J. Trop. Plant Pests Dis.
Vol. 25, No. 2, September 2025
Pages: 317–327

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.225317-327

SHORT COMMUNIC

In vitro antifungal activity of *trembesi* leaf extract [*Samanea saman* (Jacq.) Merr.] against *Colletotrichum magnum* Rossman & Allen, the causal agent of papaya anthracnose

Athalia Ratnasari[1], Anak Agung Ketut Darmadi[2], Fainmarinat Selviani Inabuy[2], & Ni Wayan Sudatri[2]

Manuscript received: 15 September 2024. Revision accepted: 5 March 2025. Available online: 1 September 2025.

ABSTRACT

Papaya fruit production in Bali has decreased over the last three years (2020-2022). One of the contributing factors is infection by *Colletotrichum* spp., which causes anthracnose. Anthracnose is an airborne disease that infects various plant commodities including papaya. The use of chemical fungicides to control anthracnose can have negative impacts if used long-term. *Trembesi* [*Samanea saman* (Jacq.) Merr.] extract has previously been reported to possess antifungal, antiseptic, antibacterial, and antidiabetic properties. This study aimed to determine the effectiveness and category of inhibitory activity, Minimum Inhibitory Concentration (MIC), Lethal Concentration 50% (LC_{50}), and the phytochemical content of *trembesi* leaves. Methanol extract of *trembesi* leaves effectively inhibited the growth of *Colletotrichum magnum* at a concentration of 5%. The experiment used 11 treatments with 4 replications, including control (0% v/v) and extract concentrations of 1% to 10% (v/v), along with a positive control. The corresponding inhibition zone diameters were: 0.00 mm, 12.00 mm, 11.87 mm, 13.62 mm, 16.00 mm, 19.62 mm, 15.75 mm, 16.87 mm, 17.87 mm, 17.87 mm, 18.25 mm, and 20.87 mm, respectively. The minimum concentration of extract showing inhibitory activity (MIC) was 0.3%, while the LC_{50} value was 0.32%. Phytochemical screening revealed the presence of phenolics, tannins, alkaloids, steroids, terpenoids, flavonoids, and saponins in the *trembesi* leaf extract.

Key words: Antifungal, anthracnose, *Colletotrichum*, phytochemicals, *trembesi* (*S. saman*).

INTRODUCTION

Papaya (*Carica papaya*) is an agricultural commodity that is rich in nutrients and relatively affordable, making it commonly consumed by the Indonesian population, including in Bali. However, papaya production in Bali has declined over the past three years, from 16,789 tonnes in 2020 to 12,554 tonnes in 2021, and then to 11,326 tonnes in 2022 (Central Bureau of Statistics, 2023). One of the factors contributing to the decline in both the quality and quantity of papaya fruit is infection by *Colletotrichum* spp., the causative agent of anthracnose (Rangkuti et al., 2017).

Anthracnose is an airborne disease caused by *Colletotrichum* spp., which infects a wide range of agricultural crops (Firmansyah et al., 2016). During the rainy season, this disease is reported to cause global mango yields losses of up to 60%–100% (Budirokhman, 2014; Uddin et al., 2018). In Indonesia, anthracnose also severely affects chili crops, leading to crop failures of up to 90% (Wakhidah et al., 2021). In Central Java, anthracnose attacks on papaya fruit have caused losses of up to 70% (Ministry of Agriculture, 2011).

Anthracnose is typically controlled using chemical fungicides, which can have negative environmental and health impacts if used continuously (Suleiman, 2010). Therefore, alternative control methods are needed, including the use of fungicides derived from natural, environmentally friendly sources (Aziziy et al., 2020).

Trembesi (*Samanea saman*) is a plant with potential as a source of botanical fungicides (Sinarsih et al., 2016). Several studies have shown that *trembesi* extract possesses antiseptic, antifungal, antibacterial, and antidiabetic properties (Sari et al., 2015; Tungadi & Abdulkadir, 2015). Pinem et al. (2023) demonstrated that methanol extracts of *trembesi* leaves can inhibit the growth of *Colletotrichum* sp. However, no studies have evaluated the effectiveness of *trembesi* leaf extract against *Colletotrichum magnum* or determined

Corresponding author:
Anak Agung Ketut Darmadi (darmadi@unud.ac.id)

[1]Undergraduate Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia
[2]Biology Doctoral Study Program, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia

its Lethal Concentration 50% (LC_{50}), which is essential for dosage recommendations.

In preliminary, several garden plants were screened for antifungal activity against papaya anthracnose, leading to the discovery that *trembesi* can inhibit *C. magnum* in vitro. Further phytochemical analysis revealed that *trembesi* contains phenols, tannins, flavonoids, steroids/terpenoids, alkaloids, and saponins, compounds known to inhibit fungal growth by disrupting the cell wall and plasma membrane (Seleem et al., 2016).

Therefore, this study aims to evaluate the antifungal activity of *trembesi* leaf extract against *C. magnum* and to determine its LC_{50} value, providing baseline data for its potential development as a botanical fungicide.

MATERIALS AND METHODS

Simplicia Preparation. *Trembesi* leaf simplicia was obtained from the Amlapura Heroes Monument area, Karangasem Regency (coordinate: 8.44636°S, 115.61167°E), Bali Province. Meanwhile, the *C. magnum* isolate used was obtained from the collection of the Biochemistry Laboratory, Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University (Sudirga et al., 2022).

Extraction of *Trembesi* Leaves. A total of 2 kg of *trembesi* leaves were thoroughly washed with running water and air-dried for approximately 72 h until reaching a constant weight. The dried leaves were then ground using a blender. About 200 g of *t*he resulting powder was macerated with 95% methanol at a 1:10 (w/v) ratio for 3 × 24 h. The extract was filtered using Whatman No.2 filter paper and evaporated using a Buchi rotary evaporator at 40 ºC.

Rejuvenation of *C. magnum.* The *C. Magnum* fungus (accession number PCS730304J) was rejuvenated by adding 10 mL of sterile distilled water to the fungal culture on slant medium. The mycelium was scraped using an inoculation loop and vortexed until homogeneous. A total of 200 µL of the fungal suspension was cultured on 10 mL of Potato Dextrose Agar (PDA) medium and incubated at 28 ºC for 5–7 days. Once a pure culture was obtained, the isolate was transferred to slant PDA tubes for further use.

Minimum Inhibiting Concentration (MIC) Test. The MIC was determined using the diffusion well method. A total of 200 µL of *C. magnum* suspension was placed into a 90 mm Petri dish containing 10 mL of PDA and mixed evenly. After solidification, wells were created using a 5 mm diameter cork borer (Usbeck, Germany). Then, 20 µL of each extract concentration was placed into each well, and the plates were incubated for 3 × 24 hours at room temperature (27 ºC ± 2). The inhibition zone diameter was calculated using the formula:

$$ID = \frac{D1 + D2}{2}$$

ID = Inhibitory diameter,
D_1 = Vertical diameter,
D_2 = Horizontal diameter.

Inhibitory Activity Test of *Trembesi* Extract Against *C. magnum.* This test also used the diffusion well method with ten different extract concentration: 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10% (v/v), along with positive and negative controls. The positive control used was a synthetic fungicide commonly used in the field, namely Benstar 50 WP. The negative control used methanol as the solvent.

Lethal Concentration 50% (LC_{50}) Test. The LC_{50} test was conducted using the colony method (Darmadi et al., 2017). The concentration tested were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%. Colony growth was observed daily until the negative control fully covered the Petri dish. Colony diameters were measured with a caliper, and the percentage of growth inhibition was calculated using the formula (Rai, 2006):

$$GI = \frac{D_1 + D_2}{D_1} \times 100\%$$

GI = Growth inhibition (%),
D_1 = Diameter of control colonies,
D_2 = Diameter of treated colonies.

A regression curve was then generated, plotting extract concentration (x) against inhibition percentage (y), to obtain a linear regression equation. The LC_{50} value (x) was derived by substituting 50% for y in the regression equation. The negative control in this test consisted of PDA media treated with methanol only.

Phytochemical Screening of *Trembesi* Leaf Extract. Phytochemical tests were conducted to identify compound classes in the active extract fraction, using specific reagents (Harborne, 1998). The tests were carried out on the extract with the highest antifungal activity. The reagents used were:

Alkaloid Test* (*Wagner's reagent): 2.7 g of iodine and 2 g of potassium iodide (KI) were dissolved in 25 mL of distilled water, then diluted to 100 mL in a volumetric flask. A few drops of Wagner's reagent were added to a small amount of extract. A brown precipitate indicates a positive result.

Terpenoid and Steroid Test (Libermann-Burchard reagent): 5 mL of anhydrous acetic acid was mixed with concentrated sulfuric acid while cooling, then added to 50% ethanol under cold conditions. A color change to green/blue indicates steroids; red to purple indicates triterpenoids.

Flavonoid Test (10% NaOH): 10 g of NaOH was dissolved in distilled water and diluted to 100 mL. A few drops of 10% NaOH were added to the extract. A specific color change indicates the presence of flavonoids.

***Tannin Test*:** 1 mL of *trembesi* methanol extract was placed in a test tube, and 2–3 drops of 1% FeCl3 was added. A change from light green to green-black indicates tannins.

***Phenol Test (1% $FeCl_3$)*:** A small amount of extract was mixed with 1% $FeCl_3$. The formation of a blackish-green color indicates the presence of phenolic compounds.

***Saponin Test (Foam Test)*:** The extract was mixed with hot water and shaken vigorously. The formation of stable foam indicates the presence of saponins.

Data Analysis. Qualitative data were presented through images and descriptions, while quantitative data were analyzed using analysis of variance (ANOVA). If significant difference were found, Duncan's multiple range test (DMRT) at the 5% significance level was used as a post hoc test. LC_{50} test data were analyzed using linear regression with Microsoft Excel 2010.

RESULTS AND DISCUSSION

Reisolation and Reidentification of *C. magnum* Isolates. Reisolation of *C. magnum* on PDA medium after 7 days of incubation produced a fungus with the following macroscopic characteristics: the top view of the colony appeared grayish white with a green center, while the underside was blackish orange. Microscopic observations showed cylindrical conidia with rounded ends, and branched, septate hyphae (Figures 1 and 2).

The reisolated *C. magnum colony* measured 35 mm in diameter and exhibited typical morphological characteristics. Microscopic observations confirmed cylindrical conidia with rounded ends and branched, septate hyphae. These features are consistent with the typical morphology of *C. magnum*, which produces transparent conidia arranged in acervuli, cylindrical in shape with rounded tips (Dickman, 1993). The mycelium consisted of hyphae with multiple septa, spreading with a size of approximately ± 150 µm, and appearing light to dark brown (Barnett & Hunter, 2003; Watanabe, 2002; Dickman, 1993).

Inhibitory Activity of *Trembesi* Leaf Extract against *C. magnum*. Crude extract of *trembesi* leaves was

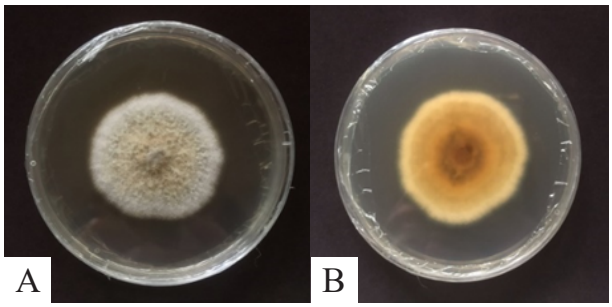

Figure 1. Macroscopic morphology of *C. magnum*. A. Top view of the colony; B. Bottom view of the colony.

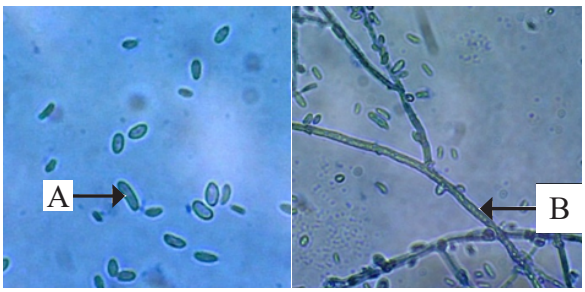

Figure 2. Microscopic morphology of *C. magnum* at 400× magnification. A. Conidia; B. Hyphae.

prepared by dissolving 1 g of thick, evaporated extract in approximately 1000 µL of methanol, yielding a concentration of nearly 50%. This test resulted in an inhibition zone diameter of 19.4 mm (Figure 3).

According to Davis & Stout (1971), this inhibition zone size indicates strong antifungal activity. This finding aligns with previous research by Pinem et al. (2023), who reported an inhibition zone of 19 mm for *Colletotrichum* sp. using *trembesi* leaf extract. The antifungal activity of *trembesi* leaves is attributed to their bioactive compound content (Pinem et al., 2023; Salni et al., 2013). Similarly, Darmadi et al. (2022) reported that cinnamon leaf extract showed a very strong inhibitory effect against *C. acutatum*, with an inhibition zone diameter of 35 mm. *C. acutatum* is known as a causal agent of anthracnose in chili plants.

Minimum Inhibiting Concentration (MIC) Test. The MIC value of *trembesi* leaf methanol extract was determined to be 0.3%, with an inhibition zone diameter of 9.37 ± 1.31 mm (Table 1 and Figure 4). This value reflects the lowest concentration capable of inhibiting *C. magnum* growth.

Previous research reported an MIC of 0.1% for *trembesi* leaf extract against *Colletotrichum* sp. (Pinem et al., 2023). Darmadi et al. (2021) reported the MIC

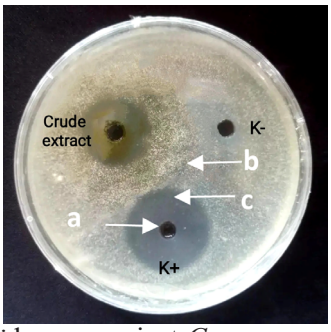

Figure 3. Test of crude extract of *trembesi* leaves against *C. magnum* fungus. A. Diffusion well; B. *C. magnum*; C. Zone of inhibition.

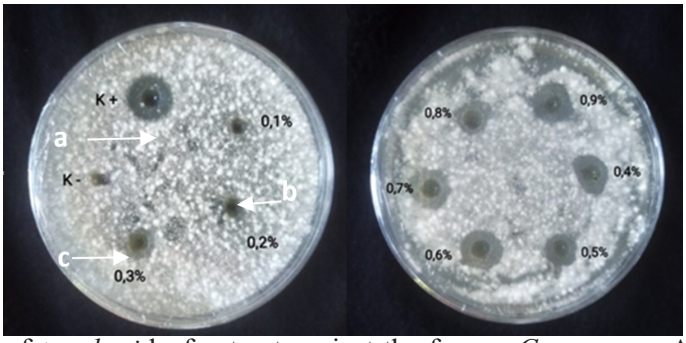

Figure 4. MIC test results of *trembesi* leaf extract against the fungus *C. magnum*. A. *C. magnum*; B. Diffusion well; C. Zone of inhibition.

Table 1. MIC test results of *trembesi* leaf extract against the fungus *C. magnum*

Treatment	Average inhibition zone diameter (mm)
0.1%	0.00 ± 0.00
0.2%	0.00 ± 0.00
0.3%	9.37 ± 1.31
0.4%	10.12 ± 0.62
0.5%	11.00 ± 0.40
0.6%	11.75 ± 0.64
0.7%	11.75 ± 1.19
0.8%	12.12 ± 0.47
0.9%	12.50 ± 0.70
Positive control	17.62 ± 1.54
Negative control	0.00 ± 0.00

of cinnamon leaf acetone extract to be 0.5% against *Colletotrichum* spp. on chili plants in Bali. The size of the inhibition zone is influenced by several factors, including extract concentration, secondary metabolite content, diffusion time and distance, and incubation temperature (Alfiah et al., 2015). Pelczar & Chan (2005) stated that higher concentrations of extract contain more dissolved bioactive compounds, thereby

Table 2. Effective concentration of *trembesi* leaf extract and the average diameter of their inhibition zones against the growth of *C. magnum*

Treatment	Average of inhibition zone diameter (mm)
Negative control	00.00 ± 0.00 a*
1%	12.00 ± 0.40 b*
2%	11.87 ± 0.62 b
3%	13.62 ± 0.62 c*
4%	16.00 ± 0.91 d*
5%	19.62 ± 1.10 eg*
6%	15.75 ± 2.53 d
7%	16.87 ± 0.62 df
8%	17.87 ± 0.25 f*
9%	17.87 ± 0.94 f
10%	18.25 ± 1.19 ef
Positive control	20.87 ± 0.85 g*

Different letters indicate significant differences based on Duncan's multiple range test (DMRT) at the 5%. Asterisks () indicate significantly different from the negative control ($p < 0.05$).*

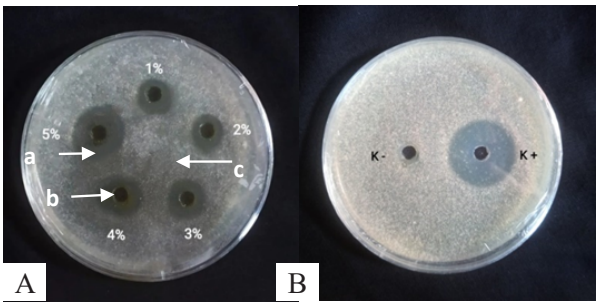

Figure 5. Results of the effective concentration test for inhibiting the growth of the fungus *C. magnum*. A. Inhibitory power of *trembesi* leaf extract at concentrations of 1%, 2%, 3%, 4%, and 5% against *C. magnum* fungus; B. Inhibitory power of *trembesi* leaf extract at a concentration of 0% or negative control (K-) and positive control (K+), against the fungus *C. magnum.*

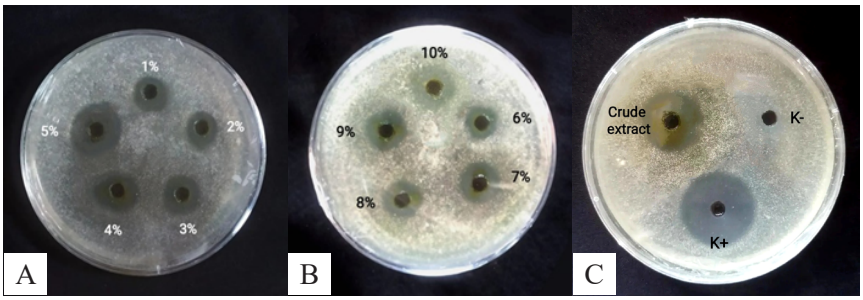

Figure 6. Comparison of inhibition zone diameters of *trembesi* leaf extract. A. Inhibitory power of *trembesi* leaf extract at concentrations of 1%, 2%, 3%, 4%, 5% against *C. magnum* fungus; B. Inhibitory power of *trembesi* leaf extract at concentrations of 6%, 7%, 8%, 9%, and 10% against *C. magnum* fungus; C. The inhibitory power of crude extract of *trembesi* leaves or the assumption of 100%, at a concentration of 0% or negative control (K-) and positive control (K+), against the fungus *C. magnum.*

increasing their inhibitory effect on microbial growth.

Determination of the Effective Concentration of *Trembesi* Leaf Extract Against *C. magnum.* *trembesi* leaf extract at concentrations of 1%, 2%, 3%, 4%, 5% and the positive control significantly inhibited *C. magnum* growth compared to the negative control. The 1% concentration produced an inhibition zone of 12 mm, while the 5% concentration gave the highest inhibitory power at 19.62 mm. Beyond this point, inhibitory activity declined as concentration increased (Table 2, Figure 5 and 6).

Table 3 shows that inhibition generally increased with concentration up to 5%, then decreased. According to Bibiana (1994), an effective antimicrobial concentration is one that shows inhibitory power at a low dose and killing power at higher doses. Duncan's test indicated that 5% *trembesi* leaf extract showed significantly higher inhibition than the negative control and concentrations of 1%, 2%, 3%, 4%, 6%, 7%, 8%, and 9%, but was not significantly different from the 10% concentration or the positive control. This suggests that 5% is the optimal effective concentration.

Tiana et al. (2016) also noted that maximum effectiveness does not always correspond to the highest concentration. Increasing extract concentration may alter the medium's pH, influencing the diffusion and bioactivity of compounds (Athaillah & Sugesti, 2020). Apart from that, if the concentration of the extract being tested exceeds a certain limit, there will be a decrease in the inhibition zone (Henaulu & Kaihena, 2020). High concentrations may result in decreased inhibition due to poor diffusion caused by increased molecular density (Allo, 2016). Conversely, lower concentrations allow better diffusion into the medium (Tiana et al., 2016).

LC_{50} Test of *Trembesi* Leaf Extract Against *C. magnum.* Colony diameter was measured on the 20th day of incubation. The LC_{50} value was found to be 0.32%. A higher concentration of *trembesi* extract corresponded to greater inhibition of fungal colony growth, based on the regression equation $y = 153.18x + 0.375$ with $R^2 = 0.9398$ (Figure 7).

This regression curve shows that the methanol extract of Trembesi leaves had an LC_{50} value of 0.32%.

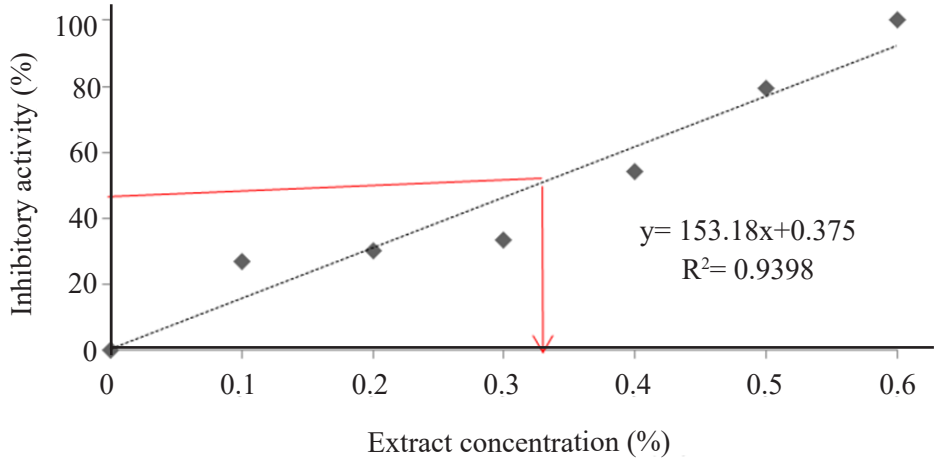

Figure 7. Regression curve of the LC_{50} concentration of *trembesi* leaf extract on the percentage of growth inhibition of the fungus *C. magnum.*

Table 3. Results of phytochemical screening of the methanol extract of *trembesi* leaves

Compound name	Visual changes	Results
Fenol	yellow → blackish purple	Positive
Tanin	A blackish green color forms	Positive
Flavonoid	yellow → red	Positive
Steroid/Terpenoid	A greenish color forms	Positive for Steroids
	A reddish color form	Positive for Terpenoids
Alkaloid	A white precipitate is formed	Positive
	A brown precipitate forms	Positive
Saponin	Forms stable foam	Positive

No inhibition was observed in the negative control (methanol only), indicating that the antifungal activity was due to the active compounds in the extract.

Phytochemical Screening. The methanol extract of *trembesi* leaves inhibited the growth of *Colletotrichum magnum* due to the presence of multiple bioactive phytochemicals. Phytochemical screening confirmed the presence of phenols, tannins, flavonoids, steroids, terpenoids, alkaloids, and saponins (Table 3). The diversity of these compounds is significant because each group contributes to antifungal activity through distinct biochemical mechanisms, making *trembesi* extract a complex and potentially potent botanical fungicide.

Tannins, steroids, terpenoids, and saponins disrupt cell membranes due to their lipophilic properties, leading to leakage of intracellular materials (Utami et al., 2022; Lutfiyanti et al., 2012). Tannins specifically interfere with iron chelation, oxidative phosphorylation, and enzymatic activity (Das & Goswami, 2019). Saponins destabilize fungal lipid membranes, while flavonoids inhibit conidial germination and fungal cell wall synthesis (Ningsih et al., 2023; Purbasari et al., 2023; Chatri et al., 2022), causing membrane depolarization, K^+ leakage, and eventual cell death (Al Aboody & Mickymaray, 2020). Alkaloids interfere with DNA and RNA synthesis, inhibit protein and phospholipid formation, disrupt respiration, and ultimately cause irreversible cell damage (Komala et al., 2020; Utami et al., 2022; Sulistyawati et al., 2019).

The present findings are novel because previous studies on *trembesi* largely focused on its inhibitory effects against general *Colletotrichum* spp. (Pinem et al., 2023) or other pathogens such as *Fusarium solani* in dragon fruit (Rita et al., 2013). In contrast, this study provides the first report of *trembesi* leaf extract effectively inhibiting *C. magnum*, the causal agent of papaya anthracnose, while also confirming its phytochemical composition and discussing the mechanistic basis of its antifungal activity. Notably, at a concentration of 50%, *trembesi* extract produced an inhibition zone of 19.4 mm, which falls into the strong inhibition category. Increasing concentrations further enhanced inhibitory activity, suggesting dose-dependent antifungal potential.

Research on botanical fungicides such as *trembesi* is highly relevant for sustainable agriculture, as plant-derived antifungals are biodegradable, environmentally safe, and non-toxic to non-target organisms (Mir et al., 2023). However, challenges remain in field application, including the need for large quantities of extract, slower action compared with synthetic fungicides, and limited stability due to rapid degradation. To address these limitations, future studies should focus on (i) fractionating and identifying the specific active compounds, (ii) testing different solvents or synergistic combinations with other plant extracts to improve efficacy, and (iii) developing formulations that stabilize active compounds for field use.

Overall, this study advances the understanding of *trembesi* as a promising source of antifungal metabolites with strong potential to be developed into botanical fungicides for papaya anthracnose management. By providing mechanistic insights and highlighting practical challenges, our work lays the foundation for translating laboratory results into field-ready applications that align with the goals of sustainable and environmentally friendly agriculture.

CONCLUSION

A 5% concentration of *trembesi* leaf extract is effective in inhibiting the growth of *C. magnum*, producing an inhibition zone with a diameter of 19.62 mm. The minimum inhibitory concentration (MIC) of the methanol extract of *trembesi* leaves is 0.3%, with an inhibition zone of 9.37 mm. The LC_{50} value of the methanol extract is 0.32%. The methanol extract of *trembesi* leaves contains phenols, tannins, steroids, terpenoids, alkaloids, flavonoids, and saponins.

ACKNOWLEDGMENTS

We would like to thank Udayana University, particularly the Institute for Research and Community Service, for providing the grant that funded this research (SP DIPA023.17.2.677526/2024, dated 24 November 2023, DIPA 2024).

FUNDING

This research was funded by Udayana University through PNBP funds under the research implementation assignment agreement No. SP DIPA023.17.2.677526/2024, dated 24 November 2023, DIPA 2024.

AUTHORS' CONTRIBUTIONS

AR, AAKD, and FSI conceived the idea, designed the study structure, and developed the

research methods. AR and NWS analyzed the data and verified the bioactive compounds. All authors participated in data collection and manuscript writing. AR and NWS proofread and finalized the manuscript. All authors have read and approved the final version.

COMPETING INTEREST

The authors declare that there are no competing interest.

REFERENCES

Al Aboody MS & Mickymaray S. 2020. Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics*. 9(2): 45. https://doi.org/10.3390/antibiotics9020045

Alfiah RR, Khotimah S, & Turnip M. 2015. Efektivitas ekstrak metanol daun sembung rambat (*Mikania micrantha* Kunth) terhadap pertumbuhan jamur *Candida albicans* [Effectiveness of methanol extract of sembung rambat (*Mikania micrantha* Kunth) leaves against the growth of the fungi *Candida albicans*]. *Protobiont*. 4(1): 52–57.

Allo MBR. 2016. Uji Aktivitas Antibakteri dari Ekstrak Air Kulit Buah Pisang Ambon Lumut (*Musa acuminata* Colla) terhadap Pertumbuhan *Staphylococcus aureus* [The Antibacterial Activity Test from Water Extract of Ambon Lumut Banana Peel (*Musa acuminata* Colla) towards the Growth of *Staphylococcus aureus*]. *Thesis*. Universitas Sanata Dharma. Yogyakarta.

Athaillah & Sugesti. 2020. Uji aktivitas antibakteri *Staphylococcus epidermis* menggunakan ekstrak etanol dari simplisia kering bawang putih (*Allium sativum* L.) [Antibacterial activity test of *Staphylococcus epidermis* using ethanol extract from dried garlic simplicia (*Allium sativum* L.)]. *Jurnal Education and Development*. 8(2): 375–380.

Aziziy MH, Tobing OL, & Mulyaningsih Y. 2020. Studi serangan antraknosa pada pertumbuhan cabai merah (*Capsicum annuum* L.) setelah aplikasi larutan daun mimba dan mol bonggol pisang [Study of anthracnose attack on the growth of red chili (*Capsicum annuum* L.) after application of the neem leaves solution and local microorganism of banana hump]. *Jurnal Agronida*. 6(1): 22–32. https://doi.org/10.30997/jag.v6i1.2668

Central Bureau of Statistics. 2023. Bali Province Papaya Fruit Production by Regency/City (Tons), 2020-2022. Bali Central Statistics Agency. https://bali.bps.go.id/id/statistics-table/2/MjA0IzI=/produksi-buah-pepaya-provinsi-bali-menurut-kabupaten-kota.html. Accessed December 12 2024.

Barnett HL & Hunter BB. 2003. *Illustrated Genera of Imperfect Fungi. Fourth Edition.* APS Press. St. Paul. Minnesota.

Bibiana WL. 1994. *Analisis Mikroba di Laboratorium*. PT Raja Grafindo Persada. Jakarta.

Budirokhman, D. 2014. Pengaruh penggunaan pupuk organik chitosan dan dosis pupuk kandang terhadap produktivitas tanaman mangga (*Mangifera indica* L.) kultivar *gedong gincu* [The effect of using chitosan organic fertilizer and manure dosage on the productivity of mango plants (*Mangifera indica* L.) *gedong gincu* cultivar]. *Jurnal Logika*. XII(3): 13–22.

Chatri M, Jumjunidang, Aini Z, & Suryendra FD. 2022. Aktivitas antifungi ekstrak daun *Melastoma malabathricum* terhadap *Fusarium oxysporum* dan *Sclerotium rolfsii* secara in vitro [Antifungal activity of *Melastoma malabathricum* leaf extract against *Fusarium oxysporum* and *Sclerotium rolfsii* with in vitro]. *Jurnal Agrotek Tropika*. 10(3): 396–401. https://doi.org/10.23960/jat.v10i3.5713

Darmadi AAK, Ginantra IK, Joni M. 2017. Uji efektivitas ekstrak aseton daun kayu manis (*Cinnamomum Burmanni* Blume) terhadap jamur *Fusarium solani* penyebab penyakit busuk batang pada buah naga (*Hylocereus* sp.) secara *in vitro* [In vitro effectiveness test of cinnamon leaf (*Cinnamomum burmanni* Blume) acetone extract on the growth of fungi *Fusarium solani,* the cause rotten stem disease of dragon fruit (*Hylocereus* sp.)]. *Jurnal Metamorfosa* 4(1): 79–86.

Darmadi AAK, Suriani NL, Sudirga SK, & Khalimi K. 2019. First Study On Fusarium Equiseti: Causes Fusarium Wilt In Tomato Crop In Bali, Indonesia. *SABRAO Journal of Breeding and Genetics.*51(4): 442–450

Darmadi AAK, Suaskara IBM, Suriani NL, Darmayasa IBG, Gari NM, Fudholi A. 2021. Cinnamon leaf extract to control anthracnose disease on chilli plants in Bali: A novel and new potential. *Int.*

J. Pharma Res. 13(1): 1006–1015. https://doi.org/10.31838/ijpr/2021.13.01.155

Darmadi AAK, Suriani NL, Ginantra IK, & Sudirga SK. 2022. Effectiveness of cinnamon leaf extract to control anthracnose disease on large chilies in Bali, Indonesia. *Biodiversitas*. 23(6): 2859–2864. https://doi.org/10.13057/biodiv/d230611

Das M & Goswami S. 2019. Antifungal and antibacterial property of guava (*Psidium guajava*) leaf extract: Role of phytochemicals. *Int. J. Health Sci. Res*. 9(2): 39–45.

Davis WW & Stout TR. 1971. Disc plate method of microbiological antibiotic assay: I. factors influencing variability and error. *Appl Environ Microbiol.* 22(4): 659–665. https://doi.org/10.1128/am.22.4.659-665.1971

Dickman MW. 1993. *The Fungi. Academic Press.* New York.

Firmansyah MY, Sastrahidayat SR, & Djauhari S. 2016. Studi identifikasi dan cara inokulasi penyakit antraknosa pada tanaman *Sansevieria trifasciata* [Study of identification and inoculation methods for anthracnose disease in *Sansevieria trifasciata* plants]. *Jurnal HPT*. 4(3): 125–133.

Harborne JB. 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Third Edition*. Chapman & Hall. London.

Henaulu AH & Kaihena M. 2020. Potensi antibakteri ekstrak etanol daun kecipir (*Psophocarpus tetragonolobus* (L.) Dc) terhadap pertumbuhan *Escherichia coli* dan *Staphylococcus aureus* in vitro [Antibacterial potential of ethanol extract of winged bean leaves (*Psophocarpus tetragonolobus* (L.) Dc) against the growth of *Escherichia coli* and *Staphylococcus aureus* in vitro]. *Biofaal Journal*. 1(1): 44–54. https://doi.org/10.30598/biofaal.v1i1pp44-54

Komala O, Yulianita, & Siwi FR. 2020. Aktivitas antijamur ekstrak etanol 50% dan etanol 96% daun pacar kuku *Lawsonia inermis* L. terhadap *Trichophyton mentagrophytes* [Activity of antifungal ethanol extract 50% and ethanol 96% leaf henna nail *Lawsonia inermis* L. against *Trichophyton mentagrophytes*]. *Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 19(1): 12–19. https://doi.org/10.33751/ekol.v19i1.1657

Lutfiyanti R, Ma'ruf WF, & Dewi EN. 2012. Aktivitas antijamur senyawa bioaktif ekstrak *Gelidium latifolium* terhadap *Candida albicans* [Antifungal activity of bioactive compounds of *Gelidium latifolium* extract against *Candida albicans*]. *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan*. 1(1): 26–33.

Ministry of Agriculture. 2011. *Budidaya Pepaya California* [California Papaya Cultivation]. Balai Pengkajian Teknologi Pertanian Jawa Tengah. Book or website? Please complete it. Example: Brogden KA & Guthmille JM. 2002. Polymicrobial Diseases. ASM Press. Washington D.C. http://www.ncbi.nlm.nih.gov/books/NBK2475/. Accessed 28 February 2014.

Mir SA, Padhiary A, Ekka NJ, Baitharu I, &Nayak B. 2023. Chapter 19 - Environmental impacts of synthetic and biofungicides. *Current Developments in Biotechnology and Bioengineering*. 487–504. https://doi.org/10.1016/B978-0-323-91900-5.00012-6

Uddin MN, Shefat SHT, Afroz M, & Moon NJ. 2018. Management of anthracnose disease of mango caused by *Colletotrichum gloeosporioides*: A review. *Acta Sci. Agric*. 2(10): 169–177.

Ningsih IS, Chatri M, Advinda L, & Violita. 2023. Senyawa aktif flavonoid yang terdapat pada tumbuhan [Active flavonoid compounds found in plants]. *Serambi Biologi*. 8(2): 126–132.

Pelczar MJ & Chan ECS. 2005. Dasar-Dasar Mikrobiologi, Jilid I Penerjemah Hadiotomo RS, Imas T, Tjitrosomo SS, dan Angka SL, [Fundamentals of Microbiology, Volume I Translator Hadioetomo RS, Limas T, Tjitrosomo SS, and Angka SL] UI-Press, Jakarta

Pinem AJH, Suprapta DN, & Suniti NW. 2023. Uji efektivitas ekstrak daun *trembesi* (*Samanea saman* (Jacq.) Merr) untuk menghambat pertubuhan jamur *Colletotrichum* sp. penyebab penyakit antraknosa pada pepaya (*Carica papaya*) [Effectiveness of rain tree leaf extract (*Samanea saman* (Jacq.) Merr) to inhibit the growth of the fungus *Colletotrichum* sp. the cause of anthracnose disease on papaya (*Carica papaya*)]. *Agrotrop.* 13(2): 207–218. https://doi.org/10.24843/AJoAS.2023.v13.i02.p05

Purbasari IGAKI, Susanti DNA, & Lestarini NKA. 2023. Efektivitas ekstrak daun *Mangifera indica* L. menghambat *Candida albicans* pada plat resin akrilik *heat-cured* [Effectiveness of

Mangifera indica L. leaf extract inhibition of *Candida albicans* on heat-cured acrylic resin plates]. *e-Gigi*. 11(2): 161–169. https://doi.org/10.35790/eg.v11i2.44596

Rai IGA. 2006. Aktivitas Fungisida Ekstrak Daun Saba (*Piper majusculum* Blume) terhadap Jamur *Fusarium oxysporum* f.sp. *vanilla* Penyebab Penyakit Busuk Batang pada Vanili. [Fungicidal Activity of Saba Leaf Extract (*Piper majusculum Blume*) Against *Fusarium oxysporum* f.sp. vanilla Fungus, the Cause of Stem Rot Disease in Vanilla].Universitas Udayana, Denpasar.

Rangkuti EE, Wiyono S, & Widodo. 2017. Identifikasi *Colletotrichum* spp. asal tanaman pepaya [Identification of *Colletotrichum* spp. originated from papaya plant]. *Jurnal Fitopatologi Indonesia.* 13(5): 175–183. https://doi.org/10.14692/jfi.13.5.175

Rita WS, Suprapta DN, Sudana IM, & Swantara IMD. 2013. First Report on Fusarium solani, a Pathogenic Fungus Causing Stem Rot Disease on Dragon Fruits (Hylocereus sp.) in Bali. *Journal of Biology, Agriculture and Healthcare*. 3(17):93–99

Rita WS, Suprapta DN, Swantara IMD, and Sudana IM. 2022. Antifungal Activity of Phenolic Compounds From Samanea saman Leaves Against Stem Rot Disease on Dragon Fruits Caused by Fusarium solani. First Asian PGPR Indonesian Chapter International e-Conference 2021, KnE Life Sciences. 621–635. https://doi.org/10.18502/kls.v7i3.11167

Seleem D, Vardi P, & Murata RM. 2016. Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro. *Arch Oral Biol.* 76: 76–83. https://doi.org/10.1016/j. archoralbio.2016.08.030

Salni, Aminasih N, & Sriviona R. 2013. Isolasi senyawa antijamur dari rimpang lengkuas putih (*Alpinia galanaga* L. Willd) dan penentuan konsentrasi hambat minimum terhadap *Candida albicans. Prosiding Semirata FMIPA*. Universitas Lampung. Lampung.

Sari PP, Rita SW, & Puspawati NM. 2015. Identifikasi dan uji aktivitas senyawa tanin dari ekstrak daun *trembesi* (*Samanea saman* (Jacq.) Merr) sebagai antibakteri *Escherichia coli* (*E. coli*) [Identification and activity testing of tannin compounds from *trembesi* leaf extract (*Samanea saman* (Jacq.) Merr) as an antibacterial for *Escherichia coli* (*E. coli*)]. *Jurnal Kimia*. 9(1): 27–34.

Sinarsih NK, Rita WS, & Puspawati NM. 2016. Uji efektifitas ekstrak daun *trembesi* (*Samanea saman* (Jacq.) Merr) sebagai antibakteri *Escherichia coli* dan *Staphylococcus aureus* [Testing the effectiveness of *trembesi* leaf (*Samanea saman* (Jacq.) Merr) as an antibacterial for *Escherichia coli* and *Staphylococcus aureus*]. *Cakra Kimia*. 4(2): 129–136.

Sudirga SK, Wijaya IMS, & Darmadi AAK. 2022. First testimony of new host plant of *Colletotrichum magnum* (S.F. Jenkins & Winstead) Rossman & W.C. Allen causing anthracnose in *Carica papaya* L. fruits in Bali, Indonesia. *Sabrao Journal of Breeding and Genetics*. 54(4): 834–841. http://doi.org/10.54910/sabrao2022.54.4.14

Suleiman MN. 2010. Fungitoxic activity of neem and pawpaw leaves extracts on *Alternaria solani*, causal organism of yam rots. *Adv. Environ. Biol.* 4(2): 159–167.

Sulistyawati D, Wiryosoendjojo K, & Puspawati, N. 2019. Uji aktivitas antijamur ekstrak etanolik daun dan daging buah berenuk (*Crescentia cujete* Linn.) terhadap *Candida albicans* ATCC 1023 [Anti-fungal activity test of ethanolic extracts of calabash's leaved and fruit meat (*Crescentia cujete* Linn.) against *Candida albicans* ATCC 1023]. *J. Biomedika*. 12(02): 217–227. http://ejurnal.setiabudi.ac.id/ojs/index.php/biomedika

Tiana O, Prasetya F, Kuncoro H, & Rijai L. 2016. Aktivitas antijamur ekstrak daun ketepeng cina (*Cassia alata* L.) [Antifungal activity of chinese ketepeng leaf extract (*Cassia alata* L.)]. *Jurnal Sains dan Kesehatan*. 1(6): 311–315. https://jsk.ff.unmul.ac.id/index.php/JSK/article/view/80

Tungadi R & Abdulkadir W. 2015. Burn wound healing effect of *trembesi* (*Samanea saman*) leaves extract gel on rats (*Rattus novergicus*). *Int. J. PharmTech Res.* 7(4): 601–605.

Utami N, Auliah A, & Dini I. 2022. Studi kandungan senyawa metabolit sekunder beberapa ekstrak tai anging (*Usnea* sp.) dan uji bioaktivitasnya terhadap (*Candida albicans*) [Study of secondary metabolites compounds content some tai anging (*Usnea* sp.) extracts and test against bioactivity test (*Candida albicans*)]. *Jurnal Chemica*. 23(1): 90–98. https://doi.org/10.35580/chemica.

v23i1.34077

Wakhidah N, Kasrina, & Bustaman H. 2021. Keanekaragaman jamur patogen dan gejala yang ditimbulkan pada tanaman cabai merah (*Capsicum annuum* L.) di dataran rendah [Diversity of pathogenic fungi and the symptoms they cause on red chili plants (*Capsicum annuum* L.) in lowlands areas]. *Konservasi Hayati*. 17(2): 63–68.

Watanabe T. 2002. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species.* 2nd Edition. CRC Press. Boca Raton.