₄	Sesquiterpene	-	2.81	-	-
Trans-Raphasatin	C ₆ H ₉ NS ₂	Organic compound isothiocyanates	-	3.68	-	2.67
(1R,1aR,2aS,6R,6aS,7aS)-1,6,6a-Trimethyldecahydro-1,2a-methanocyclopropa[b] naphthalene	C ₁₅ H ₂₄	Sesquiterpene	-	-	1.99	-
4-isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	C ₁₅ H ₂₂ O	Sesquiterpene	-	-	1.99	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	Flavonoid/ketone	-	-	-	1.99
Eudesma-4(15),7-dien-1.beta.-ol	C ₁₅ H ₂₄ O	Sesquiterpene	-	-	-	1.18
(3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine	C ₁₅ H ₂₄ O	Sesquiterpene	-	-	-	1.27
Xanthurenic acid	C ₁₀ H ₇ NO ₄	Amino acid	-	-	-	1.31
(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	C ₁₅ H ₂₄ O	Sesquiterpene	-	-	-	1.22
3-Deoxy-d-mannoic lactone	C ₆ H ₁₀ O ₅	Cyclic ester	-	-	-	8.21
Trehalose	C ₁₂ H ₂₂ O ₁₁	Disaccharide	-	-	-	1.31

From the metabolites of *duku* fruit peel, the Sumber and Woro cultivars were part of the same group, which means they have similar metabolite compounds. Moreover, from the metabolite compounds, the Matesih cultivar has a distinct metabolite type from the other cultivars, as shown in the heatmap (Table 1, Figure 1), because several fruit peel metabolites of the Matesih cultivar were not identified.

Several metabolites from the *duku* fruit peel were not detected in the Matesih cultivar, such as Phenol, 2,6-dimethoxy-, .tau.-Muurolol, .alpha.-Cadinol, while three of them were found in Sumber, Woro, and Kalikajar, cultivars. Tricyclo [5.2.2.0 (1,6)] undecane-3-ol, 2-methylene-6,8,8-trimethyl- compound conserved in Sumber, Matesih and Woro cultivars; however, it is absent in Kalikajar cultivar. The benzoic acid compound was not discovered in the Sumber cultivar, while it was found in the other three cultivars. The .alpha.-D-Glucopyranose,4-o.beta.-D-galactopyranosyl- compound was absent in the Woro and Kalikajar cultivars while discovered in the Sumber and Matesih cultivars. It is contrary to the Trans-Raphasatin compound detected in the Woro and Kalikajar cultivars; however, it is absent in the Sumber and Matesih cultivars. Each cultivar also lacks one metabolite, such as the Matesih cultivar, which was missing Phenol, 2,6-dimethoxy-. The Kalikajar cultivar was missing the Tricyclo [5.2.2.0 (1,6)] undecane-3-ol, 2-methylene-6,8,8-trimethyl- compound. .tau.-Muurolol and .alpha.-Cadinol compounds were missing from the Matesih cultivar. Those differences in metabolites detected in each cultivar imply the cultivar metabolites grouping shown in the heatmap of *duku* fruit peel metabolites (Figure 1).

The unique metabolites we have detected through our GC-MS analysis have become the hallmark of each cultivar. The Sumber cultivar, for instance, boasts twelve unique compounds, with the highest percentage of unique compounds detected being (1aR,4S,7R,7aS,7bR)-1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azule-4-ol. Similarly, the Woro cultivar has three unique compounds, with the highest concentration being 1R,3Z,9S-4,11,11-Trimethyl-8-methylenebicyclo [7,20] undec-3-ene (1R,1aR,2aS,6R,6aS,7aS). The Matesih and Kalikajar cultivars also have unique compounds, each contributing to the diversity of the *duku* fruit peel metabolites. These unique compounds of each cultivar can be utilized as a tool for the future selection of *duku* breeding.

The two-dimensional biplot of *duku* fruit peel PCA cumulative contribution was 77.65%, with F1 accounts 48.88% and F2 accounts 28.77% (Figure 2). The PCA analysis, a statistical method that simplifies complex data, was used to understand further the relationships between the metabolites of *duku* fruit peel. The principal component analysis (PCA) result is in line with the heatmap analysis through the principal analysis component (PCA) analysis in *duku* fruit peel, which shows that Matesih cultivar metabolites were distinct from the Sumber, Woro, and Kalikajar cultivar. The F1/F2 indicated four different clusters of *duku* fruit peel metabolites; the Sumber and Woro metabolites collided in the same quadrant. Therefore,

Sumber and Woro cultivars have the same *duku* fruit peel metabolites. Even though the Sumber cultivar was in a different quadrant from the Woro and Kalikajar cultivars, the Kalikajar cultivar also positively correlated with the Sumber and Woro cultivars. However, from the identification of *duku* fruit skin metabolites, the Matesih cultivar was mainly different from Sumber, Woro, and Kalikajar, which showed a negative correlation.

The fruit flesh metabolite fingerprinting of four locals' favorite *duku* in Central Java

Duku fruit flesh MeOH extract compound identification reveals the closest similarity of Matesih and Woro cultivars, which belonged to the same group based on heatmap analysis (Figure 3). In contrast, the Sumber cultivar has distinct similarities among the four cultivars identified. The similarities between the metabolites from the flesh fruit of the *duku* cultivar and those of the Woro and Matesih cultivars were identified by seven unique metabolites (Figure 3, Table 3). At the same time, the conserved metabolites of *duku* fruit flesh among four *duku* cultivars were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and 5-Hydroxymethylfurfural. The 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- found as common metabolites in *duku* flesh fruit in four cultivars, whereas in *duku* fruit peel, it was found as the unique compound of Kalikajar cultivar. The same compound can be found in the different cell tissues of plants with different amounts; the other expression or production of those metabolites in the various tissues or organs causes these. The differentiation of total metabolite amounts, such as phenol, flavonoids, and alkaloids in the leaves and fruit, is mainly caused by enzymatic reactions and genetic and environmental effects in their metabolomic pathways (Wang et al. 2019; Kuntorini et al. 2023). The highest compound percentage of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- was identified in Sumber, Woro, Matesih, and Kalikajar cultivars, respectively. At the same time, the highest 5-Hydroxymethylfurfural compound percentage was determined in Sumber, Woro, Matesih, and Kalikajar cultivars, respectively.

The metabolites identified from *duku* fruit flesh reveal each cultivar's unique compounds. Sumber cultivars have nine unique compounds, with the highest compound percentage identified being 3-Deoxy-d-mannoic lactone, which became a major compound at around 61.52%. Woro has six unique compounds, with ethanimidic acid, an ethyl ester, being the highest unique compound, identified at around 8.44%.

Matesih cultivar unique metabolites were eight compounds with 3-Hydroxy-N, N-dimethylpropanamide share around 8.00% and 2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine 7.08%. The unique metabolites of *duku* fruit flesh were identified from the Kalikajar cultivar, with ten compounds, with 1,3-methylene-d-arabitol sharing around 12.78%.

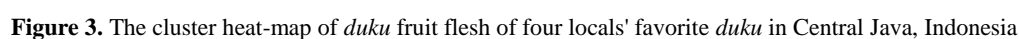
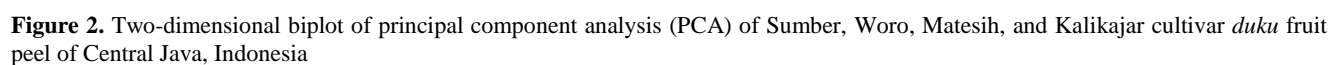


Table 3. Composition and percentage of phenolic and flavonoid on *duku* flesh fruit of Sumber, Mateseh, Woro, and Kalikajar cultivar of Central Java, Indonesia

Compound	Molecule	Compound in nature	Composition (%) /cultivar			
			Sumber	Woro	Matesih	Kalikajar
Oxalectic acid	C ₈ H ₁₀ O ₃	Keto acid	2.41	-	-	-
Isosorbide Dinitrate	C ₆ H ₈ N ₂ O ₈	Organic nitrate	2.34	-	-	-
1,3,5-Triazine-2,4,6-triamine	C ₃ N ₃ (NH ₂) ₃	Amino acid	4.42	-	-	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	Flavonoid/ Ketone	19.79	27.12	17.38	14.24
5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	Aldehyde	12.40	31.18	28.67	6.97
Monoethylmalonate monoamide	C ₄ H ₇ NO ₃	Amide	1.37	-	-	-
1-Octanamine	C ₈ H ₁₉ N	Aliphatic amine	3.78	-	-	-
3-Deoxy-d-mannonic lactone	C ₁₅ H ₂₆ O	Cyclic ester/sugar	61.52	-	-	-
d-Mannitol, 1,4-anhydro-	C ₁₅ H ₂₂	Sesquiterpene	0.20	-	-	-
1,4-Butanediamine, N-(3-aminopropyl)-	C ₁₅ H ₂₄	Sesquiterpene	0.66	-	-	-
N-Methoxy-1-ribofuranosyl-4-imidazole carboxylic amide	C ₁₅ H ₂₄ O	Sesquiterpene	0.21	-	-	-
2-Furanmethanol	C ₁₉ H ₁₂ O ₂	1-Heptanol/Terpenoid	-	15.91	-	1.94
Acetamide, N-(aminocarbonyl)-	C ₃ H ₆ N ₂ O ₃	Amide	-	3.02	-	-
2-Cyclopenten-1-one, 2-hydroxy-	C ₅ H ₆ O ₂	Cyclic ketones	-	6.44	-	-
Ethanimidic acid	C ₁₅ H ₂₆ O	Ethyl ester	-	8.44	-	-
N-Acetyl-D-glucosamine	C ₆ H ₁₁ NO ₃	Carbon-nitrogen/ amide	-	2.18	-	0.82
beta-Alanine amide	C ₃ H ₈ N ₂ O	Amino acid	-	2.07	-	0.22
2-Isopropoxyethylamine	C ₁₀ H ₁₅ NO	-	-	2.18	-	-
2-Propanone, dimethylhydrazine	C ₅ H ₁₂ N ₂	Ketone	-	1.12	27.25	30.78
Isobutyraldehyde, propyl hydrazone	C ₇ H ₁₆ N ₂	Ketone	-	5.44	-	-
5-Amino-1,3,4-oxadiazole-2-carboxamide	C ₇ H ₁₂ N ₄ O ₂	Monoamine	-	4.35	-	0.25
1,6-Anhydro-2,4-dideoxy-. beta. -D-ribo-hexopyranose	C ₆ H ₁₀ O ₃	Organic compound	-	0.31	-	-
Piperidine, 3-(bromomethyl)-	C ₇ H ₁₆ BrN	Heterocyclic organic compound	-	-	1.41	-
6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	C ₃ H ₄ N ₄ O ₂	Heterocyclic organic compound	-	-	5.98	-
3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-	C ₆ H ₁₀ N ₂ O	Verbenone	-	-	2.83	-
2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine	C ₁₀ H ₁₅ N ₅ O ₅	Guanine	-	-	7.08	-
3-Hydroxy-N, N-dimethylpropanamide	C ₅ H ₁₁ NO ₂	Amide of propanoic acid	-	-	8.00	-
Butanal, dimethylhydrazine	C ₆ H ₁₄ N ₂	Butane	-	-	0.42	-
Piperazine	C ₄ H ₁₀ N ₂	Azacyclo alkane	-	-	0.16	-
N-Aminomorpholine	C ₄ H ₁₀ N ₂ O	Heterocyclic	-	-	0.11	-
Cyclohexanone	C ₆ H ₁₀ O	Cyclic ketone	-	-	-	1.98
2,4,6,8-Tetraazabicyclo [3.3.0] octan-3-one, 7-nitroimino-	C ₄ H ₆ N ₆ O ₃	Polynitro heterocyclic	-	-	-	0.55
2(3H)-Furanone, dihydro-3-methylene-	C ₅ H ₈ O ₂	Glycin/Ketose	-	-	-	1.07
Cycloserine	C ₃ H ₆ N ₂ O ₂	D-alanine analogue	-	-	-	0.88
Furaneol	C ₆ H ₈ O ₃	Furanone cyclic ketone	-	-	-	4.60
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	Flavonoid (Ketone bodies)	-	-	-	2.82
2,5-Dimethylfuran-3,4(2H,5H)-dione	C ₆ H ₈ O ₃	Phenolic/ Ketones	-	-	-	4.60
Dimethyl trisulfide	C ₂ H ₆ S ₃	Organic trisulfide	-	-	-	1.73
1,3-Methylene-d-arabitol	C ₆ H ₁₂ O ₅	Pentitol/Carbohydrate	-	-	-	12.78
2-Carbamyl-9- [. beta. -d-ribofuranosyl] hypoxanthine	C ₁₁ H ₁₃ N ₅ O ₅	Purine	-	-	-	1.20

Woro and Kalikajar also have the same compound in fruit flesh, namely, the 2-Furanmethanol, N-Acetyl-D-glucosamine, beta-Alanine amide, and 5-Amino-1,3,4-oxadiazole-2-carboxamide, besides the two common compounds which found in all four *duku* cultivars. Furthermore, the 2-Propanone, dimethylhydrazone, was missing from the Sumber cultivar, while it was present in the Woro, Matesih, and Kalikajar cultivars. While this compound, the 2-Propanone, dimethylhydrazine was also identified as the primary compound in the Kalikajar cultivar, which is around 30.78 %, and the second primary compound (27.25%) in the Matesih cultivar. It indicates that the same metabolite is expressed differently in different cultivars.

The two-dimensional biplot of *duku* fruit flesh PCA cumulative contribution was 73.09%, with F1 accounts 41.32% and F2 accounts 31.77% (Figure 4). The PCA analysis result supported the heatmap analysis through the principal analysis component (PCA) analysis in *duku* fruit flesh; the F1/F2 indicated four distinct clusters of *duku* fruit flesh metabolites, the Sumber and Matesih metabolites were colliding in the same quadrant. Therefore, Sumber and Matesih mostly have the same *duku* fruit peel metabolites. The Kalikajar cultivar was positively correlated with the

Sumber and Matesih cultivar. While the Woro cultivar was identified, metabolites of *duku* fruit peel were mostly different from Sumber, Matesih, and Kalikajar, which showed a negative correlation.

Discussion

From the principal component analysis (PCA) (Figure 1 and Figure 3) and heatmap analysis (Figure 2 and Figure 4), the metabolites, both the *duku* fruit peel and flesh, were grouped based on metabolite percentages present in each *duku* cultivar. The metabolites identified were grouped consistently in the Sumber, Woro, Matesih, and Kalikajar cultivars. The PCA analysis and heatmap have been successfully used to group the identified metabolites in several metabolites plant analyses such as in the wild tree peony, *Schizonepetae spica*, *Eurycoma longifolia* (Liu et al. 2020; Luo et al. 2020; Serag et al. 2023). While the heatmap analysis with PCA analysis combination has been widely used to analyze the volatile metabolite compounds identification in *Camellia tetracocca*, citrus cultivars, and radish microgreen cultivars (Deng et al. 2022; Zhang et al. 2023; Zhong et al. 2023).

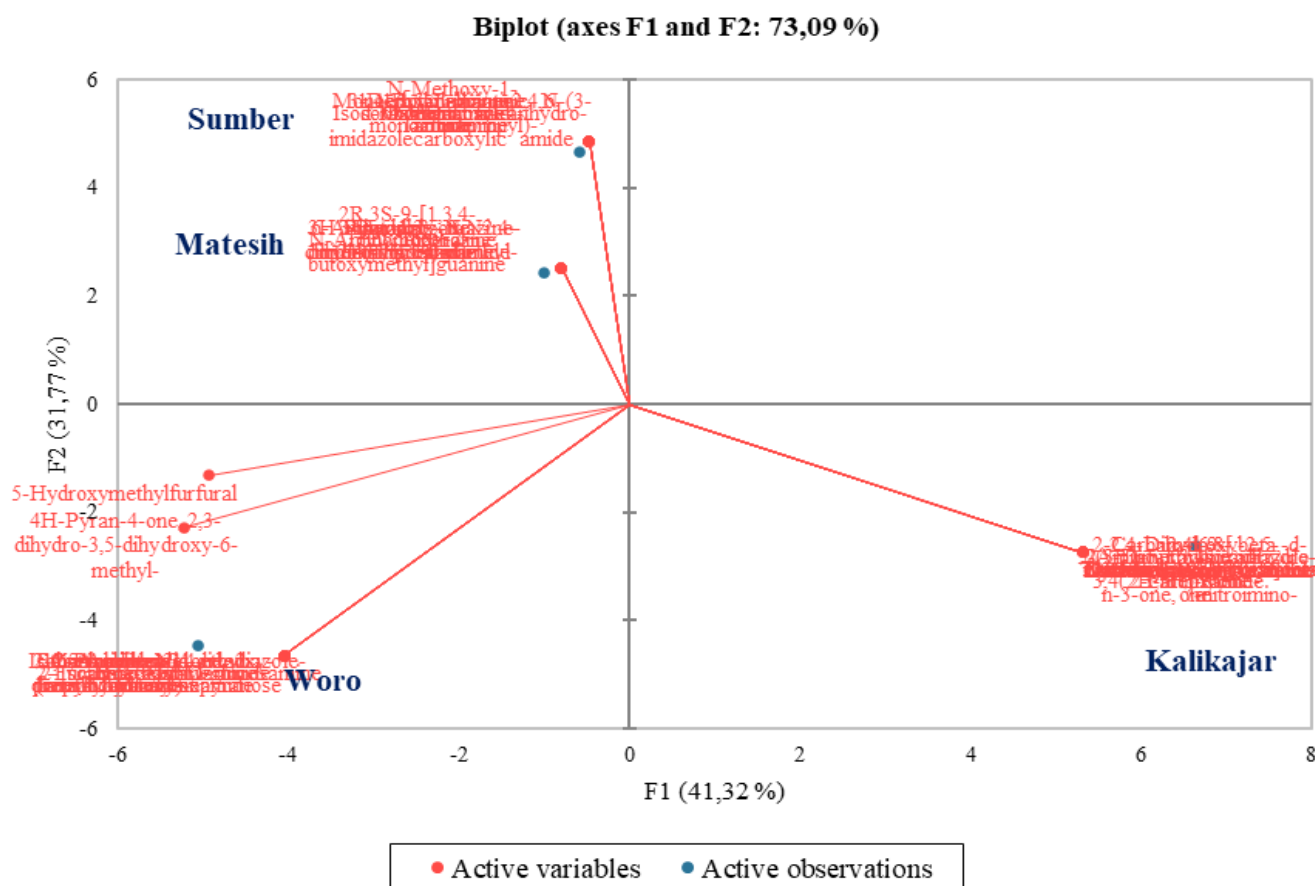


Figure 4. Two-dimensional biplot of principal component analysis (PCA) of Sumber, Woro, Matesih, and Kalikajar cultivar *duku* fruit flesh of Central Java, Indonesia

The heatmap and PCA analysis show that the Germacrene D and sphenatulenol compounds are conserved in the *duku* fruit of Sumber, Woro, Matesih, and Kalikajar cultivars. Germacrene D and sphenatulenol are part of the sesquiterpene group and were seen as major compounds in the plant's essential oil. The metabolites from the *duku* peel fruit character (Figure 1, Table 2) highlight that the 1- [-]-4-hydroxy-1-methylproline is the dominant compound of four *duku* cultivars. Matesih cultivar has a higher content of 1- [-]-4-hydroxy-1-methylproline, around the third quarter 75,01%. 1- [-]-4-hydroxy-1-methylproline, also known as (2S,4R)-4-hydroxy-1-methyl-2-pyrrolidine-carboxylic acid. It is a metabolite that indicates metabolite resistance to the leaf miner (*Liriomyza trifolii*) attack, causing anti-oviposition on *Liriomyza trifolii* so that it could not breed in chili plants (Dekebo et al. 2007). It is also found as the main constituent in *Adenocalymma marginatum* (Schmeda-Hirschmann et al. 2020), *Buchholzia coriacea* (Ojinnaka et al. 2015), and *Mangifera indica* (Bolade et al. 2021).

Germacrene-D or (7-iso-propyl-10-methyl-4-methylene-cyclo- deca-5,10-diene) is a volatile compound from the sesquiterpene group which shows as the ancient trait in several genera such as *Bursera* and *Commiphora* species (Røstelien et al. 2000; Noge and Becerra 2014), *Tanacetum sonbolii* (Firozy et al. 2012), *Hyptis mutabilis* (Silva et al. 2013); *Lindera chunii* (Liu et al. 2013), *Taxodium distichum* (Adams et al. 2014). Germacrene-D is an antibacterial compound with a wide potential function for insecticides such as anti-mosquito, anti-aphid, and anti-tick and as a precursor to producing other sesquiterpene compounds such as γ -amorphene, α -copaene, cubenene, γ -murolene, and δ -cadinene (Liu et al. 2022; Suharti et al. 2023). Germacrene-D also acts as a pheromone for insect attractants in pollination (Sharma et al. 2024). On the other hand, Sphenatulenol shows its function as a flowering metabolites compound and also as an anti-microbial (Yavuz et al. 2016; Mohebi et al. 2017; Doorandishan et al. 2021). Along with Germacrene-D, Sphenatulenol plays a pivotal role as a bioherbicide, exhibiting its allelopathy activity (Abd-elgawad et al. 2021). 5 (1H)-Azulennone,2,4,6,7,8,8a-hexahydro-3,8, -dimethyl-4-(1-methylethylidene)-, (8S-cis)- had been reported from *Cyperus rotundus* plant (Ambarwati et al. 2019). Moreover, two *Kundmannia* aerial extracts with high sphenatulenol compound rates display remarkable antimicrobial properties against several parasite microorganisms, namely

Bacillus megaterium, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter aerogenes*, and *Saccharomyces cerevisiae* (Yavuz et al. 2016). The major compound structure of *duku* fruit peel is presented in Figure 5.

Unique metabolite of the Sumber cultivar *duku* plant (Figure 6), the Alloaromadendrene, part of the phenolic group (Jang et al. 2016), was found in the *Ridolfia segetum* (Basaid et al. 2020), *Eucalyptus maculata* (Ololade et al. 2013) and *Hedyosmum cumbalense* and *Hedyosmum spectabile* plants, known for their anti-microbial activity against three Gram-positive, two Gram-negative, and two sporulated fungi (Guerrero et al. 2023). The other Sumber cultivar unique metabolites, the 6-Isopropenyl-4,8,1-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-2-ol, was found in *Senecio glaucus* ssp., *Coronopifolius* (Basaid et al. 2020), *Tripleurospermum callosum* root (Yaşar et al. 2005), *Illicium griffithii* (Vijayakumar et al. 2012) and *Lindera aggregata* essential oil (Hong 2016), which is known for its antimicrobial and antifungal activity against pathogens. (3R,4aR,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene compound in Sumber cultivar also known as (-)-Eudesma-1,4(15),11-triene is a sesquiterpene was isolated from the essential oil of *Callitris intratropica* (Oyediji et al. 1998) and *L. domesticum* stem bark (Apridamayanti and Sari 2023). The Muurola-4,10(14)-dien-1. beta. -ol, categorized as alcohol, was also found in *Citrus jambhiri* (Hamdan and El-Shazly 2014). Amberonne (isomer 2), also known as 1- [(1R*, 2R*,8aS*) -1,2,3,5,6,7,8,8a-octahydro-1,2,8,8-tetramethylnaphthalen-2-yl] ethan-one [140194-26-9], a cyclohexenyl methyl ketone intermediate with phosphoric acid has a typical woody odor (Surburg and Panten 2006; Scientific Committee on Consumer Safety 2012). Amberonne (isomer 2), a common fragrance in perfume and cosmetics, gives a woody odor. Amberonne is a trading name for tetramethyl acetyloctahydronaphthalenes- (Surburg and Panten 2006; Nicolantonio et al. 2022). It has never been found and elucidated naturally by plants and other organisms. Therefore, further confirmation of this compound's existence in *duku* fruit peel extract is needed. Beta-gurjunene is an aromadendrane sesquiterpenoid. Gurjunene derivatives have been found in species *Garcinia nigrolineata* essential oil as a minor compound.

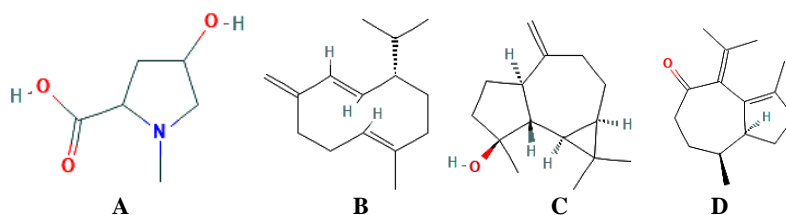


Figure 5. Major and common component structures are found in the *duku* fruit peel of Sumber, Woro, Kalikajar, and Matesih cultivars of Central Java, Indonesia. A. 1- [-]-4-hydroxy-1-methylproline; B. Germacrene D; C. Sphenatulenol; D. 5 (1H)-Azulennone,2,4,6,7,8,8a-hexahydro-3,8, -dimethyl-4-(1-methylethylidene)-, (8S-cis)-

The unique metabolites in the Woro cultivar (Figure 7), the 4(1H)-Pyrimidinobne-6-hydroxy-a compound also found in a medicinal herb plant, *Argyrea capitiformis* - suggested that methanolic extract provides anti-inflammatory effects (Obaidullah et al. 2022). While the function of (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d]naphthalene-2-carbaldehyde or Cyclopropa[d]naphthalene-2-carboxaldehyde, 1,1a,4,4a,5,6,7,8-octahydro-4a,8,8-trimethyl-, (1aR,4aS,8aS)- is a sesquiterpene found in *L. domesticum* stem bark (Apridamayanti and Sari 2023). Whereas the 1R,3Z,9S-4,11,11-Trimethyl-8-methylenebicyclo

[7,20] undec-3-ene compound was found in *Eucalyptus maculata* (Ololade et al. 2013).

Matesih's unique metabolites (Figure 8) have been found in several plants. The (1R,1aR,2aS,6R,6aS,7aS)-1,6,6a-Trimethyldecahydro-1,2a-methanocyclopropa[b]naphthalene known also as Ishwarane, that was found in *Cedrelopsis grevei* (Rakotobe et al. 2008), *Corallocarpus epigaeus* and *Aristolachia* sp. (Sati et al. 2011), *peperomia scandens* ruiz and pavon (Fernando et al. 2013). Ishwarane isolated from the *Hortonia* plant exhibits antifungal activity to *Colletotrichum gloeosporioides* (Ratnayake et al. 2008).

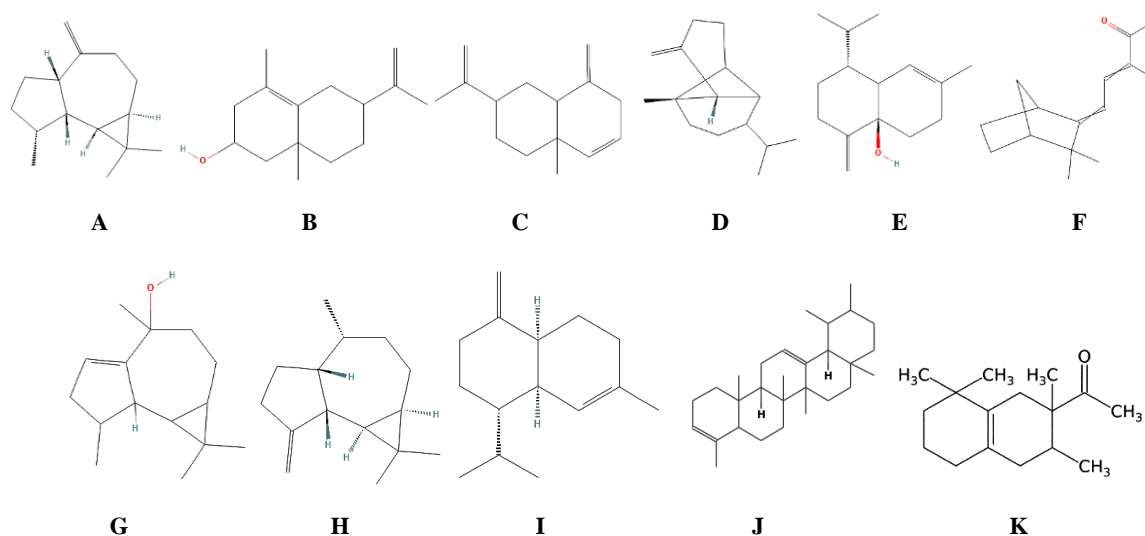


Figure 6. The unique compound structure of Sumber cultivar fruit peel of Central Java, Indonesia. A. Alloaromadendrene; B. 6-Isopropenyl-4,8,1-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol; C. (3R,4aR,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene; D. Tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol, 1-methyl-8-(1-methylethyl)-; E. Muurola-4,10m(14)-dien-1.beta.-ol; F. Benzeeneethanol,2-methoxy-.alpha.-methyl-; G. 5-(3,3-Dimethylbicyclo[2.2.2] heptan-2-yl)pent-3-en-2-one (isomer 1); H. (1aR,4S,7R,7aS,7bR)-1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azule-4-ol; I. beta.-Gurjunene; J. gamma.-Muurolene; K. 24-Norursa-3,12-diene; Amberonne (isomer 2)

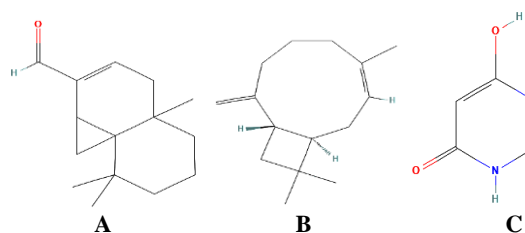


Figure 7. The unique compound structure of Woro cultivar fruit peel of Central Java, Indonesia. A. (1aR,4aS,8aS)-4a,8,8-Trimethyl-1,1a,4,4a,5,6,7,8-octahydrocyclopropa[d] naphthalene-2-carbaldehyde; B. 1R,3Z,9S-4,11,11-Trimethyl-8-methylenebicyclo [7,20] undec-3-ene; C. 4 (1H)-Pyrimidinobne-6-hydroxy-

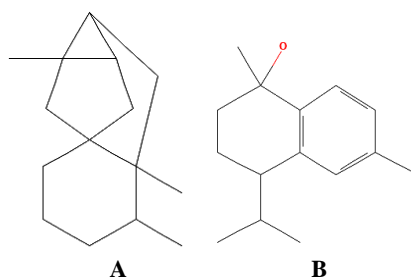


Figure 8. The unique compound structure of Matesih cultivar fruit peel of Central Java, Indonesia. A. (1R,1aR,2aS,6R,6aS,7aS)-1,6,6a-Trimethyldecahydro-1,2a-methanocyclopropa[b] naphthalene; B. 4-isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene

The 4-isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene, also known as 10 α -Hydroxycalamenene (cis-Calamenene), is part of the Calamenene group. It had been elucidated from several plant parts, such as from the stem bark of *Entandrophragma cylindricum* and *Guarea macrophylla* (Riyadi et al. 2023). Additionally, it was also found previously in *Argyrea capitiformis* (Brown 2010), genus *Eremophila* (Singab et al. 2013), and two endemic Kundmannia, *Kundmannia anatolica* and *Kundmannia syriaca* (Yavuz et al. 2016).

Kalikajar unique metabolites (Figure 9) such as Eudesma-4(15),7-dien-1- β -ol was found in *Taxodium distichum* (Adams et al. 2014) and *Eryngium* species (Klein-Júnior et al. 2016). Eudesma-4(15),7-dien-1. β -ol, found in *Magnolia compressa* var. *formosana*, a leaves, twigs and flower essential oil, shows its function against several mildew fungi, such as *Aspergillus clavatus*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum*, and *Trichoderma viride* (Su et al. 2015). It is found in *Jatropha* essential oil and shows inhibitory activity against *Escherichia coli* around 39.25 \pm 0.58% at 1000 μ g/mL concentration (El-Din et al. 2022). The (3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine has not been reported before. The (1R,7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol is an alkenol class compound and found in the *Pterocarpus pedatus*, tick berry, ginger, *Schizonepetae spica*, *Plectranthus amboinicus* essential oil and extract which serve as antifungal inhibitory against *Fusarium oxysporum* (Chen 2018; Liu et al. 2020; Mugao et al. 2020; Antonio-Gutiérrez et al. 2023).

The highest unique component in the Kalikajar cultivar was 3-Deoxy-d-mannoic lactone which is an ester or sugar found in several plant extracts: *Gynochthodes ridsdalei* extract, *Camellia japonica* petal wine, stems and roots

extract of *Codonopsis clematidea*, and *Hylocereus costaricensis* (Nair and Gangaprasad 2018; Anjarsari et al. 2020; Majumder et al. 2022). It is also found in *Aspergillus oryzae* YRA3, which decreases sorghum's root rot disease attack (Rashad et al. 2023). The 3-Deoxy-d-mannoic lactone was also detected in date palm seed extract and showed its activity to enhance the several genes involved in metabolites modulation of *Lotus arabicus* callus (Elsherif et al. 2024). Therefore, it shows that the 3-Deoxy-d-mannoic lactone has a wide role in the metabolism pathways. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- or 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone or Maltol is a flavor enhancer found as the second highest metabolites found in date palm seed, *Glycine max* and has an anti-microbial function (Sharma et al. 2016; Alghamdi et al. 2018; Ramadan et al. 2022; Elsherif et al. 2024), in fig fruit (Mopuri and Islam 2016). Trehalose compound is sucrose with activity to treat amyloidosis by preventing the deposition of amyloid protein in the body (Krishnamoorthy and Subramaniam 2014). In plants, trehalose is vital in every plant stage of metabolism from embryo development to senescence; it also drives the biotic and abiotic stress response through carbon source utility and viability integration (Grennan 2007; Ponnu et al. 2011; Sedijani 2015).

Compounds (Figure 10) that are not found in the Matesih cultivar, phenol, 2,6-dimethoxy, or syringol, as phenol derivatives, have a strong odor and sour taste. It has been identified as one of the major volatile compounds, such as in the *Canarium indicum* shell (Yusnaini et al. 2021), olive stem (Asfaw 2022), *Cyperus rotundus* (Kusuma et al. 2017), *Causonis clematidea* stems and roots (Bhardwaj et al. 2019). The other compound identified in the Matesih fruit peel is the tau-murolol compound, the oxygenated monoterpenes, which have vital roles in coping with abiotic stress.

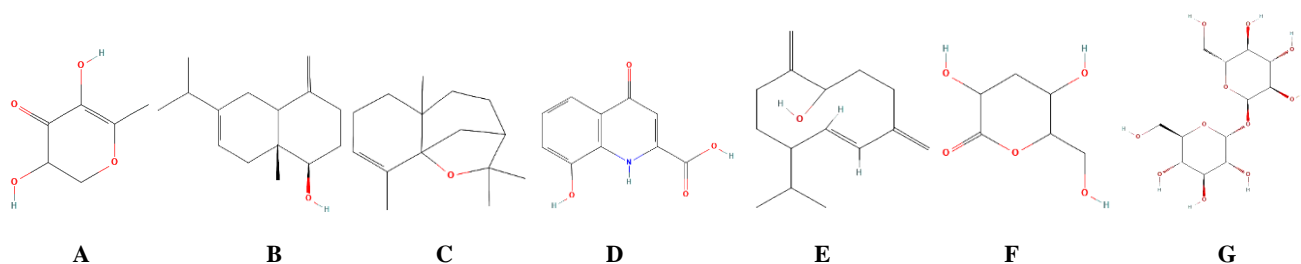


Figure 9. The unique compound structure of Kalikajar cultivar fruit peel of Central Java, Indonesia. A. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; B. Eudesma-4(15),7-dien-1. β -ol; C. (3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine; D. Xanthurenic acid; E. (1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol; F. 3-Deoxy-d-mannoic lactone; G. Trehalose

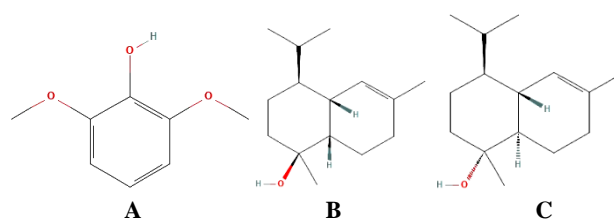


Figure 10. The structure of the same compound of Sumber, Woro, Kalikajar cultivar fruit peel of Central Java, Indonesia. A. Phenol, 2,6-dimethoxy-; B. tau-Murolol; C. α -Cadinol

The percentages of oxygenated monoterpenes, especially linalool and tau-murolol, increased under the stockosorb application. In conclusion, applying SAPs can alleviate the harmful effects of salt stress on the quantity and quality yields of sweet basil (Farsaraei et al. 2020). The alpha-cadinol is a sesquiterpene that has been found in *Illicium griffithii* fruit (Vijayakumar et al. 2012), *Solanum aculeastrum* berrie (Pender et al. 2024), and in *Mentha longifolia*, which is an antioxidant and has antimicrobial properties (Tourabi et al. 2023).

The same compound of Woro and Kalikajar cultivar (Figure 11), the trans-raphasatin is also known as (*E*)-4-Methylthio-3-butenyl isothiocyanate, the organic compound from the glucosinolate breakdown. Glucosinolate is believed to oblige as a response to a broad range of external and environmental stimuli and plant nutrition and also acts as a direct-to-plant defense system (Chhajer et al. 2019; Kitanda and Jez 2021). The compound is a pungency in radish microgreen cultivars, their roots, and several brassicas. It is well known as an antimicrobial and antifungal (Ishida et al. 2011; Hanschen et al. 2014; Zhong et al. 2023). Additionally, glucosinolate was found in several plants such as *Rhaphonticum*, *Lepidium meyenii*, *Eleutherococcus senticosus*, and *Panax ginseng*, which contain high glucosinolate compounds and possess the antioxidant, antithrombotic, antitumor, antifungal, antiallergy, photoprotective, anti-hypersensitivity properties (Todorova et al. 2022; Harvey et al. 2024; Ulloa del Carpio et al. 2024). The same compound of Sumber and Matesih cultivars (Figure 11), the alpha.-D-Glucopyranose,4-o.beta.-D-galactopyranosyl is a trisaccharide belonging to o-glycosyl compound (Okada et al. 2009), and it has never been found in the other plant compounds. Therefore, it is the first identified in the plant metabolites. However, a

similar compound to the the alpha.-D-glucopyranose,4-o.beta.-D-galactopyranosyl, the 5,7,30,40-tetrahydroxy-3-methoxy flavone-7-O-β-D-galactopyranosyl-(1-4)-O-β-D-glucopyranoside has been found in the *Acacia catechu* plant (Yadava and Sodhi 2002).

Sumber, Woro, and Matesih cultivars the Tricyclo [5.2.2.0 (1,6)] undecan-3-ol, 2-methylene-6,8,8-trimethyl (Figure 11) is found as a minor compound in leaf blade and petiole essential oil of *Toona sinensis* (Chen et al. 2014), *Phyllanthus emblica* fruit (Siregar et al. 2024). Besides, it is also found as a major compound in the n-hexane fraction fruit of *Xylopia aethiopica* (Oso et al. 2018) and *Artemisia monosperma* stem, which serves as an anticancer by decreasing 29.3% of the HepG2 cell's viability (Khan et al. 2022). Therefore, it shows that this compound is a plant metabolite commonly found in other plants, even though the function of this compound has never been reported before. Besides the Tricyclo [5.2.2.0 (1,6)] undecan-3-ol, 2-methylene-6,8,8-trimethyl, Woro, and Matesih also have the same compound, benzoic acid (Figure 11). Benzoic acid is the same metabolite compound found in Woro, Matesih, and Kalikajar cultivars. The benzoic acid compounds are found in a higher percentage in the Woro cultivar than in the Matesih and Kalikajar. Benzoic acid (BA) is a vital salicylic acid precursor. High conjugated BA form, the benzoyl-gluconate accumulation, is a response to plant defense mechanisms in tobacco plants (Chong et al. 2001) and in lettuce (Windisch et al. 2021). Plant benzoic acid has a wide range of functions as vital structural elements of primary and specialized metabolites, such as plant hormones, seed dispersers, attractants, and cofactors, which are involved in multiple stress tolerance (Senaratna et al. 2003; Sepúlveda et al. 2015; Widhalm and Dudareva 2015).

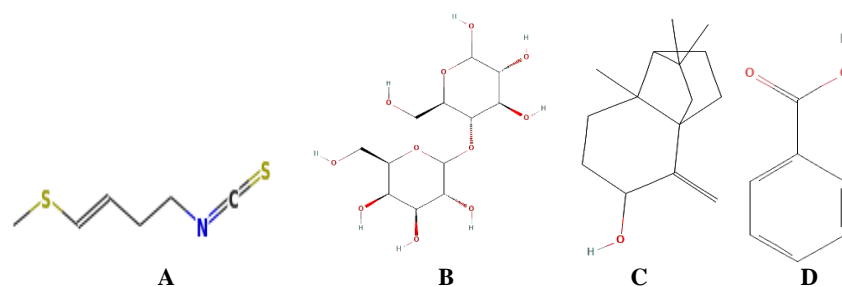


Figure 11. The same compound structure in *duku* cultivars peel fruit of Central Java, Indonesia. A. Woro and Kalikajar cultivars the trans-Raphasatin; B. Sumber and Matesih cultivars the alpha.-D-Glucopyranose,4-o.beta.-D-galactopyranosyl-; C. Sumber, Woro, and Matesih cultivars the Tricyclo [5.2.2.0 (1,6)] undecan-3-ol, 2-methyle Glucopyranose ne-6,8,8-trimethyl; D. Woro, Matesih and Kalikajar cultivars the Benzoic acid

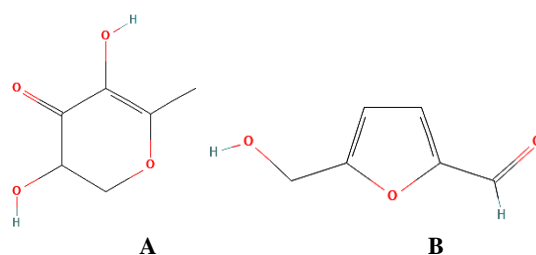


Figure 12. The Major and common compound in *duku* fruit flesh of Sumber, Woro, Matesih, and Kalikajar cultivars of Central Java, Indonesia. A. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; B. 5-Hydroxymethylfurfural

Metabolites in *duku* fruit flesh compound

Duku fruit flesh MeOH extract compound identification reveals the highest similarity between Matesih and Woro cultivars, which belong to the same group based on heatmap analysis (Figure 3). From the metabolites identified, the highest 5-Hydroxymethylfurfural compound percentage was determined in Woro, Matesih, Sumber, and Kalikajar cultivars. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and 5-Hydroxymethylfurfural was conserved in the four *duku* cultivar fruit flesh and detected as the highest component detected. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDPM), known as a strong antioxidant, and 5-Hydroxymethylfurfural were also detected in the date palm seed extract and known as a precursor for genes involved in secondary metabolism modulation (Yu et al. 2013; Chen et al. 2021; Elsherif et al. 2024).

The 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- was also found in *duku* fruit peel extract along with 3-Deoxy-d-mannonic lactone as a unique metabolite in Kalikajar cultivar. However, the 3-Deoxy-d-mannonic lactone (Figure 13) became the major unique compound in the *duku* fruit flesh of the Sumber cultivar. It was reported to be a predominant compound in *Clerodendrum viscosum* leaves MeOH extract serves as an antibacterial and antifungal in *Enicostema hyssopifolium* (Ghosh et al. 2015; Komal et al. 2020). Oxalectic acid or oxaloacetate (Figure 13) is an intermediate in Krebs's cycle that increases the NAD⁺/NADPH⁺ level and redox balance, elevating the antioxidant in the mesophyll cell chloroplast (Salin et al. 1973; Luo et al. 2021). It is also involved in many metabolic pathways, including precursors for succinic acid, amino acids, gluconeogenesis, the citric acid cycle, the glyoxylate cycle, and the urea cycle (Campos et al. 2012; Ingram and Roth 2015; Luo et al. 2021; Li et al. 2022). The Isosorbide Dinitrate (ISDN) (Figure 13), an organic nitrate, induces nitric oxide through the enzymatic action of NOS and has a relaxation function (Thomson et al. 2008; Sosa et al. 2016). The 1,3,5-Triazine-2,4,6-triamine, known as

melamine, can be used as a flame retardant in industrial production (Kanagathara et al. 2013). The monoethylmalonate monoamide (Figure 13) function is still unrecognized. 1-Octanamine (Figure 13) was discovered in the *Solanecio gigas* essential oils (EO). The EO contains mono and sesquiterpenes, a phenolic compound, as a major component, which is widely known for its advantages as an antimicrobial (Yitayeh and Wassihun 2022). When humic acid was applied to *Calendula officinalis* plants, 1-octanamine was absent; however, it was present under control and yeast suspension treatment (Essaa 2023). The myriad functions of the metabolites, especially in the major compound, show the possibility of underlining those compounds as markers for the *duku* plant as the metabolites that contribute to coping with the abiotic stress, especially in leaf function.

The unique compound of the Sumber cultivar, the d-Mannitol 1,4-anhydro-(Figure 13), has been detected as the highest metabolite in *Phoenix dactylifera* fruit or date fruit (Chinedu et al. 2019). The date fruit, known for its antioxidant, antitumor, antimicrobial, antiapoptotic, and antihyperglycemic properties, contains the unique compound d-Mannitol 1,4-anhydro-. Another unique compound, 1,4-butanedi-amine, N-(3-aminopropyl)-, also known as spermidine, is part of the most abundant polyamines (PAs) in organisms. PAs, including putrescine and spermine, play a vital role in plant biological processes and enhance stress tolerance in many plants (Prabhavathi and Rajam 2007; Tiburcio and Alcázar 2018; Alcázar et al. 2020; Song et al. 2023b; Tyagi et al. 2023). The N-Methoxy-1-ribofuranosyl-4-imidazole carboxylic amide (Figure 13) was also detected in *Moringa oleifera* (Anzano et al. 2021; Okechukwu et al. 2021), also known as the miracle tree, offers numerous benefits, including antimicrobial, antifungal, antioxidant, anti-inflammatory, for lowering cholesterol, antiepileptic, antiulcer, antihypertensive, antipyretic, and antispasmodic properties. This comprehensive range of benefits makes the miracle tree a valuable resource in various applications.

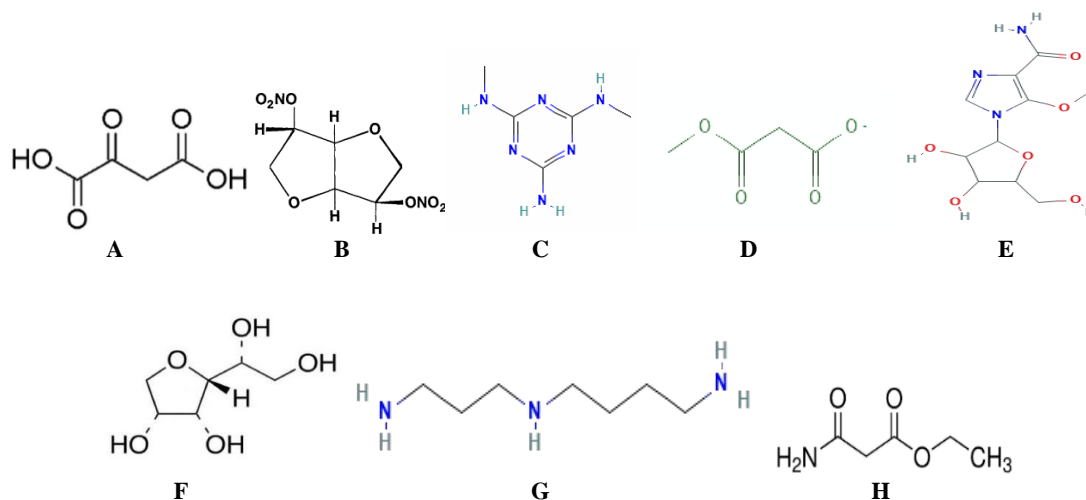


Figure 13. The unique compound structure of Sumber Cultivar flesh fruit of Central Java, Indonesia. Left to right respectively: A. Oxalectic acid; B. Isosorbide dinitrate; C. 3,5-Triazine-2,4,6-triamine; D. Monoethyl malonate monoamide; E. 1,4-Butanedi-amine, N-(3-aminopropyl)-; F. 1-Octanamine; G. 3-Deoxy-d-mannonic lactone; H. d-Mannitol, 1,4-anhydro-.

Woro unique compounds (Figure 14), the ethanimidic acid, an ethyl ester, is the highest unique compound, identified around 8.44%. In *Hibiscus×rosa-sinensis*, it was found higher, around 31.43 % (Rassem et al. 2024), in pineapple peel (Erukainure et al. 2016). Ethanimidic acid has benefits as it is a high antioxidant. Therefore, ethanimidic acid can be used as an anti-inflammatory, attenuating lipid peroxidation and antilipidemic and antibacterial activity. The Acetamide, N-(aminocarbonyl)-also known as Acetamide, N-(aminocarbonyl)-N-hydroxy-function has never been reported. However, the mixture of acetamide, glycoside, and its derivatives was identified to have strong antioxidant, anti-inflammatory, and antibacterial properties, hypocholesterolemia, and antiarthritic (Autore et al. 2010; Kakarla et al. 2016; Mohamed et al. 2020).

The 2-Cyclopenten-1-one, 2-hydroxy- or 2-Hydroxy-2-cyclopenten-1-one was detected in *Imperata cylindrica* group (Hidayat et al. 2019), *Saussurea costus* (Mammate et al. 2022), which exhibit antioxidant and anti-urolithiasis, and from the mangrove endophytic fungus *Alternaria* sp. (Wang et al. 2015). The benefit of the 2-isopropoxyethylamine compound has not yet been uncovered. The isobutyraldehyde, propylhydrazone, has antibacterial properties and had been found in *Moringa oleifera* extract, which, in low concentration, 6.25%, gave effective cytotoxicity against *Enterococcus faecalis* bacteria (Soraya et al. 2022a, 2022b). The 1,6-Anhydro-2,4-dideoxy-. Beta. -D-ribo-hexopyranose or now has been updated its name to (1S,3S,5R) 6,8-

Dioxabicyclo[3.2.1]octan-3-ol, according to PubChem (2019), has been found in *Scutellaria orientalis* (Gharari et al. 2022).

The 3-Hydroxy-N, N-dimethylpropanamide, and 2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine (Figure 15) are the Matesih unique metabolites that share the highest compound percentage among eight Matesih unique compounds, around 8.00% and 7.08% respectively. The 3-hydroxy-N, N-dimethylpropanamide is a motif present in the oxazolomycin family (Oleksak et al. 2020). Moreover, as part of the phenol group, it has antibacterial activity. 2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine (Figure 15) or 2R,3S-9-[1,3,4-trihydroxy-2-butoxymethyl] guanine with N1-(4-hydroxybutyl)-N3-methyl guanidine acetate are the main compounds produced by *Streptomyces rochei* DSM 41729 which are used to coat grapevines to combat the most dangerous post-harvest diseases, which can inhibit *Botrytis cinerea* by around 85-90% (Buzón-Durán et al. 2023) and the main antifungal compound of *Earliella scabrosa*, a biocontrol on rubberwood (Peng and Don 2013). It is also elucidated from *Citrus reticulata* plant extract, which is useful to combat *Tribolium castaneum* and *Plodia interpunctella* and exhibits 70% repellent efficacy (Lee et al. 2020). In phytochemical constituents of *Phoenix dactylifera* fruit (date fruit), it is also present and known for anti-tumor, anti-inflammatory, anti-microbial, and anti-oxidant activities (Chinedu et al. 2019).

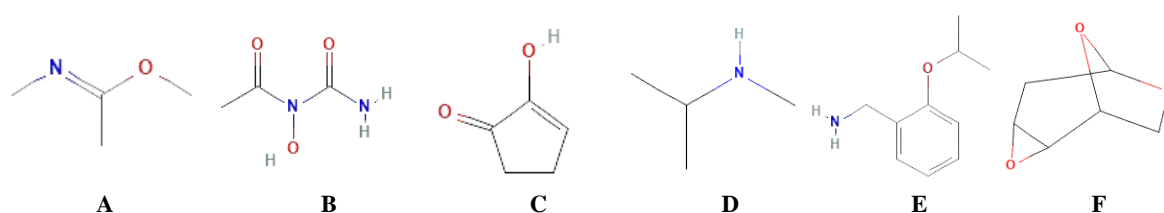


Figure 14. The unique compound structure of Woro Cultivar flesh fruit of Central Java, Indonesia. A. Ethanimidic acid; B. Acetamide, N-(aminocarbonyl)-; C. 2-Cyclopenten-1-one, 2-hydroxy-; D. 2-Isopropoxyethylamine; E. Isobutyraldehyde, propylhydrazone; F. 1,6-Anhydro-2,4-dideoxy-. Beta. -D-ribo-hexopyranose

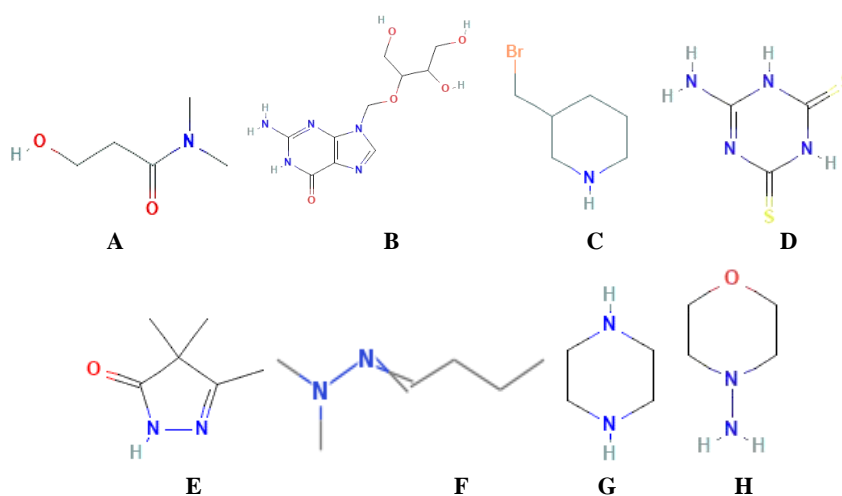


Figure 15. The unique compound of Matesih Cultivar flesh fruit of Central Java, Indonesia. A. The 3-Hydroxy-N, N-dimethylpropanamide; B. 2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl] guanine; C. Piperidine, 3-(bromomethyl)-; D. 6-Amino-1,3,5-triazine-2,4(1H,3H)-dione; E. 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-; F. Butanal, dimethylhydrazone; G. Piperazine; H. N-Aminomorpholine

Piperidine, 3-(bromomethyl)- (Figure 15) is one of the piperidine derivatives. It is an organic compound that plays a vital role in the pharmaceutical industry. Piperidine is utilized for medicinal purposes, both in synthetic and natural forms (Frolov and Vereshchagin 2023). Piperidine and its other piperidine forms extracted from *Piper longum* and *Piper nigrum* fruit exhibit potential for antimicrobial and antioxidant activity (Rawat et al. 2022). Piperidine is used for myriad purposes, such as pesticides in agriculture, medicines for tumors, inflammation, infection, obesity and diabetes, and depression or physiological disorders both in humans and animals (Xiang et al. 2016). 6-Amino-1,3,5-triazine-2,4(1H,3H)-dione (Figure 15) or dithioammelide and its derivatives administration with D-serine can inhibit the D-amino acid oxidase (DAAO), which results in the high sustain of D-serine plasma level for chronic psychiatric disorders treatment (Hin et al. 2015). 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl- detected in fig fruit (*Ficus carica*), which has numerous pharmacological characteristics such as anticancer, anti-inflammatory, antimutagen, antiviral, antiparasitic, antidiabetic, and antibiosogenic (Mopuri et al. 2018; Shahrajabian et al. 2021). Butanal dimethylhydrazone (Figure 15) has the function of antibacterial. It is found in the *Artemisia annua* as butyraldehyde or butanal (Brown 2010). The *Eleocharis dulcis* leaves extract contains the butanal derivatives, showing the antibacterial pathogenic for fish (Ramadhani et al. 2020). Piperazine (Figure 15) is an organic base known as a plant defense mechanism activator. The piperazine derivatives, trifluoromethyl pyridine piperazine derivatives (A1-A27), strengthen the tobacco antiviral activities by increasing the defensive enzyme activities of superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), also the

phenylpropanoid biosynthesis pathway (Zhang et al. 2022). 1-[(4-bromophenoxy) acetyl]-4-[(4-fluorophenyl) sulfonyl] piperazine (ASP) also activates the auxin hormone in *Arabidopsis thaliana* (Xu et al. 2020). N-aminomorpholine has been found in *Glycine max* (Alghamdi et al. 2018).

The unique metabolites from *duku* fruit flesh were identified from the Kalikajar cultivar (Figure 16), with 1,3-methylene-d-arabitol sharing around 12.78%, the highest percentage. *Clerodendrum viscosum* leaves were analyzed using GC-MS, and they showed that they contain 1,3-methylene-d-arabitol (Ghosh et al. 2015). Arabitol and its derivatives are natural sweeteners. They have 0.2 kcal g⁻¹, or twelve times lower calorie content, and are 70% sweeter than xylitol (Farias et al. 2022). Cyclohexanone derivative with anti-fungal properties has been detected in *Punica granatum* leaf extract, which protects wheat against fungus (Radhi and Khashan 2022). It is also detected in several plants, such as *Piper longum* fruit (Rawat et al. 2022), *Camellia japonica* petal (Majumder et al. 2022), Yoyo bitters (Iheagwam et al. 2021), *Allium tuberosum* (Huang et al. 2016), and *Musa* sp. (Ohiri et al. 2023). The 2,4,6,8-Tetraazabicyclo [3.3.0] octan-3-one, 7-nitroimino- is N-rich heterocyclic form that had been identified in Knobweed (*Hyptis capitata*) leaves (Xing-hui et al. 2015; To'bungan and Jati 2022). 2(3H)-Furanone, dihydro-3-methylene is a natural flavor in fruit. It is one of 3(2H)-furanone structure, part of furaneol, also known as 4-hydroxy-5-methyl-3(2H)-furanone (HMF), the key flavor compounds in numerous fruits (Schwab, 2013) and flavor for the food industry (Haag et al. 2021). It has preservative properties and has been identified in strawberries (Schiefner et al. 2013), red dragon fruit (Anjarsari et al. 2020), and *Punica granatum* (Radhi and Khashan 2022).

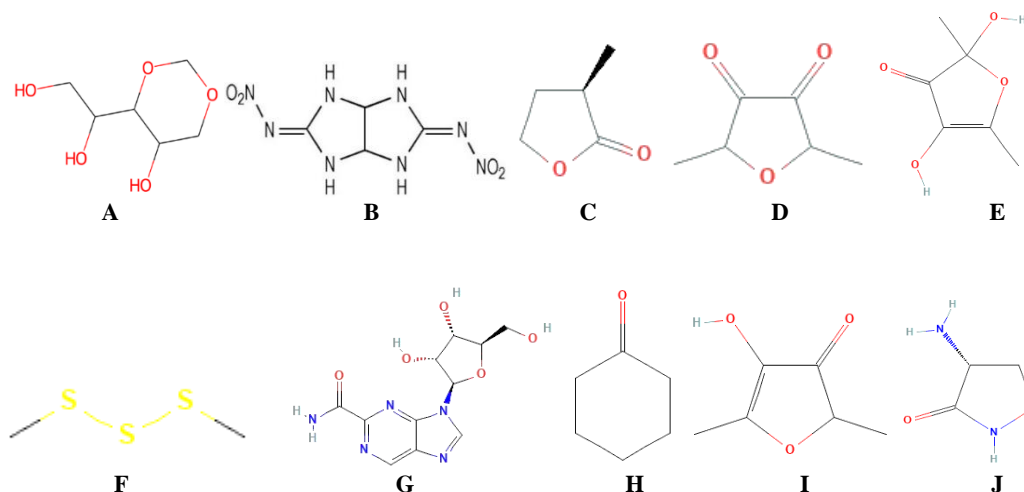


Figure 16. The unique compound of the structure of Kalikajar Cultivar flesh fruit. A. 1,3-Methylene-d-arabitol; B. 2,4,6,8-Tetraazabicyclo [3.3.0] octan-3-one, 7-nitroimino-; C. 2(3H)-Furanone, dihydro-3-methylene; D. 2,5-Dimethylfuran-3,4(2H,5H)-dione; E. 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; F. Dimethyl trisulfide; G. 2-Carbamyl-9- [. beta. -d-ribofuranosyl] hypoxanthine; H. Cyclohexanone; I. Furaneol; J. Cycloserine

As a terpenoid compound, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one has several benefits, such as a natural antioxidant, anti-microbial, and flavoring. The 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one had been detected in *Antidesma bunius* fruit (Yellianty et al. 2022), *Polygenia* sp. (Hao et al. 2015), *Pouteria caimito* (Bin Arif et al. 2021), *Taxus baccata* (Sánchez-Hernández et al. 2023), *Punica granatum* (Usha et al. 2021), *Palmyra* palm (Gummadi et al. 2016), apple cider vinegar (Singh et al. 2023), *Cyperus rotundus* kombucha (Dechakhamphu et al. 2023), *Ziziphus jujuba* (Tepe and Doyuk 2020), and vanilla extract (McCormick 2018). The 2,5-Dimethylfuran-3,4 (2H,5H)-dione is a furaneol derivate that contributes to food flavor. It was detected as a major component in day 0 black garlic processing (Wonorahardjo et al. 2023) and has antifungal properties against *Bipolaris oryzae* in rice (Rajasekar et al. 2020), also abundant in the sautéed shiitake (Sissons 2021).

Dimethyl trisulfide contributes to fishy aroma due to its highest containment sulfur, known as microbial volatile compounds (MVCs) that accelerate plant growth and pathogen defense. Dimethyl trisulfide (DMDS) is one of the highest compounds in chocolate and gives a cabbage-like odor (Seyfried and Granvogl 2019). DMDS inhibits the ring rot disease in apples (Sun et al. 2022) and displays antifungal activity against *Sclerotinia minor* (Tyagi et al. 2020) by inducing the defense-related genes. Besides being found in several plants, such as the *Allium* family, DMDS can also be found in *Burkholderia ambifaria*, which can be targeted against *Rhizoctonia solani* and *Alternaria alternata* pathogens (Thomas et al. 2020). The 2-Carbamyl-9-[β -D-ribofuranosyl] hypoxanthine has been found in the *rambutan* fruit, which has anticancer properties by inhibiting the Caspase-3, Caspase-9, -Actin, p53, and Bcl-2, the apoptotic protein (Angalammal et al. 2022). Furaneol (DHMF), a key component of the furanone volatile family, is responsible for the sweet, candy, and caramel flavor, also as the floral aroma found in berries (Gu et al. 2022; Abouelenein et al. 2023).

Woro and Kalikajar have the same compound beside the two conserved compounds, the 2-Furanmethanol, beta-alanine amide, and 5-amino-1,3,4-oxadiazole-2-carboxamide; and N-acetyl-D-glucosamine, (Figure 17). One of the metabolite compounds, the 2-propanone, dimethylhydrazone (Figure 17), was missing from the Sumber cultivar, while it was present in the Woro, Matesih, and Kalikajar cultivars. The 2-propanone dimethylhydrazone was also identified as

the major compound in the Kalikajar cultivar, around 30.78%, and the second major compound (27.25%) in the Matesih cultivar.

The 2-furanmethanol also known as 2-(1-Hydroxyprpyl) furan, 1-(furan-2-yl)propan-1-ol, α -Ethylfuran-2-methanol, Furfuryl alcohol, α -ethyl-, The research result shows that the 2-furanmethanol rate was detected higher in healthy patients than in breast cancer patients (Silva et al. 2012), which shows the possibility of 2-furanmethanol as an anticancer metabolite. Moreover, it has also been detected in the *Aspergillus carbonarius* fungal-infected grape berries plant, which is absent in healthy grape berries (Giannoukos et al. 2020). The 2-furanmethanol was also identified in several plants, such as *Hewittia malarabica* and Polygoneae (Hao et al. 2015), *Cyperus rotundus* shoots and tubers (Kusuma et al. 2017), *Achillea wilhelmsii* (Achakzai et al. 2019), leaves of *Psidium guajava* (Yelwa and Ibrahim 2022). It is also found in endophytic fungi of *Aloe dhufarensis* as the major compound against pathogenic fungi (Al-Rashdi et al. 2022). N-acetyl-d-glucosamine (N-GlcNAc) is from the amide derivatives, significantly boosting plant performance. N-GlcNAc shapes the soil microbial communities by acting as a microbial signaling molecule (Kyrychenko 2020), enhances the rhizosphere microbiome in tomato plants, strengthens plant resilience to pathogens and increases plant growth and development (Sun et al. 2022). The depletion of UDP-N-GlcNAc causes salt hypersensitivity in *Arabidopsis thaliana* during early development (Chen et al. 2022). Even though N-GlcNAc usually resulted from chitin and non-plant-based (Chen et al. 2010; Vidhate et al. 2023), the plant-based novel chitinase-like lectin from *Tamarindus indica* had been produced (Patil et al. 2013).

The other compound, the 5-Amino-1,3,4-oxadiazole-2-carboxamide compound, is 1,3,4-oxadiazole derivatives, which have been synthesized. It has myriad applications in the medicine and agriculture fields as antioxidant, antimicrobial, cytotoxic agents, and enzyme inhibitors for anticancer, antifungal, blood pressure lowering properties, plant protection against pathogens as herbicides and insecticide (Ali 2015; Wu et al. 2019; Luczynski and Kudelko 2022; Kumar et al. 2023). The beta-alanine amide is higher in the Woro cultivar than in the Kalikajar cultivar. It is an amino acid masked by carboxylates in an amide, resulting in greater stability of peptides (Kudo et al. 2014; Song et al. 2023a).

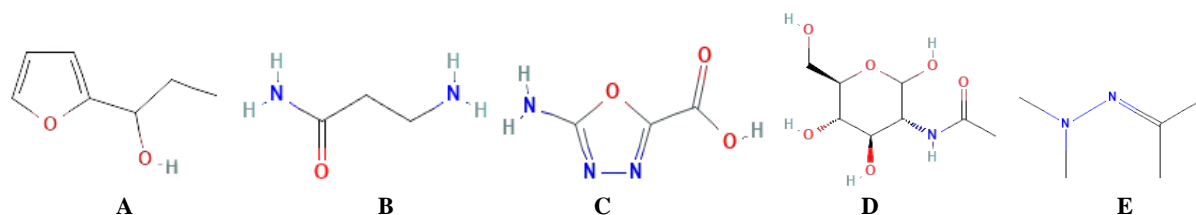


Figure 17. The same compound structure of Woro and Kalikajar Cultivar flesh fruit. A. 2-Furanmethanol; B. beta-Alanine amide; C. 5-Amino-1,3,4-oxadiazole-2-carboxamide; D. N-Acetyl-D-glucosamine and E. The same compound structure of Woro, Matesih, and Kalikajar cultivar, 2-Propanone, dimethylhydrazone (bottom centre).

β -alanine is formed from α -L-aspartate, polyamines spermine/spermidine, the carboxylic acid propionate, and the nucleotide base uracil. β -alanine is also a coenzyme A (CoA) precursor; CoA is a key cofactor in many metabolic and energy reactions (Parthasarathy et al. 2019). Therefore, it has prominent functions in plant metabolism (Perchat et al. 2022). β -alanine numerous roles in plants are protecting the plant from drought, hypoxia, extreme temperature, heavy metal stress, and several biotic stresses (Braun et al. 2015; Göttlinger and Lohaus 2024).

Identifying volatile organic compounds (VOCs) through the GC-MS of four *duku* cultivars from Central Java, Sumber, Woro, Matesih, and Kalikajar successfully reveals their harbored unique traits. Fingerprinting plants with the specific genetic characteristic of their metabolite differentiation has been conducted rapidly in numerous plants (Ouwerkerk and Meijer 2011; Yunita et al. 2019; Mayasari et al. 2021). One of the methods to conduct the fingerprinting of plant characteristics through the identification of their metabolite compounds is GC-MS analysis. GCMS utilization for plant metabolomic fingerprinting provides genetic diversity data and potential as a superior parent plant with favorable nutritional value and desired morphological and physiological traits. The metabolite identification by GC-MS is divided into targeted and non-targeted metabolite or metabolomic ways. Non-targeted metabolomic is the recent genomic approach applied as a tool to identify the plant's powerful characteristics, such as its plant defense action against biotic stress due to the pathogen and pest invade (Hu et al. 2019; Kashyap and Kumar 2021; Makhumbila et al. 2022; Al-Khayri et al. 2023; Zhang et al. 2023), nutritional value, and plant product quality (Farag et al. 2012; Kobayashi et al. 2012; Jumhawan et al. 2015; Soni and Sureshkumar 2016; Deng et al. 2022), environmental stress signalling system (Abdelrahman et al. 2018; Jin et al. 2021; Arabsalehi et al. 2022; Wu et al. 2023).

Metabolomic analysis is a powerful direct method to identify morphological and physiological genetic traits. Physiological characteristics are associated with the compounds or metabolite interaction within plants at the molecular level. Metabolites are defined into two forms: primary and secondary metabolites. The primary metabolites mainly control the plant development. In contrast, the secondary metabolites (SM) act as the plant system response to the micro and mega environmental change and pathogen attack (Pais et al. 2018). The primary metabolites consist of carbohydrates, amino acids, and organic acids compounds, while the phenolics, alkaloids, and terpenoids are secondary metabolites that form (Hill and Roessner 2013). Since the metabolite is the phenotype resulting from the genome and environment dynamic interaction as moiety from the biological system (Sheth and Thaker 2014; Pieruschka and Schurr 2019), therefore, it explains the correlation of some metabolites to the specific plant characteristics (Allwood et al. 2021). Patel et al. (2021) the metabolomic analysis is closer to the plant phenotype expression than the other genomic level. Hence, it functionally acts as a character marker and vice versa. Metabolomic analysis is the best method to identify genetic diversity

because metabolites are the end products of gene expression and the byproducts of the biological system, contributing to morphological and physiological differentiation. In this context, metabolomic compounds play a crucial role in identifying genetic diversity as they are the end products of gene expression and the byproducts of the biological system, contributing to morphological and physiological differentiation (Ch et al. 2021; Patel et al. 2021).

In conclusion, this finding shows that four *duku* cultivars, fruit peel and flesh, display each unique metabolite as metabolic fingerprinting of their traits with a wide range of functions. From its nutritional value, as biocontrol for agriculture purposes, to the massive metabolites compound plays a vital role in coping with the abiotic and biotic stress tolerance to tackle the problem in agriculture practices from climate change. The common and unique metabolites detected from Sumber, Woro, Matesih, and Kalikajar cultivars can be used to fingerprint each cultivar's identity. It is proven by certain metabolites, such as 3-Deoxy-d-mannoic lactone, which is present in the Matesih cultivar fruit peel with a small percentage and present in the Sumber cultivar fruit flesh with a high rate. Furthermore, the 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- present in Matesih fruit peel- is a common metabolite compound in four *duku* cultivar fruit flesh. Moreover, the benefit of every metabolite detected reveals fundamental traits of the Sumber, Woro, Matesih, and Kalikajar cultivars for future research and breeding decisions of *duku* plants, such as the metabolites identified for biotic and abiotic stress resilience.

ACKNOWLEDGEMENTS

The authors thank the Research and Community Services Institution (LPPM) of Universitas Muria Kudus, Kudus, Indonesia, for funding this research from the University Internal Grant Scheme.

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