

GC-MS PROFILE AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM *STROBILANTHES KALIMANTANENSIS* LEAVES: A NEW SPECIES FROM EAST KALIMANTAN, INDONESIA

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ABSTRACT

Objective: The aim was to determine the botanical, GC-MS chemical characteristics and antibacterial activity of *Strobilanthes kalimantanensis* as a new species.

Methods: Characterization of plants through microscopic and macroscopic analysis, isolation of essential oil (EO) by steam distillation, GC-MS analysis of essential oil content and antibacterial effectiveness tests.

Results: The results of the steam distillation process of essential oil produced yield 0.547%, clear and has fresh scent. GC-MS results from essential oils identified major phytochemicals, including anethole and estragole. Antibacterial activity of EO is 100 mg/ml against *Staphylococcus aureus* and 150 mg/ml against *Bacillus subtilis*, *Escherichia coli*, and *Streptococcus epidermidis*.

Conclusion: Plant with the first species reported as *Strobilanthes kalimantanensis* contains essential oils that have antibacterial activity.

Keywords: Essential oil, *Strobilanthes kalimantanensis*, Antibacterial

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INTRODUCTION

The island of Kalimantan (Borneo) is included in three countries, Indonesia, Brunei Darussalam, and Malaysia, part of Sabah-Sarawak. The largest island in most of Indonesia is located in the biogeographical subregion of Sunda, which WWF has long recognized as a center of biodiversity. The island's geography consists of tropical rainforests and rivers that create an ecosystem for biodiversity to live [1]. Kalimantan, which has a large forest area, is believed to contain many undescribed endemic species still. Most biologists in botany agree that the plant species have few collections. The botanical exploration carried out by Herbarium Wanariset in 2019 has 19 799 collection items, but only 3675 species have been determined [2]. Botanical exploration, such as medicinal plants, must be accompanied by preserving culture and local wisdom. The culture of using traditional medicine in Indonesia depended on herbs for health care needs. Efforts to preserve the biodiversity of tropical rainforest plants to avoid extinction. Threatening factors include (1) shifting knowledge about, (2) distribution of local medicinal plants and (3) degradation of traditional knowledge [3].

Strobilanthes (Acanthaceae) plant was discovered from exploration in the tropical rainforest of East Kalimantan, Indonesia. The people call it "Lintut", the leaves are unique because they have a fresh and distinctive essential oil scent. They are a native endemic species, and their uses have not been identified. The empirical benefits of the *Strobilanthes* plant are used as respiratory lozenges by inhaling the aromatic of the crushed fresh leaves. The intense aromatic produced by plant organs is due to the high content of essential oils (EO). Its growth is different from that of EO-producing plants because it grows rapidly in an aqueous environment. Several basic potentials of essential oils can be identified from their antibacterial activity. The activity of the phenylpropanoid derivatives found in EO contains many phenol groups, which are antibacterial substances. Based on the origin, it is necessary to carry out the morphological, phytochemical, and activity characteristics of the leaves of *Strobilanthes*. This can be vital information to ensure quality and sustainable sources as pharmaceutical raw materials [4, 5]. Studies on plant characteristics, chemical content and antibacterial activity are needed to reveal new species and their benefits.

MATERIALS AND METHODS

Materials

Muller Hilton agar (MHA Merck®), Sodium chloride 0.9% (Widatra®), Dimethyl sulfoxide (DMSO Merck®), Mc. Farland 0.5 standart solution, Paper disk (Macherey Nagel®), Aquadest, chloramphenicol USP Grade (Phytotechlab®), *Staphylococcus aureus* (ATCC 25923®), *Bacillus subtilis* (ATCC 6633®), *Escherichia coli* (ATCC 25922®), *Pseudomonas aeruginosa* (ATCC 9027®) and *Streptococcus epidermidis* (ATCC 12228®).

Equipment

Steam Distillation, Chiller (Buchi), gas chromatography (Agilent Technologies 7890A GC System) and mass spectrometry (Agilent® Technologies 5975C Inert XL EI), microscope (Olympus CX 23 with ObtiLab Viewer®), UV/Vis Spectrophotometer (Halo DB-20S), micrometer (Tricle®), Anova (SPSS® Statistics Version 22) and Tukey HSD (Honestly Significant Difference).

Methods

Preparation of plant

Cultivation and determination of *Strobilanthes* leaf seedlings were taken from the West Kutai area of East Kalimantan, then cultivated in a field of 10x5 m². Harvest the leaves of mature plants after reaching the age of 3 mo. Specimens of plant organs were observed as macroscopic and microscopic. Fresh samples and herbarium consisting of complete organs were determined (Number S.431/BP2TKSDA/DISP/9/2021) by the Research Institute for Natural Resources Conservation Technology Samboja, East Kalimantan, accompanied by a profile of the origin of the plant. Plant organs were analyzed macroscopic and microscopic [6].

Plant essential oils distillation

Fresh and clean *Strobilanthes sp* leaves were weighed and put into a distillation apparatus using distilled water as a solvent for the steam distillation process. The distillation process was carried out at a

temperature of 105 °C for 4-6 h [7]. The resulting distillate is then filtered and separated from the solvent. The distillation was replicated five times with grams of sample each, and the average weight of the EO obtained was calculated as the yield value of the EO.

$$\text{Yield} = \frac{\text{Essential oil value (grams)}}{\text{Essential oil yield (grams)}} 100\%$$

Essential oils content analysis

EO was analyzed using gas chromatography and Mass Spectrometry. The oil sample was diluted 10 times and then analyzed using GC-MS (Agilent®) Central Laboratory of Padjajaran University. EO sample (1 µl) under Wall Coated Open Tubular (WCOT) column conditions, length and diameter of column (30 m, 2 mm), temperature of injector, detector, beginning of the column, and end of column (270, 250, 200, and 120 °C), temperature program column (8 °C/min), detector (MS), carrier gas (helium), and flow rate (0.93 ml/min) [8].

Antibacterial activity test

The EO obtained was tested for *in vitro* bioactivity as an antibacterial. Research design with five concentration variations and at least 3 times of replication and using a control in the form of a comparison.

Test Bacteria Preparation. Rejuvenation of the test bacteria was carried out by inoculating it into MHA. The test bacteria used were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*. Bacterial cultures were incubated for 1 x 24 h at 37 °C. The results of the inoculation were suspended by adding NaCl 0.9% to obtain a suspension with an absorbance of 0.8-1 at an absorption spectrophotometer 600 nm according to Mc Farland 0.5 (1.5 x 10⁸ CFU/ml).

Disk Diffusion Method Antibacterial Activity Test. MHA (10 ml) was put into a petri dish and waited for it to solidify. Then 200 µl of the bacterial test suspension was added to the solidified MHA medium. The treatment designs consisted of test samples (concentration 50, 100, 150, 200 and 250 mg/ml), negative control (DMSO 10%) and positive control of chloramphenicol 30 µl/ml. Sample (20 µl) consisting of control and variation of test concentration in ethyl acetate solvent was inserted into a paper disk, then dried for 30 min at room temperature. Paper disks that have been treated each are placed on the surface of the test bacteria inoculated media. Then

incubated for 1 x 24 h at 37 °C. The clear zone formed was then measured using a micrometer [9]. The measurement results were analyzed using Anova SPSS Statistics Version 22 and the Tukey HSD (Honestly Significant Difference).

RESULTS

Plant new report identification

Strobilanthes (Acanthaceae) are widely distributed in eastern Nepal, spreading to China and Southeast Asia. Botanists of tropical forest plants expert have studied the origin, distribution, and characteristics of plants. Based on this botanical study of the *Strobilanthes* genus, new reports of the species as *Strobilanthes kalimantanensis*.



Fig. 1: *Strobilanthes kalimantanensis* plant

Classification of these plants as follows: kingdom *Plantae*; divisi *Magnoliophyta*; Kelas *Magnoliopsida*; subclass *Asteridae*; ordo *Scrophulariales*; family *Acanthaceae*; genus *Strobilanthes*; species *Strobilanthes kalimantanensis*.

The natural habitat of the *S. kalimantanensis* plant is in wet soil. Hydrophytic plants whose body parts are above the water surface and their roots are underground. It is suitable to grow in tropical areas that are rich in sunlight. The complete morphology of the *S. kalimantanensis* plant based on physical observations is shown in table 1 and fig. 2, while the microscopic leaf tissue is shown in fig. 3.

Table 1: Plant morphology of *Strobilanthes kalimantanensis*

No	Organ	Morphology
1	Leaves	Measuring 2-5 cm are single leaves. The shape of the leaves is oval, the edges of the leaves are serrated, the upper and lower surfaces of the leaves are slightly rough and dark green in color
2	Flowers	small, form buds, white with a height of 1-2 cm
3	stem	Wet stem type is green with a length of 25-30 cm
4	Seed	Very small, black, found in mature flower buds.
5	Root	Fibrous root type, forming stolons for reproduction.



Fig. 2: Morphology of cultivated *S. kalimantanensis* (A), flower shape (B), height and width of leaf (C), and shape and length of root (D)

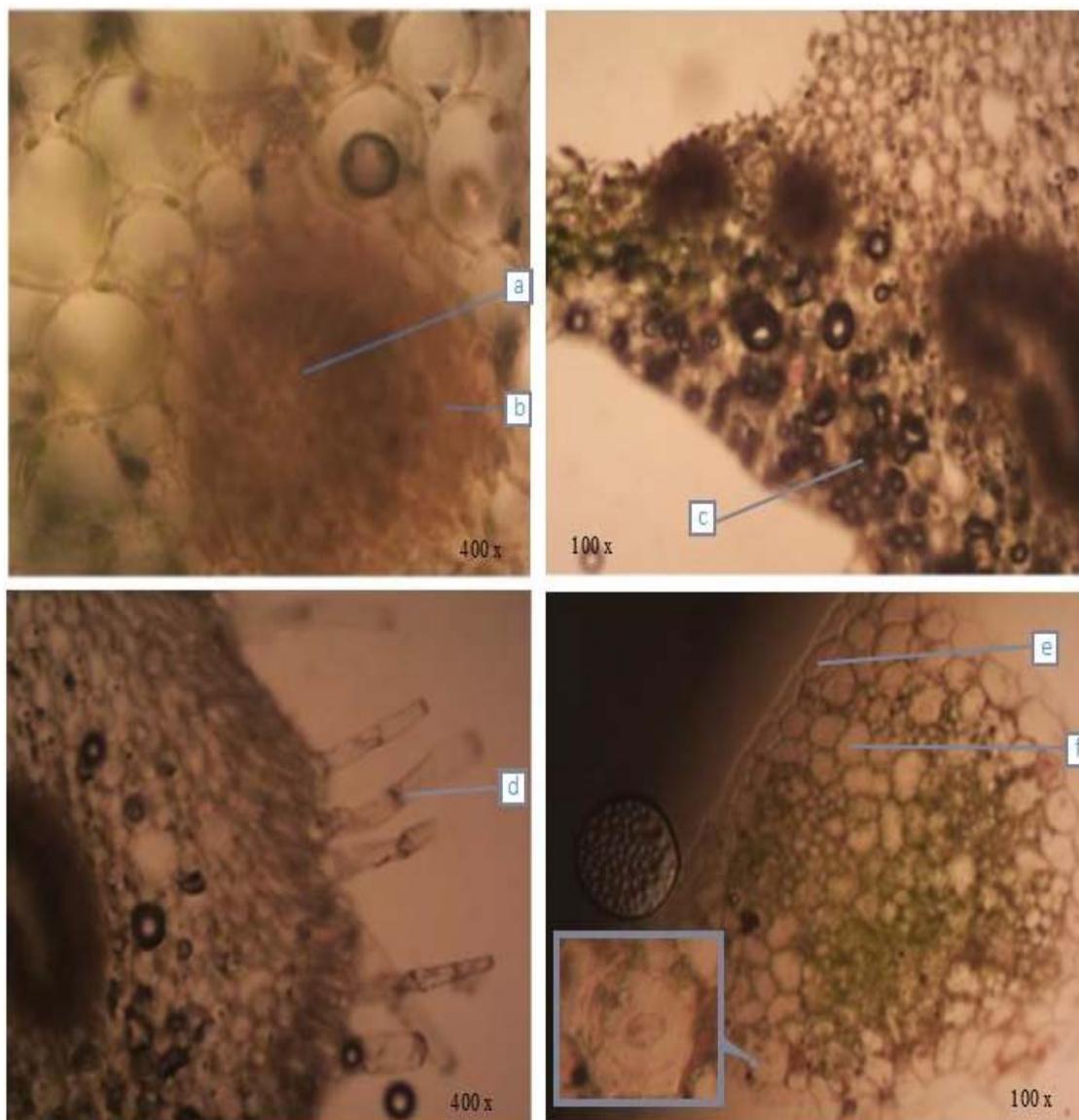


Fig. 3: Microscopic of *S. kalimantanensis* leaf tissue on (a) xylem; (b) phloem; (c) oil glands; (d) trichomes; (e) upper epidermis; (f) cortex; diacytic stomata in mark zoom

Essential oils yield from the distillation

Data analysis was obtained based on the calculated value of the percent yield of EO (table 2). The average yield of essential oils produced from the steam distillation process of the leaves of *S. kalimantanensis* is 0.547%, with the characteristics of clear yellowish color and fresh aromatic.

Separation and characteristics of essential oils

Several conventional ways to obtain commercial essential oils include water distillation and steam distillation. This method is the most widely used, because it is still considered cheaper and easier to do than using other methods [10]. The essential oils obtained and

analyzed from the GC-MS database contained several major compounds (fig. 4) (table 1).

Compounds of estragole (methyl eugenol) and anethole are major constituents of great interest. The compound is an EO component derived from phenylpropanoid. Then it will be converted into final product essential oils such as caryophyllene, phytol and linalool.

Essential oil antibacterial activity

The results of essential oil activity in an inhibition zone were analyzed using Kolmogorov-Smirnov and obtained homogeneous and normal data.

Table 2: Essential oil yield of *S. kalimantanensis* with steam distillation

Fresh leaves (g)	Essential oil yield (g)	Essential oil (%)
500	1.417	0.283
454	2.349	0.517
139	3.021	0.604
498	3.795	0.761
391	2.230	0.570
Average of essential oil		0.547

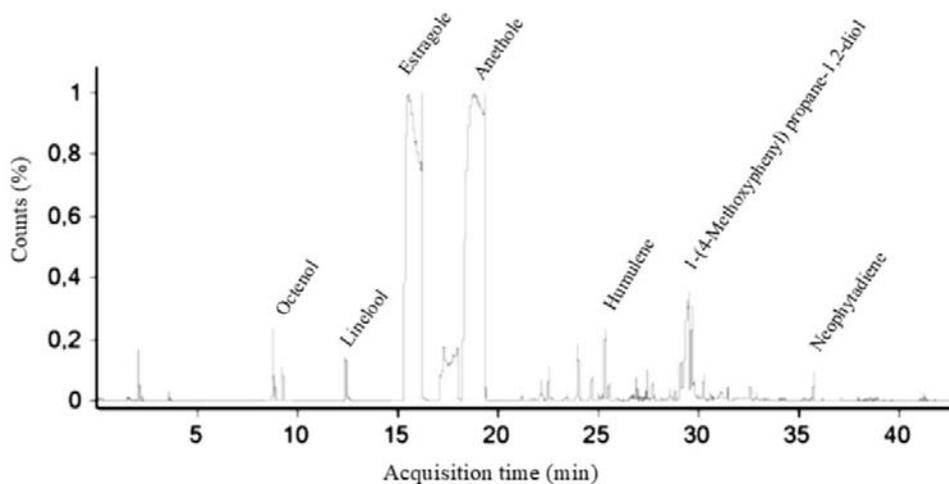


Fig. 4: Chromatogram (GC-MS analysis) of essential oil from leaves of *S. kalimantanensis*

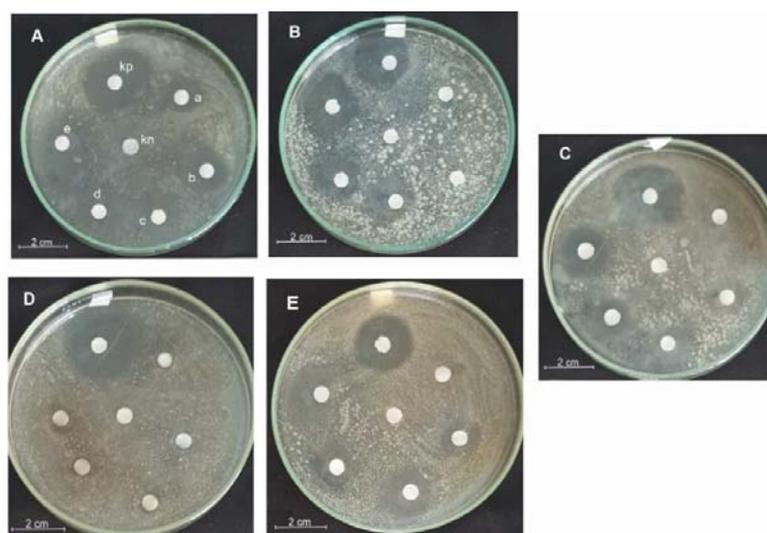


Fig. 4: Antibacterial activity of *S. kalimantanensis* leaf essential oil 50 mg/ml (a), 100 mg/ml (b), 150 mg/ml (c), 200 mg/ml (d), 250 mg/ml (e), chloramphenicol 30 µg/ml (kp), DMSO 10% (kn), against *Staphylococcus aureus* (A), *Bacillus subtilis* (B), *Escherichia coli* (C), *Pseudomonas aeruginosa* (D) and *Staphylococcus epidermidis* (E)

The EO from the leaves of *S. kalimantanensis* has activity with 100 mg/ml against *Staphylococcus aureus* and 150 mg/ml against *Bacillus subtilis*, *Escherichia coli*, and *Streptococcus epidermidis*, shown in fig. 4. The potential for EO (Tukey HSD test) with 200 mg/ml against *Bacillus subtilis* and 250 mg/ml against *Escherichia coli* was equivalent to the activity in the control chloramphenicol 30 µl/ml.

DISCUSSION

This genus in Indonesia, reportedly spread in Java, Sumatra, Bali, Flores and Sumbawa. Found some similarities in the shape of leaves and flowers in the genus *Strobilanthes*. However, judging from the distribution, it is impossible to find the possibility of spreading to the East Kalimantan area [3, 7]. The study of *Strobilanthes* found in East Kalimantan, Indonesia, has determined that this plant is a new species.

The essential oils contained in several essential oil-producing plant commodities have different levels. The average essential oil content does not exceed 1%, so the distillation process is one of the most critical steps in extraction. When compared with the essential oil content in the leaves of *S. kalimantanensis* the levels are medium. Essential oil content is influenced by several factors such as weather conditions, harvest time, storage, and distillation techniques [12]

Some EO-producing plants of the genus *Asteraceae* contain estragole with 90%-95%. From the biosynthetic pathway, the levels of these

two compounds influence each other. A high estragole content will result in a low anethole content; conversely, if the anethole content is high, the estragole content will be very low [13]. Based on chemotaxonomic research, these two compounds can be found in several essential oil-producing plants, including *Foeniculum vulgare* (Family *Apiaceae*), *Artemisia dracunculus*, *Clausena anisata* (Family *Asteraceae*) and *Tagetes pusilla*, *Pimpinella anisum* (Family *Apiaceae*) [14-18].

The essential oils contained in some plants are known to have a strong antibacterial activity such as antibacterial, antifungal and antiviral. One of the factors responsible for this activity is that the phenylpropanoid derivatives generally have a phenol group that directly affects the permeability of the walls of living things [19, 20]. Estragole and anethole compounds are the main constituents of *S. kalimantanensis* EO. These compounds play an important role as derivatives in forming other sesquiterpene compound products. The activity of these compounds is known as antibacterial, inhibition of cancer cells (MCF-7, HEP-2, and NCI-H292), sedative, anticonvulsant, antioxidant, antimicrobial, and anesthetic [19, 21-24]. In Indonesia, *S. crispus* is a genus of *Strobilanthes*, which is widely found and used in medicine. The plant is known to have cytotoxic activity against HepG2 (liver cancer), MDA-MB-231 (hormone-dependent breast cancer) and MCF-7 (hormone-dependent breast cancer). It is known that the oil from this plant also has a common toxic effect, so it can be used as a nutraceutical material that is safe for consumption [25].

It is necessary to re-examine the toxic effects to support the antibacterial activity of EO from *S. kalimantanensis* to be used as a good pharmaceutical ingredient.

CONCLUSION

The morphological characteristic of new species: *Strobilanthes kalimantanensis* is a hydrophytic plant dominated by green leaves, wet stems, and roots forming stolons and growing in watery areas. Microscopic observations found unique characteristics in leaf tissue, including xylem, phloem, oil glands, trichomes and diacytic stomata. Essential oils (EO) are characterized as containing anethole and estragole as major components. The antibacterial activity of EO is 100 mg/ml against *Staphylococcus aureus* and 150 mg/ml against *Bacillus subtilis*, *Escherichia coli*, and *Streptococcus epidermidis*.

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AUTHORS CONTRIBUTIONS

All authors have contributed according to their expertise and abilities

CONFLICT OF INTERESTS

All author declared that have no conflict of interest

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