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Research Article

Compound Analysis and Genetic Study of Selected *Plectranthus scutellarioides* Varieties from Indonesia

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ABSTRACT

Background: *Plectranthus scutellarioides* is one of medicinal plants in Indonesia, which has several hundred varieties but only one is known by local people as medicine. **Objective:** Six varieties of *Plectranthus scutellarioides* were analyzed for their total flavonoid content, chemical compound, and moleculer genetic. **Methods:** TFCs were analyzed using $AlCl_3$ colorimetric method, chemical compounds were identified using TLC-scanning densitometer, GC-MS, and FTIR, moleculer genetic were observed using DNA barcoding rbcL gene. **Results:** The TFCs of *trailing psycholeus,* and *flamingo* varieties were higher than the other varieties. TLC-scanner densitometer showed that *color blaze dark star*, *trailing psycholeus,* and *trailing queen* had similar profiles, as did *beale street*, *trailing rose,* and *flamingo*. The GC-MS results showed notable difference in *trailing psycholeus* and *trailing queen* which have 2-oleoylglycerol and 9(E),11(E)-conjugated linoleic acid in larger amounts than others, respectively. Multivariate analysis of the FTIR spectra showed the closeness of all varieties, except for *beale street* which had the lowest similarity with the others. Despite that, genetic studies using the rbcL gene and comparing the results with the *P. scutellarioides* gene in the database (MW538954.1) showed *beale street* was the most similar (99.52%). The phylogenetic analysis showed that *beale street* and *trailing psycholeus* have the highest similarity among others. **Conclusions:** There is a slight difference in chemical composition between varieties as well as the genetic. Therefore, quality control or standardisation is needed in the use of this plant as a traditional medicine.

Key words: *Coleus scutellarioides*, Densitometer, Flavonoid, FTIR, GC-MS, rbcL.

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INTRODUCTION

Plectranthus scutellarioides is a member of the Lamiaceae family, which has several hundred varieties[1]. Among all the varieties, only the variety with brownish-red leaves is used as a traditional medicine in Indonesia[2], while the rest are used as ornamental plants because of their varied leaf shapes and colours. Secondary metabolite compounds such as alkaloids, flavonoids, terpenoids and tannins were found in methanol and ethanol extracts of some varieties of *P. scutellarioides*[3]. Isolated constituents of some varieties of *P. scutellarioides* have previously been reported, such as rosmarinic acid[4], five abietane diterpene derivatives coleon O[5], the 2,16-diacetyl derivative of 2,6,11,12,14,16,17-heptahydroxy-5,8,11,13-abietatetraen-7-one[6], quercetin[7], spirocoleon 7-*O*-*β*-D-glucoside, apigenin 7-*O*-(3"-*O*-acetyl)-*β*-D-glucuronide, apigenin 5-*O*-(3"-*O*-acetyl)-*β*-D-glucuronide[8], spiroscutelones A-C[9], scutellarioidone A-D, the 6-acetyl derivative of fredericone B, scutellarioidolide A[10], sincoetsin C, and 3-hydroxyspirocoleon 7-*O*- *β*-D-glucoside[11].

The extracts of the leaves of *P. scutellarioides* have demonstrated antioxidant activity[12-13], which may be affected by flavonoids, which are natural antioxidants and another common compound in plants. The hexane, ethyl acetate, and butanol fractions of the *P. scutellarioides* variety with brownish-red leaves have total flavonoid contents (TFC) of 1.75 mg/g, 9.63 mg/g and 16.78 mg/g in equivalent with quercetin[13], respectively. The TFC also differs in both varieties and accession of *P. scutellarioides*: the variety with deep purple leaves has 4.009% TFC compared with total extracts, 5.858% for greenish-purple, 4.971% for green, and 3.451% for reddish-purple, although these values are not significantly different[14].

The phytochemistry of other varieties of *P. scutellarioides* has not been investigated even though they have the potential to be a source of medicine due to their bioactivity[6-11], rapid growth rate and easy propagation[1]. This study aims to analyse the compound using three instruments (TLC-scanner densitometer, GC-MS, FTIR) and molecular genetic using DNA barcoding rbcL of six varieties of *P. scutellarioides.*

MATERIAL AND METHODS

General experimental procedures

The absorption spectrum for calculate the total flavonoid content was measured on UV-Vis spectrophotometer X-Ma 1200 (Human corporation, South Korea) with rutin as the standard (rutin hydrate 95%, Sigma-Aldrich). TLC silica gel 60 F_{254} aluminium plates (Merck, Germany) with pore volume (N_2-isotherm): 0.74-0.84 ml/g was used to analyze the phytochemical compound with the aid of Camag TLC-scanner 3 (Camag, Switzerland) densitometer. The GC-MS was performed using ISQ 7000 mass detector (Thermo Scientific) and Chromeleon 7.0 software. The FTIR was conducted on a FTIR spectrometer (Nicolet iS10, Thermo Fisher Scientific). The amplification of the DNA was done using GeneAmp PCR system 9600 (Perkin Elmer, United States).

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Plant material

The leaves of six varieties of *P. scutellarioides* (V1: var. *colour blaze dark star*, V2: var. *trailing psycholeus*, V3: var. *trailing queen*, V4: var. *beale street*, V5: var. *trailing rose*, V6: var. *flamingo*) were collected from Rantebelu Village, Luwu, South Sulawesi between 10 and 12 a.m. on September 2020, and identified by Dr. A. Mu'nisa, Department of Biologi, Faculty of Mathematics and Natural Science, State Makassar University. A voucher specimen has been deposited in the Pharmacognosy-Phytochemistry Laboratory, Hasanuddin University (ADA-FFUH-01;02;03;04;05;06).

Extraction

Insects were removed from the leaves. The leaves were separated from the rest of the plant and cleaned with water. After the water was drained, the leaves were put in the oven for five days at 50°C until the leaves could be crushed. The dry weight of each sample was measured, and the sample was divided for the two extraction methods. The dried powdered leaves of the *P. scutellarioides* varieties were extracted gradually using two extraction methods: Soxhlet extraction and maceration, each with n-hexane and ethanol. The Soxhlet extraction was conducted at 50°C for n-hexane and 70°C for ethanol. Maceration was carried out at room temperature. The sample was stirred every 24 hours, and the solvent was filtered and replaced with the fresh solvent. Both of these methods were repeated until the solvent was clear. The extracts were concentrated using a rotary evaporator (50°C), and the remaining water was evaporated using a water bath at 70°C.

Determination of TFC

The TFC of the six varieties of *P. scutellarioides* was determined using the $AlCl_3$ colourimetric method. Rutin was used as the standard, prepared in solutions of 2, 4, 6, 8 and 10 µg/ml, and 0.005 g of the extract produced with ethanol was dissolved into 25 ml of methanol (200 µg/ml). Each aliquot (0.5 ml) was pipette into a vial and mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of CH_3COONa 1 M and 2.8 ml of distilled water. The mixture was shaken and left to stand for 30 minutes at room temperature. A blank was prepared in the same way without the addition of $AlCl_3$. The absorption spectrum was measured at 425 nm using a UV-Vis spectrophotometer, and a standard calibration curve was measured to calculate the regression equation. The measurement was repeated three times, and the TFC was expressed in RE (Rutin equivalents) per g of DWE (dry weight of extract).

Evaluation of chemical compounds

Thin Layer Chromatography (TLC)–densitometric scanning

Five µl of n-Hexane extract (10 µg/ml) and ethanol (5 µg/ml) of each varietal extract were eluted with a mixture of n-hexane and ethyl acetate (3:1) as the mobile phase onto thin layer chromatography plates (silica gel 60 F254). The plates were air-dried for 30 minutes before being evaluated using TLC-densitometric scanning at 200–700 nm.

Gas Chromatography-Mass Spectroscopy (GC-MS)

The sample was prepared for GC-MS analysis by extracting 1 mg of the crude extract with ethanol, filtering by Whatman paper no. 42 pore size 2.5 µm, and concentrating to 2 ml. The Trace 1310 GC used in the analysis employed a column packed with FactorFour (capillary column VF-5 ms, 30 m, 0.25 mm, 0.25 µm, for length, width, and height, respectively) and helium (1 ml/min) was used as the carrier gas. Up to 2 µl of the sample was injected and detected by the ISQ 7000 mass detector (Thermo Scientific) and Chromeleon 7.0 software. During the 42 minutes of the GC elution process, the GC oven was maintained at 250°C, with a 5-minute holding time where the temperature was constant . The injector temperature was set to 250°C. The ISQ 7000 mass detector settings were also standard (MS transfer line temperature: 250°C; ion source temperature: 200°C; mass range 50–500 amu. The MS detection was completed in 42 minutes.

Fourier Transform Infrared (FTIR) Spectroscopy

A small amount of *P. scutellarioides* ethanol extract was homogenised with KBr (10:1 w/w), placed in a mould, and pressed with 2 tons. The disc formed must be >50% transparent and was placed in a sample holder. The sample was scanned using an FTIR spectrometer (Nicolet iS10, Thermo Fisher Scientific) from 4000–400 cm^{-1}.

Genetic study using rbcL gene sequences

DNA isolation was performed by using a Wizard Genomic DNA Purification Kit (Promega) following standard protocols. Qualitative and quantitative measurements of isolated DNA were performed by electrophoresis using 1% agarose gel and a NanoDrop UV spectrophotometer. The primers used in the amplification process were *rbc*L1 forward (TGTCACCAAAAACAGAGACT) and *rbc*L2 reverse (TTCCATACTTCACAAGCAGC). The Polymerase Chain Reaction (PCR) cycles were performed as follows: predenaturation at 95°C for 2 minutes, denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 120 seconds. This cycle (denaturation, annealing and extension) was repeated 35 times. The post-elongation stage was performed at 72°C for 7 minutes. Sequencing was carried out by 1st base Laboratories Sdn. Bhd. (Selangor, Malaysia) and reading by BioEdit, a sequence scanner program. Identification was made using BLAST.

Statistical analysis

TFC measurements were conducted in triplicate and reported as means ± standard deviation (SD). The raw data, including position and intensity of FTIR peaks, were loaded into Minitab version 18.0 for multivariate analysis (Principal Component Analysis and Cluster Observations) to understand the differences between the varieties.

RESULTS AND DISCUSSION

Total Flavonoid Content

The dried powdered leaves of six varieties of *P. scutellarioides* (Figure 1) were extracted gradually using two extraction methods: Soxhlet extraction and maceration, each with n-hexane and ethanol in order to obtain 24 residues, which were subjected to further investigation: the total flavonoid content (TFC) examination for ethanol extract, compound analysis using TLC-densitometric scanning for both n-hexane and ethanol extract, GC-MS, and FTIR for ethanol extract. Figure 2 shows that Soxhlet extraction produces extracts with higher TFC than the extracts obtained by maceration. These results are similar to other studies of the methanol extracts of *Urtica dioica*[15-16], the ethanol extract of *Vernonia amigdalina*[17] and the methanol extract of *Rhaponticoides iconiensis*[18]. The time needed for Soxhlet extraction is much less than for maceration, which required a minimum of three days. In addition, Soxhlet extraction produced higher extraction yields[19] as well as higher TFCs. The release of flavonoids and the extraction rate can be enhanced by increasing the extraction temperature, which leads to decreased solvent density and viscosity, decreased surface contact area, and increased plant cell fractures[20]. Although the use of high temperature is not recommended for the extraction of some thermolabile compounds, such as flavonoids, the diversity of plant matrixes and chemical characteristics of flavonoids can make it the best standard method to extract the flavonoids[21]. Observing plant behaviour and growth in the wild, selecting the part of the plant used, and investigating their chemical composition could be initial steps to determine a better extraction method.

Figure 1: Six varieties of *P. scutellarioides*, **A**. *color blaze dark star* (V1), **B**. *trailing psycholeus* (V2), **C**. *trailing queen* (V3), **D**. *beale street* (V4), **E**. *trailing rose* (V5), **F**. *flamingo* (V6).

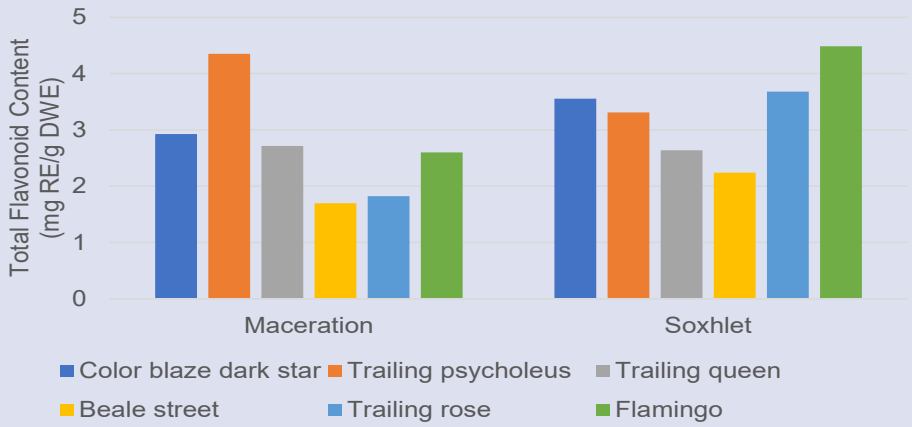

Figure 2: Total flavonoid content of extract obtained by maceration and soxhlet extraction.

Table 1: Total flavonoid content of six varieties of *P. scutellarioides*.

Method	Total Flavonoid Content (mg RE/g DWE)[a,b]					
	Color blaze dark star	Trailing psycholeus	Trailing queen	Beale street	Trailing rose	Flamingo
Maceration	2.906 ± 0.018	4.305 ± 0.045	2.696 ± 0.017	1.667 ± 0.029	1.761 ± 0.060	2.570 ± 0.030
Soxhlet	3.499 ± 0.056	3.254 ± 0.058	2.620 ± 0.017	2.222 ± 0.016	3.590 ± 0.088	4.422 ± 0.061

[a]Values are represented as mean (n = 3) ± SD
[b]TFC was expressed in RE (Rutin equivalents) per g of DWE (dry weight of extract)

Table 1 shows the *color blaze dark star* (V1), *trailing queen* (V3), *beale street* (V4), *trailing rose* (V5), and *flamingo* (V6) varieties have relatively higher TFC values in soxhletation than maceration, but not on the trailing psycholeus (V2) variety. The environment in which the trailing psycholeus variety grows tends to be shadier than other varieties. This is thought to have an influence on the flavonoid properties of each variety.

Compounds analysis

TLC-densitometric scanning

TLC-densitometric scanning was performed to examine the differences between *P. scutellarioides* varieties based on chemical compounds without the use of a standard. The number of peaks of each extract, ranging from 12 to 18, is shown in Table 2.

The ethanol extract showed more peaks and higher intensity in some peak areas than the n-hexane extract (Figure 3), indicating that some compounds had higher extraction yields in ethanol than in n-hexane. The spectra produced by different extraction methods also vary. The spectra of extracts produced by maceration contained more peaks compared to those produced by Soxhlet extractions, especially varieties 4, 5 and 6 in ethanol. This may be due to the degradation of some secondary metabolites, which are preferentially extracted using the Soxhlet method. Meanwhile, varieties 1, 2 and 3 showed similar numbers of peaks for both extraction methods. Overall the TLC-densitometric scanning spectra showed that V1, V2 and V3 had similar profiles, as did V4, V5 and V6 (Figure 4).

Gas Chromatography-Mass Spectroscopy

The GC-MS analysis of the ethanol extracts of the *P. scutellarioides* varieties revealed many components, with the most abundant constituents observed in high percentages (Table 3) being trans-13-octadecenoic acid, which was present in all extracts except in V5 and V6 by maceration and the V2 Soxhlet extract. The biological activity of trans-13-octadecenoic acid has been previously reported, including anti-inflammatory[22], anti-androgenic, antileukotriene-D4, 5-α reductase inhibition, dermatitigenic and anemiagenic activities[23]. In addition to trans-13-octadecenoic acid, other components that also have a high percentage in the extract are cis-13-octadecenoic acid, which has been reported[24] to be anti-inflammatory, hypocholesterolemic, anti-histamine, anti-acne, and anti-arthritic; oleic acid, which has been reported as antifungal[25], anti-inflammatory, antibacterial and to contain antioxidants[26]; cis-vaccenic acid, which has anti-inflammatory and anti-hypercholesterolemic activity[27]. Fatty acids like n-hexadecanoic

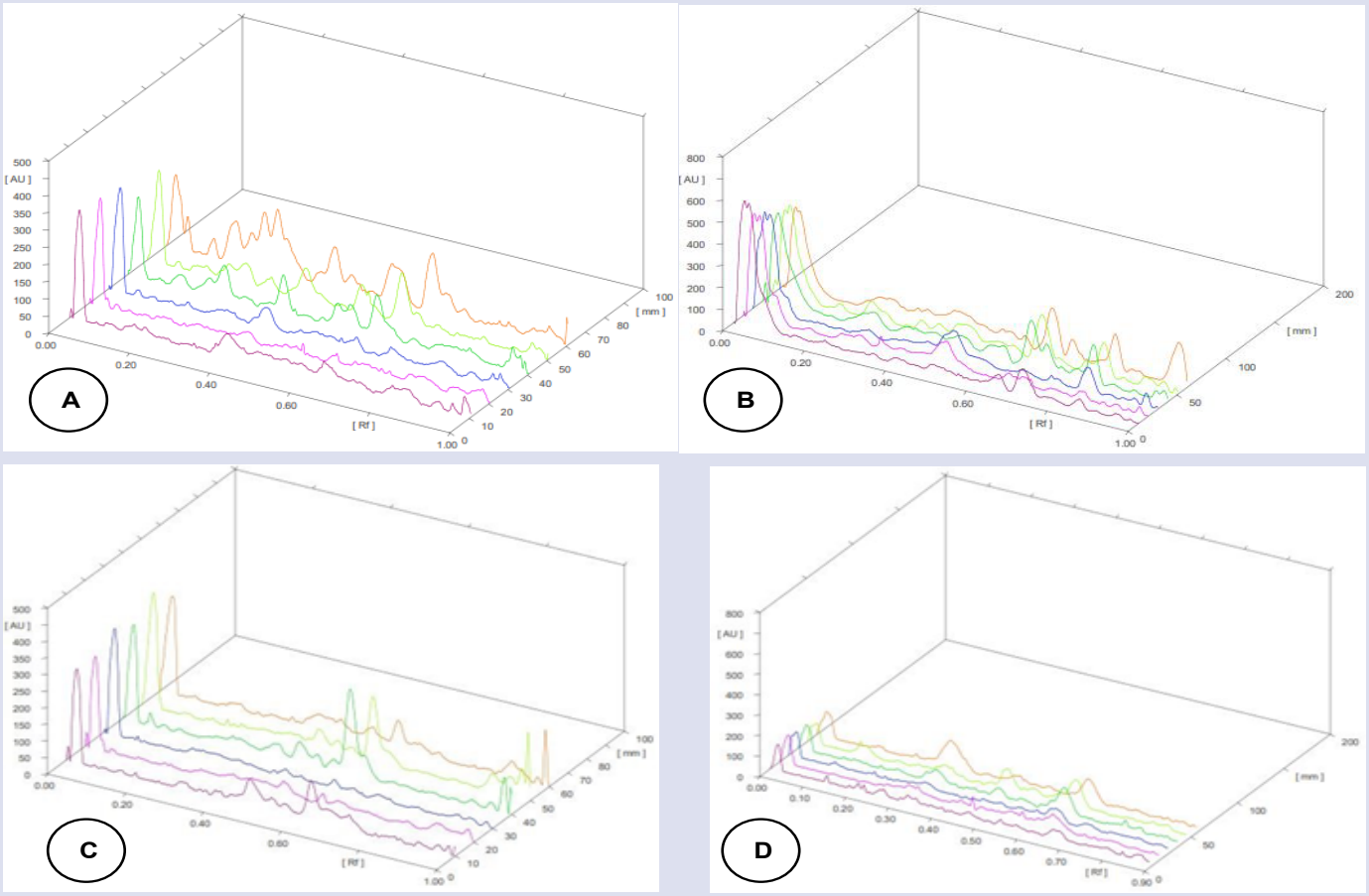

Figure 3: Densitogram of six varieties of *P. scutellarioides* (**A**. ethanol extract obtained by maceration, **B**. n-Hexane extract obtained by maceration, **C**. ethanol extract obtained by soxhlet extraction, **D**. n-Hexane extract obtained by soxhlet extraction).

Table 2: Peak of each extract from TLC-densitometric scanner.

Sample	Method	Solvent	Peak Amount
Color blaze dark star	Maceration	n-Hexane	12
		Ethanol	13
	Soxhlet extraction	n-Hexane	16
		Ethanol	16
Trailing psycholeus	Maceration	n-Hexane	15
		Ethanol	15
	Soxhlet extraction	n-Hexane	13
		Ethanol	11
Trailing queen	Maceration	n-Hexane	16
		Ethanol	14
	Soxhlet extraction	n-Hexane	11
		Ethanol	12
Beale street	Maceration	n-Hexane	17
		Ethanol	17
	Soxhlet extraction	n-Hexane	12
		Ethanol	16
Trailing rose	Maceration	n-Hexane	15
		Ethanol	17
	Soxhlet extraction	n-Hexane	14
		Ethanol	12
Flamingo	Maceration	n-Hexane	14
		Ethanol	18
	Soxhlet extraction	n-Hexane	12
		Ethanol	13

acid and oleic acid have antibacterial and antifungal activities against various species, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Candida krusei*, and *Candida tropicalis*[28].

Palmitic acid (n-hexadecanoic acid), which has anti-inflammatory activity that acts by inhibiting the enzyme phospholipase A2[29], was detected in the V3 with sufficient quantity. The GC-MS chromatogram of the V3 extract also contained 9(E),11(E)-conjugated linoleic acid (isolinoleic acid) in greater amounts than in the other varieties. Conjugated linoleic acid (CLA) has an anticarcinogenic effect on colon[30], mammary[31], prostate[32], stomach[33], and hepatic carcinomas in the early stages using a murine model[34]. Some researchers have reported that the anticarcinogenic mechanisms of CLAs were inducing apoptosis[35], promoting anti-proliferation[36], reducing reactive oxygen species (ROS) and generating peroxide[35]. Among all the CLA isomers, 9(E),11(E)-conjugated linoleic acid has been shown to have the greatest potential as an anticancer drug[37]. The compound 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, or 2-oleoylglycerol (2OG), was identified from V2 Soxhlet extract in abundant quantity. 2OG is a GPR119 agonist[38], a protein-coupled receptor that can promote glucose-stimulated insulin secretion from β-cells in the pancreas, and thus represents a new class of treatment for type 2 diabetes mellitus[39].

All six varieties of *P. scutellarioides* contain the same common phytochemical components in different quantities. This commonality could be caused by the extraction process, the environment in which the plants grew, or the genetics of the plants. These results can be used

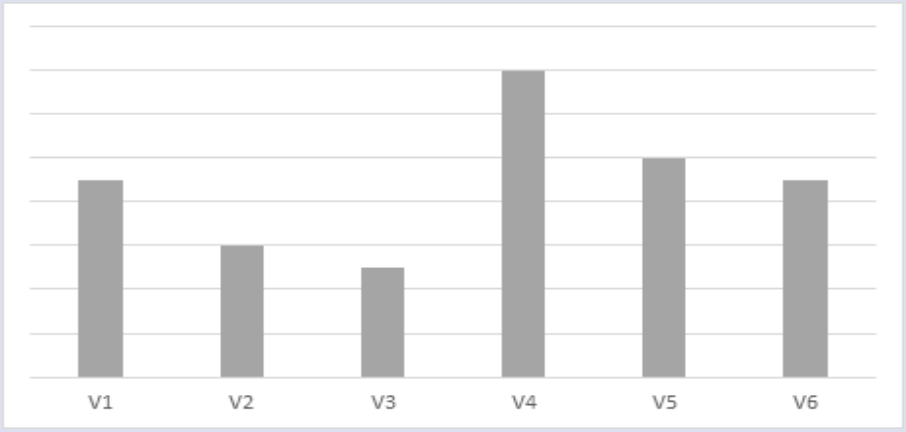

Figure 4: Peak amount of each variety observed by TLC-densitometric scanning.

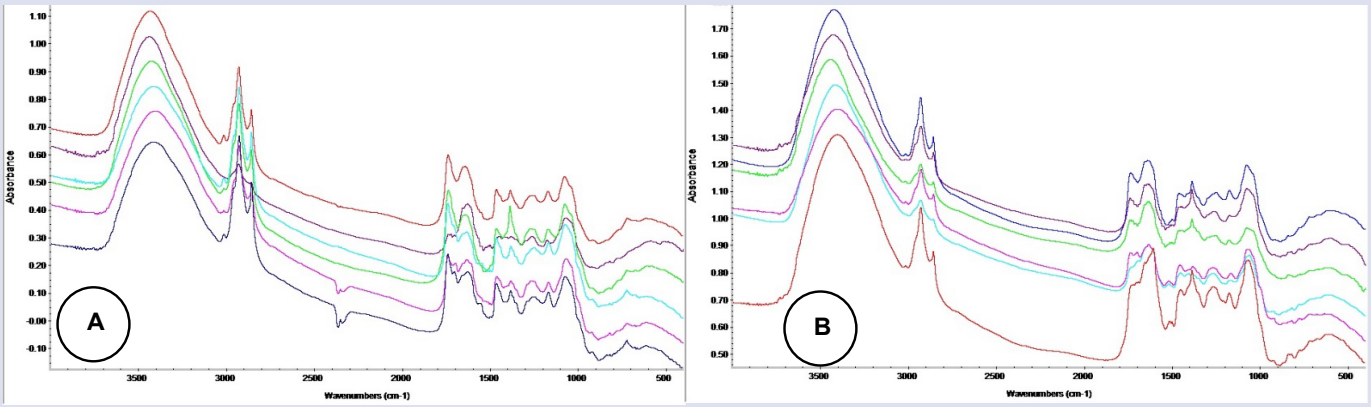

Figure 5: Spectra FTIR of *P. scutellariodes* varieties, **A**. Ethanol extract by maceration, **B**. Ethanol extract by soxhlet.

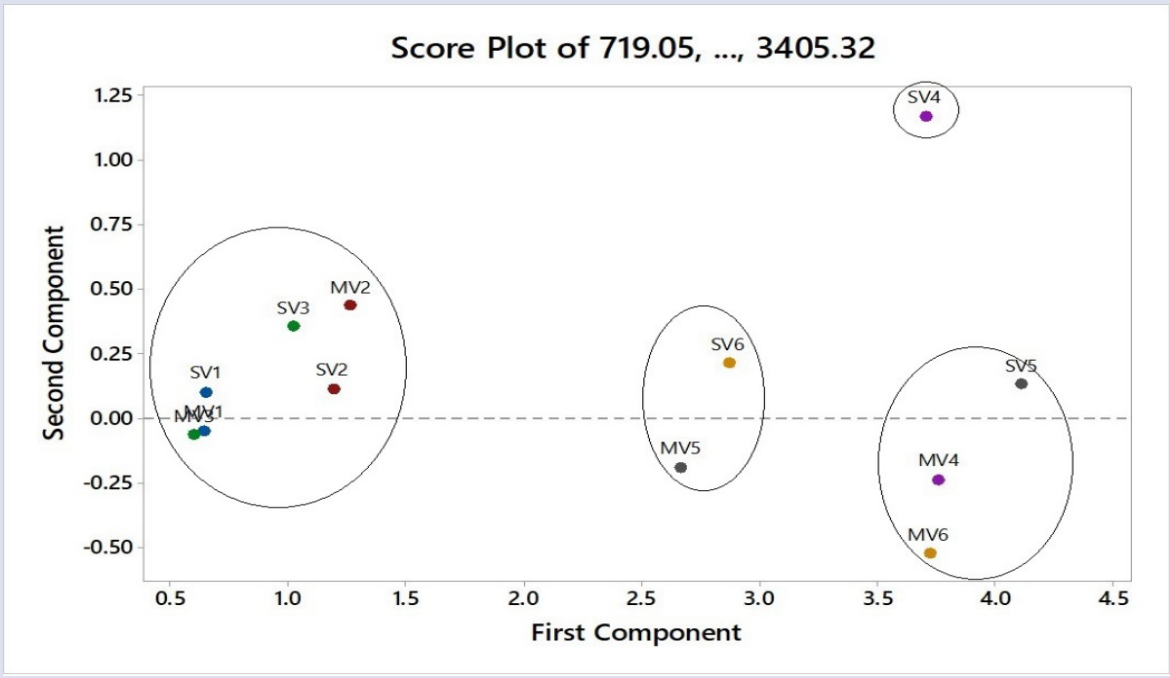

Figure 6: Score plot of ethanol extract of six varieties *P. scutellarioides* obtained by maceration (M) and soxhlet (S).

Table 3: List of major constituents detected on ethanol extract of six varieties *P. scutellarioides* by GC-MS.

Extraction Process	Variety	Total Component	Major Component	Retention Time	% Area
Maceration	V1	20	trans-13-Octadecenoic acid	32.079	55.19
			Oleic acid	32.198	15.51
			(Z)-18-Octadec-9-enolide	32.004	7.94
	V2	24	trans-13-Octadecenoic acid	32.112	53.56
			(Z)-18-Octadec-9-enolide	32.028	11.09
			Oleic acid	32.701	8.02
	V3	23	trans-13-Octadecenoic acid	32.103	64.09
			9(E),11(E)-Conjugated linoleic acid	32.025	12.18
			n-Hexadecanoic acid	30.480	8.81
	V4	23	cis-13-Octadecenoic acid	32.069	35.92
			trans-13-Octadecenoic acid	32.470	15.85
			Oleic acid	32.174	12.95
	V5	21	Oleic acid	32.066	43.20
			Tungsten, dicarbonyl-(?-4-2-methylenecycloheptanone) [1,2-bis(dimethylphosphino)ethane	1.374	17.90
			cis-13-Octadecenoic acid	32.470	7.62
	V6	15	cis-Vaccenic acid	32.082	54.14
			cis-13-Octadecenoic acid	32.470	16.73
			(Z)-18-Octadec-9-enolide	32.004	8.71
Soxhlet extraction	V1	18	trans-13-Octadecenoic acid	32.076	38.90
			cis-13-Octadecenoic acid	32.470	16.66
			Oleic acid	32.202	15.12
	V2	24	Oleic acid	32.065	45.67
			Pregn-5-en-20-one, 3,17-bis[(trimethylsilyl)oxy]-, O-(phenylmethyl) oxime, (3ß)-	1.401	18.98
			9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	32.473	12.01
	V3	20	trans-13-Octadecenoic acid	32.096	66.50
			(Z)-18-Octadec-9-enolide	32.025	11.17
			n-Hexadecanoic acid	30.480	8.15
	V4	18	trans-13-Octadecenoic acid	32.076	54.32
			Oleic acid	32.208	13.59
			ψ,ψ-Carotene, 3,3',4,4'-tetradehydro-1',2'-dihydro-1-hydroxy-1'-methoxy	1.398	10.18
	V5	21	trans-13-Octadecenoic acid	32.082	45.44
			cis-Vaccenic acid	32.198	21.87
			ψ,ψ-Carotene, 3,3',4,4'-tetradehydro-1',2'-dihydro-1-hydroxy-1'-methoxy	1.401	9.27
	V6	32	trans-13-Octadecenoic acid	32.120	39.74
			1-Heptatriacotanol	36.130	21.46
			Oleic acid	32.290	14.67

Table 4: Comparison of nucleotide compositions of the rbcL gene of six varieties of *P. scutellarioides*.

Sample Code	CRL[a]	QV20+[b]	Signal intensity				Identification (Ref. ID)	Similarity
			A	C	G	T		
V1	1143	1174	762	785	419	656	*Plectranthus scutellarioides* (MW538954.1)	98.52%
V2	1198	1191	1019	970	563	825		98.87%
V3	1184	1184	996	968	544	837		99.26%
V4	1217	1209	839	845	461	747		99.52%
V5	1206	1215	939	884	491	775		99.05%
V6	1199	1200	972	930	471	835		99.36%

[a]The Contiguous Read Length (CRL) is the longest uninterrupted stretch of bases with quality higher than a specified limit.
[b]QV20+ value is the total number of bases in the entire trace that have basecaller quality value greater than or equal to 20.

as initial screening data to isolate abundant bioactive compounds in certain varieties.

Fourier Transform Infrared

FTIR spectroscopic fingerprints based on the metabolites were used to differentiate the varieties of *P. scutellarioides*. The mid-IR spectra of the ethanol extracts are shown in Figure 5. All of the varieties show similar spectra with different peak intensities, especially V5 and V6. There were 7 peaks in the fingerprint region (500–1500 cm^{-1}), and the rest were observed in the double bond (1500–2000 cm^{-1}) and single bond regions (2500–4000 cm^{-1}).

P. scutellarioides has been reported to contain the main compound in the diterpenoid group. The peaks at 3200–3500 cm^{-1} are assigned to the hydrogen bond, confirming the presence of a hydroxyl group. The absorption peak at ~3010 cm^{-1} is assigned to alkenes (C=H), and peaks near 2925 cm^{-1} and 2854 cm^{-1} indicate the presence of alkanes. Carbonyl compounds (C=O) are associated with a peak between 1690–1760 cm^{-1}. There are also absorption bands of C=C alkenes (~1637 cm^{-1}), NO_2-nitro compounds (1570–1500 cm^{-1}), C-H alkanes (1470–1340 cm^{-1}) present in the spectra and three peaks assigned to the C-O ester carbonyl (1300–1050 cm^{-1}). All the absorption peaks support the presence of terpenoids. Two peaks at 2348 and 2286 cm^{-1} could be traces of CO_2 due to the absorption of CO_2 molecules on the KBr.

The FTIR spectra of six varieties of *P. scutellarioides* appear similar to the naked eye. However, a statistical method is needed to interpret and obtain valid measurements from data with slight differences. One possible method is multivariate analysis. The varieties of *P. scutellarioides* can be grouped as in Figure 6, based on the position and intensity of the peaks. The extraction method affected the results, especially in varieties 4, 5 and 6. From Figure 7, variety 4 has the least similarity (59.89%), while varieties 1 and 3 are very similar (96.42%).

Genetic study

Some *P. scutellarioides* varieties have such different morphologies that local people in Indonesia often mistake them for other plants. As a result, only one variety is used in traditional medicine, specifically, V1. Ribulose-biphosphate carboxylase (rbcL) gene is the DNA barcode markers with better species discriminatory power than maturase K (matK) gene for the Lamiaceae[40]. Three varieties of *Datura* can be identified using the rbcL barcode[41]. The clusters that constitute clades within the same species also can be identified with higher resolution using rbcL barcode sequences. The partial length of the amplified rbcL gene sequence with rbcL1 forward and rbcL2 reverse primer on 1% agarose gel electrophoresis was 1500 bp.

Table 4 shows the analysis of sequencing and statistical simulation of Basic Local Alignment Search Tool (BLAST) sequence homology of *P. scutellarioides* with the rbcL gene. The contignous read length (CRL) values of all varieties are not significantly different from the value of quality value larger than 20 (QV20+), which indicates good quality sequence results. The similarity shows the percentage of matches between the aligned sample and database sequences. The BLAST results showed that V4 had the highest similarity with *P. scutellarioides* (99.52%), followed by V6 (99.36%), V3 (99.26%), V5 (99.05%), V2 (98.87%), and V1 (98.52%).

The following is the genetic distance between varieties using the calculation of Pairwise distance (Table 5). The smaller the number that

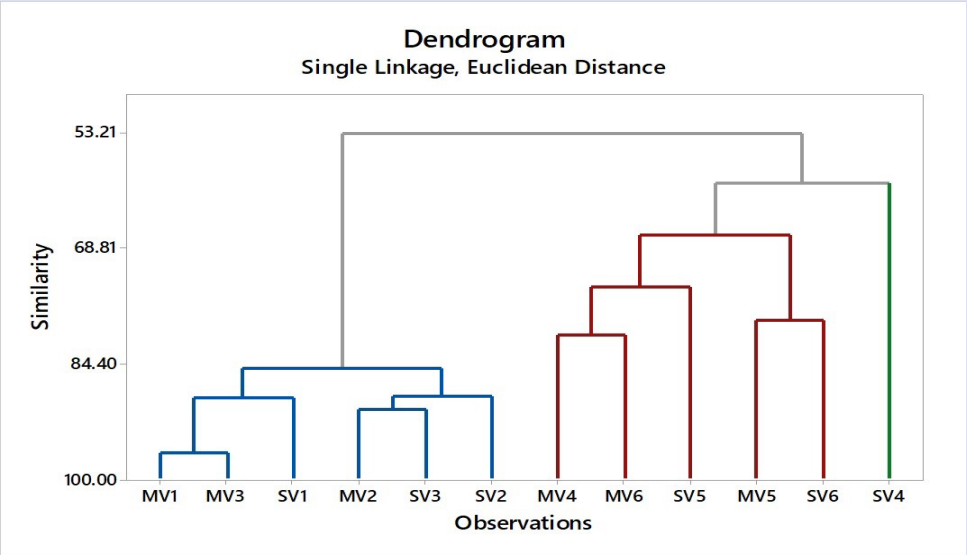

Figure 7: Dendogram of ethanol extract of six varieties *P. scutellarioides* obtained by maceration (M) and soxhlet (S).

Table 5: Genetic distance between varieties of *P. scutellarioides*.

	Trailing psycholeus	Trailing queen	Flamingo	Beale street	Trailing rose	Color blaze dark star
Trailing psycholeus						
Trailing queen	0.0050					
Flamingo	0.0058	0.0066				
Beale street	0.0033	0.0074	0.0057			
Trailing rose	0.0058	0.0058	0.0049	0.0049		
Color blaze dark star	0.0125	0.0158	0.0108	0.0149	0.0132	

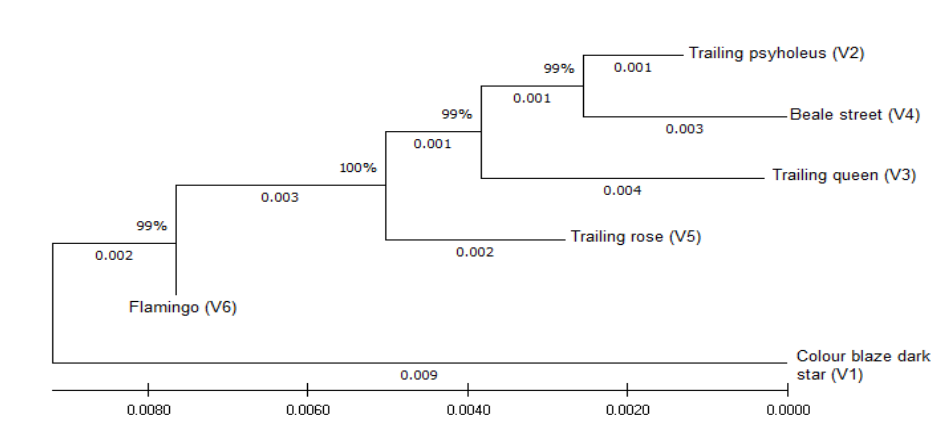

Figure 8: Phylogenetic tree of six varieties of *P. scutellarioides.*

appears, the closer the genetic distance and vice versa. From the table, it can be seen that the *color blaze dark star* variety has a far genetic distance from the other five varieties. The phylogenetic tree analysis (Figure 8) showed short branch lengths, ranging from 0.001 to 0.009. This indicates that in general the genetic changes that occur between varieties are small.

CONCLUSION

Compound analysis of six varieties of *Plectranthus scutellarioides* using three instruments and multivariate analysis showed that there were slight differences in each variety, as well as genetic results. Although only one variety is used by the Indonesian people as traditional medicine, other varieties are proven to have chemical compounds and genetic that are not much different from *color blaze dark star*. This results indicate the potential of the varieties of *P. scutellarioides* as traditional medicines, although further research is still needed in order to obtain data on its pharmacological activity. In addition, quality control and standardisation are needed in the use of *color blaze dark star* as traditional medicine in Indonesia.

SUPPORTING INFORMATION

List of constituent detected by GC-MS and visualization of 1% agarose gel electrophoresis of six varieties of *P. scutellarioides* are available as Supporting Information.

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CONFLICTS OF INTEREST

The authors declare there is no conflict of interest.

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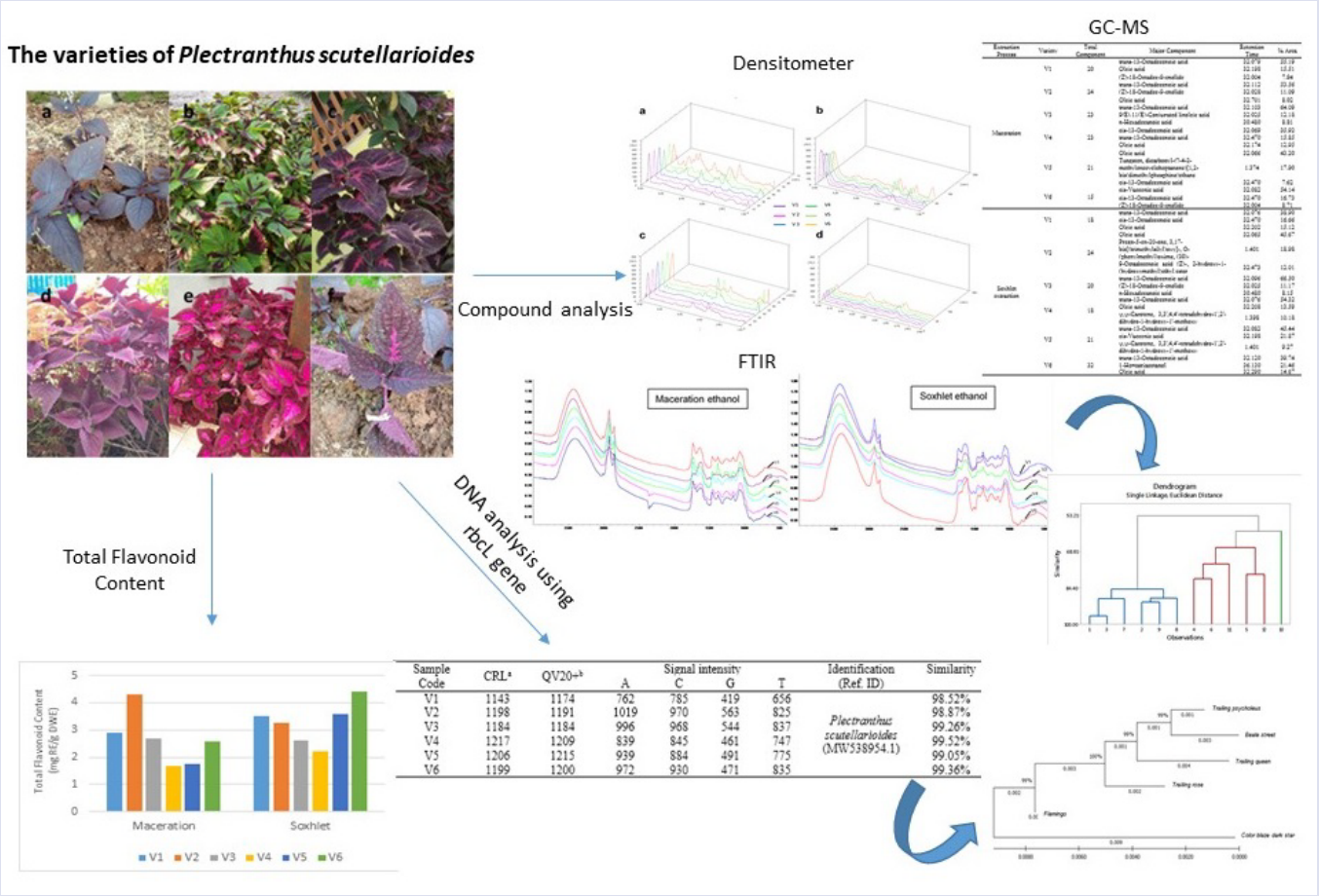
GRAPHICAL ABSTRACT



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