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Evaluation Potential of *Strobilanthes Auriculata* var. *Dyeriana* (Mast.) J.R.I. Wood as an Antioxidant and Antimicrobial Agent

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ABSTRACT

Strobilanthes belongs to the Acanthaceae family and possesses various medicinal qualities, including wound healing, antioxidant, antibacterial, antidiabetic, anticancer, and anti-inflammatory effects. One species in this genus, *Strobilanthes auriculata* var. *dyeriana*, is a popular ornamental plant that has long been used as a diuretic to alleviate rheumatism. While few are recognised, this species likely has many more benefits. This study examined the phytochemical composition, phenolic content, anthocyanin content, and antibacterial and antioxidant qualities of *S. auriculata* var. *dyeriana* leaf extract. Finding no anthocyanin, we discovered that the leaves of this species contained phenolic compounds (82.9±0.86 µg/100µg). Furthermore, vigorous antioxidant activity (IC_{50} 96.17 ppm) and growth-inhibiting action against *Salmonella typhimurium* are provided by high phenolic content. According to this study, the leaf of *S. auriculata* var. *dyeriana* may act as a potential antibacterial and antioxidant agent.

Keywords: Antibacterial; Anthocyanin; DPPH; Phytochemical screening; Total phenol content

NOMENCLATURE

ε	: Molar absorptivity of cyanidin-3-glucoside (269000 L/(mol.cm))
DF	: Solubility factor
DPPH	: 1,1-diphenyl-2picylhydrazyl
L	: Cuvette width (1cm)
MW	: The molecular weight of cyanidin-3-glucoside (449.2 g/mol)
TPC	: Total Phenol Content

1. INTRODUCTION

Strobilanthes is a genus from the Acanthaceae family that Blume first described in 1826 from samples taken in West Java. It is the most species-rich genus throughout tropical Asia and Australia. *Strobilanthes* is a well-known genus in Sri Lanka due to its diverse behaviours, gregarious distribution, and gorgeous blooms. Many *Strobilanthes* species have medicinal qualities. In several parts of the world, *Strobilanthes* extract has been used to treat illnesses caused by respiratory viruses, spider poisoning, snake bites, and cerebrospinal meningitis. Furthermore, the leaves of this plant are often used to make indigo dyes[1].

Traditionally, several *Strobilanthes* species are also used as medicinal plants in several parts of the world, particularly in the Ayurvedic medicinal system[2]. *S. ciliatus* and *S. heynianus* are used as medicinal herbs to treat several conditions such as epilepsy, paraplegia, back pain, hemiplegia, and paralysis[2,3]. *S. heynianus* also exhibits potent antioxidant potential[4].

Figure 1. ***Strobilanthes auriculata* var. *dyeriana* is widely recognized as an attractive plant in indonesia because of its magnificent learf color.**

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Strobilanthes auriculata var. *dyeriana*, known as *sembar lilin* in Indonesia, is distinguished by its dark green leaves with metallic-purple stripes radiating from the vein's center[5] (Fig. 1). Despite the popular use of foliar ornamentation plants, this species is also used as an herbal treatment for rheumatism in Indonesia. However, more information is needed on this plant. Most information is primarily about its propagation, but studying its potential as a therapeutic herb still needs to be explored. As a result, several facets of this plant, spanning from botanical features to its uses, still need to be explored. *S. auriculata* var. *dyeriana* is thought to have additional potential benefits that are not commonly known, particularly in herbal medicine. This study aimed to examine the efficacy of *S. auriculata* var. *dyeriana* extract as a natural antioxidant and assess its antibacterial activities.

2. MATERIAL AND METHODS

2.1 Leaves Extraction

The leaves of *S. auriculata* var. *dyeriana* used in this study were obtained from a non-collection ornamental plant in the Eka Karya Bali Botanic Garden area. The leaves were cleaned, thinly cut, and air-dried for five days. The leaf extraction was carried out using the maceration method, slightly modified from the procedure used by Baehaki[6], *et al.* and Andila & Hartanto[7]. In 1000 mL of methanol, 100 g of dried leaves were macerated and then incubated in the dark at 26 °C. The mixture was filtered with filter paper after three days. The crude extract was separated from the solvent using a vacuum rotary evaporator. The concentrated crude extract was then used for further analysis.

2.2 Phytochemical Screening, Total Phenol, and Anthocyanin Content

Phytochemical contents, including saponin, phenols, and steroids, were screened qualitatively according to the methods carried out by Yuniati[8] *et al.,* Putri[9] *et al.,* and Hossain[10] *et al.,* respectively.

TPC was calculated using the linear regression equation of gallic acid standards. The determined content was expressed as equivalents of gallic acid. Total phenolic analysis was performed using the modified method by Ghafoor[11] *et al.,* The sample's TPC was estimated using the gallic acid linear regression equation.

The total anthocyanin content test was carried out using the pH differential method. Sample solutions were prepared from each filtrate, and each sample was measured for its absorbance at its maximum absorption wavelength and λ700 nm (as an absorbance correction) with pH 1.0 and pH 4.5 solutions. The total anthocyanin content (%) was calculated using the following formula:

$$\% \text{ inhibition} = \frac{((A510 \pm A700)\text{pH } 1{,}0 - (A510 \pm A700)\text{pH } 4{,}5) \times MW \times DF}{\varepsilon} \times L$$

2.3 Antioxidant Activity Assay

The antioxidant activity of *S. auriculata* var. *dyeriana* leaves was performed according to the modified method by Wibawa[12], *et al.* The stock solution of plant crude extract was diluted into numerous concentration series: 50, 100, 150, 200, 250, 300, and 350 ppm. One ml of each extract concentration was mixed with 4 ml of DPPH (40 ppm). The mixture was mixed and incubated in a dark room for 30 minutes. Ascorbic acid antioxidant activity was tested in various concentrations (2, 4, 6, 8, 10, and 12 ppm) as a comparison. Following that, the absorbance of each mixture was measured at λ 517 nm with a UV-Vis spectrophotometer. The quantitative calculation was performed by determining the free radical inhibitory power of the sample, which was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100$$

2.4 Antimicrobial Activity Assay

Antimicrobial activity was assessed using the modified Kirby-Bauer disc diffusion method[13] on nutrient agar. The microorganisms tested were those that cause human diseases, including *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus mutans*.

The tested microorganisms were regenerated before the antimicrobial activity using the following protocol: one ose of the microbes tested was transferred aseptically onto a sterile slant nutrient agar, then the bacteria were incubated for 24 h at 37 °C.

For the antimicrobial activity assay, the fresh colonies were transferred into a sterile saline solution, and the turbidity was adjusted to 0.5 McFarland standards before streaking onto the surface of nutrient agar and allowed for 15 minutes before the tested discs were placed on the surface of the agar. The formation of the clear inhibition zone was observed at one to three days of incubation (37 °C).

Table 1. Chemical properties contained in *strobilanthes auriculata* var. *dyeriana* leaces extract

Chemical compounds	Presence
Phenols	√
Steroid	—
Triterpenoid	—
Saponin	—
TPC (equivalent with gallic acid) (μg/100μg extract) ± S.D.	82.9 ± 0.86
Anthocyanin (%)	—

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening, Total Phenol, and Anthocyanin Content

The results of phytochemical screening showed that the leaf extract of *S. auriculata* var. *dyeriana* only contained chemical compounds belonging to the phenol group. No detectable levels of saponin, steroid, and triterpenoid

compounds were found through qualitative testing (Table 1). According to this result, the TPC of this extract was further assayed. As a result, *S. auriculata* var. *dyeriana* leaves extract showed 82.9±0.86 µg/100µg extract of TPC (equivalent to gallic acid), with no anthocyanin detected.

Several studies reported that dark rind contains more polyphenols (flavonoids, anthocyanins, and tannins)[14, 15]. Anthocyanins are plant pigments that produce red, blue, and purple colours in several plant parts, including leaves[16, 17]. Although *S. auriculata* var. dyeriana is commonly known as a purple plant due to its leaf colour, our study showed no anthocyanin compounds in the sample, suggesting that the biosynthesis process of anthocyanin compounds does not occur in the leaf parts of this plant.

3.2 Antioxidant Activity

The present study showed that *S. auriculata* var. *dyeriana* had an IC_{50} value of 96.17 ppm, while ascorbic acid had an IC_{50} value of 5.02 ppm. Compared to the IC_{50} value of ascorbic acid, the *S. auriculata* var. *dyeriana* extract had lower activity but still had strong antioxidant ability.

Because of its high phenolic content, *S. auriculata* var. *dyeriana* was assumed to have high antioxidant potential. According to various reports, phenolic compounds exhibit several biological functions, such as antioxidant, antimutagenic, modified gene expression, cardiovascular protection, antidiabetics, vision improvement, and carcinogenesis suppression[18-20]. The number of -OH groups in the phenolic compound framework can influence antioxidant activity. Their ability as a hydrogen donor atom can neutralize free radicals and prevent oxidation[21].

The high antioxidant activity in *S. auriculata* var. *dyeriana* is also thought to be related to its anti-inflammatory properties. Previous research has linked *S. heyneanus*' high amount of antioxidant activity to its action as an anti-inflammatory medication[22]. Closely related plants, both in family and genus, tend to produce comparable metabolites, implying that their efficiency as therapeutic components is also likely to be similar. Previously, antiviral, anticancer, anti-inflammatory, and anticoagulant activities have been demonstrated from *S. tonkinensis* extract[23], while *S. barbatus* and *S. tonkinensis* have also been found to be high in antioxidants[3,4]. *S. crispus* leaves are frequently employed in traditional medicine for their blood pressure-lowering, antidiabetic, anticancer, and diuretic qualities. Scientifically, it has been proven to have potent antioxidant activity, anti-AIDS, and anticancer properties[4,24]. Furthermore, a study discovered that the aqueous extract of *S. crispus* leaves contains a high level of antioxidants and anticancer potential[25].

3.3 Antimicrobial Activity

The plant extracts' antimicrobial activities were demonstrated by establishing a clear zone in the growth media, indicating the presence of antagonistic metabolites produced by the extracts, hence inhibiting microbial development[28]. We discovered that the extract of *S. auriculata* var. *dyeriana* could only suppress the growth of *S. typhimurium* in this investigation (Fig. 2).

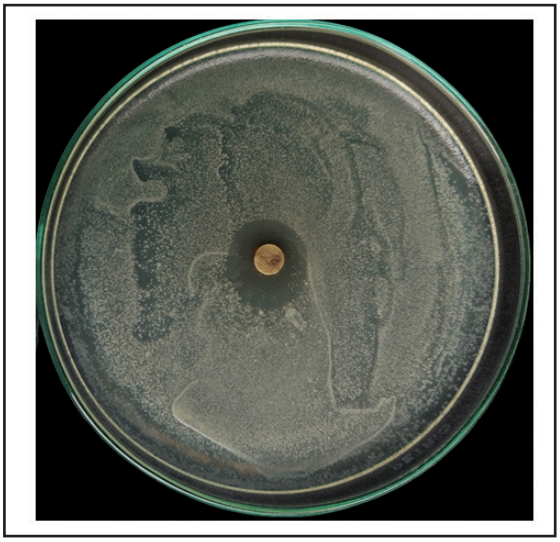

Figure 2. Methanolic leaf extract of Strobilanthes auriculata *var. dyeriana* formed a clear zone when challenged with Salmonella typhimurium. The clear zone emerged on the first day after treatment. the diameter of the clear zone increased with each passing day. this figure was taken on the thrid-day post -treatment.

The ability of the *S. auriculata* var. *dyeriana* leaf extract to inhibit bacterial growth is likely due to compounds with antibacterial properties but with a narrow or specific spectrum. The effectiveness of the extract is probably due to the phenolic compounds contained in the extract. Several studies proved phenol has potent antibacterial action against various organisms, including bacteria, yeasts, and molds[19, 27, 28]. Phenolic compounds efficiently reduced the growth of *Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*[29].The mechanism of antimicrobial activity of phenolic compounds is usually caused by hydrogen bonding from the phenolic compound, resulting in intracellular function changes that lead to changes in cell permeability. The increased lipophilic character of the phenolic compound will increase antimicrobial activity by supporting the interaction of phenolic compounds with cell membranes, causing permanent damage to the cytoplasmic membrane resulting in lysis[30].

Several *Strobilanthes* species, notably *S. formosanus* and *S. kunthiana*, have been shown to have antibacterial activity[31,32]. Additionally, *S. urticifolia* methanolic extract was also found to have antibacterial activities against *E. coli, Micrococcus luteus, S. aureus,* and *S. typhi*[33], while dichloromethane extract of *S. crispus* inhibited *B. subtilis* and *S. aureus*[34]. Recently, *S. ciliatus* was found to have antibacterial activity against *S. aureus, B. subtilis, E.coli, P. aeruginosa, Klebsiella, Cornybacterium, A. niger, C. albicans, Trichophyton rubrum, Microsporum gupseum,* and *Monascus purpureus*[2].

4. CONCLUSION

Phytochemical screening of *S. auriculata* var. *dyeriana* leaf extract showed the presence of phenols. In addition, the total phenol content is high by 82.9±0.86µg/100µg,

but no anthocyanin was found. The leaves also showed excellent antioxidant activity (IC_{50} value of 96.17 ppm) and strongly inhibited the growth of *S. typhimurium*. This study reveals that *S. auriculata* var. *dyeriana* leaves are potent antibacterial and antioxidant agents.

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According to the contribution made in this paper, the authors state that IPAHW and ASL are the main contributors, while PSA, INL, and VS are the co-contributors.