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COMPARABLE ANTIFUNGAL ACTIVITY OF BINAHONG (*Anredera cordifolia*) LEAF AND STEM ETHANOL EXTRACTS AGAINST *Colletotrichum gloeosporioides* ISOLATED FROM ANTHRACNOSE-INFECTED CURLY RED CHILI

AKTIVITAS ANTIJAMUR YANG SEBANDING ANTARA EKSTRAK ETANOL DAUN DAN BATANG BINAHONG (*Anredera cordifolia*) TERHADAP *Colletotrichum gloeosporioides* YANG DIISOLASI DARI CABAI MERAH KERITING (*Capsicum annuum* L. var. Longum) TERINFEKSI ANTRAKNOSA

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Abstract

Colletotrichum gloeosporioides is one of the pathogens that causes anthracnose in curly red chili (*Capsicum annuum* L. var. Longum). Anthracnose in red chile is marked characterized by fruit damage and consequently reduced market value. *Anredera cordifolia* (binahong) contains various bioactive compounds with presumed antifungal activity. Most binahong studies focus on the leaves, whereas this study compared the antifungal activity ethanol extracts from the leaves and stems against Colletotrichum gloeosporioides isolated from anthracnose-infected curly red chili fruits. An experimental method was used by varying extract concentrations in the binahong stem and leaf treatment groups. Fungus from anthracnose-infected fruit was isolated, purified, and identified as *Colletotrichum gloeosporioides*. Binahong leaves and stems were extracted with 96% ethanol and tested in two sequential stages at various concentrations, both showing that the highest concentrations exhibited the greatest inhibitory activity with no significant difference between leaf and stem extracts (P >0.05). Therefore, the leaf and stem extracts of binahong have similar biofungicidal potential that increases proportionally with concentration, against *Colletotrichum gloeosporioides* from curly red chili fruit.

Keywords: *Anredera cordifolia*; Anthracnose; Binahong; *Capsicum annuum*; *Colletotrichum gloeosporioides*; Curly red chili

Abstrak

Colletotrichum gloeosporioides merupakan salah satu patogen penyebab penyakit antraknosa pada tanaman cabai merah keriting (Capsicum annuum L. var. Longum). Penyakit antraknosa pada cabai merah ditandai dengan kerusakan buah, dan akibatnya penurunan nilai jual di pasaran. Anredera cordifolia (binahong) mengandung berbagai senyawa bioaktif, termasuk yang terduga antifungi. Sebagian besar penelitian binahong menyasar pada daunnya, sedangkan penelitian ini membandingkan antijamur ekstrak etanol daun dan batang terhadap Colletotrichum gloeosporioides. Metode eksperimental digunakan dengan memvariasikan konsentrasi ekstrak pada kelompok perlakuan batang dan daun binahong. Kapang dari buah cabai merah keriting terduga antraknosa diisolasi, dimurnikan, dan teridentifikasi sebagai Colletotrichum gloeosporioides. Daun dan batang binahong diekstraksi dengan etanol 96% dan diuji dalam dua tahap berurutan pada berbagai konsentrasi, keduanya menunjukkan bahwa konsentrasi tertinggi menunjukkan aktivitas penghambatan terbesar tanpa perbedaan signifikan antara ekstrak daun dan batang (P >0.05). *Dengan demikian, ekstrak daun dan batang binahong memiliki potensi biofungisida yang serupa seiring dengan peningkatan konsentrasinya, terhadap Colletotrichum gloeosporioides asal buah cabai merah keriting.*

***Kata Kunci**: Anredera cordifolia; Antraknosa; Binahong; Cabai merah keriting; Capsicum annuum; Colletotrichum gloeosporioides*

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INTRODUCTION

Chili is a horticultural food crop and a vegetable commodity with high economic value. This food crop is essential for domestic demand, with the remainder used as raw material for the processing industry, food and beverages, seeds, and export needs. In the industrial sector, chili is used to produce chili flavor, a market dominated by the Asia Pacific region, driven by chili-based culinary traditions rooted in India, Thailand, China, and Indonesia. Red chili held the largest global sales in 2023, and is estimated to reach USD 697.04 million by 2032 (Fortune Business Insights, 2025).

Anthracnose is the most common disease affecting chili in Asia (de Silva et al., 2019), attacking chili plants throughout their growth stages, and causing infections on the stems, leaves, and fruits. The disease is caused by fungi belonging to the genus *Colletotrichum*. *C. gloeosporioides* was among the most common species causing anthracnose of *Capsicum annuum* in Asia, which was identified in three of four *Colletotrichum* isolates (de Silva et al., 2019; Wartono et al., 2024). When the fruit is infected, black spots appear that gradually develop into soft rot; severe infection may cause the entire fruit to dry out, shrivel, and eventually fall off (Yanty et al., 2024; Yulia et al., 2016).

In managing anthracnose, farmers typically apply synthetic fungicides by spraying them on affected plants, as this method provides a rapid visible result. However, continuous, long-term use of fungicides may target site alteration, efflux pump activation, and mutation of the plant pathogenic fungi that lead to resistance and negatively impact the surrounding environment (Islam et al., 2024). One approach to controlling plant diseases that is widely recognized for its safety is the use of biofungicides. These environmentally friendly agents are degraded naturally, helping to maintain ecological balance in agricultural ecosystems.

Binahong is a plant with potential antagonistic activity against *Colletotrichum* species, making it a promising candidate for a biofungicide agent. Its extract contains various bioactive compounds that have the potential to fight various diseases (Astuti et al., 2011; Dadiono et al., 2025; Ilmiyah et al., 2025; Purwaningsih et al., 2025; Sidhartha et al., 2024). Water extract of binahong leaves has antifungal activity that suppresses the growth of *Colletotrichum capsici* mycelia (Yulia et al., 2016), as does the ethanol extract (Aminatuzzifah et al., 2024). Ethanol extract of binahong stem has antifungal activity against *Candida albicans* (Kumalasari & Sulistyani, 2011). Binahong is a fast-growing climbing plant that has vigorous growth and abundant stems. Comparative studies evaluating antifungal properties of binahong leaf and stem extracts at different concentrations against *C. gloeosporioides* isolated directly from anthracnose-infected red chilli fruits under the same experimental conditions are limited. Therefore, this study examined the inhibitory effects of binahong extracts against *C. gloeosporioides* isolated directly from red chili fruits showing anthracnose symptoms while comparing leaf and stem extracts. In addition, each extract was examined in various concentrations compared to a chemical fungicide using the same comparative method. The results of this study are expected to contribute information on environmentally friendly biofungicides that also support the prevention of anthracnose disease in red chilies.

MATERIALS AND METHODS

Research Design

This study used an experimental method with a completely randomized design, which examined the inhibitory effect of binahong plant extract on the growth of *C. gloeosporioides* using the agar plate diffusion method (Kirby-Bauer). The test consisted of two stages, namely an initial test that examined 12 treatments and a further test that examined 8 treatments. The initial test was carried out to determine the concentration range of binahong extract, namely 6.25; 8.84; 12.50; 17.70; and 25% (Yulia et al., 2016), accompanied by the fungicide 3 mg/mL Antila 80 WP (containing 80% Mancozeb) as a positive control and distilled water as a negative control. Each treatment was repeated 3 times. Based on the initial test result, a further experiment was conducted to confirm and find the most effective concentration of binahong. Concentrations of 15; 25; and 35% were tested, using both stem and leaf extracts. All tools and materials were sterilized using an autoclave at 121 °C for ± 15 minutes before use.

Isolation of *Colletotrichum*

The fungus was isolated from red chili fruits showing symptoms of anthracnose. The infected fruits were observed and selected for anthracnose symptoms, which include the appearance of black spots on the fruit, which then progress to soft rot. Five large, ripe, red chillies infected with anthracnose were sampled. The isolation procedure followed Harahap et al. (2013). The fruits were washed thoroughly with running water, and the fruit skin showing anthracnose symptoms was cut into 1 × 1 cm pieces. The pieces were then surface-sterilized by soaking in 0.5% sodium hypochlorite for 30 seconds, rinsed with sterile water, and air-dried. The underside of the chilli skin was then inoculated onto the surface of potato dextrose agar in a Petri dish and incubated at 28 °C for 7 days. The fungal isolate that grew was purified and identified.

Identification of *Colletotrichum gloeosporioides*

The fungal isolate was identified morphologically. Morphological observations were primarily conducted by examining the colony colour as well as the shape and size of conidia. Microscopic observations were performed by placing a loopful of fungal mycelium on a glass object, adding a drop of methylene blue solution, and then covering it with a cover glass. The observation results were compared with various references (Bedsole et al., 2024; Rangkuti et al., 2017; Sari & Kasiamdari, 2021; Wicaksono & Kafiya, 2022; Zhang & Fan, 2025; Zhang et al., 2023). Microscopic characteristics of *C. gloeosporioides* include dark red conidia that are cylindrical with blunt tips. Furthermore, the identified isolates were multiplied and stored on slant potato dextrose agar.

Extraction of the Binahong Plant (*Anredera cordifolia*)

Binahong plants were obtained from Patrasana Village, Kresek District, Tangerang Regency, before their leaves and stems were extracted. The selected plant samples were in good condition and fresh, with uniform green leaves and stems. The plant material preparation included the following steps: wet sorting, washing, chopping, drying, dry sorting, grinding, and sieving. At a ratio of 1:6 w/v, 80 g of the medicinal plant powder was soaked in 480 mL of 96% ethanol, shaken on a shaker at 150 rpm at room temperature for 24 hours. The solution was then filtered, and the filtrate was evaporated using a rotary evaporator at 50 °C and 120 rpm to obtain a thick extract (Susanty & Bachmid, 2016). The examined extracts were diluted with sterile distilled water to 6.25; 8.84; 12.50; 17.70; and 25% (Yulia et al., 2016) for the initial examination, and 15; 25; and 35% for the further experiment.

Inhibitory Test of Binahong Extract Against *Colletotrichum gloeosporioides* Isolate

The *C. gloeosporioides* inoculum was prepared by mixing conidia from a 5-day-old (Than et al., 2008) working culture of the isolate with a sterilized saline (0.9% NaCl) solution to achieve a turbidity of 0.5 McFarland solution containing about 10^6 CFU/mL (Toigo et al., 2022). The inoculum was inoculated onto the surface of 15 mL of PDA media using the spread plate method and allowed to dry for 5 minutes at room temperature. Meanwhile, the binahong extract was diluted with sterile distilled water to become 6.25; 8.84; 12.50; 17.70; and 25% for the initial examination. Each 100 µL of binahong extract was added to the paper discs and air-dried. The paper disc containing the extract was placed aseptically on the agar surface that had been inoculated with *C. gloeosporioides*. The culture was incubated at room temperature. The same method was also carried out for the positive and negative controls. After incubation for 24 hours, the diameter of the inhibition zone around the paper disc was measured with a digital calliper. Measurements were also carried out on the positive and negative controls. The further test examined three concentrations, referring to the highest concentration from the initial examination results, and 10% below and above that concentration, namely 15; 25; and 35%. The test method was the same as in the initial examination.

Data Analysis

The results of the fungal isolate responsible for anthracnose in curly red chili are presented in figures. The inhibition zone diameters were calculated as a percentage relative to that of the chemical fungicide. These percentages were analyzed using two-way analysis of variance, followed by the

Tukey-HSD test, all at the 5% significance level. Statistical analysis and boxplot generation were performed using (Statistic Kingdom, 2025).

RESULTS AND DISCUSSION

Isolation and Identification of *Colletotrichum gloeosporioides*

Chili fruits with partial rot (Figure 1) were the target for the isolation of *C. gloeosporioides*. The rotted fruit, which is characteristic of anthracnose caused by *C. gloeosporioides*, is a circular or concave-angled lesion on ripe chili, characterized by the presence of black spots in concentric circles when ripe. This symptom is typical of anthracnose in chili, and the diameter of the lesion will increase with the age of the plant (Saxena et al., 2016; Sharma & Kulshrestha, 2015).

Figure 1. Chili fruits showing rotting symptoms (a) and the excised rotting part (b)

The mold isolated from rotting chili fruits on potato dextrose agar exhibited typical characteristics of *C. gloeosporioides* colonies. The colonies were grayish-white on the front, with a brownish-white surface, and orange on the back (Figure 2). These characteristics resemble those described in several other studies. The morphology of the *C. gloeosporioides* isolate colony is characterized by the color of the upper colony varying from gray, cream, or dark green, while the lower colony is gray, and the formation of an orange acervulus containing a mass of conidia is clearly visible in the center of the colony (Rangkuti et al., 2017; Sari & Kasiamdari, 2021). The size of the colony on potato dextrose agar also follows that described by Zhang et al. (2023), which is 75 mm in diameter after 7 days at 25 °C, with regular edges, white, villous, dense, and prominent aerial mycelium, pink to white edges, or orange-tinted conidial masses.

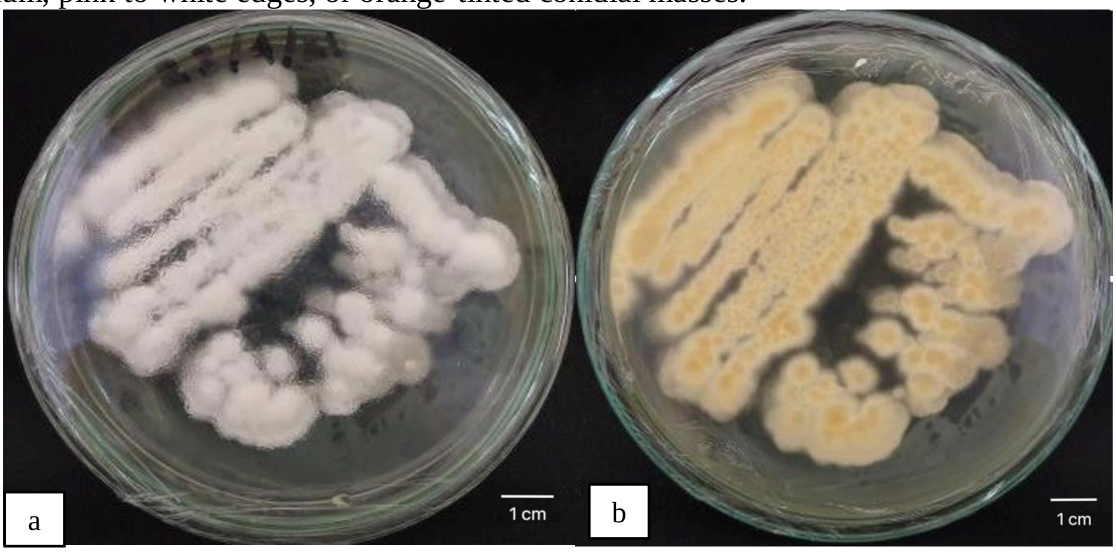


Figure 2. *Colletotrichum gloeosporioides* colonies on 7-day-old potato dextrose agar plates, isolated from rotting chili fruit (a= top view; b= bottom view)

Microscopic observations of *C. gloeosporioides* isolates showed the presence of hyaline hyphae, septate, and branched growth. Conidia were cylindrical (oval) with both blunt, rounded ends, 16.49 μm long, and 3.92 μm wide (Figure 3). These characteristics resemble those of *C. gloeosporioides* isolates described in other studies, also isolated from *Capsicum annum*, namely hyaline conidia with an average length of 13.5 μm and a cylindrical shape (Wicaksono & Kafiya, 2022). The cylindrical shape of *C. gloeosporioides* conidia distinguishes it from other types of *Colletotrichum*, which are falcate-shaped and oval (Bedsole et al., 2024). In addition, the conidia appeared to contain granular, smooth, or flat walls, no oil droplets, and approached the average size characteristics = 16.1 × 4.6 μm and L/W ratio = 3.4 according to Zhang and Fan (2025). Thus, the fungal isolate originating from rotting chili fruit was confirmed as *C. gloeosporioides,* after which it was subjected to treatment with binahong extract.

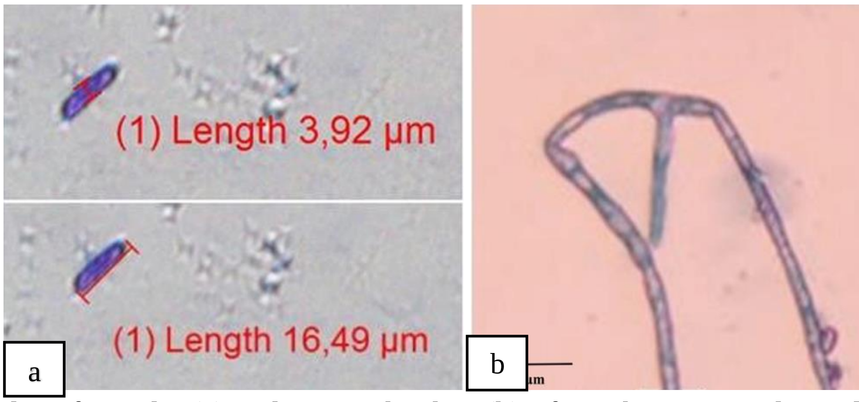


Figure 3. Examples of conidia (a) and septate hyphae (b) of *C. gloeosporioides* isolates

Inhibitory Activity Examination of Binahong Ethanol Extract Against *C. gloeosporioides* isolate

The inhibition zone is the area where fungal mycelium growth is inhibited due to the presence of toxic compounds capable of preventing conidia germination. In the initial examination, all concentrations of binahong extract applied to paper discs diffused across the agar surface, inhibiting the growth of *C. gloeosporioides* in the surrounding area. The extract solution gradually diffused away from the filter paper. Observations were made after 24 hours of incubation due to concerns that the extract's volatile content would slowly release its chemical components into the air over time, resulting in lower concentrations in the paper disc (Danh et al., 2021).

The initial examination result showed that ethanol extracts of binahong leaves and stems at concentrations of 6.25; 8.84; 12.50; 17.70; and 25% inhibited the growth of *C. gloeosporioides*, as indicated by the formation of inhibition zones, and calculated as the relative percentage to the chemical fungicide (Figure 4). The results of two-way analysis of variance ($P < 0.05$) with plant organ and concentration factors showed a significant difference in the inhibition percentage at different concentrations ($P = 0.001184 < 0.05$), but there was no significant difference between binahong leaves and stems ($P = 0.07018 > 0.05$). The Tukey HSD test revealed significant differences in the inhibition percentage of binahong extract in various concentrations. The 25% concentration of binahong extract produced the greatest inhibition, for both the leaf and stem extracts, although the inhibitory effects were not yet comparable to the fungicide Antila. Furthermore, the inhibition percentages of the 17.7% stem extract were not significantly different from those of the 25% stem and leaf extracts. The leaf extract showed a sharper increase, i.e., a larger difference between concentrations, compared to the stem extract. Low concentrations (6.25–8.84%) tended to differ significantly from high concentrations, indicating a dose-responsive trend in concentration, which was particularly evident in the leaf extract. In general, increasing concentrations increased inhibition in both leaves and stems.

Although the differences were not significant, at the same concentration, 8.84–25% stem extract was slightly more effective than leaf extract. The higher variation (wider boxplot) in the leaf extract results indicates the possibility of more complex or dominant active compounds that are not yet fully homogenized in the extract solution. Meanwhile, the smaller variation between replicates in the stem extract (narrower boxplot) indicates that the stem extract is more stable between replicates and its diffusion is more even (Figure 4).

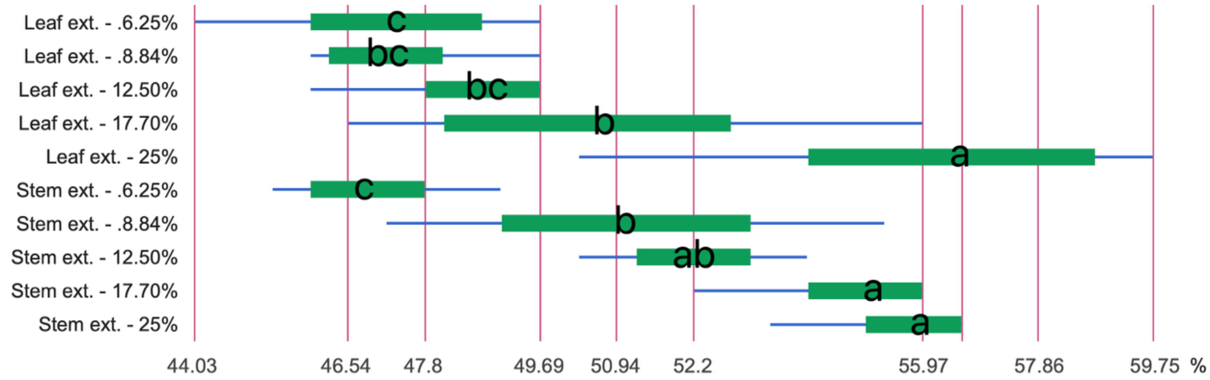
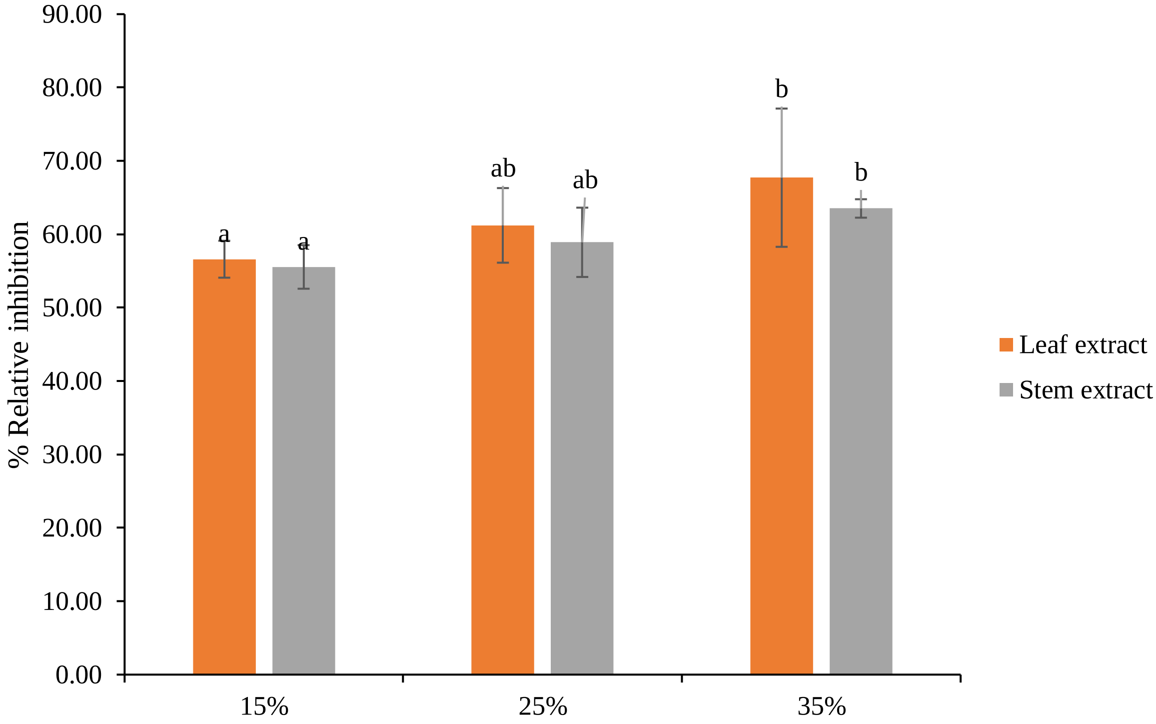


Figure 4. Percentage of inhibition zone diameter of binahong extract in various concentrations against *Colletotrichum gloeosporioides* in the initial examination. The percentage on the x-axis is the percentage of the positive control (Antila fungicide 3 mg/mL) with an inhibition zone diameter of 15.90 ± 1.84 mm. Significant differences are indicated by letters (Tukey HSD test, $P < 0.05$)

After the concentration showing the greatest inhibition was identified as 25% in the initial examination, further experiment was conducted to confirm that the 25% leaf and stem extracts were the most effective concentrations for inhibiting *C. gloeosporioides*. By taking a comparison of concentrations 10% below and 10% above 25%, the concentrations tested were 15; 25; and 35%. The results of two-way analysis of variance with plant organ and concentration factors showed a significant difference ($P = 0.0218 < 0.05$) in the inhibition percentages according to the concentration factor, but again not significant ($P = 0.3141 > 0.05$) between the leaves and stems of binahong extract (Figure 5). These results are consistent with the results of the initial examination, which showed a trend of increasing inhibition according to the concentration of binahong extract. In addition, the absence of significant differences provided by the leaf and stem extracts at the three concentrations implies that the content of antifungal compounds in both organs may have relatively similar levels.



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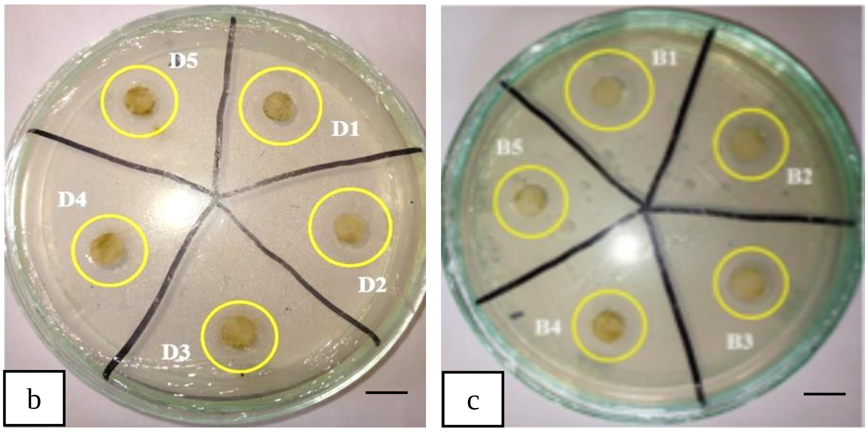

Figure 5. Percentage of inhibition zone diameter of binahong extract against *Colletotrichum gloeosporioides* in the further experiment (a). The percentage on the y-axis is the relative percentage to the positive control (Antila fungicide 3 mg/mL) with an inhibition zone diameter of 15.90 ± 1.84 mm. Significant differences are indicated by letters (Tukey HSD test, $P < 0.05$). Examples of inhibition zones (highlighted by yellow circles) produced by binahong leaf extract (b) and stem extract (c); bar= 1 cm

Consistent with these results, the growth of *Colletotrichum* spp. colonies were inhibited by up to 70% by a 2.5% ethanol extract of binahong leaves (Aminatuzzifah et al., 2024). These results differ from other studies that tested binahong leaf aqueous extract against *C. capsici*. Using a different method than this current study, 6.25% binahong leaf aqueous extract produced the greatest inhibition of *C. capsici* growth compared to higher concentrations (up to 25%), and even tended to increase the growth of the fungus at higher concentrations (Yulia et al., 2016). Thus, antifungal compounds can be extracted effectively using 96% ethanol as a solvent.

The antimicrobial function of binahong leaves in other studies is often targeted as an antibacterial; rarely as an antifungal. Binahong leaves contain 98% butylated hydroxytoluene compounds, which have antibacterial properties (Dadiono et al., 2025), and endophytes from binahong leaves are also known to produce antibacterial compounds (Nxumalo et al., 2020). Among the few recent antifungal studies, binahong leaf extract inhibits the yeast *Candida albicans* (Kumalasari & Sulistyani, 2011; Sidhartha et al., 2024) and *Pyricularia oryzae*, which caused rice blast disease (Yulia et al., 2019).

Furthermore, most studies on the active compounds focus on binahong leaves, while studies examining the stems are still very rare. A review of the functional properties of binahong in over 30 journal articles by Tedjakusuma and Lo (2022) showed that phytochemical screening studies of binahong have all focused on the leaves. Research by Kumalasari and Sulistyani (2011) suggested that the bioactive compounds contained in binahong stems are lower than in the leaves, and no alkaloids or tannins were detected in the stems. Saponins, which exert antibacterial and antifungal effects, are higher in binahong leaves than in the stems (Astuti et al., 2011). The lower bioactive compounds in the stems compared to the leaves, while their antifungal effects are not significantly different, highlight the significant potential of binahong stems. The lack of significant differences between the inhibitory effects of binahong leaves and stems on fungi in this current study suggests that the stems are just as important as the leaves.

Various studies have revealed the antimicrobial properties of binahong extracts, suggesting that the use of binahong leaves is more common. The more common use of binahong leaves is thought to arise from local wisdom, which relies on leaves that are easy to harvest and process. Although the most organ reported to contain flavonoids, tannins, saponins, and steroids in binahong was the leaf (Sidhartha et al., 2024), the binahong stems are also noteworthy for their phytochemicals. Both the stems and leaves of binahong contain steroidal and alkaloid phytochemicals, which are not necessarily found in tubers and flowers (Astuti et al., 2011). Flavonoids, tannins, and saponins are also found in both leaves and stems of binahong (Purwaningsih et al., 2025). The presence of these bioactive compounds in both organs explains the similarity in the inhibitory activity of binahong stems and leaves (Figure 5). This finding suggests that binahong stem may contain antifungal-active compounds that have not yet been characterized, highlighting the need for targeted phytochemical analysis in future studies.

The phytochemical compounds in binahong are thought to be responsible for its antifungal properties. The flavonoids and tannins responsible for the antifungal properties of binahong leaves can be extracted by ethanol, but not by methanol (Sidhartha et al., 2024). Flavonoids disrupt plasma membrane integrity, inhibit the electron transport chain in mitochondria, inhibit cell wall formation, inhibit cell division, inhibit efflux pump activity, and inhibit transcription and translation enzymes, thus inhibiting protein synthesis essential for fungal growth (Aboody & Mickymaray, 2020). Meanwhile, tannic acid has antifungal properties by disrupting cell wall integrity and plasma membrane permeability, inhibiting spore germination, and chelating iron ions, thus inhibiting enzymes that require it (Zhu et al., 2019).

Binahong contains a higher concentration of saponins than other plants, and these compounds are present in almost all binahong organs, especially tubers and leaves (Astuti et al., 2011). Research on tea saponins has shown that this group of compounds can disrupt cell membrane structure, causing leakage of cell contents and inhibiting mycelial growth, reducing cell adhesion and aggregation, and effectively inhibiting *Candida albicans* biofilm formation (Yu et al., 2022).

Alkaloids are known to penetrate fungal cell and mitochondrial membranes, affecting membrane integrity and causing oxidative stress (Sulaiman et al., 2022). Alkaloid compounds inhibit the biosynthesis pathway of ergosterol, the most abundant sterol in fungal cell membranes, thus disrupting fungal survival (Wong-Deyrup et al., 2021). Examples of alkaloids identified in binahong leaves are betalains (Ilmiyah et al., 2025). These compounds have antifungal activity against various types of yeast and mold (Sadowska-Bartosz & Bartosz, 2021).

In addition to saponins, flavonoids, tannins, and alkaloids, the ethanol extract of binahong contains polyphenolic compounds. The dominant phenolic compound detected in binahong leaf extract is butylated hydroxytoluene (BHT), which is thought to be responsible for its antibacterial effect (Dadiono et al., 2025). BHT is also known to have fungicidal effects that can inhibit post-harvest leaf rot caused by *Botrytis cinerea* and bitter rot caused by *Colletotrichum gloeosporioides* (Huang et al., 2021). Polyphenols have also been detected in binahong stem extract (Kumalasari & Sulistyani, 2011).

There are very limited studies focused on the antifungal activities of binahong stem extract.. Numerous phytochemical studies on binahong indicate that its aerial parts contain bioactive compounds such as flavonoids, phenolics, and saponins, which are commonly associated with antimicrobial activity (Astuti et al., 2011; Miladiyah & Prabowo, 2012). Although most reports focus on leaves, these secondary metabolites are generally distributed throughout plant tissues, suggesting that stems may also possess similar chemical constituents (Harborne, 1998). Therefore, the stem of binahong is likely to exhibit comparable bioactivity to the leaves due to the presence of these shared phytochemical compounds.

CONCLUSION

The results on inhibitory activities and their analysis indicated that the effectiveness of binahong extract against *C. gloeosporioides* is primarily determined by the extract concentration instead of the organ extracted. Both leaf and stem extracts have relatively similar antifungal potential in various concentrations. Based on these findings, future studies are recommended to characterize the antifungal compositions of stem extract and to evaluate combined leaf-stem extracts to explore potential synergistic effects, to reduce reliance on leaf material alone, and to optimize formulation for biofungicide development.

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