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Analysis of the Morphology and Secondary Metabolite Content of Several Katuk (*Sauropus androgynus*) Accessions

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Abstract

Katuk (*Sauropus androgynus*) is a species valued for its nutritional and medicinal properties, as well as its potential applications in food, herbal medicine, livestock feed, and cosmetics. This study aimed to evaluate the morphological diversity and secondary metabolite profiles of four katuk accessions. Two analyses were conducted: (1) morphological characterization using cluster analysis and (2) qualitative and quantitative assessment of secondary metabolites. Cluster analysis revealed that all accessions showed high similarity with the “Paris”, an accession widely grown in West Java, Indonesia (similarity coefficient = 1.00), and moderate similarity with accessions “K1”, “K2”, “K4”, “Bastar”, and “Zanzibar” (distance coefficient = 0.68). Metabolite profiling indicated variations in compound abundance among accessions. “Ciaruteun Ilir 1” exhibited the highest vitamin E content; “Ciaruteun Ilir 2” had the highest fatty acid and phenolic content; “Pager Jangkung 1” accumulated the most flavonoids, while “Pager Jangkung 2” contained the most terpenoids and carboxylic acids. The leaves of katuk accessions in this study contains 14-17% squalene; this is an important finding for the pharmaceutical and health industries.

Keywords: “Ciaruteun Ilir 2”, katuk, “Pager Jangkung 2”, secondary metabolites

Introduction

Katuk (*S. androgynus*) is a perennial leafy vegetable extensively utilized in Indonesia as food and a source of biopharmaceutical raw materials due to its rich nutrient and metabolite profile. Katuk leaves contain proteins, crude fibre, vitamins A, B, C, and K, as well as β-carotene and essential minerals, surpassing spinach in fibre and vitamin C content (Ministry of Health, 2020; Dolang et al., 2021). They are also reported to enhance lactation, support immune and bone health, and prevent anaemia, cancer, and digestive disorders (Saras, 2023; Fransisca, 2021). Phytochemical analyses reveal the presence of tannins, flavonoids, alkaloids, saponins, and terpenoids/steroids (Fadilah et al., 2022), while ethanol extracts show strong antioxidant activity with an IC_{50} of 55.83 ppm (Usman et al., 2024). Such bioactivities highlight katuk’s potential for nutritional, pharmaceutical, and cosmetic applications.

Katuk exhibits significant morphological and chemical diversity across Indonesia, influenced by local environmental conditions. According to HRDC (2014), diversity sources include cultivated fields, home gardens, markets, and wild populations. Current katuk germplasm exists as accessions, as no official varieties have been released (MoA RI). Phenotypic variation, especially in leaf morphology, has been documented among accessions “K1”, “K2”, and “K4” from Ciaruteun Ilir, Bogor (Safha, 2021), which show morphological similarities to previously described accessions “Bastar”, “Paris”, and “Zanzibar” (Maslahah et al., 2005). Since accession-specific variation affects metabolite composition (Santana et al., 2021), detailed profiling using Gas Chromatography–Mass Spectrometry (GC–MS) is essential for characterizing phytochemical diversity across katuk germplasm.

Materials and Methods

The experiment was conducted at Cikabayan Atas Experimental Farm, Jakarta Regional Health Laboratory, Ecotoxicology Laboratory, Testing

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Laboratory, and Post-Harvest Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University. The experiment was conducted from June to November 2023.

The materials used in this experiment include katuk plants in the form of cuttings with four accessions: “Ciaruteun Ilir 1”, “Ciaruteun Ilir 2”, “Pager Jangkung 1”, and “Pager Jangkung 2”, growth regulators made from auxins (Rootone-F®), agricultural lime in the form of dolomite, manure, Urea (45% N), SP-36 (36% P_2O_5), KCl (60% K_2O). Bio fungicide with active ingredient *Trichoderma hamatum* SY-03 (Tricho Wish®), biofertilizer with active ingredients *Bacillus polymixa* and *Pseudomonas fluorescens* (RhizomaX®), and insecticide with active ingredient carbofuran.

Experiment 1. Analysis of Morphological Characteristics of Katuk Accessions

This experiment was conducted using the cluster analysis method (clustering), a method of grouping data or objects based on their characteristics, so that they form a group with distinct characteristics from other groups (Sidiq and Manaf, 2020). Ahyati et al. (2023) noted the relevance of character similarity analysis, which can serve as a reference for grouping data based on relatively similar (homogeneous) characteristics. A high similarity coefficient value characterizes close kinship and results in narrow genetic diversity. On the other hand, a distant relationship that produces a wide diversity is obtained when the similarity coefficient value is low. Plant morphological observation data are presented in the form of scores, then grouped on matrix data (Cluster Analysis) and continued with the creation of dendrograms using Unweighted Pair Group Method Arithmetic Average (UPGMA) through the Multi-Variate Statistical Package (MVSP) version 3.22 program.

Cluster analysis was conducted using qualitative data, namely the presence of spots, the proportion of spots, and the shape of the leaf tip. Qualitative characters were scored, including the shape of the leaf tip (1= pointed, 2= tapered, 3= rounded), spots on the leaf surface (1= spread, 2= in the center), and the proportion of spots based on the percentage of spots and qualitative observations. The spot proportion was classified into three categories: little (<25% of the leaf area), medium (25–50%), and many (>50%) (Safha, 2021). Characteristics were compared across 12 accessions of katuk from three studies, namely four accessions with the names “Bastar”, “Kebo”, “Paris”, and “Zanzibar” (Maslahah et al., 2005); four accessions with the names “K1”, “K2”, “K3”, and “K4” (Safha 2021); and four accessions with the names “Ciaruteun Ilir 1”, “Pager Jangkung 1”, “Ciaruteun Ilir 2”, and “Pager Jangkung 2”

Growth measurements were conducted during the first harvest period (10 weeks after transplanting, WAT) by taking five leaf samples in each replication, resulting in 20 leaves per accession. The morphological characteristics, including leaf shape, shape of leaf tips, and leaf base, were described in Figures 1, 2, and 3 based on Tjitrosoepomo (2007).

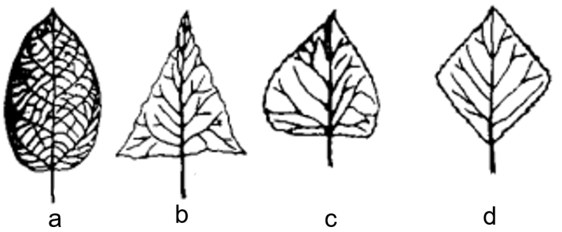

Figure 1. Leaf shapes: (a) ovoid; (b) triangular; (c) delta; (d) rhombic.

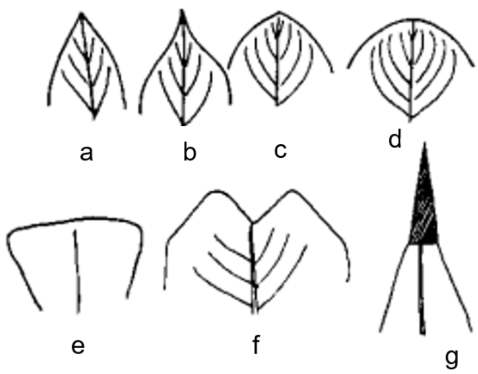

Figure 2. Leaf tip shapes: (a) pointed; (b) tapered; (c) blunt; (d) rounded; (e) vine; (f) split; (g) spiny.

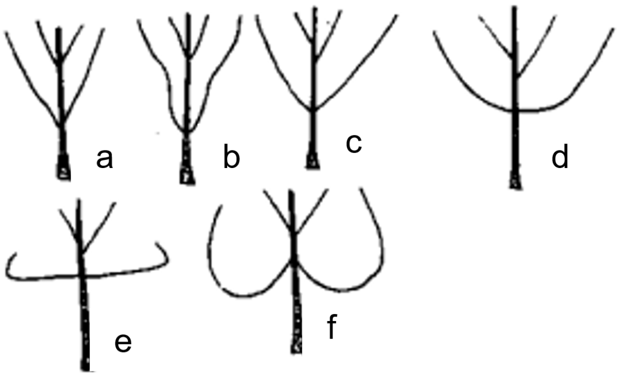

Figure 3. Leaf base shapes: (a) pointed; (b) tapered; (c) blunt; (d) rounded; (e) flat; (f) notched.

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Leaf color variables were measured using the Leaf Color Chart (BWD), then grouped into three scales: 2-3 (light green), 4 (green), and 5 (dark green)

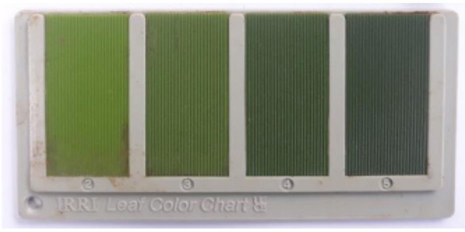

Figure 4. Leaf colors: (1) 2-3= light green; (2) 4= green; (3) 5= dark green.

The variable of spot location on the leaf surface was recorded based on the method by Maslahah et al. (2005)

(1) (2)

Figure 5. Spot locations: (1) diffused; (2) centered.

Experiment 2. Analysis of Secondary Metabolite Compound Content of Four Katuk Accessions Based on Morphological Characteristics

Katuk leaf samples were oven-dried at 60°C for 24 hours, ground into a fine powder, and macerated in methanol (p.a.) for 5 days. A 10 mL aliquot of the extract was pipetted into a test tube and dried at 60°C for 1 hour. The residue was re-dissolved in 200 μL of extract and injected into the GC–MSD. Each sample was repeated ten times.

GC–MSD conditions were set as follows: oven program—initial temperature 80°C (0 min hold), ramped at 3°C per min to 150°C (1 min hold), then at 20°C per min to 280°C (26 min hold); carrier gas—helium at 1.2 mL per min (constant flow); injection port temperature, 250°C; ion source, 230°C; interface, 280°C; quadrupole, 140°C; ionization mode, electron impact (70 eV); injection volume, 5 μL; split ratio, 8:1. Compound identification was confirmed using PubChem, NIST Chemistry WebBook, and KEGG Pathway Database to determine molecular identity, class, and biological function.

Total phenolic and flavonoid contents were determined following the method of Vongsak et al. (2013) leaf samples were washed, oven-dried at 60°C for 24 h, pulverized, and sieved (20 mesh). Powdered samples were macerated in 70% ethanol for 72 hours at 28±2°C with intermittent shaking. The combined filtrates were used for quantitative analysis.

Phenolic content was determined via the Folin–Ciocalteu method. A 200 μL sample (1000 $\mu g.mL^{-1}$) was mixed with 500 μL Folin–Ciocalteu reagent (1:10 dilution) and 800 μL sodium bicarbonate (7.5% w/v). The mixture was incubated for 30 minutes at room temperature with intermittent shaking, and the absorbance was read at 765 nm using a UV–VIS spectrophotometer (PerkinElmer, USA). Results were expressed as grams of chlorogenic acid equivalent (CAE) per 100 g dry extract (mean ± SD, n= 3).

Flavonoid content was determined colorimetrically: 500 μL samples were mixed with 500 μL of 2% $AlCl_3$ solution and incubated for 10 min at room temperature. Absorbance was measured at 415 nm against a blank without $AlCl_3$. Results were expressed as grams of isoquercetin equivalent (IQE) per 100 g dry extract (mean ± SD, n= 3)

Results and Discussion

Experiment 1. Analysis of Morphological Characteristics of Katuk Accessions

The leaf morphology of the katuk accessions is described in Table 1, while the results of the clustering analysis are presented in Figure 6.

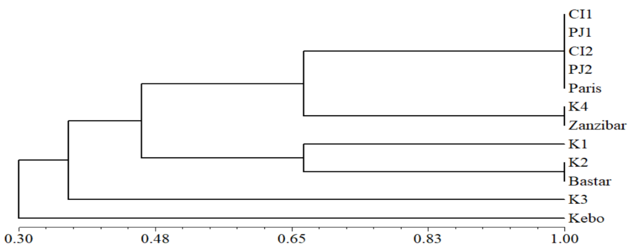

Figure 6. Dendrogram of 12 katuk accessions. 4 accessions based on Maslahah et al. (2005), namely accessions “Bastar”, “Kebo”, “Paris”, and “Zanzibar”, 4 accessions based on Safha’s research (2021), namely accessions “K1”, “K2”, “K3”, and “K4”, and 4 accessions in the current research, namely accessions “Ciaruteun Ilir 1” (CI 1), “Ciaruteun Ilir 2” (CI 2), “Pager Jangkung 1” (PJ 1), and “Pager Jangkung 2” (PJ 2).

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Table 1. Leaf morphological characteristics of 12 katuk accessions

Accession	Spot presence	Spot percentage (%)	Spot abundance	Leaf length (cm)	Leaf width (cm)	Leaf shape	Leaf tip shapes	Leaf base shapes	Leaf color	References
“Bastar”	Scattered	30-75	Many	3.56-5.45	2.21-2.70	-	Pointed	Blunt slightly pointed	Light green -dark green	Maslahah et al. (2005)
“Kebo”	None	0.00	None	4.83-5.70	2.11-2.30	-	Pointed	Pointed	Light green - dark green	
“Paris”	Scattered	0-10	Little	3.25-4.15	2.00-2.15	-	Pointed	Blunt slightly pointed	Light green - dark green	
“Zanzibar”	In the center	10-25	Little	4.56-5.75	2.00-2.40	-	Pointed	Blunt slightly pointed	Light green - dark green	
“K1”	Scattered	19.42	Little	5.71	2.70	Ovate	Tapered	Rounded	Dark green	Safha (2021)
“K2”	Scattered	13.01	Little	5.41	2.49	Ovate	Pointed	Rounded	Dark green	
“K3”	In the center	5.03	Little	4.59	2.34	Ovate	Pointed	Rounded	Green	
“K4”	In the center	10.96	Little	4.96	1.19	Ovate	Pointed	Rounded	Green	
“Ciaruteun Ilir 1”	In the center	7.91	Little	4.60	2.26	Ovate	Pointed	Rounded	Green	The current study
“Ciaruteun Ilir 2”	In the center	8.70	Little	4.31	2.29	Ovate	Pointed	Rounded	Light green	
“Pager Jangkung 1”	In the center	9.67	Little	4.73	4.50	Ovate	Pointed	Rounded	Dark green	
“Pager Jangkung 2”	In the center	3.23	Little	3.86	1.12	Ovate	Pointed	Rounded	Light green	

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Dendrogram analysis in Figure 6 indicates that, qualitatively, the four accessions observed exhibit the highest similarity with the “Paris” accession, with a coefficient of 1.00. Additionally, there is a similarity between the four accessions observed and other accessions, such as “K1”, “K2”, “K4”, “Bastar”, and “Zanzibar”, with a distance coefficient of 0.68. This finding aligns with the results of Safha’s research (2021), where accessions with the highest similarity are “K2” and “Bastar”, and “K4” and “Zanzibar”, both with a similarity coefficient of 1.00. Accessions within the same group exhibit high similarity.

Experiment 2. Analysis of Secondary Metabolite Compound Content of Four Katuk Accession Based on Morphological Characteristics

GC-MS analysis revealed the presence of various compounds in four samples of katuk leaf accessions (Table 2). These compounds are classified into two categories, namely general and specific compounds. General compounds are compounds that are contained in all accessions. There are 10 of the same compounds found in all accessions.

Among the 16 bioactive compounds identified in the four katuk accessions examined, there are specific compounds that are produced only by certain accessions. The compound Stigmasta-5,24(28)-dien-3-ol, (3. beta, 24Z)- was only produced by accession “Ciaruteun Ilir 1”, the compound 4-Fluoro-N-(2-hydroxy-5-methylphenyl) b-enzamide, ITMS derivative was only made by “Pager Jangkung 1”, and the specific compound 9,12-Octadecadienoic acid (Z, Z)- was only found in accession “Ciaruteun Ilir 2”. Octadecanoic acid, and ethyl ester compounds were only produced by accessions “Pager Jangkung 1” and “Ciaruteun Ilir 2”. The compound 9,12,15-Octadecatrienoic acid, 2,3-dihydroxy propyl ester, (Z, Z, Z)- was only produced by accessions

Table 2. Characterization and abundance of secondary metabolite compounds in katuk leaves by GC-MS

Compounds	Formula	Abundance (%)			
		CI1	CI2	PJ1	PJ2
Terpenoid group					
- Neophytadiene	$C_{20}H_{38}$	10.22	10.53	9.65	13.06
- Phytol	$C_{20}H_{40}O$	8.80	5.98	5.89	5.56
- Squalene	$C_{30}H_{50}$	17.16	14.18	15.45	14.27
- 3,7,11,15-Tetramethylhexadec-2-ene	$C_{20}H_{40}$	1.97	2.28	1.87	2.75
9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	$C_{20}H_{34}O_2$	14.94	17.75	18.02	19.06
Fatty acid group					
- Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	6.16	6.55	7.08	4.89
- Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	3.61	3.92	3.71	3.01
Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	-	1.37	2.62	-
- 9,12-Octadecatrienoic acid (Z, Z)-	$C_{18}H_{30}O_2$	-	2.12		-
- 9,12,15-Octadecatrienoic acid, - 2,3-dihydroxypropyl ester, (Z, Z, Z)-	$C_{21}H_{36}O_4$	11.01	10.47	-	13.67
Carboxylic acid group					
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	1.63	1.52	1.36	1.84
Vitamin E group					
- Beta-tocopherol	$C_{28}H_{48}O_2$	1.12	1.11	1.15	1.09
- Gamma-tocopherol	$C_{28}H_{48}O_2$	1.11	1.35	1.08	-
Vitamin E	$C_{12}H_7Cl_3O_2$	14.40	13.23	12.83	11.70
Steroid group					
Stigmasta-5,24 (28)-dien-3-ol, (3. beta.,24Z)-	$C_{29}H_{48}O$	1.13	-	-	-
Flavonoid group					
4-Fluoro-N-(2-hydroxy-5-methylphenyl) b enzamide, 1TMS derivative	$C_{14}H_{12}FNO_2$	-	-	1.25	-

Notes: CI1: “Ciaruteun Ilir 1”, CI2: “Ciaruteun Ilir 2”, PJ1: "Pager Jagkung 1", PJ2: “Pager Jangkung 2”.

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"Ciaruteun Ilir 1", "Ciaruteun Ilir 2", and "Pager Jangkung 2". Gamma-tocopherol is only found in accessions "Ciaruteun Ilir 1", "Pager Jangkung 1", and "Ciaruteun Ilir 2" (Table 3).

According to Syahadat and Siregar (2020), katuk leaves contain a diverse array of phytochemical constituents, including alkaloids, tannins, flavonoids, saponins, and triterpenoids. Among the observed accessions, the terpenoid content followed the descending order of "Pager Jangkung 2" > "Ciaruteun Ilir 2" > "Pager Jangkung 1" > "Ciaruteun Ilir 1". Terpenoid compounds identified included neophytadiene, phytol, squalene, 3,7,11,15-tetramethylhexadec-2-ene, and 9,12,15-octadecatrienoic acid ethyl ester (Z, Z, Z)-. The highest total abundance of terpenoids (54.74%) was recorded in "Pager Jangkung 2". These compounds exhibit significant biological activities, acting as anti-inflammatory, antibacterial, antifungal, and antiamnesic agents, which are widely utilized in medicine.

Fatty acid constituents comprised hexadecanoic acid ethyl ester, linoleic acid ethyl ester, octadecanoic acid ethyl ester, 9,12-octadecatrienoic acid (Z, Z)-, and 9,12,15-octadecatrienoic acid 2,3-dihydroxypropyl ester (Z, Z, Z)-. The total fatty acid abundance ranked as "Ciaruteun Ilir 2" > "Pager Jangkung 2" > "Ciaruteun Ilir 1" > "Pager Jangkung 1". These fatty acids contribute to anti-inflammatory, antimicrobial, and antioxidant defenses, aid in cardiovascular health, and suppress tumor progression. Supporting this, Kusuma and Putri (2020) reported that fatty acids demonstrate antibacterial and antifungal properties.

Carboxylic acids were represented by hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, showing relative abundances of "Pager Jangkung 2" > "Ciaruteun Ilir 1" > "Ciaruteun Ilir 2" > "Pager Jangkung 1", with "Pager Jangkung 2" exhibiting the highest total (1.84%). These compounds serve cosmetic functions as pH regulators, exfoliants, moisturizers, flavouring agents, and antioxidants. Vitamin E derivatives, identified as β-tocopherol and tocopherol, exhibited decreasing abundance from "Ciaruteun Ilir 1" > "Ciaruteun Ilir 2" > "Pager Jangkung 1" > "Pager Jangkung 2". The highest concentration (16.63%) occurred in "Ciaruteun Ilir 1". Basuki and Devitasari (2022) noted that vitamin E compounds act as antioxidants, maintain skin hydration, provide photoprotection, and accelerate wound healing.

Flavonoids were present in all accessions, decreasing in abundance as "Pager Jangkung 1" > "Ciaruteun Ilir 2" > "Pager Jangkung 2" > "Ciaruteun Ilir 1". "Pager Jangkung 1" recorded the highest total flavonoid content (3.93%). These compounds function as potent antioxidants, mitigating oxidative stress by neutralizing free radicals. Ullah et al. (2020) further highlighted the broad pharmacological potential of their compound, including anticancer, antimicrobial, antiviral, antiangiogenic, and antiproliferative activities.

Phenolic compounds were most abundant in "Ciaruteun Ilir 2" (8.26%), followed by "Pager Jangkung 1" > "Pager Jangkung 2" > "Ciaruteun Ilir 1". As reported by Majid et al. (2023), phenolics possess a strong antioxidant capacity, which is beneficial for managing degenerative diseases, including diabetes, hepatic injury, inflammation, cancer, cardiovascular disorders, and neurodegenerative disorders, as well as the aging process.

The specific compound found in accession "Ciaruteun Ilir 1" is (3β,24Z)-stigmasta-5,24(28)-dien-3-ol, a compound belonging to the steroid group that is present in abundance at 1.13%. The only compound found in accession "Pager Jangkung 1" is 4-fluoro-N-(2-hydroxy-5-methylphenyl with an abundance of 1.25%. The specific compound in accession "Ciaruteun Ilir 2" is 9,12-Octadecadienoic acid (Z,Z), a component of the fatty acid group, with an abundance of 2.12%. The other content found in katuk leaves, which has a specific function, is the squalene compound. Squalene serves as an important biochemical precursor in the biosynthesis of sterols,

Table 3. Abundance (%) of secondary metabolite compounds in katuk leaves

Compounds (%)	"Ciaruteun Ilir 1"	"Ciaruteun Ilir 2"	"Pager Jangkung 1"	"Pager Jangkung 2"
Terpenoid*	53.09	50.72	50.88	54.70
Fatty acids*	20.78	24.43	13.41	21.57
Carboxylic acid*	1.63	1.52	1.36	1.84
Vitamin E*	16.63	15.69	15.06	12.79
Steroid*	1.13	0.00	0.00	0.00
Total flavonoids*	2.56	3.50	3.93	2.84
Total phenol+	7.56	8.26	7.72	7.70

Notes: *: compounds analyzed using GC-MS method, +: compounds analyzed using Vongsak method.

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including cholesterol and steroid hormones. Selvi and Basker (2012) have researched katuk leaves using gas chromatography-mass spectrometry (GC-MS) analysis, with results indicating that the ethanol extract of katuk leaves contains phytol and squalene. Squalene functions as an antioxidant, anticancer agent, and auxiliary material for vaccines and drug carriers, playing a significant role in skin care products (Suhendra et al., 2021). The highest squalene content of the four accessions observed was “Ciaruteun Ilir 1” at 17.16%. The squalene content is higher than that in olive oil, which is only 0.7% (Tiastuti, 2018). However, the results of the squalene content in katuk are still below the squalene value contained in bidara (*Ziziphus mauritiana*) leaves, which is 27.17% (Badai et al., 2022), and the squalene content in agarwood plants, which is as high as 98% (Jayuska et al., 2015). Santoso (2021) mentioned that squalene has several benefits for human health, including serving as a hepatitis C vaccine adjuvant and lowering cholesterol levels (Santoso, 2021). Squalene neutralizes reactive oxygen species generated by UV exposure, preventing lipid peroxidation and premature aging of the skin; this gives it a significant role in sunscreens, serums, and anti-aging products. Additionally, squalenoylation is employed in cancer chemotherapy and widely used in the beauty industry to protect the skin. Therefore, our study, which shows that katuk leaves contain squalene, is an important finding because it shows that squalene can be sourced from plants instead of sharks, ensuring renewable and ethical use in therapeutic and cosmetic applications.

Conclusions

The katuk accessions “Ciaruteun Ilir 1”, “Ciaruteun Ilir 2”, “Pager Jangkung 1”, and “Pager Jangkung 2” exhibited a high similarity to the widely grown “Paris” accession, with a similarity coefficient of 1.00. The second cluster, consisting of accessions “K1”, “K2”, and “Bastar”, showed a similarity coefficient of 0.67. Despite their genetic relatedness, the accessions displayed variation in secondary metabolite composition. “Ciaruteun Ilir 1” produced the highest vitamin E content (16.63%) and contained a unique steroid compound (1.13%). “Ciaruteun Ilir 2” recorded the highest fatty acid concentration (25.95%), while “Pager Jangkung 1” exhibited the highest total flavonoid content (3.39%). Meanwhile, “Pager Jangkung 2” had the highest terpenoid content of 54.70%. Based on the chemical compound analysis, the katuk accessions suitable for cultivation as cosmetic ingredients are "Ciaruteun Ilir 1" and "Pager Jangkung 1".

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