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PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF YOUNG KAWISTA FRUIT (*Limonia acidissima Groff*) EXTRACTS

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ABSTRACT

Kawista fruit (*Limonia acidissima Groff*) has been traditionally used for its medicinal properties, with its bioactive compounds offering potential health benefits. Among these, antioxidants play a crucial role in neutralizing free radicals, which are associated with oxidative stress and various diseases. Exploring the antioxidant potential and metabolite profile of Kawista fruit could contribute to the development of natural therapeutic agents. This study investigated the antioxidant potential and metabolite profile of young Kawista fruit peel and pulp extracts. The plant material was extracted using ethanol and analyzed for quality parameters based on the Indonesian Herbal Pharmacopoeia standards. Antioxidant activity was assessed using the DPPH method, while metabolite profiling was performed using LC-MS/MS. The results showed that both extracts met the quality standards, with the peel and pulp extracts exhibiting IC_{50} values of 41.398 ± 0.055 µg/mL and 47.816 ± 0.020 µg/mL, respectively, indicating moderate antioxidant activity compared to vitamin C (3.909 ± 0.017 µg/mL). LC-MS/MS analysis identified 16 compounds in the peel extract, with 5-tetradecanoyloxolan-2-one (31.66%) and Buscopan (14.68%) as the major components, while 11 compounds were identified in the pulp extract, including 3,5-dicaffeoylquinic acid (15.64%) and phospholipid derivatives (26.36-28.2%). The presence of various bioactive compounds, such as flavonoids, alkaloids, saponins, polyphenols, and tannins, supports the traditional therapeutic uses of young Kawista fruit and suggests its potential for development as a natural antioxidant agent.

Keywords: *Limonia acidissima Groff*, Antioxidant Activity, DPPH Method, Metabolite Profiling, Bioactive Compound.

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INTRODUCTION

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the damage they cause. This imbalance results in oxidative damage to cells, which can disrupt their normal functions and contribute to disease progression.[1,2] Antioxidants play a vital role in neutralizing ROS, thus preventing cellular damage and maintaining physiological balance.[3] The impact of oxidative stress is particularly evident in cardiovascular diseases (CVD), which are among the leading causes of mortality worldwide. According to the World Health Organization (WHO), about 31% of all global deaths are attributed to CVD, underscoring its significant health burden.[4,5] Oxidative stress contributes to CVD by damaging the endothelium, promoting plaque formation in arteries, and exacerbating risk factors such as diabetes and hypertension. Beyond CVD, oxidative stress has been implicated in other chronic conditions, including cancer and neurodegenerative diseases.[6] This highlights the need for effective antioxidant interventions to mitigate its impact on human health. The young Kawista fruit (*Limonia acidissima Groff*) has shown promising antioxidant potential due to its rich content of bioactive compounds. These compounds, including phenols, flavonoids, and terpenoids, are known for their ability to neutralize free radicals and prevent oxidative damage at the cellular level.[7] Traditional medicine in South Asia has long utilized Kawista for various therapeutic purposes, such as treating gastrointestinal issues and infections.[8]

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Despite its traditional use, scientific research on Kawista's antioxidant properties remains limited, particularly in understanding the secondary metabolite profiles that contribute to its bioactivity. Secondary metabolites are crucial in determining the therapeutic potential of natural products, yet the profiles of these compounds in Kawista remain underexplored.[9] The antioxidant activity of Kawista suggests it could serve as a natural source of agents for preventing oxidative stress-related diseases. Moreover, identifying the specific bioactive compounds in Kawista can help in developing targeted antioxidant therapies.[10] Understanding these compounds' roles is essential for advancing pharmacological applications of Kawista as a functional food or natural medicine. Advanced techniques such as LC-MS/MS (Liquid Chromatography-Mass Spectrometry/Mass Spectrometry) provide a powerful tool for identifying and analyzing secondary metabolites in plant extracts. This analytical method enables the detailed profiling of bioactive components, which is critical for understanding their antioxidant mechanisms.[11,12] Using LC-MS/MS, this study aims to evaluate the antioxidant potential of ethanol extracts from the peel and flesh of young Kawista fruit. By identifying the secondary metabolites present in these extracts, the study seeks to uncover the compounds responsible for its antioxidant activity.[13] This research not only addresses the gap in scientific knowledge about Kawista but also contributes to the broader field of natural product pharmacology. The findings are expected to highlight the therapeutic potential of Kawista as a natural antioxidant source. Additionally, the study may pave the way for future applications of Kawista in developing preventive and therapeutic strategies for oxidative stress-related conditions. Ultimately, this work aims to advance the utilization of natural resources in addressing global health challenges.

EXPERIMENTAL

Plant Collection and Identification

The young Kawista (*Limonia acidissima Groff*) fruit was collected from Subang Regency, West Java, Indonesia, as part of this study on its antioxidant potential. The plant was identified and authenticated at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia. The identification process ensured the sample met the required taxonomic standards, with the specific sample registered under number 46/HB/11/2023. The plant parts used in this research were the fruit peel and pulp of young Kawista, which are known for their traditional medicinal applications. Accurate identification of the plant is critical to ensure consistency and reliability in the subsequent experimental processes. Kawista has long been recognized for its potential bioactive compounds, but rigorous taxonomic confirmation is necessary for scientific research. This verification process provides a foundation for evaluating the antioxidant and therapeutic properties of the plant. These preliminary steps ensure the validity of results in further analyses and experiments.

Extract Preparation

The preparation of the young Kawista fruit extracts involved distinct methods for the peel and pulp to maximize the yield and preserve the bioactive compounds. Initially, 200 g of Kawista pulp was weighed and placed in a Soxhlet apparatus for extraction with 96% ethanol as the solvent, using a ratio of 1:10. The extraction was conducted for seven cycles, followed by two repetitions to ensure comprehensive extraction of bioactive compounds. Similarly, 200 g of Kawista fruit peel powder was extracted using the reflux method with 96% ethanol at a ratio of 1:5 for three hours. The filtrates obtained from both methods were filtered and subjected to a repeat extraction process to maximize yield. The concentrated extracts were obtained using a rotary evaporator and a water bath, ensuring minimal loss of volatile components. The thick extracts of the peel and pulp were weighed to calculate the extraction yield, expressed as the ratio of extract weight to the initial simplicia weight. Additionally, phytochemical analysis was conducted qualitatively to identify the chemical composition of the extracts. Proper storage of these extracts in a refrigerator ensures their stability for subsequent assays and analyses.

Antioxidant Assay

The antioxidant activity of the young Kawista fruit extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a reliable method for evaluating radical scavenging activity. A series of extract concentrations were prepared and mixed with a 50 µg/mL DPPH solution at a 1:1 volume ratio to initiate the reaction. Methanol was used as a blank control, and DPPH alone was used as a control to establish a baseline absorbance. Ascorbic acid was included as a standard to provide a reference for

antioxidant activity. After a 30-minute incubation period, the absorbance of each sample was measured at 516 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The experiment was conducted in triplicate for each sample and the standard to ensure the accuracy and reproducibility of the results. The radical scavenging activity was calculated using the formula: $I\%=Abs_0(Abs_0-Abs_1)\times100$. Here, Abs_0 represents the absorbance of the control, and Abs_1 represents the absorbance of the sample at each concentration. The IC_{50} value, which indicates the concentration required to inhibit 50% of DPPH radicals, was determined from a calibration curve plotting inhibition percentage against concentration.[14] This assay provides insights into the antioxidant capacity of the extracts, enabling comparison with standard antioxidants such as ascorbic acid.

Metabolite Profiling using LC-MS/MS

The bioactive compounds in the young Kawista fruit extracts were analyzed using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). This advanced analytical technique consists of three main components: the ion source, mass analyzer, and detector, which work together to identify and quantify secondary metabolites. Before analysis, the samples were prepared using the solid-phase extraction (SPE) method to remove impurities and concentrate the bioactive compounds. The prepared samples were then injected into the LC-MS system for analysis. Electrospray ionization (ESI) was employed as the ionization method to convert molecules into ions, a crucial step for accurate mass spectrometry. This process generates a high number of ions through charge exchange in solution, forming complete molecular ions that serve as the basis for identification.[15] The mass analyzer separates these ions based on their mass-to-charge ratio, providing detailed information about the molecular structure of the compounds. The data obtained is further refined and interpreted to identify the specific secondary metabolites present in the extracts. This method allows for precise identification of compounds such as phenols, flavonoids, and terpenoids, which contribute to the antioxidant activity. The results from LC-MS/MS profiling not only enhance the understanding of Kawista's bioactive components but also provide a basis for developing its pharmacological applications. Advanced metabolite profiling is essential for exploring the therapeutic potential of natural products and their role in addressing oxidative stress-related diseases.

RESULTS AND DISCUSSION

Phytochemicals and Standard Quality of Ethanol Extract

The selection of an appropriate solvent is critical for extracting secondary metabolites efficiently and preserving their bioactivity. In this study, ethanol was chosen as the solvent due to its polarity and versatility, which make it suitable for extracting a wide range of bioactive compounds. Ethanol is widely recognized for its ability to solubilize both polar and non-polar compounds, making it ideal for comprehensive phytochemical extraction.[16] The ethanol extracts from the peel and pulp of young Kawista fruit (*Limonia acidissima Groff*) were analyzed to ensure their quality met the standards set by the Indonesian Herbal Pharmacopoeia, as presented in Table-1. Phytochemical screening of these extracts confirmed the presence of several key secondary metabolites, including flavonoids, alkaloids, saponins, polyphenols, and tannins. These compounds are well-documented for their antioxidant, antimicrobial, and anti-inflammatory properties, highlighting the therapeutic potential of Kawista fruit. The presence of these metabolites validates the use of Kawista fruit in traditional medicine and supports its pharmacological applications. Ensuring high-quality extraction and adherence to standard guidelines is essential for future research and development of natural products based on Kawista fruit.

Antioxidant Activity of the Extract

Based on the data presented in Table-2, the ethanol extracts of the peel and pulp of young Kawista fruit showed moderate antioxidant activity, as evaluated using the DPPH assay. The IC_{50} values for the peel and pulp extracts were recorded as 47.816 ± 0.055 µg/mL and 47.816 ± 0.020 µg/mL, respectively. These results suggest that the peel extract demonstrated slightly higher antioxidant activity than the pulp extract, as lower IC_{50} values indicate greater antioxidant efficacy. In comparison, vitamin C (ascorbic acid), used as the standard antioxidant, exhibited an IC_{50} value of 3.909 ± 0.017 µg/mL, significantly lower than those of the young Kawista fruit extracts. This vast difference highlights the superior free radical scavenging ability of ascorbic acid. Despite this, the moderate antioxidant activity of young Kawista extracts suggests their

potential as natural antioxidants. The difference in IC_{50} values between the extracts and the standard also indicates room for further optimization or potential combination with other bioactive compounds. Overall, these findings support the potential application of Kawista fruit in natural health products or as complementary antioxidant sources.

Table-1: The Quality of the Ethanol Extract from the Peel and Pulp of Kawista Fruit (*Limonia acidissima Groff*) Meets the Standards Required by the Indonesian Herbal Pharmacopoeia.

Parameter	Fruit Peel (%)	Fruit Pulp (%)	Standard Quality Required by the Indonesian Herbal Pharmacopoeia (%)
Loss on drying	8.4	6.3	≤ 10
Total ash	2	5	≤ 7.5
Moisture content	8	6	≤ 10
Water-dissolved essence	9	39	≥ 18
Ethanol-dissolved essence	10	44	≥ 10

Table-2: Antioxidant Activity of the Ethanol Extract from the Peel and Pulp of Kawista Fruit (*Limonia acidissima Groff*)

Parameter	DPPH IC_{50} (µg/mL)
Fruit Peel of Kawista fruit (*Limonia acidissima Groff*)	41.398 ± 0.055
Fruit Pulp of Kawista fruit (*Limonia acidissima Groff*)	47.816 ± 0.020
Vitamin C	3.909 ± 0.017

The linear regression equation used in the calculation, $y = 6.9712x + 22.842$ with an R^2 value of 0.9993, indicates a high level of accuracy and reliability in the data fit for the calibration curve. This suggests a strong correlation between the concentration of the young Kawista fruit extracts and their DPPH scavenging activity. The extracts exhibit promising antioxidant potential, but their performance is moderate compared to the ascorbic acid standard, which demonstrated superior free radical scavenging ability. The difference highlights the need to better understand the mechanisms driving the antioxidant properties of Kawista fruit. Future studies could focus on optimizing the extraction process to improve the concentration of bioactive compounds responsible for antioxidant activity. Additionally, exploring the synergistic effects of Kawista extracts in combination with other natural antioxidants could enhance their efficacy. Investigating their role in oxidative stress prevention and related health benefits may open new applications in functional foods or natural medicine. In addition, these findings provide a foundation for further research into Kawista fruit as a source of antioxidant agents.

Metabolite Profiling via LC-MS/MS

The metabolite profiling of ethanol extracts from the peel and pulp of young Kawista fruit was carried out using LC-MS/MS. The total ion chromatograms (TIC) in negative ion mode for the peel and pulp extracts are illustrated in Fig.-1 and Fig.-2, respectively. In the peel extract, 16 chromatographic peaks were annotated, while the pulp extract revealed 11 peaks. The classification of compounds in these extracts was initially based on ultraviolet (UV) absorption spectra obtained from the total scan PDA chromatograms. Most of the UV-absorbing compounds in both extracts exhibited maximum absorption between 200–300 nm, a range typical of phenolic compounds and other secondary metabolites associated with antioxidant and antimicrobial properties. The LC-MS/MS analysis was performed in both positive and negative ion modes, with the negative ion mode proving more effective for ionizing the majority of compounds. This mode was therefore chosen as the preferred method for identification. The analysis demonstrated a robust ability to identify bioactive compounds across the extracts, providing an important foundation for further research.[17]

The profiling revealed a diverse array of bioactive compounds, including flavonoids, alkaloids, and phenolic glycosides. Notable compounds detected in the peel extract included Peonidin-3-glucoside and Petunidin-3-glucoside, which are known for their antioxidant properties. Similarly, key compounds identified in the pulp extract included 3,5-dicaffeoylquinic acid and Fumitremorgin C, which also contribute

to bioactivity. The detailed structural information provided by LC-MS/MS offers valuable insights into the therapeutic potential of these compounds, particularly in combating oxidative stress. These findings highlight the medicinal value of Kawista fruit, supporting its traditional use in herbal remedies. Moreover, the results underscore its potential for development into modern therapeutic agents targeting oxidative stress and related diseases. This study further strengthens the growing body of evidence for the pharmacological applications of Kawista fruit. It opens avenues for future research to optimize extraction methods and explore its broader health benefits.

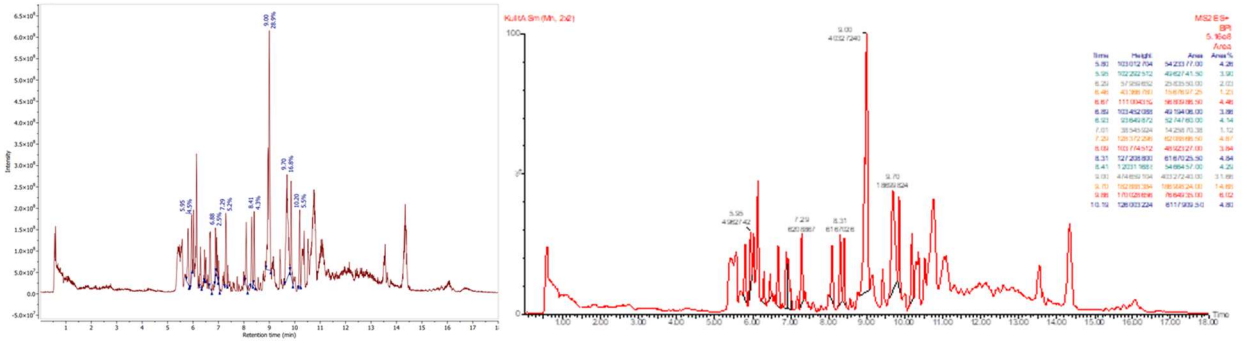

Fig.-1: Total Ion Chromatogram (TIC) In Negative Ion Mode of the Peel Extract from Kawista Fruit (*Limonia acidissima Groff*)

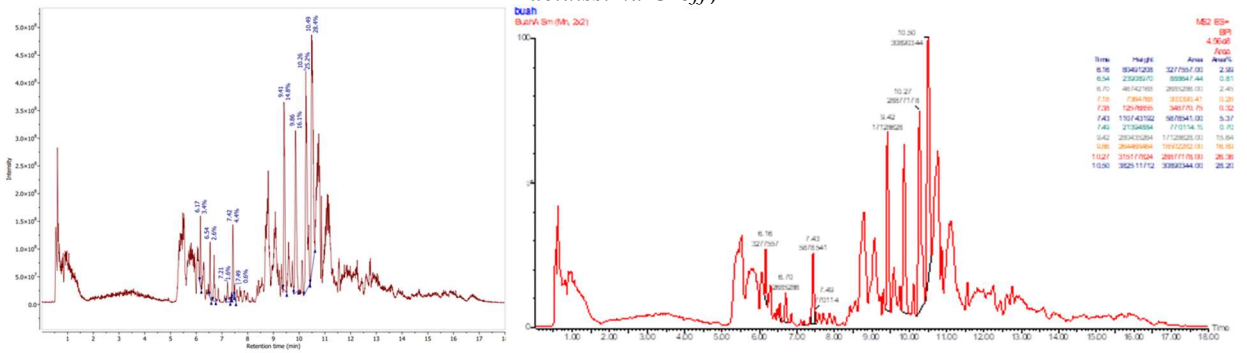

Fig.-2: Total Ion Chromatogram (TIC) in Negative Ion Mode of the Pulp Extract from Kawista Fruit (*Limonia acidissima Groff*)

Annotated Compound from Kawista Fruit Extract

The LC-MS/MS analysis of the peel extract from young Kawista fruit identified 16 distinct compounds, as shown in Table-3. These compounds were annotated based on their retention times (RT), molecular formulas, and fragment ion masses. Among the bioactive compounds identified, Peonidin-3-glucoside and Petunidin-3-glucoside were notable, with respective compositions of 4.26% and 2.03%, both known for their antioxidant properties. Another significant compound was Osthole, contributing 3.84%, which has been widely studied for its anti-inflammatory and antimicrobial activities. The compound with the highest composition, 5-tetradecanoyloxolan-2-one, accounted for 31.66%, suggesting its potential role as a major bioactive constituent. Additionally, Buscopan, a compound known for its antispasmodic effects, contributed 14.68% to the peel extract. The molecular diversity observed in the peel extract highlights its potential pharmacological benefits. The combination of phenolic and nitrogen-containing compounds further supports its therapeutic value. These findings validate the traditional use of Kawista peel in herbal remedies and provide a foundation for further exploration of its bioactivity.

Table-3: Annotated Compounds from the Peel Extract of Kawista Fruit (*Limonia acidissima Groff*), Identified using LC-MS/MS and MN

No	RT	Calculated Mass (m/z)	Molecular Weight	Molecular Formula	Prediction Compund	%Composition
1	5.81	464.1311	463.41	$[C_{22}H_{23}O_{11}]^+$	Peonidin-3-glucoside	4.26
2	5.95	361.2170	359.46	$C_{21}H_{29}NO_4$	3,5-dihydroxy-4-(1,3,6-trimethyl-2-propyl-	3.90

					4a,5,6,7,8,8a-hexahydro-2h-naphthalene-1-carbonyl)pyrrol-2-one	
3	6.29	480.1266	479.41	$[C_{22}H_{23}O_{12}]^+$	Petunidin 3-glucoside	2.03
4	6.45	412.2104	411.49	$C_{24}H_{29}NO_5$	6,7-bis(3,4-dimethoxyphenyl)-2,3,5,8-tetrahydro-1h-indolizin-8a-ol	1.23
5	6.68	344.1131	343.33	$C_{18}H_{17}NO_6$	(2s)-2-amino-3-(4-{[(2e)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}phenyl)propanoic acid	4.46
6	6.88	257.0800	256.25	$C_{15}H_{12}O_4$	Pinocembrine	3.86
7	6.93	390.1340	389.40	$C_{23}H_{19}NO_5$	1-{24-methyl-5,7,18,20-tetraoxa-24-azahexacyclo[11.11.0.0^{2},^{10}.0^{4},^{8}.0^{14},^{22}.0^{17},^{21}]tetracosa-1(13),2,4(8),9,11,14(22),15,17(21)-octaen-23-yl}propan-2-one	4.14
8	7.01	220.1333	219.28	$C_{13}H_{17}NO_2$	2-(1H-indol-3-yl)-3-methylbutane-1,3-diol	1.12
9	7.29	242.1180	241.29	$C_{15}H_{15}NO_2$	N-[2-(4-hydroxyphenyl)ethyl]benzenecarboximidic acid	4.87
10	8.09	245.1108	244.29	$C_{15}H_{16}O_3$	Osthole	3.84
11	8.31	256.0977	255.27	$C_{15}H_{13}NO_3$	1-hydroxy-3-methoxy-10-methylacridin-9-one	4.84
12	8.41	253.1055	252.26	$C_{13}H_{16}O_5$	6-(2,4-dihydroxy-5-methylphenyl)-6-oxohexanoic acid	4.29
13	9.00	297.2422	296.45	$C_{18}H_{32}O_3$	5-tetradecanoyloxolan-2-one"	31.66
14	9.70	304.1544	303.35	$C_{17}H_{21}NO_4$	Buscopan	14.68
15	9.86	252.2690	251.45	$C_{17}H_{33}N$	(2s,5s)-2-butyl-5-(non-8-en-1-yl)pyrrolidine	6.02
16	10.20	342.1775	341.40	$C_{20}H_{23}NO_4$	(9s)-4,15,16-trimethoxy-10-methyl-10-azatetracyclo[7.7.1.0^{2},^{7}.0^{13},^{17}]heptadeca-1(16),2,4,6,13(17),14-hexaen-3-ol	4.8

Table-4: The Annotated Compounds from the Pulp Extract of Kawista Fruit (*Limonia acidissima Groff*), Identified Through LC-MS/MS and MN

No	RT	Calculated Mass (m/z)	Molecular Weight	Molecular Formula	Prediction Compund	%Composition
1	6.17	521.0901	520.40	$C_{23}H_{20}O_{14}$	3,4,5-trihydroxy-6-{[9-hydroxy-6-(3-hydroxy-4-methoxyphenyl)-8-oxo-2h-[1,3]dioxolo[4,5-g]chromen-7-yl]oxy}oxane-2-carboxylic acid	2.99
2	6.54	344.1130	343.33	$C_{18}H_{17}NO_6$	(2s)-2-amino-3-(4-{[(2e)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}phenyl)propanoic acid	0.81

3	6.70	266.0555	265.18	$C_9H_7N_5O_5$	3-(4-hydroxy-2-imino-6-oxo-1,5-dihydropteridin-7-yl)-2-oxopropanoic acid	2.45
4	7.21	380.1977	379.45	$C_{22}H_{25}N_3O_3$	Fumitremorgin c	0.28
5	7.36	309.1333	308.33	$C_{16}H_{20}O_6$	Monocerin	0.32
6	7.42	325.1439	324.37	$C_{20}H_{20}O_4$	Isobavachin	5.37
7	7.49	277.0881	226.23	$C_{13}H_{10}N_2O_2$	5,10-dihydrophenazine-1-carboxylic acid	0.70
8	9.41	517.1336	516.45	$C_{25}H_{24}O_{12}$	3,5-dicaffeoylquinic acid	15.64
9	9.86	520.3403	519.33	$C_{26}H_{50}NO_7P$	1-(9Z,12Z-Octadecadienoyl-2-hydroxy-sn-glycero-3-phosphocholine	16.89
10	10.26	496.3401	495.63	$C_{24}H_{50}NO_7P$	2-{[(2r)-3-(hexadecanoyloxy)-2-hydroxypropyl phosphonato]oxy}ethyl)trimethylazanium	26.36
11	10.49	522.3778	521.70	$C_{26}H_{47}N_7O_4$	N-{3-[(3-{[3-({4-[(3-aminopropyl)amino]butyl}amino)propyl](hydroxy)amino}propyl)(hydroxy)amino]propyl}-2-(4-hydroxy-1h-indol-2-yl)ethanimidic acid	28.2

The LC-MS/MS profiling of the pulp extract from young Kawista fruit identified 11 bioactive compounds, as detailed in Table-4. Notable among these is 3,5-dicaffeoylquinic acid, which constituted 15.64% of the extract and is well-known for its strong antioxidant and anti-inflammatory activities. The compound with the highest abundance, 2-{[(2r)-3-(hexadecanoyloxy)-2-hydroxypropyl phosphonato]oxy}ethyl)trimethylazanium, accounted for 26.36% and may contribute to the pulp's functional properties. Another major component, 1-(9Z,12Z-Octadecadienoyl-2-hydroxy-sn-glycero-3-phosphocholine, comprised 16.89%, and its lipid-derived nature suggests a role in cellular protection and repair. Additionally, Fumitremorgin C, although present in a smaller amount (0.28%), is recognized for its antifungal properties. The diversity of phenolic, lipid-based, and nitrogenous compounds in the pulp extract suggests a broad spectrum of biological activities.

These results emphasize the pulp extract's potential as a source of natural antioxidant agents. While the composition differs from the peel, the pulp extract remains a valuable component for therapeutic applications, particularly in oxidative stress management. Further studies on the synergistic effects of these compounds could reveal additional pharmacological uses.

The use of ethanol as the extraction solvent for young Kawista fruit highlights its versatility and effectiveness in extracting a broad range of bioactive compounds, aligning with established findings on its suitability for natural product extraction.[18,19] Meeting the Indonesian Herbal Pharmacopoeia standards further reinforces the quality of the extraction process and the extracts' potential for therapeutic applications.[20] The observed antioxidant activity, while moderate compared to standard vitamin C, demonstrates the potential of these extracts in addressing oxidative stress-related conditions.

The slightly higher activity of the peel extract compared to the pulp underscores the importance of considering different plant parts in maximizing bioactive compound yields, a finding consistent with previous studies emphasizing the phenolic richness of fruit peels. The phytochemical diversity of flavonoids, alkaloids, saponins, polyphenols, and tannins suggests possible synergistic effects that could enhance the biological activity of the extracts beyond what individual compounds might achieve alone.[21] These findings validate traditional uses of Kawista fruit and offer a foundation for its development into functional foods or natural antioxidant agents. Moving forward, further studies could focus on optimizing extraction techniques and exploring the extracts' efficacy in complex biological systems to unlock their full pharmacological potential. By leveraging these insights, Kawista fruit extracts can be positioned as valuable natural resources in combating oxidative stress and associated diseases.

CONCLUSION

This study on young Kawista (*Limonia acidissima Groff*) fruit extracts has highlighted several key findings with potential pharmaceutical applications. Both peel and pulp extracts adhered to the Indonesian Herbal Pharmacopoeia standards, confirming their quality and suitability for further development. The ethanol extraction method effectively isolated a broad spectrum of bioactive compounds, as evidenced by high water-soluble and ethanol-soluble essence values, especially in the pulp extract (39% and 44%, respectively). Antioxidant activity assessments revealed moderate free radical scavenging capacity, with the peel extract (IC_{50} 41.398 ± 0.055 µg/mL) showing slightly higher activity than the pulp extract (IC_{50} 47.816 ± 0.020 µg/mL). The LC-MS/MS analysis identified 16 compounds in the peel and 11 in the pulp, including high concentrations of 5-tetradecanoyloxolan-2-one (31.66%) and phospholipid derivatives (26.36–28.2%). These findings, coupled with the presence of flavonoids, alkaloids, saponins, polyphenols, and tannins, scientifically validate the traditional therapeutic uses of Kawista fruit. While the extracts demonstrate promising antioxidant potential, further optimization of extraction techniques and formulation strategies could enhance their efficacy. These results provide a solid foundation for the phytochemical characterization and therapeutic potential of *Limonia acidissima Groff*.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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