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Research Article

Unveiling Bioactive Profiles: Comparative Analysis of Soluble and Insoluble Compounds in Katokkon Chili Landraces of South Sulawesi, Indonesia

Rahmansyah DERMAWAN*[1], Tandu RAMBA[2], Abdullah Bin ARIF[3], Kaimuddin KAİMUDDİN[4] Amir YASSI[5], Hari ISWOYO[6], Sulfikar SULFIKAR[7], Sylvia SJAM[8], Muhammad FARIED[9]

[1,4,5,6,9]Department of Agronomy, Agriculture Faculty, Hasanuddin University, Makassar 90245, Indonesia
[2]Community Development Motivator (MPM), Indonesia
[3]Research Center for Agroindustry, National Research and Innovation Agency, Indonesia
[7]Chemistry Department, Faculty of Mathematics and Natural Science, Universitas Negeri Makassar, 90224, Makassar, Indonesia
[8]Department of Plant Protection, Agriculture Faculty, Hasanuddin University, Makassar 90245, Indonesia

[1]https://orcid.org/0000-0003-4206-5673, [2]https://orcid.org/0000-0001-9281-8130, [3]https://orcid.org/0000-0002-3552-2875
[4]https://orcid.org/0000-0003-4007-4007, [5]https://orcid.org/0000-0001-6940-014X, [6]https://orcid.org/0000-0001-8994-1015
[7]https://orcid.org/0000-0002-2902-7309, [8]https://orcid.org/0000-0002-2881-1871, [9]https://orcid.org/0000-0001-6326-1724

*Corresponding author e-mail: radesya09@gmail.com

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Abstract: Katokkon chili is a type of local chili from Toraja, South Sulawesi, Indonesia, which has a unique character, both in shape and spicy taste. This research will reveal the bioactive compounds in three Katokkon chilies' landraces: Limbong Sampolo Sampolo, Leatung 1, and Leatung 2. Dissolved and undissolved bioactive compounds were identified using the gas chromatography-mass spectrometry (GC-MS) method. The findings were that these three Katokkon chili landraces had different bioactive compound compositions. However, the Limbong Sampolo Sampolo landrace has the most bioactive compounds, namely 58 types, compared to the Leatung landraces 1 (36 compounds) and 2 (32 compounds). The most soluble bioactive compound in the Limbong Sampolo Sampolo landrace is n-hexadecanoic acid, while in the Leatung 1 and 2 landraces, it is capsaicin. Then, the insoluble bioactive compounds were dominated.

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1. Introduction

Horticultural products are a crucial food source for the global population, providing a wide range of essential nutrients that are vital for maintaining human health and well-being. These products, which include fruits, vegetables, and spices, play an integral role in addressing nutritional needs and supporting food security worldwide. In addition to their nutritional significance, horticultural products hold substantial economic value, representing a dynamic market segment that benefits producers, distributors, and consumers. In Indonesia, horticultural products are a significant contributor to the agricultural economy, supporting livelihoods and driving economic growth at both local and national levels (Dewi et al., 2015).

Among the various horticultural products, chili stands out as one of the most prominent and widely consumed. Chili, belonging to the genus *Capsicum*, is an indispensable ingredient in both household and industrial food preparation, renowned for its ability to enhance flavor and provide a distinct spicy kick to dishes (Alonso-Villegas et al., 2023). The demand for chili is so significant in Indonesia that its unavailability in the market often triggers inflationary pressures, highlighting its critical role in the country's food supply chain (Nugrahapsari and Arsanti, 2019).

Chili's popularity stems from its unique and intense spiciness, a sensory characteristic that many consumers find highly appealing. The compound responsible for this spicy sensation is capsaicin, a bioactive component that not only defines chili's heat but also contributes to its health benefits. Chili is an excellent source of essential nutrients, including provitamin A, vitamins E and C, carotenoids, and various phenolic compounds such as capsaicinoids, luteolin, and quercetin, also flavonoid (Ergün, 2021). These compounds are widely recognized for their antioxidant properties and other beneficial biological activities, making chili a valuable addition to a healthy diet (Batiha et al., 2020).

Indonesia is home to a diverse range of chili species, both local and introduced, which are cultivated across the archipelago. Among these, the Katokkon chili is particularly noteworthy. Originating from the Tanah Toraja Regency in South Sulawesi, the Katokkon chili is a unique local variety known for its distinct shape, taste, and aroma. Resembling miniature bell peppers, these chilies possess a pungent, spicy, and savory flavor profile that sets them apart from other chili varieties. Due to their distinctive qualities, Katokkon chilies hold significant potential for the development of value-added products such as sauces, chili powders, and other processed ingredients, offering opportunities for business and industrial growth (Warisno and Dahana, 2010).

The spiciness of Katokkon chilies is attributed to their high capsaicin content, a compound characteristic of plants in the *Capsicum* genus. In addition to capsaicin, these chilies contain other valuable bioactive compounds, including alkaloids, carotenoids, flavonoids, and capsaicinoids, which contribute to their health-promoting properties (Basharat et al., 2021). Notably, among the *Capsicum* species, *Capsicum chinense* is recognized as the spiciest, containing the highest levels of capsaicin and related compounds (Chapa-Olivier and Mejia-Teniente, 2016).

Within Toraja, Katokkon chilies are further categorized into several landraces, each exhibiting unique morphological and biochemical traits. For instance, landraces from Limbong Sampolo Sampolo Village and Leatung Village differ in terms of leaf structure, fruit shape, and other physical characteristics. These variations reflect the rich genetic diversity of Katokkon chilies, which is a valuable resource for breeding, conservation, and agricultural development.

This research focuses on three distinct landraces of Katokkon chili to analyze the bioactive compounds present in their fruit. The findings are expected to provide critical insights for farmers, policymakers, and industry stakeholders, facilitating the sustainable development and commercialization of this unique local chili variety. By leveraging the genetic potential and unique properties of Katokkon chilies, Indonesia can further strengthen its position in the global horticultural market while preserving its agricultural heritage.

2. Material and Methods

2.1 Study location

The research was carried out from January to May 2020. Experimental materials were taken from farmers' gardens in Tao Pao Village, Toraja Regency. Bioactive compounds were analyzed at the Development Laboratory and Testing Laboratory of the Center for Agricultural Postharvest Research and Development, Bogor, Indonesia. The research used three (3) Katokkon chili landrace landraces: Limbong Sampolo Sampolo, Leatung 1, and Leatung 2. Limbong Sampolo Sampolo is a Katokkon chili landrace from Limbong Sampolo Village, while Letaung 1 and 2 come from Leatung Village.

2.2 Fruit collecting and sample preparation

The fruit sampled from each fresh Katokkon chili landrace was prepared in 50 g wet weight and cleaned of adhering dirt. The fruit flesh of the three Katokkon chili landraces was dried using the freeze-drying method. The drying aims to remove the water content from these materials. Drying using a freeze dryer starts by placing the ingredients in the freezer for 12 hours. Next, the material was freeze-dried

using a freeze dryer at -55°C for 24 hours. The ingredients resulting from the freeze dryer drying process are then floured using a mixer at a speed of 13,000 rpm until they become flour.

Figure 1. Fruit of three katokkon chili landrace.

2.3 Bioactive compounds analysis

At this stage, the content of the three landrace Katokkon chili bioactive compounds was analyzed using a GC-MS tool (Agilent Technologies 7890 B, USA). Then, the material was soaked using methanol and hexane solvents. Soaking with methanol solvent aims to determine the water-soluble content of the active ingredients in the three Katokkon chili landraces (polar compounds). Meanwhile, soaking with hexane solvent seeks to determine the active ingredient content in the three Katokkon chili landraces, which are not water-soluble (non-polar compounds) (Awotedu et al., 2020; Anwar and Lewar, 2023). The stages of the analysis process using a GC-MS tool followed the method of Maddo et al. (2021) with slight modifications. Approximately 5 g of Katokkon chili extract was soaked in methanol and hexane solvents for 24 hours. Then, 2 ml of the resulting extract was analyzed using a GC-MS tool to determine the bioactive compounds (both polar and non-polar) present in the three Katokkon landraces.

3. Results and Discussion

3.1 Limbong Sampolo landrace

A total of 58 bioactive compounds were discerned from the extraction of the chili flesh of the Katokkon landrace Limbong Sampolo (Table 1); however, only 14 of these compounds exhibited a peak area percentage exceeding 1%. The concentration of soluble compounds in the Limbong Simpulu landrace was more abundant than that of insoluble compounds. The soluble compound with a retention time of 83.098 minutes was identified as n-Hexadecanoic acid, showing an area percentage of 22.084%. The second-highest soluble bioactive compound appeared at a retention time of 98.839 minutes, with an area of 3 382 142.06 and a height of 509 472.88 resulting in an area percentage of 14.367%. Additionally, the compound capsaicin, an essential component in chili flesh, was detected with the third-highest area percentage at a retention time of 95.927 minutes, showing an area percentage of 13.998%. The insoluble compounds of 6-Hepten-3-one, and 5-hydroxy-4-methyl exhibited a significant area percentage of 76.167% and were detected at a retention time of 1.507 minutes.

Table 1. Soluble and insoluble bioactive compounds in Limbong Sampolo landrace

No	Compound	Molecular Form	Retention time (min)	Height	Area	Area (%)
			Dissolved			
1	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	83.098	248 412.73	2 450 461.62	22.084
2	9,12-Octadecadienoic acid (Z,Z)-	$C_{16}H_{32}O_2$ $C_{19}H_{34}O_2$	89.010 98.839	423 629.69 509 472.88	2 748 215.70 3 382 142.06	14.367
3	Capsaicin	$C_{18}H_{27}NO_3$	95.927	397 634.10	3 295 221.58	13.998
4	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	93.827	111 912.63	1 116 624.76	4.744
5	1,1,6-trimethyl-3-methylene-2-(3,6,9,13tetramethyl-6-ethenyl-10,14-dimethylenepentadec-4-enyl) cyclohexane	-	111.748	339 58.88	1 114 468.41	4.734
6	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	97.134	159 224.53	1 051 840.15	4.468
7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-	101.099	130,020.72	942 670.08	4.004
8	Palmitoleic acid	$C_{16}H_{30}O_2$	87.847	79 353.92	545 864.02	2.319
9	Oleic Acid	$C_{18}H_{34}O_2$	81.232	50 590.31	426 511.74	1.812
10	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	92.654	35 533.62	384 593.40	1.634
11	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	72.792	64 159.15	350 171.31	1.487
12	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	96.201	50 925.15	333 259.51	1.415
13	6-Octadecenoic acid	$C_{18}H_{34}O_2$	90.336	31 228.38	304 839.99	1.294
14	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	98.645	55 297.00	283 648.54	1.205
15	Others (44 compounds)	-				20.436
			Undissolved			
1	6-Hepten-3-one, 5-hydroxy-4-methyl-	$C_8H_{14}O_2$	1.507	13 153 583.86	26 420 287.27	76.167
2	Cyclopentane, methyl-	C_6H_{12}	1.616	6 764 521.91	6 172 592.08	17.793
3	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	98.862	76 618.79	621 288.63	1.790
4	Vitamin E	$C_{29}H_{50}O_2$	115.307	15 121.44	487 669.68	1.409
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	89.135	48 270.53	355 963.18	1.028
6	Others (14 compounds)	-				1.813

3.2 Leatung 1 landrace

A cumulative total of 36 bioactive compounds were identified from the extraction of the chili flesh belonging to the Katokkon landrace Leatung 1 (Table 2); nevertheless, merely 19 of these compounds demonstrated a peak area percentage surpassing 1%. The concentration of soluble compounds in the Leatung 1 landrace was significantly higher compared to insoluble compounds. Capsaicin, a soluble compound identified at a retention time of 98.851 minutes, exhibited the highest area percentage of 28.156%. In contrast, the Limbong Simpulu race contained n-Hexadecanoic acid as the compound with the highest area percentage. In the Leatung 1 chili landrace, n-Hexadecanoic acid had the second-highest area percentage at 17.843%, detected at a retention time of 89.117 minutes. Additionally, the compounds 6-Hepten-3-one and 5-hydroxy-4-methyl constituted an area percentage of 79.114% among the insoluble compounds.

Table 2. Soluble and insoluble bioactive compounds in Leatung 1 landrace

No	Compound	Molecular Form	Retention time (min)	Height	Area	Area (%)
			Dissolved			
1	Capsaicin	$C_{18}H_{27}NO_3$	98.851	155 922.85	1 216 874.82	28.156
2	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	89.117	112 653.40	771 082.72	17.843
3	Oleic Acid	$C_{18}H_{34}O_2$	97.134	44 134.13	326 000.95	7.543
4	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	101.111	40 455.53	294 536.91	6.814
5	Propanoic acid, butyl ester	$C_7H_{14}O_2$	27.199	79 93.35	57 677.62	4.860
		$C_7H_{14}O_2$	29.362	37 386.00	210 013.63	
6	Geranyl vinyl ether	$C_{12}H_{20}O$	87.870	27 628.22	171 951.78	3.978
7	Undec-10-ynoic acid, butyl ester	$C_{15}H_{26}O_2$	2.600	67 172.87	169 712.04	3.928
8	Methyl 12,13-tetradecadienoate	$C_{15}H_{26}O_2$	0.100	13 816.49	93 331.00	2.160
9	Pentanoic acid	$C_5H_{10}O_2$	23.686	96 35.53	87 077.63	2.016
10	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	96.831	17 947.45	86 387.21	1.999
11	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	72.804	13 464.54	73 553.44	1.701
12	n-Decanoic acid	$C_{10}H_{20}O_2$	81.604	6 784.89	65 026.55	1.504
13	15,15'-Bi-1,4,7,10,13pentaoxacyclohexadecane	$C_{22}H_{42}O_{10}$	96.218	9 193.31	60 051.34	1.388
14	2-Methoxy-3-methyl-butyric acid, methyl ester	$C_7H_{14}O_3$	2.823	25 455.66	58 928.95	1.363
15	Propanoic acid, butyl ester	$C_7H_{14}O_2$	27.199	7 993.35	57 677.62	1.335
16	9-Decenoic acid	$C_{10}H_{18}O_2$	80.220	7 895.48	51 928.04	1.202
17	3-Hydroxy-4-methoxybenzyl alcohol	$C_8H_{10}O_3$	84.368	7 645.75	50 673.67	1.171
18	Methyl 16-hydroxy-hexadecanoate	$C_{17}H_{34}O_3$	87.344	10 650.03	47 813.16	1.107
19	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$	61.354	6 345.77	47 476.14	1.098
20	Others (17 compounds)	-				8.853
			Undissolved			
1	6-Hepten-3-one, 5-hydroxy-4-methyl-	$C_8H_{14}O_2$	1.507	13 111 073.92	26 448 638.29	79.114
2	Cyclopentane, methyl-	C_6H_{12}	1.616	6 743 204.88	6 337 774.35	18.956
3	Others (11 compounds)	-				1.930

3.3 Leatung 2 landrace

The pulp of the Leatung 2 landrace of Katokkon chili contains 32 bioactive compounds, the lowest count compared to the Limbong Sampolo and other Leatung 2 landraces of Katokkon chili (Table 3). The capsaicinoid group makes up approximately 80.869% of the Leatung 2 Katokkon chili extracts, detected at a retention time of 99-103 minutes. Within this group, capsaicin accounts for 75.379%, and dihydrocapsaicin comprises 5.490%. The compounds capsaicin and dihydrocapsaicin are important compounds in chilies that affect the level of spiciness. Additionally, among the insoluble compounds, 6-Hepten-3-one, and 5-hydroxy-4-methyl represent an area percentage of 73.687%.

Table 3. Soluble and insoluble bioactive compounds in Leatung 2 landrace

No	Compound	Molecular Form	Retention time (min)	Height	Area	Area (%)
			Dissolved			
1	Capsaicin	$C_{18}H_{27}NO_3$	101.111	104 131.89	4 338 655.43	75.379
			103.016	111 740.78	3 066 625.42	
2	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	99.680	21 933.71	539 276.29	5.490
3	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	98.862	60 985.60	425 075.65	4.328
4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	89.134	47 694.69	353 311.92	3.595
5	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	2.594	53 760.87	157 136.76	1.599
6	3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17 hexaoxacyclononadecane	$C_{16}H_{32}O_8$	97.151	19 777.55	128394.33	1.307
7	2-Buten-1-ol, propanoate	$C_7H_{12}O_2$	29.351	21 154.81	126 235.54	1.285
8	Others (25 compounds)	-				7.017
			Undissolved			
1	6-Hepten-3-one, 5-hydroxy-4-methyl-	$C_8H_{14}O_2$	1.507	13 133 816.24	26 482 443.46	73.687
2	Cyclopentane, methyl-	C_6H_{12}	1.616	6 514 654.91	6 189 363.43	17.221
3	7-Oxabicyclo [4.1.0] heptane, 1-(1,3-dimethyl1,3-butadienyl)-2,2,6-trimethyl-,	$C_{15}H_{24}O$	101.655	25 159.56	914 816.62	2.542
4	Others (14 compounds)	-				6.551

3.4 Comparative bioactive profile of Katokkon Chili Landraces

The radar charts illustrated hereinafter (Figures 2 and 3) serve to visually delineate these profiles. Figure 2 elucidates the dissolved compounds that are distinctive to each Katokkon landrace, thereby exemplifying the variations in concentration that characterize their prospective applications and health advantages, as similarly depicted in Figure 3, which highlights the undissolved compounds that are unique to each Katokkon landrace.

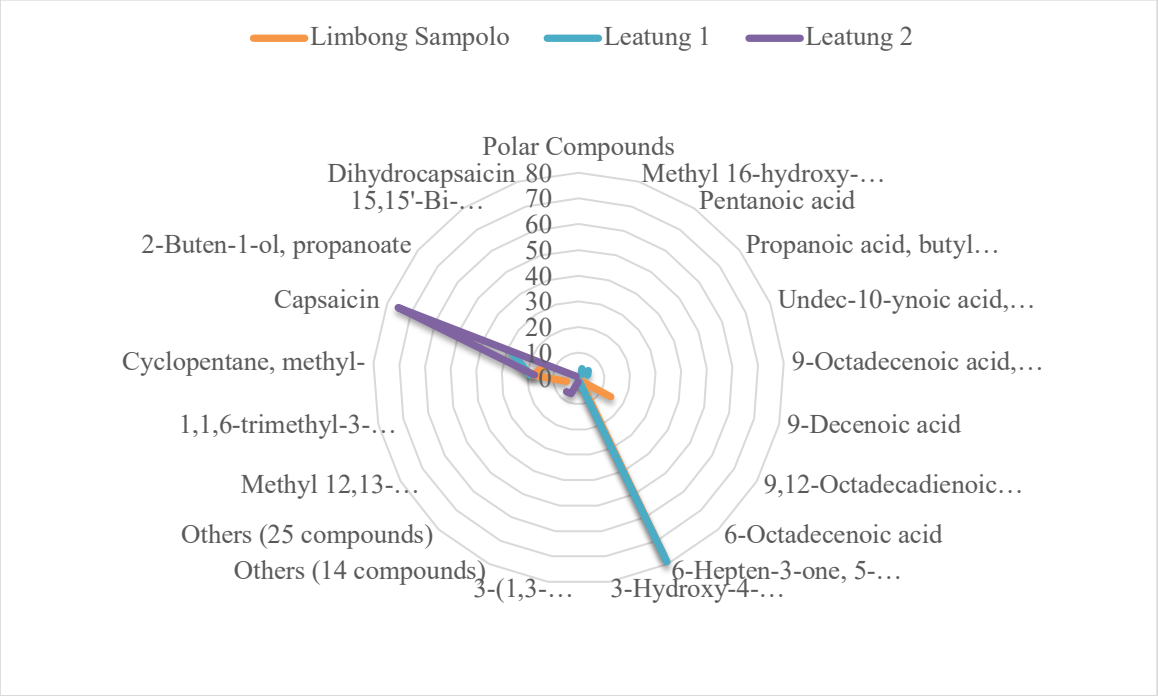

Figure 2. Radar chart for dissolved compound.

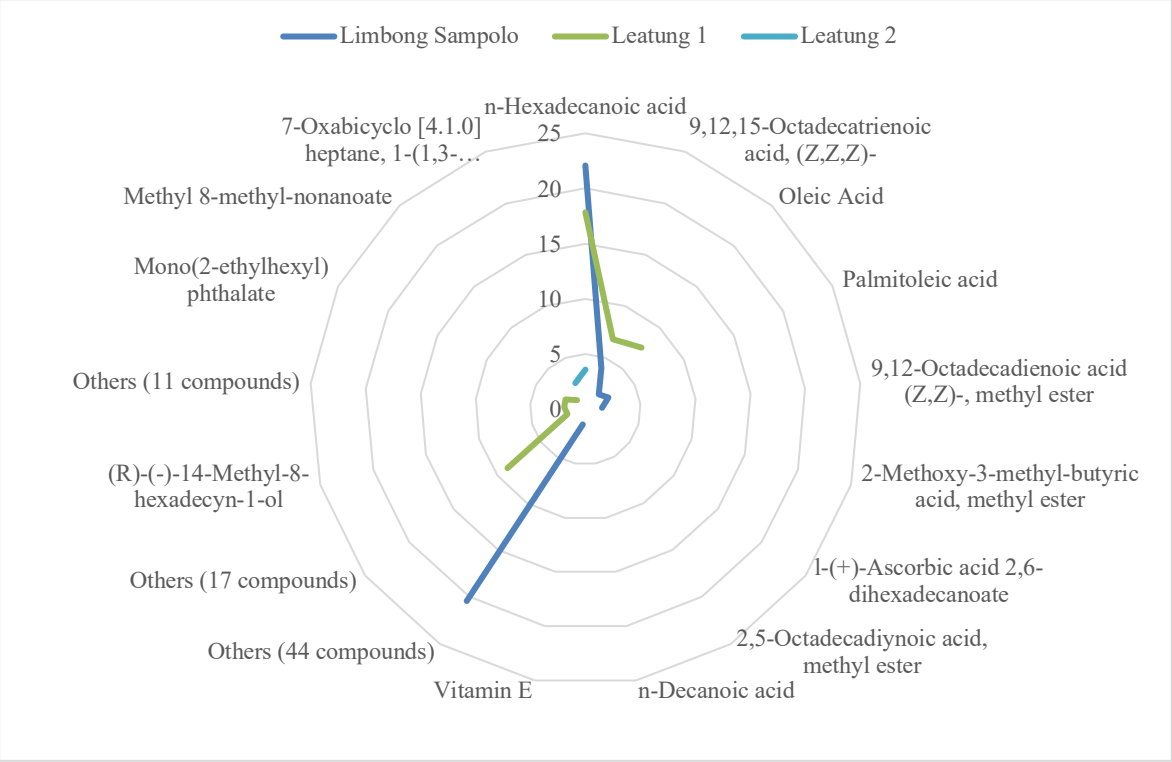

Figure 3. Radar chart for undissolved compound.

Discussion

The analysis of the bioactive compounds in the Katokkon chili landraces—Limbong Sampolo, Leatung 1, and Leatung 2—highlights their diverse chemical profiles and underscores their potential applications in health, nutrition, and industry. The Limbong Sampolo landrace was found to contain 58 bioactive compounds, with 14 exceeding 1% in peak area percentage. Among these, n-hexadecanoic acid was the most abundant. This compound, with the molecular formula $C_{16}H_{32}O_2$, this compound functions as an antioxidant, hypocholesterolemic, antiandrogenic, flavor, and nematicide hemolytic 5-alpha reductase inhibitor (Easwaran and Ramani, 2014). The compound with the second highest area percentage is 9, 12-Octadecadienoic acid. The compound 9, 12-Octadecadienoic acid is known as an omega-6 fatty acid that is important for average cell growth, for lowering cholesterol levels in the blood (Igwe and Okwu, 2013), and for supporting the quality of skin lubrication (Okwu and Morah, 2006). 9,12-Octadecadienoic acid (Linoleic acid) is known for its anti-inflammatory properties, linoleic acid is essential for heart health and skin barrier function. Studies have linked linoleic acid intake with reduced cardiovascular risks, which could enhance the culinary or nutraceutical applications of *Limbong Sampolo* extracts (Das, 2006; De Roos and Calder, 2018). 9-Octadecenoic acid (Oleic acid) is a primary component of olive oil and is beneficial for reducing inflammation and promoting cardiovascular health. It has been linked to improved blood lipid profiles and reduced risk of coronary heart disease (Poudyal and Brown, 2011; Carrillo et al., 2012). Oleic acid is also reported as an anti-inflammatory fatty acid that plays a role in activating competent immune cell pathways (Carrillo et al., 2012). Foods rich in oleic acid benefit inflammation-related diseases (Santa-Maria et al., 2023). Palmitoleic acid recognized for its anti-inflammatory and metabolic health benefits, palmitoleic acid may improve insulin sensitivity and support cardiovascular health. Its presence in Limbong Sampolo offers potential for use in skin care or wellness products (Mozaffarian and Wu, 2012; Yang et al., 2021). Vitamin E a well-known antioxidant can support immune health and protects cells from oxidative damage. Its presence in Limbong Sampolo could be advantageous for creating antioxidant-rich supplements (Traber and Stevens, 2011).

N-hexadecanoic acid, commonly known as palmitic acid, is a saturated fatty acid predominantly found in palm oil. Despite its limited nutritional benefits due to its saturated nature, palmitic acid holds significant value in the cosmetic industry, where it is widely appreciated for its moisturizing properties and its ability to improve skin hydration and texture (Raederstorff et al., 2015). Another notable compound is 9,12,15-octadecatrienoic acid, or alpha-linolenic acid, a well-known omega-3 fatty acid typically sourced from flaxseed. Renowned for its anti-inflammatory properties and its role in promoting cardiovascular health, the presence of alpha-linolenic acid in Leatung 1 highlights this chili variety's potential for nutraceutical applications. Although its concentrations in Leatung 1 are lower compared to conventional sources like flaxseed or fish oil, its inclusion underscores the diverse bioactive profile of this chili (Rodriguez-Leyva et al., 2010). Additionally, Leatung 1 contains unique esters and alcohols, such as methyl 8-methyl-nonanoate and 3-hydroxy-4-methoxybenzyl alcohol. These rare compounds are highly valued for their roles in enhancing fragrance and flavor, opening avenues for applications in food flavoring and cosmetic formulations. The distinct combination of these bioactive compounds not only enriches the functional profile of Leatung 1 but also positions it as a versatile ingredient for multiple industries.

Leatung 2 stands out due to its high concentration of capsaicin, a bioactive compound renowned for its diverse therapeutic properties. Capsaicin is widely recognized for its pain-relief capabilities, making it a key ingredient in topical creams designed to alleviate discomfort from conditions such as arthritis and neuropathy. Additionally, it exhibits potent anti-tumor, antioxidant, analgesic, alpha-amylase inhibitor, and anti-fungal. These well-documented benefits make Leatung 2 a promising candidate for use in both pharmaceutical and nutraceutical applications, including metabolic health supplements and anti-inflammatory products (Surh, 2002; Reyes-Escogido et al., 2011; Maokam et al., 2014; Levono and Prasad, 2017). Capsaicin is also an anti-microbial that plays a positive role in several mechanisms to kill microbes, like pepper extract can be used to inhibit bacteria growth (Taolin, 2019; Ergün et al., 2024). Complementing capsaicin is the presence of 2,5-octadecadiynoic acid, methyl ester, a compound with emerging potential as an anti-inflammatory agent (Alrabie et al., 2023). Although research on this compound is still in its early stages, its inclusion in Leatung 2 suggests synergistic possibilities when combined with capsaicin, particularly in the formulation of advanced anti-

inflammatory products. Together, these bioactive compounds highlight the unique therapeutic potential of Leatung 2, making it a valuable resource for the development of innovative health and wellness solutions. Leatung 1, Leatung 2, and Limbung Sampolo exhibit distinct biochemical characteristics, reflecting the genetic diversity among these genotypes. Similar findings were reported by Öntürk and Çürük (2019), who observed variations in growth and pungency levels across different local chili pepper populations. This highlights that both biochemical and morphological diversity are common in chili populations originating from different regions.

Conclusion

In conclusion, the study of these Katokkon chili landraces underscores their value as sources of bioactive compounds with diverse and significant applications. The presence of antioxidants, fatty acids, and capsaicinoids across the landraces highlights their potential to address various health challenges, from oxidative stress and inflammation to microbial infections and cancer. Moreover, the distinct chemical profiles of each landrace provide opportunities for targeted applications in food, pharmaceuticals, and nutraceuticals. Future research should explore the bioavailability and synergistic interactions of these compounds to fully harness their therapeutic potential. Additionally, the sustainable cultivation and selective breeding of these landraces could enhance their bioactive content, ensuring their continued relevance in modern health and industry applications. This study exemplifies the intersection of traditional agricultural practices and scientific innovation, offering a roadmap for leveraging indigenous crops in global markets.

Ethical Statement

This study was conducted ethically, adhering to all relevant guidelines and regulations, with no involvement of human participants, animal testing, or endangered plant species.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

Each author made an equal contribution to the article.

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