BIODIVERSITAS
Volume 26, Number 7, July 2025
Pages: 3623-3633

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260748

Botanical, phytochemical, and bioactivity characterization of *Coleus scutellarioides*

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Manuscript received: 13 March 2025. Revision accepted: 31 July 2025.

Abstract. *Kartini K, Shevira RA, Setiawan F, Pradana AT, Azminah A, Sukweenadhi J, Rosyidah A, Widyowati R. 2025. Botanical, phytochemical, and bioactivity characterization of* Coleus scutellarioides*. Biodiversitas 26: 3623-3633. Coleus scutellarioides*, commonly known as *miana* in Indonesia, is a versatile ornamental plant with a longstanding history of traditional medicinal use, particularly for treating inflammation and metabolic disorders. This study focuses on the purple variety due to its prevalence in traditional medicine and its distinctive features, including deep purple foliage with serrated margins. A comprehensive characterization was conducted, including botanical identification, physicochemical tests, and spectroscopic analysis (TLC, ATR-FTIR) to support its standardization as a medicinal crude drug. Biological activities were assessed for in vitro antioxidant (DPPH, NO) and enzyme inhibition (α-glucosidase, α-amylase, xanthine oxidase) assays. Botanical analysis confirmed distinct morphological and microscopic traits, aiding in varietal identification. The physical evaluation yielded acceptable standardization values: loss on drying (8.86% w/w), total ash (9.35% w/w), and acid-insoluble ash (2.57% w/w). The thick extract exhibited a moisture content of 20.04% w/w, with total and acid-insoluble ash contents of 5.44% w/w and 3.72% w/w, respectively. Thin layer chromatography and spectral analysis confirmed the presence of flavonoids and phenolic acids, with total flavonoid contents of 0.59 mg QE/g (crude drug, i.e., dried plant material prior to extraction) and 1.64 mg QE/g (concentrated extract). Extractive values indicated that water-soluble constituents dominate. Biological assays demonstrated significant antioxidant activity (IC_{50} = 70.06 µg/mL), moderate α-glucosidase (IC_{50} = 630 µg/mL), weak xanthine oxidase (IC_{50} = 900 µg/mL) and nitric oxide (IC_{50} = 2.52×10^3 µg/mL) inhibition, but no α-amylase inhibition activity. These findings support the traditional use of *C. scutellarioides* and provide scientific evidence for its potential as a natural remedy for oxidative stress and metabolic disorders.

Keywords: Antioxidant activity, botanical characterization, *Coleus*, enzyme-inhibitory properties, phytochemical profiling

INTRODUCTION

Coleus scutellarioides (L.) Benth., known in Indonesia as *miana* or *iler*, is a flowering plant from the Lamiaceae family widely distributed across Southeast Asia, including Indonesia, the Philippines, and Malaysia (Suva et al. 2015; Astuti et al. 2021; Subositi et al. 2021). It displays a wide variety of leaf shapes, colors, and patterns, making it valuable both as a traditional medicinal plant and as an ornamental. Studies in India and Indonesia have described its visual characteristics, chemical composition, and genetic variation across multiple varieties (Suva et al. 2015; Astuti et al. 2021). Field observations in Indonesia have identified 11 varieties, including purple, random pattern, neat pattern, middle green-white, middle purple-green, red feathers, finger red, middle yellow-red, colorful middle, middle large purple, and small purple (Figure 1). *C. scutellarioides* thrives in lowland areas up to 1,500 meters above sea level, commonly growing in moist, open environments such as rice field embankments, rural roadsides, and gardens. It is a soft-stemmed plant, making its stems easily broken. Although there are hundreds of varieties, in Indonesia only the purple-leaf variety is used in traditional medicine, while others are cultivated for ornamental purposes due to their attractive leaf shapes and colors. The purple variety is especially popular in traditional remedies for various ailments and holds significant potential for phytopharmaceutical research and development (Suva et al. 2015; Subositi et al. 2021).

Coleus scutellarioides is traditionally used to treat hemorrhoids, pain, menstrual irregularities, asthma, cough, worm infections, poisoning, diarrhea, abscesses, ear and eye inflammation, diabetes, stomachaches, constipation, and fever. A historic remedy combining *C. scutellarioides*, *Graptophyllum pictum*, and *Tadehagi triquetrum* has long been used for hemorrhoids, with scientific studies confirming its ability to reduce grade II and III symptoms, leading to its certification as a *Scientific Jamu* (Mardisiswojo and

Rajakmangunsudarso 1987). The Toraja ethnic group also uses its leaves for pulmonary tuberculosis. The plant is typically applied fresh, boiled, or as an infusion (Astuti et al. 2019). Pharmacological research supports its anti-inflammatory activity via COX-1, COX-2, nitric oxide, and NF-κB inhibition, as well as analgesic effects through suppression of prostaglandin synthesis (Levita et al. 2016). Additionally, it exhibits antibacterial effects against Gram-positive and Gram-negative bacteria, along with antidiabetic and cholesterol-lowering properties. These findings validate its wide ethnomedicinal use and highlight its potential for further development as a standardized herbal medicine (Salaeh et al. 2018; Yoppi et al. 2018; Aziz et al. 2021; Bismelah et al. 2022).

C. scutellarioides leaves contain flavonoids, terpenoids, alkaloids, and tannins, with flavonoids as the dominant constituents. Isolated compounds include quercetin-3-glucoside, quercitrin, several acetylated quercetin and apigenin derivatives, and luteolin glucoside (Cretton et al. 2018; Kubínová et al. 2019; Bismelah et al. 2022; Sutrisno and Kartini 2025; Kubínová et al. 2019; Bismelah et al. 2022). Previous studies rarely specify the plant variety, highlighting the need for targeted research on the purple-leafed type, which is widely used in Indonesian traditional medicine. Its distinctive anthocyanin pigmentation is linked to antioxidant potential, and it is commonly employed to treat wounds, inflammation, and digestive disorders. Morphologically, the purple variety features deep violet leaves, serrated margins, an ovate shape, and symmetrical venation, setting it apart from green or variegated forms. This study focuses on its botanical identification, bioactive profile, and therapeutic relevance to support standardization and potential pharmaceutical development.

Although *C. scutellarioides*, particularly the purple-leafed variety, is widely used in Indonesian traditional medicine for treating inflammation, wounds, and metabolic disorders, detailed studies on its botanical identity, physicochemical characteristics, and comprehensive bioactivity remain limited. Previous research has primarily focused on general phytochemical screening or pharmacological activities without specifying varietal differences (Kubínová et al. 2019; Bismelah et al. 2022). Moreover, standardized data on its crude drug properties are lacking, which hinders its potential development as a scientifically validated herbal medicine and quality-controlled raw material for industry (Astuti et al. 2021; Subositi et al. 2021).

This study investigates the purple-leafed variety of *C. scutellarioides*, lacking detailed data on its identity, physicochemical traits, and phytochemical profiles despite wide ethnomedicinal use. It characterizes morphology, composition, antioxidant, and enzyme inhibition activities, providing novel insights for differentiation, quality control, and potential therapeutic development against oxidative stress and metabolic disorders, thereby supporting its standardization and pharmaceutical application.

Figure 1. Eleven varieties of *Coleus scutellarioides* were observed at several locations in Indonesia. Panels A-K represent the following varieties: A. Purple, B. Random pattern, C. Neat pattern, D. Middle green-white, E. Middle-purple green, F. Red feathers, G. Finger red, H. Middle yellow-red, I. Colorful middle, J. Middle large purple, and K. Small purple

MATERIALS AND METHODS

Plant materials and chemicals

The plant material used in this study consisted of dried leaves of the purple variety of *Coleus scutellarioides*, obtained from the Functional Service Unit (UPF) for Traditional Health Services, Dr. Sardjito Hospital, Tawangmangu, Indonesia, in December 2023. The plant was authenticated by the institution, as documented in Plant Identification Certificate No. TL.02.04/D.XI.5/16536.491/2023. The selected variety was identified by its uniformly purple leaves, ovate shape, and slightly serrated margins. All samples were collected from mature, disease-free plants grown in shaded, well-drained soil, which reflects common cultivation conditions. Identification was carried out through macroscopic and microscopic examination, a widely accepted pharmacognostic approach. As this study focused on morphological characterization, DNA barcoding or sequencing methods were not applied. Instead, diagnostic features such as trichomes, venation patterns, and pigmentation were used to ensure accurate varietal identification.

Chemicals and enzymes were procured from Sigma Aldrich Co., including quercetin, rutin, xanthine, allopurinol, acarbose, trolox, 3,5-dinitrosalicylic acid, 4-nitrophenyl β-D-glucopyranoside, aluminum chloride ($AlCl_3$), sodium acetate, sodium carbonate, 2-aminoethyl diphenylborinate, PEG 4000, DPPH, phosphate buffer (pH 7.0), xanthine oxidase, α-amylase, and α-glucosidase. TLC Silica Gel 60 F_{254} plates and analytical-grade solvents such as HCl, methanol, ethanol, n-butanol, acetic acid, phloroglucinol, and chloral hydrate were purchased from Merck KGaA. The Griess reagent was obtained from Promega Corp.

Preparation of *Coleus scutellarioides* crude drug powder and concentrated extract

The dried *C. scutellarioides* leaves were powdered using a blender (Philips HR 2222) and sieved through a No. 20 mesh sieve (850 µm), yielding particles smaller than 850 µm. The powdered leaves were then stored in a dry, tightly sealed container. A concentrated extract of *C. scutellarioides* leaves was prepared by stirring-assisted maceration using 70% ethanol as a solvent, following these steps: 500 g of leaf powder was mixed with 3 L of 70% ethanol, stirred using an overhead stirrer (IKA RW 20; IKA-WERKE) at 500 rpm for 1 hour, left to stand overnight, and then filtered. The residue was mixed with 1.5 L of 70% ethanol and extracted again using the same method. This process was repeated twice. The extracts were combined and evaporated using a rotary evaporator (Buchi R-300; BÜCHI Labortechnik AG) at 60°C and 175 mmHg, followed by further evaporation in a water bath (WBB 22; Memmert GmbH) at 60°C until a concentrated extract was obtained (Kartini et al. 2023). The thick extract refers to a semi-solid extract that is concentrated under reduced pressure and not dried to a constant weight. Moisture content was measured as part of physical characterization relevant to semi-solid herbal preparations.

Determination of botanical characteristics

Organoleptic characteristics

The organoleptic characteristics of *C. scutellarioides* leaf crude drugs were observed using the senses to describe their shape, size, color, surface characteristics, texture, cracking characteristics, odor, and taste (Kumar et al. 2022; Obika and Obika 2023). Meanwhile, the organoleptic characteristics of the concentrated extract include consistency, color, odor, and taste.

Microscopic characteristics

Fragments of *C. scutellarioides* leaves were observed under a binocular microscope (Olympus CX-23; Olympus Corp., Shinjuku City, Tokyo, Japan) at a magnification of 10×40, using phloroglucinol, chloral hydrate, and water as reagents (Khan et al. 2020).

Determination of the physical characteristics of crude drugs and concentrated extracts

Loss on drying and water content

Loss on Drying (LOD) of the crude drugs was determined according to the protocol of the WHO (WHO 1998) and the Indonesian Herbal Pharmacopoeia Edition 2 (Health 2017) as follows: 1-2 g of crude drugs (finely ground, mesh size No. 8) was placed into a preheated short-neck weighing bottle at 105°C. The crude drug was evenly spread by shaking the bottle to form a layer approximately 5-10 mm thick, then heated in a drying oven at 105°C for 1 hour until a constant weight was achieved. The water content of the concentrated extract was determined by weighing 10 g of the extract and then heating it in a drying oven at 105°C for 5 hours until a constant weight was achieved. The LOD of the crude drug and the water content of the concentrated extract were calculated using the following formula, where W_0 and W_1 are the weight of the material before and after heating, respectively.

$$\text{LOD or water content (\%w/w)} = \left(\frac{W_0 - W_1}{W_0}\right) \times 100\%$$

Total ash and acid-insoluble ash

The total ash and acid-insoluble ash content were determined gravimetrically, referring to the WHO (WHO 1998) and the Indonesian Herbal Pharmacopoeia Edition 2 (Health 2017). 2-3 g of crude drug or concentrated extract was placed in a pre-ignited silica crucible. The sample was then gradually heated (800±25°C) in a furnace until all the carbon was burned off. The acid-insoluble ash was determined as follows: the total ash was boiled with 25 mL of diluted HCl for 5 minutes. The acid-insoluble part was filtered through ash-free filter paper, washed with hot water, and ignited until a constant weight was achieved. The total ash and acid-insoluble ash content were calculated using the following formula, where W_0, W_1, and W_2 represent the sample's initial weight, total ash weight, and acid-insoluble ash weight, respectively.

$$\text{Total ash (\%w/w)} = \left(\frac{W_1}{W_0}\right) \times 100\%$$

$$\text{Acid} - \text{insoluble ash (\%w/w)} = \left(\frac{W_2}{W_0}\right) \times 100\%$$

Determination of chemical characteristics

Thin Layer Chromatography (TLC) profile

The TLC profile of *C. scutellarioides* leaves was developed using Silica Gel 60 F_{254} as the stationary phase. *C. scutellarioides* leaf extract (1% in absolute ethanol) and reference solutions (0.1% quercetin in absolute ethanol and 0.1% rutin in absolute ethanol) were each applied by spotting 4 µL and 2 µL, respectively. The plate was then eluted with a mobile phase of n-butanol: acetic acid: water (4:1:3) with an elution distance of 8 cm. After elution, the plate was derivatized with 2-aminoethyl diphenylborinate and PEG (NP/PEG) and observed under UV light at 366 nm (Wagner and Bladt 1996; Sultana et al. 2023). This reagent system forms fluorescent complexes with flavonoids and phenolic compounds, allowing their visualization under UV light at 366 nm. The fluorescence intensity and Rf values were used to characterize flavonoids and phenolics in the sample.

FTIR spectrum

ATR-FTIR analysis was conducted on the powdered crude drug of *C. scutellarioides* using the Agilent Cary 630 FTIR Spectrometer (Agilent Technologies, Inc., CA, USA), equipped with a Diamond ATR (Attenuated Total Reflectance) accessory for sample collection and MicroLab software. The spectrum was recorded over the wavenumber range of 4000-650 cm^{-1}, with the response expressed as percent transmittance (%T) (Kartini et al. 2024, 2025). ATR-FTIR enabled direct profiling of the solid plant material without solvent extraction.

Water-soluble and ethanol-soluble extractive values

The water-soluble and ethanol-soluble extractive values were determined according to the WHO (WHO 1998) and the Indonesian Herbal Pharmacopoeia Edition 2 (Health 2017), as follows: 5 g of crude drug powder (with a mesh size of 4/18) was placed in a stoppered flask, and 100 mL of chloroform-saturated water or 70% ethanol was added. The mixture was shaken periodically for the first 6 hours and left to stand for 18 hours. Afterward, the extract was filtered, and 20.0 mL of the filtrate was evaporated to dryness in a preheated porcelain dish at 105°C. The residue from evaporation was then dried in an oven (105°C) until a constant weight was reached. The water-soluble or ethanol-soluble extractive value was calculated using the following formula, where W_1 and W_2 represent the weight of the crude drug and the weight of the extract after drying, respectively.

$$\text{Extractive value (\%w/w)} = \left(\frac{W_2}{W_1}\right) \times {}^{100}/_{20} \times 100\%$$

Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) of *C. scutellarioides* leaf crude drug and thick extract was determined using UV-Vis Spectrophotometry (Shimadzu UV 1900; Shimadzu, Kyoto, Japan) regarding the Indonesian Herbal Pharmacopoeia Edition 2 and a previous study, using quercetin as the standard (Health 2017; Kartini et al. 2019b; Sutrisno and Kartini 2025). 1.5 mL of absolute ethanol, 0.1 mL of 10 %w/v $AlCl_3$ in absolute ethanol, 0.1 mL of 1 M CH_3COONa in demineralized water, and 2.8 mL of demineralized water were added to 0.5 mL of extract. The mixture was then homogenized and incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at λ 425 nm. A series of quercetin concentrations (40, 60, 80, 100, 110, 120, 140, 160, 180, and 200 µg/mL) was prepared as the standard and reacted with the reagents in the same procedure as the sample to generate a calibration curve (y = bx + a). TFC (mg QE/g crude drug or mg QE/g concentrated extract) was calculated using the calibration curve.

Determination of the in-vitro biological activity of extracts

DPPH free radical scavenging activity

The DPPH scavenging activity of the concentrated extract was analyzed using a method similar to that employed in a study using rutin as a standard (Kartini et al. 2019a; Sukweenadhi et al. 2020). Serial concentrations of the extract (7.8-125 µg/mL) and rutin (5-25 µg/mL) were prepared. 100 µL of the extract or rutin was added to each well of a 96-well microtiter plate, followed by 50 µL of 0.026% DPPH in methanol. The mixture was then incubated in the dark for 15 minutes, and the Absorbance (A) was measured at λ 517 nm using a microplate reader (UVM 340 Biochrom; Biochrom Ltd., Cambridge, UK). A control was prepared using the same procedure, except that methanol was used instead of the extract. The DPPH scavenging capacity was calculated using the following equation, and IC_{50} was determined through linear regression (y = bx + a) between the test substance concentration (x) and the percentage inhibition (y).

$$\text{\% Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

Nitric Oxide (NO) inhibition activity

The determination of NO inhibition activity refers to previous studies with several modifications (Tsai et al. 2007; Alam et al. 2013; Kartini et al. 2019c). A 40 µL aliquot of serial concentrations of the extract (1.5×10^3, 1.75×10^3, 2×10^3, 3×10^3 µg/mL) was pipetted into the wells of a 96-well microtiter plate. Then, 60 µL of 20 mM sodium nitroprusside in phosphate-buffered saline (PBS) at pH 7.0 was added to each well, and the plate was incubated at room temperature for 180 minutes. After incubation, 100 µL of Griess reagent was added to each well, and the nitrite content was measured using a microplate reader at 540 nm. The same procedure was followed for the control, except the extract was replaced with PBS. Trolox (0.1-0.5 × 10^3 µg/mL) was used as a positive control. The percentage of NO inhibition was calculated using the following equation. The IC_{50} value was determined through linear regression analysis (y = bx + a), where *x* represents the sample concentration, and *y* represents the percentage of inhibition.

$$\text{\% Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

Xanthine oxidase (XO) inhibition activity

The XO inhibition activity was measured spectrophotometrically at 288 nm, referring to previous studies (Chen et al. 2023). Serial concentrations of the extract (200-800 µg/mL) or the reference compound allopurinol (15.6-31.25 µg/mL) were pipetted, each in 60 µL, into a reaction mixture. The solution was then supplemented with 30 µL PBS and 90 µL XO enzyme (0.2 U/mL from bovine milk). The mixture was incubated at 25°C for 15 minutes. After incubation, 30 µL of xanthine solution (0.3 mg/mL) was added, followed by an additional 30-minute incubation at 25°C. The reaction was terminated by adding 30 µL of 1 N HCl. The Absorbance (A) of the mixture was measured at 288 nm using a UV spectrophotometer. A control was prepared using the same procedure, except the extract was replaced with PBS. Allopurinol (100-500 µg/mL) was used as a positive control. XO inhibition activity was determined by calculating the percentage of inhibition using the following equation. The IC_{50} value was determined using linear regression analysis (y = bx + a), where *x* represents the concentration and *y* represents the percentage of inhibition.

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100\%$$

α-amylase and α-glucosidase inhibition activity

The inhibition of α-amylase and α-glucosidase was determined by referring to previous studies (Klomsakul and Chalopagorn 2024). α-Amylase from *Bacillus licheniformis* (1 mg/mL) was prepared in PBS. A pre-incubation mixture consisting of 250 µL of α-amylase and 500 µL of the extract was incubated at room temperature (25°C) for 60 minutes. Then, 250 µL of 1% starch solution in PBS was added, and the mixture was incubated at 25°C for 10 minutes. The reaction was stopped by adding 500 µL of 3,5-Dinitrosalicylic Acid (DNS) solution (96 mM) and further incubated in a boiling water bath (100°C) for 15 minutes. After cooling, 4.5 mL of distilled water was added to the mixture. The Absorbance (A) of the solution was measured using a spectrophotometer at a wavelength of 540 nm. A control was prepared using the same procedure, except that the extract was replaced with PBS. Acarbose was used as a positive control.

The inhibition of α-glucosidase was determined using α-glucosidase from *Saccharomyces cerevisiae* (4 IU/mL) prepared in PBS. The pre-incubation mixture consisted of 20 µL of α-glucosidase, 60 µL of the extract, and 50 µL of PBS. The mixture was incubated at 25°C for 15 minutes. Subsequently, 20 µL of 20 mM *p*-nitrophenyl-α-D-glucopyranoside in PBS was added, followed by an additional 10-minute incubation at 25°C. 100 µL of 0.1 M sodium carbonate was added to each well to stop the reaction. The plate was then read using a microplate reader at 399 nm. A control was prepared using the same procedure, except the extract was replaced with PBS. Acarbose (2-15×10³ µg/mL) was a positive control.

The inhibition activities of α-amylase and α-glucosidase were calculated using the following equation: A, B, C, and D represent the absorbance of the control, control blank, sample, and sample blank, respectively. The IC_{50} value was determined using linear regression analysis (y = bx + a), where *x* represents the sample concentration, and *y* represents the percentage of inhibition.

$$\% \text{ Inhibition} = \left\{\frac{(A - B) - (C - D)}{(A - B)}\right\} \times 100\%$$

Data analysis

Unless stated otherwise, all experiments were performed in triplicate, and data were reported as mean±Standard Deviation (SD) using descriptive statistics.

RESULTS AND DISCUSSION

Botanical characteristics of *Coleus scutellarioides* crude drugs

The purple variety of *C. scutellarioides* leaves (Figure 2) exhibited distinct morphological features, including elliptical-shaped leaflets with a pointed base, serrated edges, and a tapering tip. The upper and lower surfaces are rough, with pinnate venation, and the leaves are brittle. The leaves are purple-brown, have a characteristic odor, and are tasteless.

The microscopic characteristics of *C. scutellarioides* leaves show the characteristics of multicellular trichomes, epidermis with papillae, upper epidermis, mesophyll, lower epidermis with diacytic stomata, parenchyma with idioblasts in the form of sclereid cells, vascular bundles with spiral thickening, and sclerenchyma fibers (Figure 3).

Physical characteristics of *Coleus scutellarioides* crude drugs and concentrated extract

The high water or moisture content in crude drugs can promote the growth of microorganisms, such as bacteria, fungi, and insects, as well as hydrolysis reactions (Bansal et al. 2016). Therefore, a permissible moisture content must be established for each plant material. It is important for materials that readily absorb water or deteriorate quickly when exposed to moisture. The Loss On Drying (LOD) assessment can be used to determine the total water content and volatile substances in crude drugs. The obtained LOD value was 8.86% w/w (Table 1). The LOD value of crude drugs can be influenced by several factors, including the type of plant organ, drying method, temperature used, size of the crude drugs (whole, cut, or powdered), and storage conditions (Kim et al. 2011; Thamkaew et al. 2021). In general, the LOD of crude drugs should be less than 10% w/w. For example, the LOD for the leaves of *Ocimum sanctum*, *Eucalyptus globulus*, and *Psidium guajava* should not exceed 10% w/w. *Azadirachta indica* leaves must have a LOD of no more than 3% (Solimene et al. 2002; Health 2017). Therefore, the LOD value of 8.86% w/w for *C. scutellarioides* leaves in this study is acceptable, as it complies with the maximum limit of 10% w/w stated in the Indonesian Herbal Pharmacopoeia Edition II (Health 2017). Loss on drying (LOD) may slightly overestimate moisture content due to the potential loss of volatile compounds, particularly in species from the Lamiaceae family. Future studies may apply azeotropic or Karl Fischer titration methods for more accurate moisture determination.

The concentrated extract of *C. scutellarioides* leaves possessed the following characteristics: a thick, paste-like consistency, a dark brown color, a characteristic odor, and an astringent taste (Figure 4), with an extract water content of 20.04% w/w (Table 1). According to the Indonesian Herbal Pharmacopoeia Edition II (2017), the water content in concentrated extracts can be determined by the Azeotropic method (toluene distillation) or the gravimetric method (Health 2017).

Total ash is the accumulation of physiological ash (from plant tissue) and non-physiological ash (from extrinsic materials such as soil or sand adhering to plant surfaces). Acid-insoluble ash reflects the amount of silica-based material, such as sand or soil particles, which are indicators of contamination. Although these values are useful for assessing the cleanliness and quality of the crude drugs, medicinal plant materials must comply with regulatory standards. They should be thoroughly cleaned to remove foreign matter prior to extraction or use (WHO 1998). The total ash content in the crude drugs of *C. scutellarioides* and the concentrated extract is 9.35% w/w and 5.44% w/w, respectively, while the acid-insoluble ash content is 2.57% w/w and 3.72% w/w, respectively (Table 1).

The ash content of a material is influenced by several factors, including the mineral content of the plant. Plants with a high content of silica or calcium carbonate produce higher ash content. In addition, extrinsic factors such as soil fertility and fertilizer use, soil conditions, the geographical location of the plant, the cleanliness of raw materials, drying processes, extraction, and storage, as well as contamination by foreign materials such as insects and fungi, can affect the ash content of plant materials (WHO 1998; Kokate et al. 2007; Mandal et al. 2017).

Figure 2. Macroscopic characteristics of *Coleus scutellarioides* leaves

Figure 4. The visual appearance of the crude drug powder and concentrated extract of *Coleus scutellarioides.* Difference in color and physical characterization

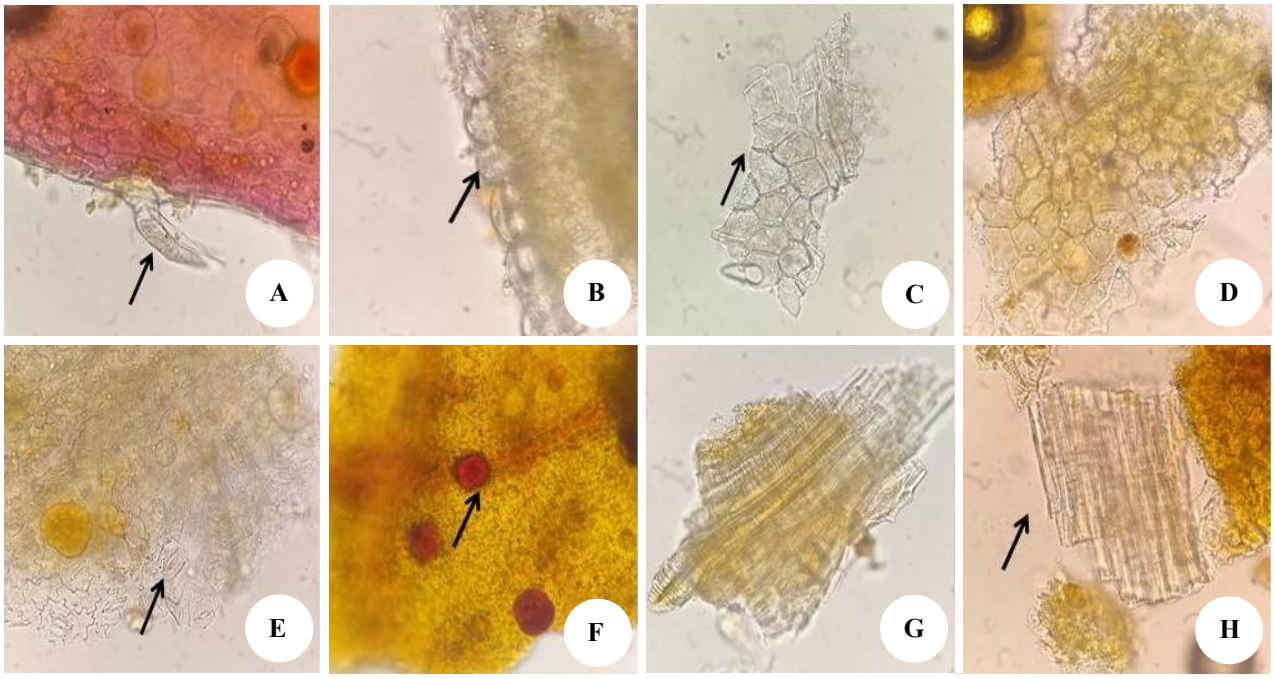


Figure 3. The microscopic characteristic of *Coleus scutellarioides* shows the following features: A. Trichomes, B. Papillae, C. Upper epidermis, D. Mesophyll, E. Lower epidermis with stomata, F. Parenchyma with idioblasts in the form of sclereid cells, G. Vascular bundles, and H. Sclerenchyma

Table 1. LOD value, water content, total ash, and acid-insoluble ash of the *Coleus scutellarioides* crude drug and concentrated extract

Parameter	Crude drugs	Concentrated extract
LOD (% w/w)	8.86±0.11	ND
Water content (% w/w)	ND	20.04±1.18
Total ash (% w/w)	9.35±0.01	5.44±0.06
Acid-insoluble ash (% w/w)	2.57±0.19	3.72±0.10

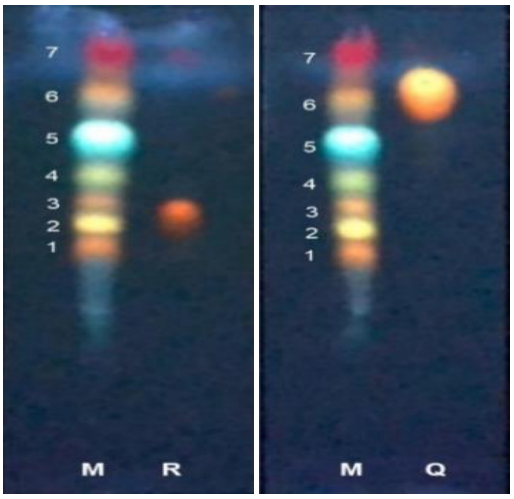
Note: ND: Not Determined

Chemical characteristics of *Coleus scutellarioides* crude drugs and extract

The chemical characteristics of *C. scutellarioides* leaves were evaluated using TLC profile, ATR-FTIR spectra, water and ethanol soluble extractive content, and total flavonoid content. The extract was assessed based on total flavonoid content. The *C. scutellarioides* leaves produced 7 fluorescent spots (Figure 5) with orange (spots no. 1, 2, 3, 6; Rf = 0.40, 0.46, 0.52, 0.77, respectively), yellow (spot no. 4; Rf = 0.57), light blue (spot no. 5; Rf = 0.67), and red (spot no. 7; Rf = 0.87) colors. Spots no. 1, 2, 3, 4, and 6 are predicted to be flavonoids; spot no. 5 is predicted to be a phenolic acid, and spot no. 7 is predicted to be chlorophyll (Wagner and Bladt 1996). Spot no. 5 exhibits a powerful intensity and a relatively wider area than the other spots, indicating that the compound is in higher amounts than the compounds predicted to be flavonoids. Multiple flavonoid-related spots suggest a diversity of flavonoid compounds in *C. scutellarioides* leaves.

The ATR-FTIR spectrum of *C. scutellarioides* leaf powder, observed in the range of 4000-650 cm^{-1}, shows absorption bands at 3267.01 cm^{-1} (O-H stretching), 2918.51 and 2860.73 cm^{-1} (C-H stretching), and 1595.30 cm^{-1} (C=C or C=O stretching) (Figure 6). This FTIR spectrum indicates that *C. scutellarioides* leaves contain hydroxyl groups (-OH), associated with phenolic or flavonoid compounds; alkyl groups (-CH_3, -CH_2-), suggesting the presence of lipids or carbon chains; and aromatic or carbonyl groups (C=C, C=O), supporting the presence of aromatic compounds such as polyphenols. These findings align with secondary metabolites such as flavonoids, tannins, or lipid compounds commonly found in *C. scutellarioides* leaf extracts (Hematian et al. 2022; Kartini et al. 2024). The results of this study are consistent with previous research, which concluded that the *C. scutellarioides* variants *Color blaze, Dark star*, and *Trailing queen* contain flavonoids, tannins, alkaloids, and terpenoids (Astuti et al. 2019).

The TLC profile and ATR-FTIR spectrum of the crude drugs provide a qualitative representation of the chemical content in *C. scutellarioides* leaves. The chemical composition of the crude drugs and extract, the water-soluble and ethanol-soluble extractive content, and the total flavonoid content were also determined. The extractive content represents the total amount of active compounds that can be extracted from the crude drugs using a specific solvent. The water-soluble and ethanol-soluble extractive contents of *C. scutellarioides* leaves were 19.97% w/w and 4.38% w/w, respectively (Table 2), indicating that the compounds in *C. scutellarioides* are predominantly water-soluble, such as polyphenols, flavonoids, tannins, and saponins. Flavonoids have been reported as the primary secondary metabolites in *C. scutellarioides* and are considered potential marker compounds for standardization. Previous studies have identified compounds such as quercetin and luteolin derivatives in the leaves. The total flavonoid content (TFC) was 0.59 mg QE/g of dry weight of crude drugs and 1.64 mg QE/g of concentrated extract (Table 2). Previous studies have reported that extracts of different *C. scutellarioides* variants, including *Color blaze, Dark star*, *Trailing psycholeus*, *Trailing queen*, *Bale street*, *Trailing rose*, and *Flamingo,* contain Total Flavonoid Content (TFC) of 2.91, 4.31, 2.70, 1.67, 1.76, and 2.57 mg RE/g Dry Weight Extract (DWE), respectively (Astuti et al. 2019).



A B

Figure 5. The TLC profile of *Coleus scutellarioides* using a stationary phase of Si Gel 60 F_{254} and a mobile phase of n-butanol: Acetic acid: water (4:1:3). Detection: NP/PEG under UV light at 366 nm. M, R, and Q represent the ethanolic extract of *C. scutellarioides* leaves, rutin, and quercetin

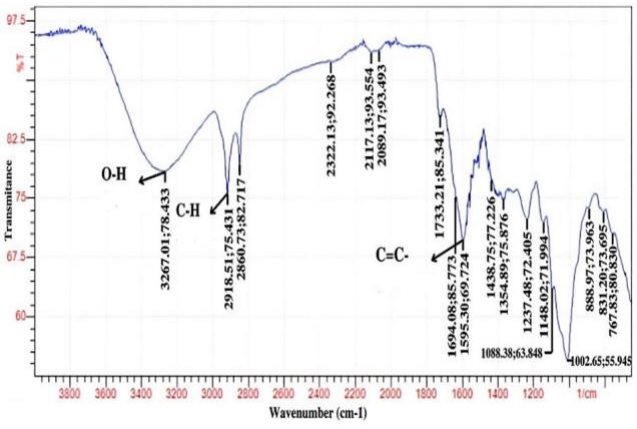


Figure 6. ATR-FTIR spectrum of the powdered crude drug of *Coleus scutellarioides*, showing characteristic peaks of major functional groups in the plant material

Bioactivity profile of *Coleus scutellarioides* leaf extract

DPPH scavenging activity

A DPPH radical scavenging assay was conducted to evaluate the antioxidant activity of *C. scutellarioides* leaf extract. The IC_{50} values of extract and standard rutin were 70.06 µg/mL and 23.16 µg/mL, respectively (Table 3). Antioxidant activity is considered strong when the IC_{50} value is less than 50 µg/mL, moderate activity when the IC_{50} value is between 50-100 µg/mL, and weak antioxidant activity when the IC_{50} value is >100 µg/mL (Thaipong et al. 2006). Thus, the *C. scutellarioides* extract has moderate antioxidant activity. The antioxidant capacity of *C. scutellarioides* leaf extract is considerably higher than that of other plant extracts reported in previous studies, such as *Phyllanthus niruri* (102 µg/mL), *Orthosiphon stamineus* (132 µg/mL), *Curcuma domestica* (361 µg/mL), *Curcuma xanthorrhiza* (538 µg/mL), *Sonchus arvensis* (1118 µg/mL), and *Apium graveolens* (2221 µg/mL) (Sukweenadhi et al. 2020). In comparison with other Lamiaceae plants widely used as traditional medicines with antioxidant or enzyme-inhibitory activities, the purple *C. scutellarioides* extract demonstrated moderate antioxidant activity (IC_{50} = 70.06 µg/mL). It is slightly weaker than *O. sanctum* (holy basil) leaf extracts, which have been reported to exhibit strong antioxidant activity with an IC_{50} value of 10 µg/mL (Chaudhary et al. 2020). *Mentha×piperita* (peppermint), another Lamiaceae species, showed antioxidant IC_{50} values of around 13 µg/mL (Hudz et al. 2023), indicating stronger than *C. scutellarioides*. However, the antioxidant activity of *C. scutellarioides* is higher than that of *Rosmarinus officinalis* (rosemary), which has been reported to have an IC_{50} value between 272 and 534 µg/mL, depending on the extraction solvent used (Kamli et al. 2022). These findings support the potential of *C. scutellarioides* leaves as a promising natural source of antioxidants.

Nitric oxide scavenging activity

Nitric Oxide (NO) is a free radical synthesized from arginine by Nitric Oxide Synthase (NOS) in biological systems. Excessive levels of NO can lead to various pathophysiological conditions such as cancer, diabetes, kidney disease, and atherosclerosis (Daenen et al. 2019; Can et al. 2022). Previous studies on NO radical scavenging activity showed that green tea extract has a strong inhibition activity against NO (IC_{50} values below 200 µg/mL), and rosemary, sweet osmanthus, rose, and lavender exhibit moderate activity (IC_{50} values between 200-400 µg/mL); jasmine, lemongrass, and aster are less effective (IC_{50} values above 600 µg/mL) (Tsai et al. 2007). The Nitric Oxide (NO) scavenging activity of *C. scutellarioides* leaf extract was evaluated to support its traditional anti-inflammatory use. The extract inhibited NO production with an IC_{50} value of 2.52×10^3 µg/mL, compared to 286.31 µg/mL for the standard trolox (Table 4). Although the extract showed relatively weak NO inhibitory activity, it provides additional insight into its potential anti-inflammatory properties.

Xanthine oxidase inhibitor

Xanthine Oxidase (XO) is an enzyme that catalyzes the formation of Uric Acid (UA) through the oxidation of purines, leading to elevated UA levels. Inhibition of XO can block the biosynthesis pathway of uric acid and reduce its production. Decreasing uric acid levels can reduce the risk of hyperuricemia and is a potential therapeutic approach for treating hyperuricemia (Azmi et al. 2012). The *C. scutellarioides* extract at 200-1000 µg/mL concentrations showed weak XO inhibitory activity, with an IC_{50} value of 900.30 µg/mL (Table 5), indicating negligible inhibition. This activity is much lower compared to *Perilla frutescens* (Lamiaceae), which exhibits more potent XO inhibitory activity with an IC_{50} value of 88 µg/mL (Ha et al. 2022), and is also weaker than several Indonesian plants such as *Sida rhombifolia*, *Acalypha indica*, *Sonchus arvensis*, and *Stelechocarpus burahol*, which have reported IC_{50} values of 91.2, 77.6, 119.0, and 128.4 µg/mL, respectively (Sianipar et al. 2022). In contrast, the standard allopurinol demonstrated a strong XO inhibitory effect with an IC_{50} value of 29.32 µg/mL. Polyphenols, flavonoids, and coumarins are compounds commonly found in plants with XO inhibition activity (Mathew et al. 2015; Fais et al. 2018; Liu et al. 2020; Xue et al. 2023). Although the XO inhibitory activity of *C. scutellarioides* was negligible, this result does not rule out its potential therapeutic properties with other mechanisms, supporting its traditional use in inflammatory conditions.

Table 2. The chemical compound in the *Coleus scutellarioides* crude drugs and concentrated extract

Chemical content parameters	Crude drugs	Concentrated extract
Water-soluble extractive content (% w/w)	19.97±0.34	ND
Ethanol-soluble extractive content (% w/w)	4.38±0.11	ND
TFC (mg QE/g sample)	0.59±0.00	1.64±0.01

Note: ND: Not Determined

Table 3. The IC_{50} value of the *Coleus scutellarioides* leaf extract as a DPPH radical scavenger

Concentration (µg/mL)		Inhibition (%)	IC_{50} (µg/mL)
Extract	7.81	6.08±0.58	70.06
	15.63	14.71±0.41	
	31.25	24.18±0.56	
	62.50	51.20±0.94	
	125.00	83.22±0.41	
Rutin	5.00	11.88±1.79	23.16
	10.00	22.76±0.77	
	15.00	35.55 ±0.39	
	20.00	45.43±0.47	
	25.00	51.42±0.63	

α-amylase and α-glucosidase inhibitor

α-amylase and α-glucosidase are two enzymes that play important roles in carbohydrate digestion and glucose absorption. Excessive activity of these enzymes can lead to a significant increase in blood sugar levels. Inhibition of these enzymes is a practical approach to managing diabetes, particularly for controlling postprandial hyperglycemia (Aryaeian et al. 2017).

C. scutellarioides extract showed only a slight inhibition against α-amylase inhibition (Table 6) even at high concentrations (10^4 µg/mL). In contrast, the standard acarbose had an IC_{50} value of 91.15 µg/mL and had inhibitory activity at 25-200 µg/mL concentrations. On the other hand, *C. scutellarioides* extract had inhibitory activity against α-glucosidase at concentrations of 200-1000 µg/mL (Table 7), with an IC_{50} value of 630 µg/mL, while the IC_{50} value for the standard acarbose is 1.7×10^4 µg/mL. These results indicate that *C. scutellarioides* extract has higher activity against α-glucosidase than α-amylase.

In terms of enzyme inhibition relevant to metabolic disorders, the α-glucosidase inhibitory activity of *C. scutellarioides* extract was more potent than that of peppermint, which showed an IC_{50} value of 1180 µg/mL for α-glucosidase inhibition (Arslan and Çam 2022). The absence of α-amylase inhibitory activity by *C. scutellarioides* extract in this study is consistent with previous findings indicating that several other Lamiaceae plants, such as peppermint, spearmint, sage, thyme, lavender, and lemon balm, also lack α-amylase inhibitory activity (Arslan and Çam 2022).

The strong antioxidant activity observed (IC_{50} = 70.06 µg/mL) is likely attributable to the high flavonoid content, as flavonoids possess multiple hydroxyl groups capable of donating hydrogen atoms to neutralize free radicals and chelate metal ions, thereby preventing oxidative damage. The NO scavenging activity further supports the role of flavonoids and phenolic acids in modulating inflammatory pathways, as excessive NO production is a known contributor to inflammatory processes. Terpenoids detected in the extract may also contribute to antioxidant and anti-inflammatory effects by inhibiting pro-inflammatory mediators, although their specific quantitative contribution was not assessed in this study. In enzyme inhibition assays, moderate α-glucosidase inhibitory activity (IC_{50} = 630 µg/mL) suggests potential utility in delaying carbohydrate digestion and glucose absorption, aligning with traditional uses of *C. scutellarioides* for metabolic regulation and diabetes management. However, the relatively low inhibitory activity against α-amylase and xanthine oxidase indicates that the extract may not be effective as a standalone inhibitor for these targets. Overall, the correlation between phytochemical classes and bioactivities observed supports the ethnomedicinal use of *C. scutellarioides* purple variety as an antioxidant and metabolic regulatory agent. Further targeted isolation and mechanistic studies are warranted to confirm which specific compounds within these classes are responsible for each biological effect.

The total ash and acid-insoluble ash fall within acceptable values for crude plant drugs, indicating low contamination and high-quality plant samples. These values, along with the extractive yields, provide essential reference data for standardizing *C. scutellarioides* as a herbal raw material. Additionally, the predominance of water-soluble extractives suggests that hydrophilic compounds such as flavonoids and phenolic acids may contribute to the biological activities.

Table 4. The IC_{50} of a Nitric Oxide (NO) scavenger of the *Coleus scutellarioides* leaf extract

Concentration (µg/mL)		Inhibition (%)	IC_{50} (µg/mL)
Extract	1.50×10^3	37.21±1.27	$2.52\times10^3\pm0.04\times10^4$
	1.75×10^3	41.07±0.53	
	2.00×10^3	43.70±1.88	
	3.00×10^3	55.65±0.04	
Trolox	100	7.32±0.39	286.31±1.98
	200	27.80±0.89	
	400	85.04±2.18	
	500	92.24±0.22	

Table 5. The IC_{50} of *Coleus scutellarioides* leaf extract as Xanthine Oxidase (XO) inhibitor

Concentration (µg/mL)		Inhibition (%)	IC_{50} (µg/mL)
Extract	200	6.78±0.25	900.30±2.12
	400	18.40±0.11	
	600	32.08±0.66	
	800	44.30±0.07	
	1000	55.70±0.26	
Allopurinol	15.6	3.31±0.12	29.32±0.25
	20.0	11.17±0.44	
	25.0	33.03±1.29	
	31.3	59.04 ±2.01	

Table 6. The IC_{50} value of α-amylase inhibitor activity of *Coleus scutellarioides* extract

Concentration (µg/mL)		Inhibition (%)	IC_{50} (µg/mL)
Extract	10^3	1.47±0.05	NO
	10^4	10.12±0.34	
Acarbose	25	26.82±0.70	91.15±1.80
	50	47.10±1.02	
	100	57.69±1.54	
	200	70.64±0.35	

Note: NO: Not Observed

Table 7. The IC_{50} value of the α-glucosidase inhibitor activity of *Coleus scutellarioides* extract

Concentration (µg/mL)		Inhibition (%)	IC_{50} (µg/mL)
Extract	200	17.38±0.04	630±0.50
	400	33.44±0.18	
	600	46.95±0.04	
	800	63.56±0.04	
	1000	78.13±0.11	
Acarbose	2×10^3	6.26±0.28	$1.7\times10^4\pm0.026\times10^4$
	5×10^3	12.85±0.54	
	7×10^3	23.07±0.88	
	10^4	36.61±0.71	
	1.5×10^4	45.98±1.15	
	2.0×10^4	53.92±1.63	

In conclusion, this study comprehensively characterizes the purple variety of *C. scutellarioides*, encompassing its botanical identity, microscopic features, physical properties, phytochemical composition, and biological activities. The extract demonstrated acceptable crude drug quality, a rich phytochemical profile including flavonoids and phenolic acids, and distinct morphological characteristics that support its authentication and standardization. Biological evaluations revealed promising antioxidant activity and moderate α-glucosidase inhibition, while inhibition of NO, XO, and α-amylase was relatively weak. These findings support the traditional use of *C. scutellarioides* in managing oxidative stress and postprandial hyperglycemia, suggesting its potential application as a natural antioxidant supplement or as an ingredient in herbal formulations for metabolic health. Furthermore, its strong ornamental appeal combined with bioactivity indicates an opportunity for development as a dual-purpose plant with both aesthetic and medicinal value. This study offers novel insights by providing the first integrated botanical, physicochemical, and bioactivity data specifically for the purple-leafed variety of *C. scutellarioides*, supporting its standardization and distinguishing its medicinal potential from other varieties commonly cultivated as ornamentals. However, further studies, including in vivo pharmacological evaluation, toxicity assessment, and formulation development, are needed to elucidate its mechanisms of action and ensure its efficacy and safety as a standardized herbal medicine. Additionally, future research comparing different varieties and collecting samples from multiple locations is warranted to comprehensively assess the pharmacological potential of this compound and support its commercial utilization.

ACKNOWLEDGEMENTS

This research was supported by the RIIM LPDP Grant and BRIN, grant numbers No. 104/IV/KS/10/2024 and No. 129/LPPM-01/PKS/X/2024. We thank the Universitas Surabaya, Indonesia, and the Research Center for Vaccine and Drug, National Research and Innovation Agency, Indonesia.

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