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Short Communication: Phytochemistry and chemical fingerprint of *katuk* (*Sauropus androgynus*) leaves extract from four varieties

FACHRUDDIN[1], AGIK SUPRAYOGI[2,♥], NOVRIYANDI HANIF[3,4]

[1]Department of Biology, Faculty of Science and Technology, Institut Teknologi dan Kesehatan Avicenna. Jl. Y. Wayong By-Pass, Lepo-Lepo, Kendari 93116, Southeast Sulawesi, Indonesia

[2]Division of Physiology, School of Veterinary Medicine and Biomedical Sciences, Institut Pertanian Bogor. Jl. Agatis, Darmaga Campus, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8629462, ♥email: agiksu@apps.ipb.ac.id

[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Darmaga Campus, Bogor 16680, West Java, Indonesia

[4]Tropical Biopharmaca Research Center, Institut Pertanian Bogor. Jl. Taman Kencana No. 3, Bogor 16128, West Java, Indonesia

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Abstract. *Fachruddin, Suprayogi A, Hanif N. 2025. Short Communication: Phytochemistry and chemical fingerprint of* katuk *(*Sauropus androgynus*) leaves extract from four varieties. Biodiversitas 26: 824-830. Katuk* (*Sauropus androgynus*) is a medicinal plant known to consist of four varieties based on the presence and distribution of white spots on the leaf surface. These characteristic differences affect the content of active compounds and their biological activities. This study aimed to determine the phytochemical content of crude ethanol extracts and to analyze the chemical fingerprints of the n-hexane fractions from the leaves of four *S. androgynus* varieties. The extraction process was conducted using ethanol to obtain the crude extract, followed by partitioning with n-hexane to separate the non-polar fractions. Qualitative phytochemical tests were performed on the crude ethanol extract, while chemical fingerprint analysis was carried out on the n-hexane fraction using LC-MS instruments. The results showed that the crude ethanol extract contained flavonoids, saponins, tannins, and steroids. LC-MS analysis revealed that all *S. androgynus* varieties shared similarities in their chemical fingerprint patterns, particularly the Zanzibar-Bastar varieties (six peaks on the chromatogram had the same retention times). The major compound groups found in the n-hexane fractions included triterpenoid glycosides (Zanzibar), diterpenoids (Bastar and Paris), and steroids (Kebo). These similar fingerprints and slight variations in chemical composition provide insights that are likely to produce subtly different pharmacological effects, thereby sparking further intrigue and eagerness for future research in the field of medicinal plant studies.

Keywords: Chemical fingerprint, *katuk* variety, LC-MS, phytochemistry, *Sauropus androgynus*

INTRODUCTION

Katuk (*Sauropus androgynus* (L). Merr. is a significant perennial shrub with considerable nutraceutical and pharmaceutical potential (Swain et al. 2024). This leafy vegetable contains nutrient-rich and antioxidant compounds (Khoo et al. 2015; Anju et al. 2022). Its leaves contain protein (22.0 g/100g), fiber (34-36%), calcium, iron (3.89-4.50 mg/100g), zinc (1.26-1.48 mg/100g), niacin, beta-carotene, and vitamin E (Platel and Srinivasan 2017; Nguyet et al. 2022). *S. androgynus* also contains ascorbic acid (Andarwulan et al. 2012) and various phytochemicals such as sterols, phenols, flavonoids, tannins, alkaloids, terpenoids, lignans, glycosides, catechols, cardiac glycosides, and acidic compounds (Arif and Shetty 2020) along with other active compounds that contribute to a range of pharmacological properties such as antioxidant, analgesic, antimicrobial, and antidiabetic (Sujila et al. 2016; Kuttinath et al. 2019).

Traditionally, the application of *S. androgynus* in South and Southeast Asia has focused on various medicinal and dietary purposes (Zhang et al. 2020). One of the most well-known traditional uses, particularly in Indonesia, is for promoting lactation. Indonesians commonly believe that *S. androgynus* leaves possess galactagogue properties (Rahmawaty and Padmasari 2023; Mien et al. 2024). However, it's crucial to note that its use in crude extract form raises concerns about safety due to the potential for significant side effects when consumed in large quantities or over an extended period. These side effects, such as respiratory system disturbances (Bunawan et al. 2015; Lorensia et al. 2015) and growth inhibition (Suprayogi et al. 2015), underscore the importance of caution when using *S. androgynus* leaves.

Previous research has explored methods to obtain forms of *S. androgynus* leaves with minimal side effects through the depolarization of dried *S. androgynus* leaves or fractionation of crude extracts, primarily using n-hexane as a solvent (Suprayogi et al. 2013, 2015). Incorporating the n-hexane fraction of *S. androgynus* leaves into feed has been reported to significantly increase milk production while reducing the harmful effects compared to other fractions (Suprayogi et al. 2015). Further investigation is required to identify the bioactive compounds in the n-hexane fraction of *S. androgynus* leaves through a chemical fingerprinting analysis approach. Generally, this approach identifies unique bioactive compounds in medicinal plants, aids in authentication, assesses quality, and evaluates the therapeutic potential of herbal medicines (Kharyuk et al. 2018). This method employs Liquid Chromatography-Mass Spectrometry (LC-MS) due to its ability to provide high selectivity and specificity, analyze compounds across various polarity levels through positive and negative electrospray ionization, and facilitate the integration of chemometric techniques (Beccaria and Cabooter 2020; Bhole et al. 2020).

All plants produce unique natural products that are important in their ecological and medicinal significance (Máthé 2014). One of these plants is *S. androgynus*, commonly known as *katuk*. This plant has extensive traditional uses, both as a medicinal plant and as a food source (Mien et al. 2024). These uses are closely linked to its nutritional composition and active compounds. The types and quantities of natural products it produces can vary depending on environmental factors and genetic variation (Burns et al. 2012; Wang et al. 2013; Olanrewaju et al. 2021). It is crucial to understand and preserve the ecological significance of *S. andogynus* for the balance of our natural ecosystems.

In the Bogor region of West Java, researchers have identified four morphophysiological varieties of *S. androgynus* based on phenotypic differences, particularly the presence and distribution of white spots on the leaf surface. These varieties are Zanzibar (white spots concentrated in the center of the leaf), Bastar (white spots covering 30-75% of the leaf surface), Paris (thinly distributed white patches covering 0-10% of the leaf surface), and Kebo (no white spots, plain green) (Maslahah et al. 2005). This phenotypic variation, a result of underlying genetic differences, adds a layer of complexity to the study of *S. andogynus*. These genetic differences may influence the chemical composition and pharmacological efficacy of the plant, sparking further research and exploration.

To date, research on these varieties has primarily focused on their morphological and physiological characteristics. This study aimed to expand that knowledge by investigating their phytochemical profiles and chemical fingerprints. By doing so, it seeks to provide a deeper understanding of their chemical compound profiles. The findings are expected to support the identification of superior varieties and their potential development as medicinal products.

MATERIALS AND METHODS

Materials

The four varieties of *Sauropus androgynus* (*katuk*) as shown in Figure 1 were collected from the cultivation center in Cinangneng, Ciampea District, Bogor Regency (6.55849° S, 106.70384° E). These plants were grown under uniform biotic and abiotic conditions. The chemicals used included 80% ethanol and n-hexane. The filtrate was evaporated using a rotary vacuum evaporator (Ogawa, Japan). Additionally, the LC-MS instrument used was an LC-ESI-QTOF located in the forensic chemistry division of PUSLABFOR, Indonesian National Police Headquarters. The LCMS data analysis was performed using MassLynx V4.1 SCN884 software (Waters Laboratory Informatics).

Figure 1. Four varieties of *S. androgynus* plants. A. Zanzibar; B. Bastar; C. Paris; D. Kebo

Extraction and fractionation of *S. androgynus* leaves

After sorting the *S. androgynus* leaves based on their morphological characteristics, they were cleaned to remove any adhering dirt. Fresh *S. androgynus* leaves were air-dried until wilted and detached from the twigs, flowers, and fruits. The drying process was continued using an oven set at 50°C for 48 hours. The dried leaves were then ground using a blender and sieved through an 80 mesh sieve to obtain powdered simplicia. The extraction and fractionation were performed in accordance with our previous study (Fachruddin et al. 2017). In separate containers, 300 g of *S. androgynus* leaf powder from four different varieties was soaked in 80% ethanol (ratio 1:6 w/v). The mixture was manually stirred for 30 minutes and then allowed to stand for 24 hours at room temperature. Filtration was performed using flannel cloth and filter paper. The same procedure was repeated three times. The filtrate was evaporated using a rotary evaporator at 50°C. A total of 10 g of the ethanol crude extract was dissolved in 200 mL of 80% ethanol, then placed into a 500 mL separatory funnel, and 200 mL of n-hexane was added to the same container. The mixture was shaken and allowed to stand until separation occurred. The n-hexane fraction was collected. The fractionation procedure was repeated five times. The n-hexane fraction solution was evaporated using a rotary evaporator at 40°C. The extraction and fractionation yields were calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of raw material}} \times 100\%$$

Phytochemical testing of ethanol extract and chemical fingerprinting analysis of n-hexane fraction of *S. androgynus* leaves

The qualitative phytochemical test of the crude ethanol extract of *S. androgynus* leaves from four varieties was conducted following the protocol by Balamurugan et al. (2019). The chemical fingerprint analysis was performed using the following procedure: 5 µL of the n-hexane fraction was injected into the LC-ESI-QTOF instrument. The LCMS analysis utilized UPLC-MS equipped with a binary pump. This liquid chromatography system was coupled to a QTOF mass spectrometer (Quadruple-Time of Flight). The mass spectrometer was the Xevo G2-S QTOF with a positive ionization method. ESI parameters included a capillary temperature of 150°C, a nebulizer gas flow of 50 L/hour, and a source voltage of +3.0 kV. The full scan mode ranged from m/z 50-1500, with a source temperature of 50°C. The UPLC column was Acquity UPLC BEH C18 1.7 µm, 2.1 × 150 mm. The eluent was methanol, set at a 0.3 mL/min flow rate. The elution system was operated isocratically from minute 0-0.5 with a ratio of 90:10, from minute 0.5-8 with a linear gradient elution of the solvent from 90% to 10%, from minute 8-9 with isocratic elution at a ratio of 10:90, and from minute 9-10 with a linear gradient elution of the solvent from 90% to 10%. The data obtained from the LC-MS chromatogram readings included retention time, mass-to-charge ratio (m/z), molecular formula, and relative area percentage. Compound class identification was performed by comparing mass spectrum peaks with ChemSpider's online chemical database.

RESULTS AND DISCUSSION

Extraction and fractionation of *S. androgynus* leaves

The results of extraction and fractionation of *S. androgynus* leaves from the four varieties are presented in Table 1. The yield of crude ethanol extract ranged from 20.34% (Bastar) to 27.56% (Kebo). This yield indicates that the Kebo variety contains relatively more bioactive compounds than other varieties. The yield of the Kebo variety in this study is higher than the 11.56% yield and 24.42% yield reported by Mustarichie et al. (2019) and Awaludin et al. (2020), respectively. The differences in extraction results may be influenced by the morphophysiology of the *S. androgynus* leaves from various varieties. The larger leaf size and solid green color without white spots in the Kebo variety may enhance photosynthetic capacity, increasing photosynthates and, consequently, a higher amount of soluble extractable compounds in organic solvents (ethanol). The high yield in the Kebo variety is also attributed to its low water content of 6.16% (Maslahah et al. 2005). Winaliani and Sari (2024) state that water content affects extraction efficiency. High water content can reduce the efficiency and effectiveness of the extraction process.

Meanwhile, the yield of the n-hexane fraction from the four varieties showed relatively similar values; however, the yield of the Zanzibar variety was slightly higher (8.65%) than the others. The Zanzibar variety is characterized by large white spots in the middle of the leaves, which are waxy layers, a nonpolar component. This waxy substance, which dissolves well in nonpolar solvents such as n-hexane, is a key factor in the Zanzibar variety's higher n-hexane fraction yield. The extensive white spots on the leaf surface of the Zanzibar variety, due to this waxy substance, result in a relatively higher n-hexane fraction yield compared to other varieties with fewer or no white spots. For example, the Kebo variety had only a 7.60% yield. According to Srivastav et al. (2021), natural waxes extracted using nonpolar solvents provide optimal extraction results.

Phytochemistry of crude ethanolic extract of *S. androgynus* leaves

The qualitative phytochemical analysis of the crude ethanol extract from various varieties of *S. androgynus* leaves is presented in Table 2. All four varieties contained flavonoids, steroids, tannins, and saponins, while alkaloids, quinones, and triterpenoids were undetected. These findings contrast with previous studies, which reported the presence of alkaloids in ethanol extracts of *S. androgynus* leaves (Kuttinath et al. 2019; Mustarichie et al. 2019; Hikmawanti et al. 2021), and the absence of steroids (Mustarichie et al. 2019). Such discrepancies may be attributed to several factors, including geographical location, biotic and abiotic stress, seasonal variations, agricultural practices, and post-harvest handling (Huang et al. 2020; Addo et al. 2021; Xu et al. 2022; El-Beltagi et al. 2023; Gfeller et al. 2023; Qaderi et al. 2023).

This study reported that they are significant as they highlight the presence of bioactive compounds that may contribute to the plant's pharmacological potential. Detecting secondary metabolites across all *S. androgynus* leaf varieties confirms their broad pharmacological potential. For instance, flavonoids possess antioxidant, anticancer, and anti-inflammatory properties (Rha et al. 2019). Steroids also exhibit activity as anti-inflammatory agents (Patel and Savjani 2015) and act as regulators of reproductive and endocrine functions (Dean et al. 2017).

Table 1. Extraction and fractionation results of *S. androgynus* leaves from four varieties

S. androgynus varieties	Yield (%)	
	Ethanol crude extract	n-heksane extract fraction
Zanzibar	20.85	8.65
Bastar	20.34	8.62
Paris	22.19	8.35
Kebo	27.56	7.60

Table 2. Phytochemical composition of ethanol crude extracts of *S. androgynus* leaves from four varieties

Secondary metabolites	Ethanol crude extract of *S. androgynus* leaves varieties			
	Zanzibar	Bastar	Paris	Kebo
Alkaloids				
Mayer	-	-	-	-
Wagner	-	-	-	-
Dragendorf	-	-	-	-
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Quinones	-	-	-	-
Triterpenoids	-	-	-	-
Tannins	+	+	+	+
Saponins	+	+	+	+

Notes: The "+" sign indicates the presence of secondary metabolites in the sample, while no secondary metabolites were observed for the "-" sign

The presence of saponins and tannins indicates potential anticancer properties (Yildirim and Kutlu 2015) and the amelioration of renal dysfunction (Abu and Ikponmwosa-Eweka 2022). Additionally, saponins have been reported to possess various other pharmacological activities, such as anti-cholesterolemic, antiparasitic, antiviral (Patel and Patel 2016), antitumor, antidiabetic, and neuroprotective effects (Nguyen et al. 2020). On the other hand, the absence of alkaloids in all *S. androgynus* varieties presents an opportunity for their development as raw materials for pharmaceuticals, as alkaloids in *S. androgynus* leaves have been reported to cause specific undesirable side effects. Papaverine is one of the main alkaloid compounds in *S. androgynus* leaves (Bunawan et al. 2015). The compound is strongly suspected to be related to the incidence of bronchiolitis obliterans organizing pneumonia, a disorder of the respiratory organs characterized by inflammation and stiffness of the lung area with organized tissue (Yang et al. 2017).

Chemical fingerprinting of n-hexane extract fractions from four *S. androgynus* varieties

The chemical compounds in the n-hexane fraction of *S. androgynus* leaves from different varieties exhibit distinct fingerprinting patterns. The LC-MS chromatograms of the n-hexane extract fractions from the four varieties are presented in Figure 2. These chromatograms are visualized with Retention Time (RT) on the X-axis and peak intensity on the Y-axis. The study results indicate that the chromatographic profiles of the four varieties share similarities in their chemical fingerprint patterns. Two varieties (Zanzibar-Bastar) demonstrate the highest similarity in fingerprint patterns (six peaks with the same retention time). In comparison, three varieties (Zanzibar-Bastar-Kebo) and (Zanzibar-Paris-Kebo) show moderate (four peaks with the same retention time) and low (two peaks with the same retention time) similarities, respectively, as presented in Table 3. However, differences in peak intensities were observed, representing the relative amounts of the compounds detected from each variety. The similarities in the chemical fingerprint profiles may be attributed to the close phylogenetic relationships of the four *S. androgynus* varieties, as they belong to the same species (intraspecific). The similarities may also be due to the plants originating from environments with similar biotic and abiotic conditions and comparable altitudes. Similar findings have been reported in other plant species; for instance, Da Silva Antonio et al. (2024) observed that three *Ocotea delicata* Vicent. specimens collected from the same growing environment exhibited similar chemical features. Ehlers et al. (2016) explained that genetic variation among individuals within the same plant species can create minor differences in the types of chemical compounds present.

Based on Figure 2, the n-hexane fraction of *S. androgynus* leaves from four varieties exhibited the highest peaks within the 9th to 10th minute retention time range. The Zanzibar variety had the highest peak with a retention time of 9.37 minutes, followed by Bastar at 9.20 minutes, Paris at 9.75 minutes, and Kebo at 9.40 minutes. The highest peaks indicate strong signals of the presence of certain compounds in the sample at higher concentrations. Therefore, the highest peaks often indicate major compounds within the analyzed sample. The major compounds identified in the n-hexane fraction of *S. androgynus* leaves for each variety are presented in Table 4. The major compounds in the n-hexane fraction of *S. androgynus* leaves from the four varieties were identified by comparing the LC-MS results with the ChemSpider chemical database. These major compounds belong to the triterpenoid glycosides, diterpenoids, and steroids groups for the Zanzibar, Bastar, Paris, and Kebo. Terpenoid compounds have been widely reported in *S. androgynus* leaves (Awaludin et al. 2020; Hikmawanti et al. 2021; Ekasari et al. 2022). However, further analysis is required to identify the specific compounds present in these varieties.

The presence of these significant compounds aligns with *S. androgynus* plant's pharmacological roles. Triterpenoid glycosides have been reported to exhibit cytotoxic effects on tumor cell lines (Careaga et al. 2009). Mahardiani et al. (2020) reported that the terpenoid content in *S. androgynus* leaf extract shows cytotoxic activity against Huh7it cells. Diterpenoids possess a broad range of biological activities, including antimicrobial, anti-inflammatory, anticancer, antimicrobial, cardiovascular, and others (Veneziani et al. 2017), which is consistent with the therapeutic effects of *S. androgynus* leaves, acting as anti-inflammatory, antibacterial, anticancer agents, and more (Zhang et al. 2020; Anju et al. 2022). Plant steroid compounds are known to be useful in treating diseases caused by viruses (Yerlikaya et al. 2023). Joshi et al. (2023) revealed through in silico studies that beta-sitosterol (a steroid) from *S. androgynus* leaves can act as an antiviral by blocking viral entry through the inhibition of fusion processes.

Table 3. Similarity levels of active compounds based on the number of peaks detected in the LC-MS chromatogram of n-hexane extracts fraction from four varieties of *S. androgynus* leaves

S. androgynus varieties	Number of peaks with the same RT	RT (minutes)
Zanzibar-Bastar	6	0.77; 5.49; 6.55; 7.22; 7.64; and 9.20
Zanzibar-Bastar-Kebo	4	0.77; 5.49; 6.55; and 7.64
Zanzibar-Paris-Kebo	2	5.49 and 6.55

Table 4. Major compound groups in the n-hexane extract fraction of *S. androgynus* leaves from four varieties

S. androgynus varieties	Retention time	m/z		Molecular formula	Compound group	% relative area
		Measured	Theoretical			
Zanzibar	9.37	424.2174	424.4922	$C_{22}H_{32}O_8$	Triterpenoid glycoside	1.297
Bastar	9.20	592.2774	592.6880	$C_{34}H_{40}O_9$	Diterpenoid	3.157
Paris	9.75	606.2947	606.7149	$C_{35}H_{42}O_9$	Diterpenoid	2.955
Kebo	9.41	374.2539	374.5215	$C_{23}H_{34}O_4$	Steroid	3.184

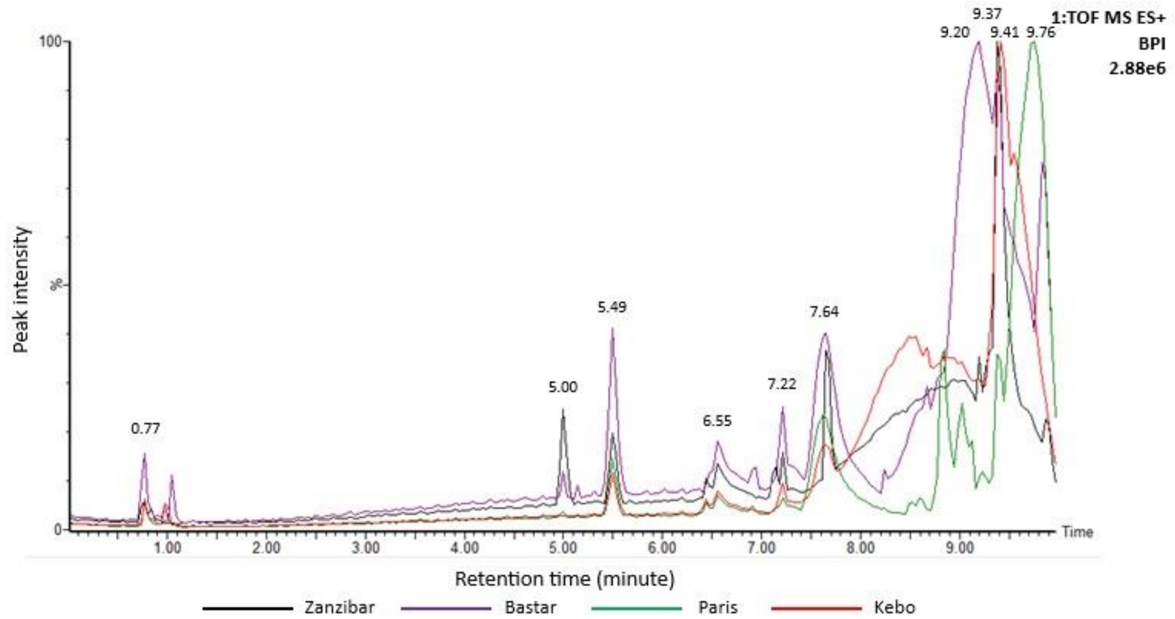

Figure 2. LC-MS chromatogram of the n-hexane extract fraction of *S. androgynus* leaves from four varieties

In conclusion, there is no difference in the phytochemical content of ethanol extracts from the four varieties of *S. androgynus*, all of which contain flavonoids, steroids, tannins, and saponins. Furthermore, the n-hexane fractions of the four *S. androgynus* varieties also exhibit similar chemical fingerprint patterns, although with varying peak intensities. The major compound groups represented by the highest peaks of each variety are terpenoid glycosides (Zanzibar), diterpenoids (Bastar and Paris), and steroids (Kebo). Thus, their application, whether individually or in combination with a mixed variety of *S. androgynus*, is likely to produce pharmacological effects that are also similar or comparable. This opens up exciting possibilities for further research and exploration.

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