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Phytochemical Analysis of the Roots of *Solanum diphyllum* L. as a Potential Exotic Weed in Purwosari Village

Studi Fitokimia Akar pada (*Solanum diphyllum* L). sebagai Gulma Eksotis yang berpotensi di Kalurahan Purwosari

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Abstrak

Penelitian ini bertujuan untuk menganalisis kandungan fitokimia pada akar *Solanum diphyllum* L., suatu spesies eksotik yang telah menaturalisasi di Kalurahan Purwosari. Akar tanaman diekstraksi menggunakan metode maserasi, kemudian dilakukan skrining fitokimia secara kualitatif dengan metode uji tabung *(Tube Test)* untuk mendeteksi keberadaan alkaloid, flavonoid, dan fenol. Hasil penelitian menunjukkan bahwa ekstrak akar *S. diphyllum* memberikan reaksi positif terhadap uji alkaloid dan flavonoid, sedangkan uji fenol menunjukkan hasil negatif. Temuan ini mengindikasikan bahwa akar *S. diphyllum* mengandung metabolit sekunder tertentu yang berpotensi berperan dalam aktivitas biologis tanaman. Hasil penelitian ini diharapkan dapat menjadi informasi awal dalam pengembangan kajian fitokimia lebih lanjut serta sebagai dasar pertimbangan dalam pemanfaatan dan pengelolaan spesies eksotik *S. diphyllum* di lingkungan tropis.

Kata kunci: *Solanum diphyllum,* fitokimia, akar, alkaloid, flavonoid

Abstract

This study aimed to analyze the phytochemical constituents of the roots of Solanum diphyllum L., an exotic species that has naturalized in Purwosari Village. Root samples were extracted using the maceration method, followed by qualitative phytochemical screening using tube tests to detect alkaloids, flavonoids, and phenolic compounds. The results showed positive reactions for alkaloids and flavonoids, while phenolic compounds were not detected in the root extract. These findings indicate that the roots of S. diphyllum contain specific secondary metabolites with potential biological relevance. This study provides preliminary information that may support further phytochemical investigations and contribute to considerations for the utilization and ecological management of the exotic species S. diphyllum in tropical environments.

***Keywords**: Solanum diphyllum, phytochemical, root, alcaloid, flavonoid*

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INTRODUCTION

Indonesia is one of the countries rich in biodiversity, especially in tropical plant groups such as Solanaceae [1]. The archipelago's tropical climate and ecosystem diversity enable various plant species, including exotic ones, to adapt and spread. Exotic species are species that originate from outside their natural range and are introduced to a new area, either intentionally or unintentionally, thereby potentially affecting the local ecosystem [2]. Land-use changes and human activities can accelerate the spread of alien species, which may become invasive and disrupt local ecological balance [3]. The presence of dominant exotic plants can suppress native species through competition for resources and habitat modification [4]. Therefore, scientific studies on alien plants in Indonesia are crucial to support efforts to sustainably manage biodiversity. Alien plants are plant species that originate from outside their natural range and then appear and grow in a new area as a result of human-mediated movement, so their presence is not naturally formed in the history of the local ecosystem [5].

Systematic scientific research on traditional medicinal plants plays an important role in the discovery of new drugs through rigorous investigation. In recent years, various reports have shown that understanding the chemical characteristics and biological activities of medicinal plants can provide initial clues for a more comprehensive interpretation [6]. In addition, these studies also serve as a basis for identifying the presence of major compound groups in medicinal plants and their potential pharmacological value [7]. Most medicinal plants can produce secondary metabolites in abundance under various environmental conditions. These compounds generally do not play a direct role in growth or primary metabolic processes, but they serve an important function in helping plants interact with their environment and other organisms [8]. In recent years, attention to the commercial value of secondary metabolites has increased, particularly through the use of tissue culture technology to optimize the production of bioactive plant metabolites [9].

Solanum diphyllum L. is commonly known as two-leaved *nightshade* and belongs to the Solanaceae family [10]. This species originates from Mexico and Central America but has now spread beyond its cultivation area as an ornamental plant and grows wild in various locations around the world. Its presence was first reported in India, specifically in Kolkata, by Paul and Biswas. During the revision of the Solanaceae family for the Flora of India project, this species was recorded growing in Maharashtra, Karnataka, and Tamil Nadu [1]. This study aims to compare the phytochemical content of the roots *of S. diphyllum* (Figure 2). This species is a member of the Solanaceae family and is a potent medicinal herb, commonly found in the dry, deciduous areas of the Eastern Ghats in Karnataka [11]. In addition,

S. diphyllum is reported to occur in several countries, including India, China, Sri Lanka, and Malaysia [12].

S. diphyllum L. is one of the exotic species of the Solanaceae family that has been detected to have naturalized in several regions of Indonesia. Naturalization is a condition in which an exotic species that enters a new area is able to live, grow, and reproduce on its own in the wild to form a permanent population without human assistance [2]. *S. diphyllum* has been collected from several locations on the island of Java, such as Bogor, Sumedang, and Semarang, indicating that this species is not only present sporadically but has become established [1]. This distribution record raises concerns that *S. diphyllum* could become an invasive weed in the future. The adaptability of this species, coupled with its morphological and biological characteristics that support its spread, warrants further attention from the scientific community and land managers [20]. One aspect that has been minimally researched is the phytochemical content of *S. diphyllum* roots. Bioactive compounds have been identified in other parts of *S. diphyllum*, but the focus has been on general phytochemical and histochemical analyses rather than on the roots specifically. In many plant species, the roots are the site of accumulation of secondary metabolites such as alkaloids, flavonoids, and glycoalkaloids, which have potential biological activity [21].

METHODS

Research Location and Time

Sampling of *Solanum diphyllum* L. roots was conducted in the Purwosari Village area on Sunday, October 19, 2025. The samples were then taken to the laboratory for preparation. Phytochemical testing was carried out at the B2P2TOOT Laboratory on Thursday, October 23, 2025. In addition to laboratory testing, this study was supplemented with a literature review to obtain quantitative data on total phenolics, antioxidant activity, and cytogenetic information *on S. diphyllum* from previous publications. The literature was reviewed through indexed sources such as SINTA, Google Scholar, ScienceDirect, and PubMed.

Research Tools and Materials

The equipment used in this study included an analytical balance for weighing samples, a grinder or mortar and pestle for grinding materials, and measuring cups, Erlenmeyer flasks, and beakers for preparing and mixing solutions. In addition, test tubes and test tube racks, droppers and measuring pipettes for taking and transferring solutions, filter paper for filtration, and airtight containers for sample storage were used. Research documentation was carried out using a camera, and a water bath was used to heat the solution at a controlled temperature.

The materials used in this study consisted of *Solanum diphyllum* L. roots as the main sample. The solvents used were ethanol or methanol. Several reagents were used for phytochemical analysis, namely Dragendorff's or Mayer's reagent for alkaloid testing, 1% $FeCl_3$ solution for phenol testing,

and Shinoda's reagent or dilute NaOH solution for flavonoid testing. Distilled water was used as a solvent and rinse. In addition, scientific articles on the phytochemistry, antioxidants, and cytogenetics of *Solanum diphyllum* were used as supporting materials for the literature review.

Research Procedure

During the sample preparation stage, the roots were cleaned of soil, dried at room temperature away from sunlight to prevent metabolite degradation, then ground into a fine powder and stored in an airtight container.

Extraction was performed by maceration in ethanol or methanol at a ratio of approximately 1:10. The mixture was soaked for 48–72 hours, with occasional stirring, to maximize the transfer of bioactive compounds. The filtrate was then filtered, evaporated at low temperature to obtain a concentrated extract, and stored at low temperature before phytochemical testing.

The parameters observed in this study included qualitative phytochemical parameters of *Solanum diphyllum* L. roots, namely the presence of alkaloids, flavonoids, and phenols. Each parameter was determined by color-change reactions or by the formation of characteristic precipitates in *tube* tests with specific reagents. Additional parameters, in the form of supporting data, included total phenolic content (mg GAE/g), DPPH antioxidant activity (% inhibition), reducing power (OD), and cytogenetic data (number of chromosomes, pollen fertility, and meiosis abnormalities) obtained from selected studies in the literature. These parameters were used to strengthen the interpretation of bioactivity and consistency of secondary metabolite biosynthesis in *S. diphyllum* roots.

This study employed an exploratory descriptive research design, combining a qualitative laboratory approach with comparative literature studies. Phytochemical analysis was performed in vitro through tube tests to detect the main secondary metabolite groups in *S. diphyllum* roots. This design was chosen to provide an initial overview of the phytochemical profile of roots as a relatively unexplored underground organ. The empirical data from the laboratory tests were then compared and contextualized alongside quantitative and cytogenetic data from previous publications to provide a more comprehensive interpretation of the bioactive potential and stability of this species' secondary metabolites.

The phytochemical test data were analyzed descriptively and qualitatively using visual indicators, such as color changes or precipitate formation, to indicate positive or negative reactions to each reagent. The results were presented as phytochemical screening tables and visual documentation to enhance the validity of the findings. Furthermore, the qualitative results were compared with quantitative data from relevant studies in the literature, including

total phenolic content, antioxidant activity, reducing power, and cytogenetic data.

The final stage of analysis was conducted by comparing the qualitative results of laboratory tests with quantitative data from literature studies, namely total phenolic content of 26 mg GAE/g root, antioxidant activity of 40.3%, and reducing power value of 0.15 OD, as well as cytogenetic data in the form of chromosome number 2n = 24, pollen fertility of 93.51%, and a meiotic abnormality rate of 2.98%. These data were used to reinforce the interpretation of the phytochemical profile of *S. diphyllum* roots. In this way, the study produced a combination of empirical and literature data that could describe the bioactive characteristics of the roots more comprehensively.

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RESULTS AND DISCUSSION

The initial stage of this study involved identifying and documenting samples as a basis for further analysis. This documentation was necessary to provide an objective description of the samples' physical condition before entering the phytochemical testing stage, so that readers could better understand the study's initial context. The following section presents illustrations showing the condition of the samples at the pre-analysis stage (Figure 1).

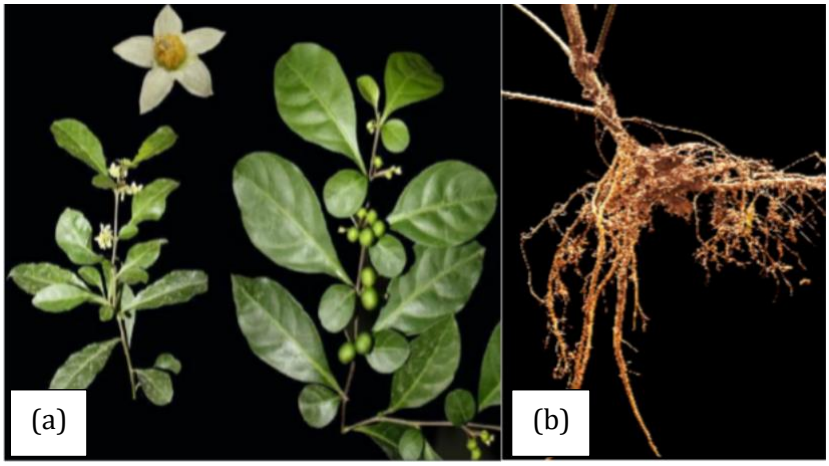

Figure 1. (a) Plant Form of *S. diphyllum* Stem, Leaves, Flowers, and Fruit; (b) Root Structure of *S. diphyllum*

The structure of the *Solanum diphyllum* stem, which undergoes mild lignification, serves to support branching and facilitate water transport in the vascular tissue [22]. The major and minor leaves that appear in pairs indicate a more efficient light distribution pattern in the photosynthesis process [23]. The relatively smooth leaf surface supports reduced gas diffusion resistance for physiological activities [24]. Small flowers arranged in clusters maximize the chances of pollination by small insects. Berry-shaped fruits with abundant seeds serve to increase the success of generative dispersal.

The growth of bifoliate leaves in the early vegetative phase illustrates the characteristic heteroblastic development pattern in the genus *Solanum* [25]. The difference in size between major and minor leaves becomes more pronounced in the adult phase as a result of meristem tissue development regulation[26] . Flowering that occurs in the leaf axils reflects the acropetal inflorescence mechanism in the *Solanum* group [27]. The change in fruit color from green to yellow-orange indicates the accumulation of carotenoids during the physiological ripening process. The overall development of this organ shows a close relationship between morphogenesis and plant reproductive strategies.

Paired leaves of different sizes regulate transpiration through different radiation distribution on each leaf blade [28]. The widespread fibrous root system adapts to shallow soil conditions to maximize water absorption [29]. The fruit's bright color is an ecological adaptation to attract seed-dispersing fauna, such as birds. Abundant seed production supports the survival of the species in disturbed habitats. This adaptive ability explains why *S. diphyllum* easily naturalizes in various regions [20].

The bifoliate leaf characteristic of *S. diphyllum* distinguishes it from other species such as *S. nigrum*, which has single leaves per node [30]. The relatively small flower size differs from *S. americanum*, which has larger flowers and is less densely clustered. The fruit's color change to yellow-orange is a distinctive characteristic not shared by many other *Solanum* species, which predominantly bear black or purple fruit [31]. This difference indicates varying adaptive pressures among members of the genus. This diversity also reflects the broad morphological evolutionary patterns within Solanaceae. The large fruit production makes *S. diphyllum* potentially aggressive in spreading through open ecosystems [32].

As a follow-up to the sample identification process, this study conducted a series of phytochemical tests to detect the presence of secondary metabolites, including alkaloids, phenols, and flavonoids. Visual documentation of each test was required to reinforce the validity of the findings through evidence of color changes that indicated a reaction. The visual results of the three tests are shown in the following section (Figure 2).

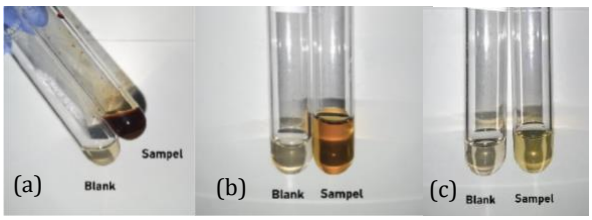

Figure 2. Documentation of (a) Alkaloid Test Results; (b) Phenol Test Results; (c) Flavonoid Test Results

The alkaloid test results in Figure 2(a) show a significant color change in the sample tube relative to the blank, indicating the presence of alkaloids in the *S. diphyllum* extract [33]. The dark color reaction observed results from the formation of a complex between the alkaloid and the reagent, a reaction common in qualitative alkaloid tests [34]. The presence of alkaloids in the *genus Solanum* has been widely reported, particularly tropane and steroidal

alkaloids [35]. These alkaloid compounds play a role in the plant's defense mechanism against herbivores and pathogens. This visual finding supports the literature stating that *Solanum* is one of the families with high alkaloid content.

The phenol test in Figure 2(b) shows a brown color change in the sample tube that does not appear in the blank, indicating the presence of phenolic compounds in the *S. diphyllum* root extract [36]. This color reaction is caused by the formation of a complex between the phenolic group and the $FeCl_3$ reagent, which produces a brownish color as a positive indicator [37]. Phenolic compounds are known as natural antioxidants that play a role in capturing free radicals in plant tissues [38]. The phenolic content in *Solanum* has been reported to contribute to antioxidant and antidiabetic activities in several species [39]. Therefore, the visualization of these test results shows the high bioactivity potential of *S. diphyllum* roots.

The flavonoid test results in Figure 2(c) show a difference in color intensity between the sample and the blank, which indicates the presence of flavonoids in the extract [40]. A positive flavonoid reaction usually occurs due to metal reduction or complex formation with the reagent aluminum chloride, which produces a yellowish color [41]. Flavonoids are an important group of secondary metabolites that function as antioxidants, antimicrobials, and protectors against environmental stress [42]. The presence of flavonoids in the *Solanum* genus has been associated with pharmacological activities such as anti-inflammatory and hepatoprotective [43]. The visual results of this test reinforce the potential *of S. diphyllum* as a source of bioactive flavonoid compounds.

Table 1. Results of the Phytochemical Screening Test

Phytochemical	Result	Test Method
Alkaloid	+	Tube Test
Flavonoid	+	
Fenol	-	

Positive results in the alkaloid test indicate that *Solanum diphyllum* roots contain important bioactive components, given that alkaloids are known to have analgesic, antimicrobial, antimalarial, and even anticancer activities [44]. The detection of alkaloids in Solanaceae species is also in line with previous reports stating that the roots and leaves of several *Solanum* species, such as *S. torvum* and *S. nigrum*, are rich in alkaloid compounds such as solasodine and solanine [39]. This suggests that *S. diphyllum* may have a similar pattern of secondary metabolite biosynthesis.

In the genus *Solanum*, steroidal alkaloids and glycoalkaloids, such as solasodine, solasonine, and solamargine, have been widely reported as the dominant compounds contributing to the bioactive pharmacological activity [45]. Recent studies show that steroidal alkaloids from Solanum can inhibit cancer cell proliferation by inducing apoptosis, disrupting the cell cycle, and modulating cellular signaling pathways, including p53 and caspase-dependent pathways [46]. In addition, several glycoalkaloids exhibit broad-spectrum

antimicrobial activity against Gram-positive and Gram-negative bacteria, reinforcing their role as plant chemical defense compounds [47].

The detection of alkaloids in the roots of *S. diphyllum* is consistent with reports on other Solanum species, such as *S. torvum* and *S. nigrum*, which are known to be rich in alkaloids in their roots and leaves, particularly solasodine and its derivatives [48]. This pattern similarity suggests that *S. diphyllum* likely shares a biosynthetic pathway for secondary metabolites, given that the biosynthetic pathway for steroidal alkaloids in Solanaceae is relatively genetically conserved [49]. The stability of this biosynthetic pathway allows for consistent alkaloid production between individuals, especially in species that have undergone naturalization and ecological adaptation.

The positive results for flavonoids reinforce the assumption that *Solanum diphyllum* has potential antioxidant activity relevant to mitigating oxidative stress in biological systems [50]. Flavonoids are a group of secondary polyphenols known to have the ability to suppress free radicals through electron donation and transition metal binding, thereby playing a role in reducing oxidative stress levels, inhibiting lipid peroxidation [51], and exhibiting anti- inflammatory activity through modulation of the NF-κB pathway and pro-inflammatory cytokine production [52]. Recent studies also indicate that flavonoids contribute to cytoprotective activity and can enhance endogenous antioxidant capacity in plant tissues and other biological systems [53].

Interestingly, while previous studies have primarily focused on measuring flavonoids in the leaves or fruits of other *Solanum* species, the discovery of flavonoids in the roots of *S. diphyllum* indicates that flavonoid accumulation is not limited to photosynthetic organs alone. This is consistent with reports that plant roots, despite minimal light exposure, can accumulate flavonoids as an adaptive response to underground environmental stresses, such as pathogen attacks and abiotic stress conditions [54]. Flavonoid compounds detected in roots generally exhibit variations in aglycone and glycoside profiles that differ from those found in leaf parts, which may influence their bioactivity and pharmacological properties [55].

Conversely, negative results in the phenol test using the $FeCl_3$ reagent method indicate that total phenolic compounds were not detected qualitatively in roots using this simple screening approach. This may occur because the phenol concentration is below the detection limit of the method or because the phenolic profile in underground organs tends to be qualitatively different, with lower proportions compared to light-exposed organs such as leaves and fruits, where flavonoid and phenolic acid biosynthesis is more intensive [56]. The literature also indicates that bound phenolics or complex forms may be dominant in root tissues, requiring more sensitive quantitative techniques such as HPLC or mass spectrometry for accurate detection and quantification [57].

The phytochemical research on *Solanum diphyllum* roots in this study is even stronger than the molecular characterization findings from previous studies. The root extract (SDR) had a total phenolic content of 26 mg GAE/g extract, making it the plant part with the lowest phenolic content compared to the leaves (68.1 mg GAE/g), stems (48.6 mg GAE/g), and fruits (38 mg GAE/g). This relatively low phenolic value in the roots is consistent with the phenol test results, which were negative with the $FeCl_3$ reagent. This difference reinforces the understanding that the phenolics in *S. diphyllum* roots are indeed at low concentrations. Roots have lower antioxidant activity than leaves and stems, as indicated by a DPPH *scavenging activity* value of 40.3% at a concentration of 0.1 mg/ml and a reducing power of 0.15 OD, which is lower than that of leaves (0.24 OD) and stems (0.20 OD) [58]. These data indicate that although roots contain flavonoids, their antioxidant bioactivity intensity remains weaker than that of photosynthetic organs. This is in line with the theory that leaves and stems have higher oxidative stress exposure, thus accumulating more phenols and flavonoids.

Enzymatic studies also provide important molecular insights. In addition, *S. diphyllum* stem extract (SDS) exhibited the highest α-amylase inhibitory activity, namely 20.3% (500 µg/ml), 28.0% (1000 µg/ml), and 38.8% (2000 µg/ml), while the roots showed much lower and statistically insignificant activity [58]. This study supports the interpretation that secondary metabolites, such as alkaloids and flavonoids, in roots primarily function as structural defense and antibacterial compounds, rather than as metabolic enzyme inhibitors. This further confirms that the bioactivity of roots is selective toward certain types of biological stress. The molecular and micromorphological aspects of *S. diphyllum* also provide important insights into the variation of secondary metabolites. Micromorphological research by Murugan et al. reported that *S. diphyllum* leaves have glandular and non-glandular trichomes, anisocytic stomata, and a thick epicuticular wax layer, which are genetic and stable features in species identification [59]. The presence of glandular trichomes is particularly relevant given that this structure acts as a site for the biosynthesis and storage of secondary metabolites such as flavonoids, terpenoids, and alkaloids. This explains why phenolic and flavonoid content is higher in leaves and stems than in roots.

Molecular data were also reinforced by FTIR analysis conducted by Murugan et al. The FTIR spectrum *of S. diphyllum* showed characteristic peaks at 1041 cm^{-1} (*aliphatic amines*), 2360 cm^{-1} (*nitriles*), 3118 cm^{-1} (*aromatic alkenes*), and a strong peak in the range of 3700–3800 cm^{-1} associated with the N–H amide group, indicating the presence of certain alkaloid compounds and structural proteins [59]. Alkaloids are among the dominant components in the roots. The FTIR analysis can be interpreted as reflecting the characteristic chemical profile of *S. diphyllum*, reinforcing the

concept that alkaloid metabolites originate from genetically stable biosynthetic pathways.

Meanwhile, previous cytogenetic data indicate that *S. diphyllum* has a chromosome number of 2n = 24 and a stable meiotic configuration, thereby supporting relatively consistent metabolite expression among individuals. A stable genome also reinforces the record of this species' naturalization and invasion in several regions, including Indonesia. The relationship between genetic stability and plants' ability to produce secondary metabolites, such as alkaloids and flavonoids, explains why these compounds were found in roots in this study, albeit at lower concentrations than in photosynthetic organs.

When evaluated as a whole, the integration of molecular, phytochemical, micromorphological, and enzymatic data shows that the roots of *S. diphyllum* have a characteristic composition of secondary metabolites, with a predominance of alkaloids and flavonoids in moderate to low levels. The low phenolic content of the roots, both qualitatively and quantitatively, is supported by statistical findings in previous studies. These findings provide a strong basis for the conclusion that differences in metabolite distribution between parts are influenced by physiological function, tissue structure, the presence of trichomes, and the stability of genetically inherited biosynthetic pathways. This study provides a comprehensive overview of the phytochemical profile *of S. diphyllum* and opens opportunities for further exploration of the roots' bioactive potential, particularly in antibacterial, anti-inflammatory, and tissue-protective activities.

Dimensions of *Solanum diphyllum* L

This species is reported as an exotic plant that has naturalized in various tropical regions, including Indonesia, due to its adaptive nature, high reproductive capacity, and attractive bioactive potential [1], [15]. Therefore, the interpretation of the research results cannot be separated from the ecological and evolutionary framework underlying this species' behavior.

1. Morphology and Ecology of *Solanum diphyllum*

The morphology *of S. diphyllum* shows strong adaptation to tropical environmental conditions and disturbed habitats. Bifoliate leaves consisting of large major leaves and small minor leaves on a single node are a characteristic that distinguishes this species from other members of the Solanaceae family [30]. This structure increases light capture efficiency and allows for optimal growth in both shaded and open habitats. The large quantity of yellow–orange fruits produced facilitates seed dispersal by birds and other fruit-eating animals [60]. The bright color and small size of the fruits indicate an ecological strategy to maximize generative dispersal. The flexible stems, extensive root system, and ability to grow on disturbed soil support rapid colonization of new areas [15]. In Purwosari Village, this adaptive morphology explains why *S. diphyllum* can thrive in open land, yards, or areas with high anthropogenic pressure.

Abundant seed production increases the likelihood of invasion if control measures are not implemented.

2. Anatomy and Micromorphology

Previous anatomical and micromorphological studies have shown that *S. diphyllum* leaves contain important secretory structures, including glandular and non-glandular trichomes, tannin pockets, and thick cuticles [59]. Glandular trichomes are the main site of biosynthesis of secondary metabolites such as flavonoids, terpenoids, and alkaloids [61]. This explains why the leaves and stems of many Solanaceae species contain higher levels of secondary metabolites than the roots. The presence of tannin pockets and mucilage exudates indicates a strong chemical defense system against herbivores and surface pathogens. A thick cuticle layer also increases tolerance to environmental stress such as UV radiation or drought [62]. In roots, no special secretory structures such as trichomes are found; therefore, phenolic and flavonoid levels are generally lower than in photosynthetic organs. The results of this study, which showed negative phenol tests in roots, are consistent with these micromorphological characteristics.

3. Cytogenetics and Genomic Stability

Cytogenetic results show that *S. diphyllum* has a diploid chromosome number of 2n = 24 with 12 bivalents that are perfectly formed in metaphase I of meiosis. The neat pairing pattern indicates that chromosome recombination occurs normally, signifying a stable and well- organized genome [63]. The very high average pollen fertility (93.51%) confirms that gamete formation is effective. Minor meiotic abnormalities found, such as *stray chromosomes*, *chromosome bridges*, and *unequal separation*, only ranged from 1.53 to 4.43%, which is within the normal physiological limits for Solanaceae [64]. These small variations are a consequence of recombination dynamics, not an indicator of genomic instability. This genomic stability has important implications for secondary metabolism. The biosynthetic pathways of alkaloids and flavonoids are highly dependent on consistent gene expression, making meiotic stability a prerequisite for stable metabolite expression in natural populations [65]. Cytogenetic data support the finding that *S. diphyllum* roots continue to produce alkaloids and flavonoids despite low phenolic levels.

4. Root Phytochemistry: Secondary Metabolite Patterns

The results of the study show that there are positive alkaloids, positive flavonoids, and phenols with negative results. These findings are consistent with studies reporting that *S. diphyllum* roots have the lowest phenolic content among roots, leaves, stems, and fruits [58]. The low phenolic content also explains why the $FeCl_3$ reaction did not show a significant color change. The presence of alkaloids in the roots is highly relevant because many Solanaceae accumulate steroid, tropane, and glycoalkaloid alkaloids in underground tissues as a defense mechanism against soil pathogens [66]. Research [63] even isolated

cytotoxic glycosylated alkaloids from *S. diphyllum* roots, demonstrating this species' pharmacological potential. The presence of flavonoids in roots also has biological functions, particularly in oxidative stress, allelopathic interactions, and microbial defense. Although flavonoid levels are lower than in leaves, their presence remains significant for root physiological functions

CONCLUSION

This study shows that the roots of *Solanum diphyllum* L. contain two main groups of secondary metabolites, namely alkaloids and flavonoids, while phenolic compounds were not detected through qualitative $FeCl_3$ testing. This is consistent with quantitative data from previous studies, which state that roots have the lowest phenolic content and antioxidant activity compared to leaves, stems, and fruit. Integration of phytochemical test results with cytogenetic literature studies confirms that *S. diphyllum* has a stable genome, as indicated by a chromosome number of 2n = 24, high pollen fertility, and minimal meiotic abnormalities. This genomic stability supports consistent secondary metabolite biosynthesis in roots, particularly the alkaloids and flavonoids identified in this study. Overall, *S. diphyllum* roots have moderate bioactive potential for phytopharmaceutical exploration and provide important information for ecological studies and for assessing the invasion potential of this species in the Purwosari Village area.

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CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in the writing of this scientific paper.

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