ORIGINAL ARTICLE

Botanical and pharmacognostic investigation of *Strobilanthes kalimantanensis*

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ABSTRACT

Under a hidden waterfall in the interior of the tropical rainforest of East Kalimantan, a new medicinal plant that produces essential oil (EO) was found with the name *Strobilanthes kalimantanensis*. The aim was to investigate the botanical and evaluate the pharmacognostic characteristics of *S. kalimantanensis* leaves from East Kalimantan, Indonesia. Pharmacognostic studies can provide recommendations for establishing quality control standards or guidelines for cultivating, harvesting, and processing *S. kalimantanensis* to ensure the consistent and reliable quality of medicinal products. Characteristic methods of *S. kalimantanensis* leaves include botanical macroscopic, fluorescence, physicochemical, and phytochemical evaluation. The plant characteristics of this plant are similar to *S. kunthia* and *S. reptans* but can be differentiated in the leaves and flowers. Fluorescence assay with sodium hydroxide 5% shows unique characteristics of secondary metabolites based on their ability to form dark green with black precipitate in Ultraviolet 365 nm. The physicochemical characteristics showed yield, water content, water-soluble, ethanol soluble, total ash value, and acid-insoluble ash. Phytochemicals showed the presence of alkaloids, polyphenols, terpenoids, and EO containing 23% trans-anethole. This evaluation report details the chemical composition, identity, and safety of *S. kalimantanensis* leaves.

Key words: Acanthaceae, East Kalimantan, essential oil, new *Strobilanthes*

INTRODUCTION

Borneo Island is the largest island in Indonesia. It has the third-largest tropical rainforest in the world, so it has a biodiversity that has the potential to be used in the development of new drugs.[1] Biodiversity in the form of medicinal plants has often been used as traditional medicine for generations. Most people use traditional medicines with proven efficacies empirically to justify their use scientifically and not solely on experience. Drug development efforts are needed to see which components are responsible for providing benefits to society.

Pharmacy standardization refers to a set of criteria, practices, and measurement techniques whose outcomes are components associated with standard requirements (chemical, biological, and pharmaceutical), such as stability assurances for pharmaceutical products. In other words, standardization also means guaranteeing that the final drug product (drug or extract product) has fixed, unchanging values for specific parameters.[2] Identifying

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botanical species or varieties is very important in the case of new medicinal plants. Documentation and collections can be used to identify medicinal and essential oil (EO) plants from the country of origin.

Strobilanthes kalimantanensis EO plant was found in the Inar waterfall forest, Temula Village, West Kutai Regency, East Kalimantan. The Dayak Tunjung and Dayak Benuaq traditionally use them as a medicine for fever and asthma of breath. Its natural habitat grows on the cliffs around the waterfall, which has a wet area. Based on the source, it is necessary to investigate the pharmacognostic, botanical, and activity of *S. kalimantanensis*. This information can be essential to guarantee sustainable and high-quality sources, such as pharmaceutical raw materials. To learn more about new species and their advantages, research is required on plant properties, chemical composition, and activity. The need for raw materials and distribution in the world is very high. There must be a guarantee of the quality of the product, and also, the scientific data, mainly pharmacognostic character, is limited. This research aims to evaluate the pharmacognostic characteristics of *S. kalimantanensis* from the Acanthaceae family to guarantee the marketed plants' quality and authenticity.[3]

MATERIALS AND METHODS

Material

The equipment used in this study was a rotary evaporator (Buchi®), grinder, D7100 Nikon®, furnace, ultraviolet (UV) light (254 and 365 nm), azeotropic distillation (Phyrex®), and gas chromatography/mass spectrometry (GC-MS) 7890A (Agilent®). The materials used in this study consisted of Fluorescence reagents (NaOH 5%, HCl 5%, NH_4OH 25%, and H_2SO_4 5%) (Merck®), phytochemical reagent (Dragendorf, Mayer, Wagner, glacial-HCl, magnesium powder, $FeCl_3$ 1%, glacial-H_2SO_4) (Merck®), toluene (Merck®), ethanol 96%, chloroform (Merck®), and aquadest.

Collection and plant preparation

S. kalimantanensis seeds were collected from West Kutai and bred in Samarinda City, East Kalimantan. After 3 months, the fresh leaves are ready to be harvested. Number sample: S.431-BP2TKSD-DISP-9-2021 was collected at the Research and Development Institute for Natural Resources Conservation Technology in Samboja, Indonesia. Leaf samples were dried, powdered, and then macerated with 96% ethanol. EO extraction was performed using the hydrodistillation technique.

Macroscopic evaluation

The botany of *S. kalimantanensis* leaves was investigated using visual organ observations. Fresh leaf samples were identified visually based on organoleptic characteristics, including color, shape, and specific components inside the leaf samples.

Fluorescence evaluation

In earlier research, a process was followed to evaluate the fluorescence of the dried powder of the leaf sample.[4] The powder and crude ethanol extract were put into a drip plate and dripped with a reagent solution. Color changes were observed using visible light and ultraviolet at 254 and 365 nm.

Physicochemical evaluation

Physicochemical analysis, including water content, water-soluble, alcohol-soluble, ash value, and acid-insoluble ash value. The water content of azeotropic distillation using toluene. Ash content analysis uses temperatures at 500°C to remove organic matter. The acid-insoluble ash content can be used to calculate the inorganic content.[5]

Phytochemical evaluation

Phytochemical investigation of ethanol extract of *S. kalimantanensis* leaves was performed using chemical reagents based on the literature to identify phytoconstituents such as alkaloids, flavonoids, polyphenols, terpenoids, and saponin.[6] Trans-anethole investigation of EO content, diluted with chloroform (1/100). It was analyzed utilizing GC-MS (Agilent®). The EO (1 µL) using 30 m open tubular column, inner diameter 2 mm, gradient injector temperature (120°C, 200°C, 250°C, and 270°C), temperature in 8°C/min, elution with helium, and flow rate in 0.93 mL/min, comparing compound with 1% trans-anethole standard.

$$\text{Trans-anethole content} = \frac{\text{Area sample}}{\text{Area standartd}} \times \text{Concentration standartd} \times \text{Dilution factor}$$

RESULTS

Authentication and macroscopic investigation

The authentication of *S. kalimantanensis* through the identification process at the Research Institute for Natural Resources Conservation Technology (BKSDA-Wanariset) Samboja, East Kalimantan, Indonesia. Extraordinary institution for the conservation of tropical rainforest plants in East Kalimantan. The classification is shown in Table 1.

Table 1: Classification of *Strobilanthes kalimantanensis*

Division	Magnoliophyta
Class	*Magnoliopsida*
Sub-class	*Asteridae*
Ordo	*Scrophulariales*
Family	*Acanthaceae*
Genus	*Strobilanthes*
Species	*Strobilanthes kalimantanensis*

Fluorescence evaluation

The fluorescence test shows the characteristics of metabolites in the sample that can fluoresce in UV light after adding reagents. Table 2 displays the findings of fluorescence experiments on leaf extracts and powders using various reagents.

Physicochemical evaluation

The physiochemical parameters of simplicia powder are essential indicators for determining the quality and purity of herbal medicines. The physiochemical analysis includes [Table 3] water content, water-soluble, ethanol-soluble, total ash value, and acid-insoluble ash.

Phytochemical evaluation

A sample of *S. kalimantanensis* EO extracted by hydrodistillation contains Trans-anethole as a marker in Figure 1. The results of GC-MS analysis of trans-anethole content in EO was 23%.

DISCUSSION

The Acanthaceae family of *Strobilanthes* is widely distributed from South to East Asia. Botanists have identified plants from tropical rainforests and examined their distribution, origins, and specific characteristics. *S. kalimantanensis* has never been found anywhere else, based on the reports of this botanical investigation of the new species. Confirmation has been strengthened by registration and storage of the Wanariset Herbarium collection (Number: WCP-01), which was discovered at the coordinates map as shown in Figure 2. Authentication of *S. kalimantanensis* is based on literature studies of several of its related species. The macroscopic study was carried out based on similarities to *S. kunthia* and *S. reptans*. Table 4 and Figure 3 show the complete physical observations-based morphology characteristics of the *S. kalimantanensis* plant. The habitat of this plant is in East Kalimantan, Indonesia, like many other *Strobilanthes* genera spread across Indonesia and Southeast Asia.[6] Based on a comparison of organ morphology against *S. kunthia* and *S. reptans*. *S. kalimantanensis* plant has smaller plant, leaf, and flower sizes. Its main characteristics can be seen in the abaxial leaf.

Inflorescence with four purple lobes in the form of broad uninterrupted spikes, densely covered with short yellow hairs. The base of the flower petals has a distinctive pattern of a combination of red and white lines. Wet soil is the *S. kalimantanensis* plant's natural habitat. Hydrophyte plants have roots and stems in the water, and their leaves grow upward. It can be planted in tropical climates with lots of sunlight, as shown in Figure 4. Powder characteristics such as color and physical texture are essential in delivering raw materials. The physiochemical parameters of simplicia are important indicators for determining the quality and purity of herbal medicines.

Physiochemical values can later help determine the authenticity, purity, and contamination of natural medicinal plants or EO plants. Fluorescence is a phenomenon of chemical elements in secondary metabolites owned by plant material. Special characteristics of secondary metabolites occur in the fluorescence test using NaOH 5%. A dark green precipitate with black is formed when exposed to UV 365 nm, as shown in Table 2. The color resulting from the reaction with sodium hydroxide is related to the content of secondary metabolites such as anthocyanins, quinones, or

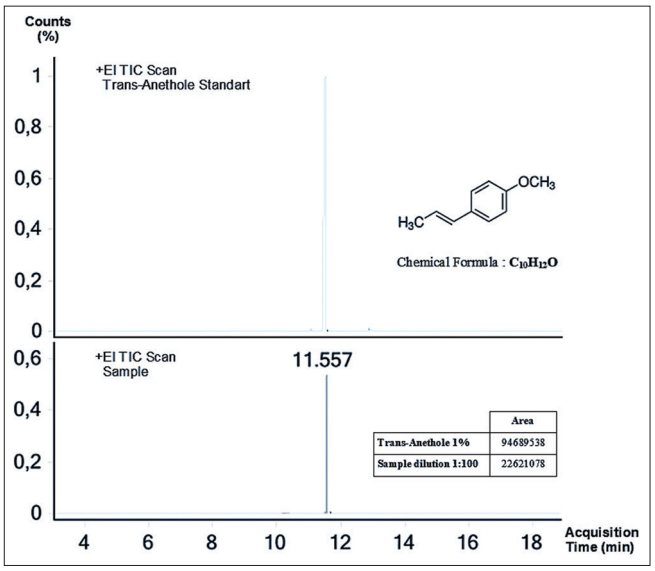

Figure 1: Gas chromatography mass spectrometry analysis of trans-anethole from essential oil sample

Table 2: Fluorescence character of extract

Treatment	Visible	UV light	
		254 nm	365 nm
Ethanol extract	Light green	Light green	Black with bright-red
Aquadest	Dark black	Light green	Dark green
+ NaOH 5%	Dark green	Light green	Dark green with black precipitate*
+ HCl 5%	Yellowish green	Light green	Reddish black
+ H_2SO_4 5%	Dark green	Light green	Reddish green
+ NH_4OH 25%	Yellowish-light green	Light green	Reddish black

*Special characteristic. UV: Ultraviolet light

Figure 2: Map coordinates of *Strobilanthes kalimantanensis*

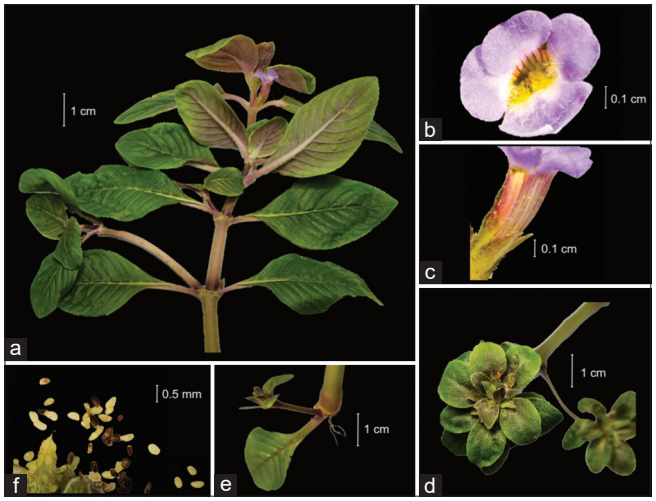

Figure 3: (a) Type opposite decussate leaf, the lower midrib, and the veins are red; (b) Purple axillaries flos with four lobes; (c) Calyx segments fused into a tube; (e) New leaves and brace roots at nodes; (d) Leaves and stems in old plants; (f) Pollen grains are oval from young to mature (green-yellow-black)

coumarins. This compound is known to have antioxidant, antimicrobial, and anti-inflammatory activity.[9,10] This method can be an essential parameter of pharmacognostic evaluation in the field.[6] A more measurable analysis can also use Fourier-transform infrared spectroscopy for metabolomic profiling.[11] Phytochemical and physicochemical values are essential for agriculture quantitative standards.

Physicochemical values [Table 3] are important for evaluating crude drugs. The water content of the

Table 3: Physiochemical parameters of simplicia

Parameter	Average (% w/w)
Water content	1.41±0.050
Water soluble	4.60±0.748
Ethanol soluble	11.00±1.414
Total ash value	7.83±0.001
Acid insoluble ash	3.04±1.247

S. kalimantanensis extract is essential to maintain stability from the threat of contamination. The solubility of the extract in ethanol is better than water, making it easier to use. The acid-insoluble ash content with a value of 3.04% ± 1.247% indicates the presence of small silica or silicate contamination in the soil. A balanced silica content in the soil will help the absorption of beneficial minerals into the roots, which are used in metabolic processes.[12]

This EO plant is known to have a content of 0.547%.[13] GC-MS compounds identified in EO are octenol (attractants and traps),[14] linalool (antidepressant),[15] estragole, humulene (antioxidant, anti-inflammatory, and antimicrobial),[16,17] threo-anethole glycol, neophytadiene (anxiolytic and anticonvulsant),[18] and the majority compound of trans-anethole (antibacterial).[19] Major compounds can be markers for the activity of EO as pharmaceutical raw materials. Trans-anethole in *S. kalimantanensis* leaves can be an alternative to the primary commodity of this component, which is currently sourced from the flowers of the *Pimpinella anisum* and *Illicium verum*.[20]

Table 4: Comparison of characteristics of *Strobilanthes kalimantanensis* with *Strobilanthes kunthia* and *Strobilanthes reptans*

Character	*Strobilanthes kalimantanensis*	*Strobilanthes kunthia*[7]	*Strobilanthes reptans*[8]
Habit	Grassy; ca 1 m high, hydrophytic	Shrubs; ca 2 m high, branches stout	Grassy; ca 1 m high
Distribution	Indonesia (East Borneo)	India, South East Asia	South East Asia
Size of leaf (mm)	30×50	39×65	35×60
Leaf shape	Elliptic oblong-ovate; apex obtuse or obtusely acuminate	Elliptic oblong-ovate; apex very obtusely acuminate	Elliptic oblong-ovate; very obtuse or obtusely acuminate
Leaf margin	Entire to distinctly serrate	Entire to distinctly serrate	Sub-entire to repand or shallowly crenate
Abaxial leaf	Dense white woolly indumentum; ventrally light red	Sparsely to densely white farinate	Sparsely pubescent; ventrally red
Adaxial leaf	Green; glabrous	Green; glabrous or sparsely covered with short	Dark green; glabrous, ventrally red
Inflorescence	Broad uninterrupted spikes, densely covered in short yellow hairs; 4–5 mm × 8–10 mm	Broad uninterrupted spikes, densely covered in short white hairs; 6–12 mm × 12–43 mm	Broad uninterrupted spikes, dense woolly indumentum; 6–7 mm × 13–15 mm
Calyx fusion	Fused from the base for 0.2–0.3 of total length at anthesis; 4 lobes	Fused from the base for 0.3–0.4 of total length at anthesis; 5 lobes	Fused from the base for 0.5–0.6 of total length at anthesis; 5 lobed
Bract: Calyx	Equal or shorter	Longer	Equal
Corolla	Ovate equally divided; single lipped; 4–6 mm	Orbicular or suborbicular; clear 2-lipped; 20–30 mm	Equally divided; slightly 2-lipped; sub-orbicular, 11–12 mm
Stamens	Present	Present	Present
Ovary apex	Pubescence	Pubescence	Pubescence

Table 5: Phytochemical screening of the extract

Constituent	Test	Result	Information
Alkaloid	Dragendorff	+	Orange-red precipitate
	Mayer	+	White precipitate
	Wagner	+	Brown
Flavonoid	HCl + Mg	−	Yellowish green
Polyphenolic	$FeCl_3$ 1%	+	Dark green
Terpenoid	H_2SO_4	+	Purplish red
Saponin	Water with shaken + HCl	−	Green without foam

+: Detected, −: Not detected

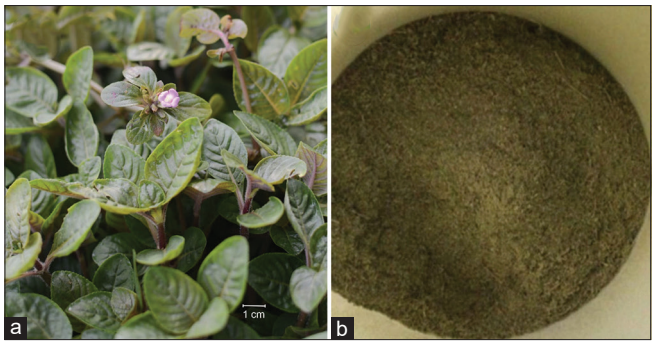

Figure 4: (a) *Strobilanthes kalimantanensis* plant can be cultivated in wet soil, producing many branches with dense growth. (b) Simplicia leaf powder has a soft texture and light green color

The ethanol extract of *S. kalimantanensis* leaves was detected to contain alkaloids, polyphenols, and terpenoids [Table 5], which are used therapeutically to treat hypertension, antimicrobial, and anti-inflammatory.[21] In the Acanthaceae family, several genera of *Strobilanthes* are known to have anticancer activity (*S. crispus*),[22] antimicrobial (*S. glutinous*),[23] antioxidant (*S. erectus*),[24] anti-inflammatory (*S. formosanus*),[7] antidiabetic (*S. cordifolia*),[25] antidepressant (*S. japonici*),[16] and analgesic (*S. ciliatus*).[26] This activity proves the potential for activity in the genus *Strobilanthes*, including the leaves of *S. kalimantanensis*. Physiochemical content and value are helpful in determining the authenticity, activity, purity, and contamination of natural medicinal plants or EO plants.

CONCLUSION

The preliminary phytochemical, physicochemical, and botanical investigation confirmed *S. kalimantanensis* as the new species. The assessment offers details on the botanical investigation, chemical ingredients of this simplicia and ethanol crude extract, and quantity of trans-anethole in EO for marker compound.

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Conflicts of interest

There are no conflicts of interest.

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