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Exploration of *keji beling* leaves (*Strobilanthes crispa*) from East Kalimantan as a Natural Antibacterial Agent

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Abstract

Keji beling (*Strobilanthes crispa*) is a traditional plant recognised by the community for its possible medicinal properties. This study sought to examine the secondary metabolite chemicals in the leaves of *keji beling* from East Kalimantan and their ability to suppress the growth of harmful bacteria. Maceration was conducted utilising ethanol as the solvent, which is classified as a polar solvent. Qualitative approaches were employed to assess the content of plant secondary metabolites. Antibacterial activity was assessed through the well diffusion method against the bacteria *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Streptococcus sobrinus, Streptococcus mutans*, and *Salmonella typhi*. The phytochemical research indicates that the ethanol extract of *keji beling* leaves comprises flavonoids, tannins, saponins, and steroids. The antibacterial test findings indicated that the ethanol extract of *keji beling* leaves may inhibit seven bacterial strains, exhibiting an inhibition zone of 7-9 mm at a dosage of 50-800 µg/well. The research indicates that the ethanol extract of *keji beling* leaves (*Strobilanthes crispa*) possesses potential as a natural antibacterial agent.

Keywords: Antibacterial; East Kalimantan; Phytochemicals; *Strobilanthes crispa*.

INTRODUCTION

Natural products and herbs are valuable sources of medicinal ingredients, and their therapeutic benefits have long been known. Many studies were conducted on the properties and medicinal benefits of natural bioactive chemicals, particularly those derived from plant sources such spices. Important substances called phytochemicals are present in medicinal plants and are not necessary for the body to operate normally, but they are active and have positive effects on health or the treatment of illnesses. Despite the fact that several phytochemicals are already recognized, many more have not yet been identified.

Strobilanthes crispa (L.) Blume, referred to as *keji beling* or *kecibeling*, is a perennial shrub extensively utilized in Southeast Asia as a traditional herbal treatment. Traditionally, the leaves are utilized in decoctions or teas to address kidney stone illness, urinary tract diseases, wounds, appendicitis, and diabetes mellitus, demonstrating its extensive ethnomedicinal acceptance within local communities. The plant is frequently grown as an ornamental hedge species because of its appealing foliage and blossoms, which allows for convenient household access for therapeutic purposes. Recent literature reviews and experimental research indicate that *Strobilanthes crispa*comprises numerous bioactive ingredients, including as flavonoids, phenolic compounds, and other secondary metabolites linked to antioxidant, anti-inflammatory, and anticancer effects. These chemicals validate the traditional applications of the plant and indicate that *Strobilanthes crispa*may be a significant source of lead molecules for the creation of innovative medicinal medicines (Silalahi, 2020; Sukendi et al., 2025). Keji Beling (*Strobilanthes crispa*) possesses abundant advantages and a diverse array of phytochemical substances, including potassium, sodium, calcium, silicic acid, salicylic acid, calcium carbonate crystals, alkaloids, saponins, flavonoids, polyphenols, and tannins. These compounds may subsequently serve as a source of mild alkali and stabilisers for the synthesis of metal oxide composites. A potential active molecule for development in herbal medicine is flavonoid content (Isrianto et al., 2021).

Targeted antibacterial assessments have been performed against certain microorganisms. Investigations into the ethanol extract of *keji beling* leaves revealed inhibitory effects against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, significant opportunistic pathogens in skin and wound infections. Additional studies have demonstrated the efficacy of *Strobilanthes crispa* preparations against oral pathogens, including

Fusobacterium nucleatum and *Streptococcus mutans*, particularly in formulations like herbal toothpaste, suggesting a potential application in oral health products. Furthermore, the amalgamation of *Strobilanthes crispa*extract with other therapeutic flora has been investigated to augment antibacterial effectiveness, demonstrating encouraging synergistic interactions against various bacterial species (Adriana et al., 2023; Wantenia et al., 2020; Wijaya et al., 2024). However these promising findings, numerous deficiencies persist in the existing corpus of information. Numerous research vary in extraction solvents, concentrations, and bacterial strains, complicating direct comparisons of antibacterial efficacy and the establishment of standardized therapeutic dosages. The majority of research is confined to in vitro assays, like disc diffusion, microdilution, and bioautography, with a paucity of in vivo evaluations or formulation studies that replicate authentic clinical or consumer product circumstances. Moreover, the precise active chemicals that confer antibacterial activity and their intricate methods of action remain inadequately clarified, especially concerning their interactions with bacterial cell targets and the possibility for the development of resistance. Consequently, additional systematic investigations on *Strobilanthes crispa* as a natural antibacterial agent are required, encompassing the fractionation and identification of active compounds, optimization of extraction and formulation, and assessment of safety and efficacy in more sophisticated models (Adriana et al., 2023; Junardin et al., 2024; Wangloan et al., 2025). Despite such considerations, *Strobilanthes crispa* emerges as a viable choice for the formulation of natural antibacterial agents that could supplement or serve as alternatives to synthetic antibiotics.

MATERIALS AND METHODS

Plant area and research study material

The leaves of *keji beling* (*Strobilanthescrispa*) were collected from Marga Rahayu Village, Loa Kulu District, Kutai Kartanegara, East Kalimantan. The samples were meticulously cleansed with water to eliminate extraneous material and subsequently air-dried for approximately two days in a laboratory with air conditioning set to 20-25ºC. The materials used in this study are Nutrient Broth (Himedia), *Chloramphenicol*, acetone, 1-naphthol, bismuth (III) nitrate, H2SO4 (sulfuric acid), HCl (hydrochloric acid), CH3COOH (acetic acid), NaOH, lead acetate (Pb CH3COO2), potassium iodide. The equipment used includes test tubes, Erlenmeyer flasks, petri dish, corkborer 7 mm, glass funnels, beakers, 1000 µL micropipettes, 200 µL micropipettes, yellow tips, blue tips, cuvettes, and rotary vacuum evaporators. Antibacterial used in this study such as *Propionibacterium acnes* (ATCC-6919), *Staphylococcus epidermidis* (ATCC-12228), *Staphylococcus aureus* (ATCC-29213), *Eschericia coli* (ATCC-25922), *Streptococcus sobrinus* (ATCC-33478), *Streptococcus mutans* (ATCC-25175), *Salmonella thypi* (ATCC-6539).



Figure 1. *Strobilanthes crispa* collected by author in Marga Rahayu Village.

Procedures

Maceration

The raw materials, comprising the leaves of *Strobilanthes crispa*, are initially rinsed with running water to eliminate any residual dirt, thereafter dried in a climate-controlled environment at a temperature of 20-25 ºC for 2-5 days until desiccated. The desiccated plant specimens are subsequently pulverised with a blender and prepared for maceration in ethanol solvent at ambient temperature, undergoing agitation on a shaker for approximately 48 hours. Following filtration, the crude extract of the leaves of *S. crispa* was evaporated utilising a rotating vacuum evaporator at 38-40 ºC.

Preliminary Phytochemical Analysis

The ethanol extract of the leaves of *S. crispa* was analyzed for secondary metabolites, including alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins, using qualitative testing methods (Sari et al., 2023).

Alkaloids determination: About 5 ml of the ethanol extracts from leaves of *Strobilanthes crispa* were added of 2 ml Hydrochloride Acid, then 1 ml of Dragendorff

solution was added. The color changes in the solution were indicated the presence of alkaloids.

Flavonoids determination: About 1 ml of the ethanol extracts from leaves of *S. crispa* were drops of 1% Sodium Hydroxide. The presence of yellow color at extracts solution and were colorless after addition of 1 % Hydrochloride Acid was indicated the presence of flavonoids.

Triterpenoids determination: About 1 ml of the ethanol extracts from leaves of *S. crispa* were drops about 10 Acetic Acid Anhydride and 2 drops of Sulfuric Acid, sequentially. The red or purple color changes in the solution was indicated the presence of triterpenoids.

Steroids determination: About 1 ml of the ethanol extracts from leaves of *S. crispa* were drops about 10 Acetic Acid Anhydride and 2 drops of Sulfuric Acid, sequentially. The green or blue color changes in the solution was indicated the presence of steroids.

Tannins determination: About 10 ml of the ethanol extracts from leaves of *Strobilanthes crispa* were added 1 % Lead II Acetate. The yellow precipitate reaction at solution was indicated the presence of tannin.

Saponins determination: About 10 ml of hot distilled water were added 1 ml of of the nethanol extracts from leaves of *Strobilanthes crispa*. The solution then cooled and shaken vigorously (10 seconds). A stable froth upon standing for 10 minutes after added 1 drop of Hydrochloride Acid 2N was indicated the presence of saponins.

Antibacterial analysis

Antibacterial activity of the leaves of *S. crispa* were test using agar diffusion method with slight modification (Kuspradini et al., 2018). About 20 ml aliquots of sterile media were transferred to Petri dishes and allowed to solidify. This investigation utilised several bacterial strains. Approximately 20 mL of sterile agar media solution was placed into a petri dish that had been sterilised for 30 minutes at 121°C in an autoclave. Subsequently, in a sterile environment (under laminar flow), let the medium to solidify until it is cold and firm, then inoculate with 100 µL of the bacterial suspension, spreading it uniformly across the test medium, and permit it to dry for approximately 30 minutes. The medium was pierced with a 5 mm sterile punch for each sample. In each test hole, 20 µL of a sample dissolved in acetone was added as a negative control, whereas chloramphenicol served as a positive control in the experiment. Tests were conducted with test concentrations of 50 µg, 100 µg, 200 µg, 400 µg, and 800 µg.

RESULTS AND DISCUSSION

Yield of extracts

The leaves of *S. crispa* L. were macerated by ethanol solvent at room temperature. The weight of extracts maceration was 3.93 g and the yielded was 14.83% extracts on the basic of sample dry weight.

Phytochemical analysis

Phytochemical analysis was conducted to ascertain the concentration of secondary metabolites present in plant extracts. Informed by the local community regarding the use of the *S. crispa* plant, commonly referred to as Daun Keji Beling in Marga Rahayu Village, it is imperative to analyse the secondary metabolite content prior to undertaking additional bioactivity assessments.

Table 1. Phytochemical analysis of *Strobilanthes crispa* leaves.

Sample	Compounds	Results
Strobilanthes crispa L.	Alkaloids	-
	Flavonoids	+
	Triterpenoids	-
	Steroid	+
	Tannin	+
	Saponins	+

The results showed that ethanol extract of *S. crispa* leaves contains flavonoids, steroids, tannin and saponin. Recent investigations consistently demonstrate that *S. crispa* leaves possess a diverse array of secondary metabolites that support its pharmacological properties. Phytochemical analysis of ethanol extracts has identified flavonoids, alkaloids, saponins, tannins, phenolic compounds, and other polyphenols, however certain investigations indicate inconsistent detection of terpenoids based on extraction circumstances and analytical techniques employed. The prevalence of polyphenolic chemicals, especially flavonoids and phenolic acids, corroborates previous findings and reinforces the classification of *S. crispa* as a therapeutic plant abundant in polyphenols. The results suggest that the bioactivities associated with *S. crispa*, including antioxidant, antibacterial, anticancer, and antiurolithic properties, are intricately connected to its varied phytochemical makeup. A study employing 70% ethanol and ultrasonic-assisted extraction indicated positive results for flavonoids, alkaloids, saponins, and tannins, whereas terpenoids were undetected at the specified conditions. Comparable screening methodologies in other studies also validated the existence of alkaloids, carbohydrates, phenolic compounds, tannins, saponins, flavonoids, polyphenols, and terpenoids when various extraction solvents and protocols were utilised. A distinct phytochemical investigation revealed that leaves extracted with 80% ethanol and subsequently separated using liquid–liquid partition produced an ethyl acetate fraction rich in phenolic and flavonoid compounds. The elevated flavonoid concentration and intricate polyphenolic composition further endorse the application of *S. crispa* as a natural source of antioxidants and antibacterial agents in pharmaceutical or nutraceutical formulations (Hasnaeni et al., 2025; Mahyantika et al.,

2025; Ng et al., 2021; Ramadhani et al., 2021; Rubiyanti et al., 2026).

Antibacterial activity

Antibacterial testing was performed utilising seven bacterial strains: *S. aureus*, *S. epidermidis*, *S. sobrinus*, *S. mutans*, *E. coli*, *S. typhi*, and *P. acnes*. The inhibition zone diameter of essential oil compared with chloramphenicol (CHP) as positive controls are shown in Table 2.

Table 2. Inhibition zone and activity indices of *Strobilanthes crispa* leaves extracts against antibacterial.

Bacteria	Doses (µg/well)	Inhibition Zone (mm)	Activity Index (%)
S. aureus	CHP	27±0.69	100
	50	7±0.00	26
	100	7±0.00	26
	200	7±0.00	26
	400	7±0.00	26
	800	8±0.38	29
S. epidermidis	CHP	28±0.69	100
	50	7±0.00	25
	100	7±0.00	25
	200	7±0.00	25
	400	7±0.00	25
	800	8±0.96	27
S. sobrinus	CHP	33±1.02	100
	50	7±0.19	22
	100	7±0.19	22
	200	7±0.00	22
	400	7±0.00	23
	800	8±0.00	24
S. mutans	CHP	28±0.19	100
	50	7±0.00	25
	100	7±0.00	25
	200	7±0.00	25
	400	8±0.19	27
	800	9±0.33	31
E. coli	CHP	28±0.38	100
	50	7±0.19	26
	100	7±0.19	26
	200	7±0.38	26
	400	7±0.19	26
	800	8±0.51	30
S. thypi	CHP	28±0.33	100
	50	7±0.00	25
	100	7±0.00	25
	200	7±0.00	25
	400	7±0.19	26
	800	8±0.19	28
P. acnes	CHP	28±0.38	100
	50	8±0.58	27
	100	8±0.38	27
	200	8±0.19	28
	400	8±0.38	29
	800	8±0.58	30

*Results are reported as the mean ± SD of three experiments.

The results of antibacterial activity showed that *S. crispa* leaves show that the extract can inhibit bacterial growth with inhibition concentrations ranging from 50-800 µg/well. The *bacteria S. aureus, S. epidermidis, S. sobrinus, E. coli, S. thypi, S. mutans*, and *P. acnes* can inhibit bacterial growth by 7-9 mm and is included in the medium category.

Recent investigations indicate that *S. crispa* leaves have extensive antibacterial activity against both Gram-positive and Gram-negative bacteria, encompassing various clinically significant oral and systemic infections. Ethanolic and aqueous extracts of *S. crispa* comprise flavonoids, anthraquinones, tannins, and other polyphenols, which are thought to significantly impede bacterial proliferation by disrupting cell membranes,

blocking enzymes, and interfering with nucleic acid production. These findings establish a robust pharmacological foundation for the traditional application of S. crispa in infection treatment and endorse its advancement as a natural antibacterial agent. A thorough TLC-bioautography investigation of the ethanol extract from keji beling leaves demonstrated that *S. crispa* possesses inhibitory effects against a broad spectrum of pathogenic bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Shigella dysenteriae*, and *Vibrio cholerae*. This study revealed distinct inhibition zones on bioautography plates, identifying two primary active spots: one at Rf 0.74, effective against *S. aureus* and P. *aeruginosa*, and another at Rf 0.83, active against *E. coli*, *S. typhi*, *S. epidermidis*, *S. mutans*, *Shigella dysenteriae*, and *Vibrio cholerae*. The phytochemical analysis of these active patches verified the existence of flavonoid and anthraquinone chemicals, suggesting that these secondary metabolites significantly contribute to the antibacterial efficacy of *S. crispa* (Aditama et al., 2025; Junardin et al., 2024b; Hanafiah et al., 2023; Sukendi et al., 2025). The presence of secondary metabolites, including tannins, flavonoids, saponins, and steroids, in the ethanol extract of *Strobilanthes crispa* contributes to its antimicrobial properties, enabling it to inhibit bacterial growth.

CONCLUSIONS

The ethanol extract of *Strobilanthes crispa* leaves from East Kalimantan has natural bioactivity and holds potential for further development as an antibacterial agent, hence providing a scientific foundation for the traditional applications of the studied plant.

Authors' Contributions: In this research, Nur Maulida Sari designed the study, supervised all processes, conducted the data analysis, and authored the report. Gebing, Arief Rahman and Sri Indayani was conducting observations and collecting research samples. Adelia Juli Kardika, Christine Elia Benedicta and Sri Indarti were overseeing the analysis of study data. Kiamah Fathirizki Agsa Kamarati and Risna Nona oversaw the samples and material preparation, as well as the processing of study findings. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there is no conflict of interest in this research.

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