

Leaf Extract of *Cinnamomum burmanni* Blume Effectively suppress the growth of *Fusarium oxysporum* f.sp. *lycopersici* the cause of *Fusarium* wilt disease On tomato

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Abstract

Productivity of tomato in Indonesia is still relatively lower than the productivity of tomato in other countries. One of the factors for the low productivity is occurrences of *Fusarium* wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici*. To find an alternative method to control the disease, in this study the antifungal activity of *Cinnamomum burmanni* was tested against the growth of *F. oxysporum* f.sp. *lycopersici* under *in vitro* condition on potato dextrose agar (PDA) and potato dextrose broth (PDB) media. Extraction was done using pro analysis (PA) grade of acetone. Results of this study showed that the leaf extract of *C. burmanni* effectively suppressed the radial growth, biomass formation and spores formation of *F. oxysporum* f.sp. *lycopersici*, with the minimum inhibitory concentration (MIC) by 1% (w/v). The inhibitory activities of this extract at concentration of 1% (w/v) against fungal radial growth, biomass formation and spores formation were respectively 41.66%, 43.68%, and 48.43% when compared to control. This leaf extract containing steroid, flavonoid, phenolate and tannin which probably responsible for the antifungal activity against *F. oxysporum* f.sp. *lycopersici*. Further study is still necessary to be done in order to identify the main substance that responsible for the antifungal activity as well as the effectiveness of the leaf extract to control *Fusarium* wilt disease on tomato plant.

Keywords: leaf extract, *Cinnamomum burmanni*, inhibitory activity, *Fusarium* wilt disease

1. Introduction

Tomato is one of important horticultural crops in Indonesia which has high economic value and wide market potential. The main tomato growing areas in Indonesia are West Java, Central Java, North Sumatera, East Java, and Bali. Productivity of tomato in Indonesia is about 6.3 ton/ha, which is relatively lower when compared to tomato productivity in other countries, such as Taiwan, Saudi Arabia and India respectively account for 21 ton/ha, 13,4 ton/ha and 9,5 ton/ha (Buntaran *et al.*, 2011). One of the factors for the low tomato productivity in Indonesia is *Fusarium* wilt disease. This disease is caused by *Fusarium oxysporum* f.sp. *lycopersici* (Subramanian, 1970). The yield losses caused by the disease may reach to 20-30% (Wibowo, 2005). In Malang, East Java the yield losses was 10.25% (Djauhari, 1987), while in Lembang and Pacet, West Java, the yield losses was 16.7% (Semangun, 2007).

Based on the preliminary survey done in early March, 2013 at the main tomato growing areas in Bali such as Petang, Badung Regency, Kintamani, Bangli Baturiti, Tabanan Regency, the incidence of the *Fusarium* wilt disease on tomato was ranged from 15 -20%.

The *Fusarium* wilt disease on tomato is mainly occurred when the soil temperature ranged between 21 to 33°C with the optimum temperature by 28°C. The cause of the disease can survive in a wide pH range viz. between 3.6 – 8.4. Most of tomato varieties cultivated by the farmers in Indonesia are susceptible to the *Fusarium* wilt disease, and only few are resistant (Semangun, 2007). Consequently, it is favorable for the outbreak of the disease.

Until recently, no effective measure has been developed to control the disease, because the pathogen can survive for a long time in the soil in the absence of the host plant. Once the soil is contaminated with this fungus, it is difficult to be sterilized. The infection by the pathogen is started from the root through wounds, and then persists and developed in the vascular bundles. Consequently, the transportation of water and nutrients are inhibited (Walker, 1952).

To control the disease, the farmers are still mainly relying on the use of synthetic fungicides; however this measure could not effectively control the disease. On the other hand, the improper use of synthetic fungicides may cause negative impacts to the environment such as resistance of pathogen to fungicide, environmental pollution, the death of non-target organisms (Oka, 1995). In addition, the residue of fungicide may be toxic to the animal and human. The toxicity caused by direct contact with synthetic fungicide may occur during fungicide's application (Djunaedy, 2009).

Efforts to reduce the negative impact of synthetic fungicide are necessary to be done in order to establish environmentally friendly sustainable agriculture. One of them is the use of botanical fungicide developed from the extracts of the higher plants. According to Djunaedy (2009) there are 37,000 species of Indonesian plants have been identified, but only one percent of them that have been used for botanical pesticide.

The secondary metabolites produced by the higher plants may act as anti-microbial agent, such as phenolic, caffeic acid, chlorogenic acid, scopoletin are toxic to the pathogens (Sinaga, 2006). Several studies have been done and showed that extracts of several tropical plants possess antifungal activities against plant pathogens (Suprpta and Khalimi, 2012; Suprpta *et al.*, 2008; Suprpta *et al.*, 2005; Astiti and Suprpta, 2012). Four species of plants namely *Eugenia aromatica*, *Piper bettle*, *Alpinia galanga* and *Sphaeranthus indicus* have been studied for their antifungal activities against *Fusarium oxysporum* f.sp. *vanilae*, the cause of stem rot disease on vanilla (Suprpta and Khalimi, 2009). The leaf extract of *Pometia pinnata* has been proven to inhibit the development of late blight disease on potato (Suprpta *et al.*, 2002). Rinrin (2011) tested 15 plant species of the Family Compositae for their antifungal activities, and found that ethanol leaf extract of *Pluchea indica* Less. Showed the highest inhibitory activity against the growth of *Candida albicans*. According to Rachma (2012) *Cinnamomum burmanni* showed antifungal activity against *Candida albicans* under *in vitro* test. This study was done to test the antifungal activity of the leaf extract of *Cinnamomum burmanni* against *Fusarium oxysporum* f.sp. *lycopersici* the cause of the *Fusarium* wilt disease on tomato.

2. Methods

2.1. Extraction

The leaves of *C. burmanni* that were used in this study were taken from the plants grown at Belok Village, Petang, Badung Bali. The leaves were washed with clean water, and chopped off into small size and then air dried for three days under room temperature. The dried leaves were powdered using blender and then were soaked in acetone (PA grade). One hundred grams of dried leaves were soaked in one liter of acetone and kept for 48 hours in the dark under room temperature. Filtrate were obtained through filtration using four layers of cheese cloth and followed by filtration using Whatman No.1 filter paper. The debris were then soaked again with one liter of acetone and kept for 48 h in the dark under room temperature. Filtration was done to obtain filtrates. All filtrates were combined and evaporated using vacuum rotary evaporator (Iwaki, Tokyo) with water bath at temperature 40°C to get crude extract. This crude extract is ready for further test.

2.2. Antifungal activity test

The crude extract of *C. burmanni* was tested for its antifungal activity against *F. oxysporum* f.sp. *lycopersici*. Spore's suspension of *F. oxysporum* f.sp. *lycopersici* (200 µl) was poured onto Petri dish and added with 10 ml of PDA (potato dextrose agar) medium and shaken horizontally gently to mix the spore's suspension and the medium. Two diffusion wells were made on a Petri dish with cork borer (5 mm diam.). A 20 µl crude extract of *C. burmanni* was put into the well. The formation of inhibition zone around the well was observed. The diameter of inhibition zone was used to determine the level of inhibitory activity. According to Ardiansyah (2005), when the diameter of inhibition zone ≥ 20 mm means the inhibitory activity is very strong, between 10–20 mm : strong, between 5-10 mm: intermediate, and < 5 mm: weak.

Determination of Minimum Inhibitory Concentration (MIC) was also done based on this method using the crude extract at concentration (w/v): 0.0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 2%, 3%, 4% and 5%.

2.3. Test for inhibitory activity against fungal radial growth

Six extract concentrations of *C. burmanni* viz. 0%, 1.0%, 1.25%, 1.5%, 1.75%, and 2.0% were tested for antifungal activity on radial growth of *F. oxysporum* f.sp. *lycopersici* PDA medium. The extract was put in the center of Petri dish and then was added with 10 ml melted PDA medium. The volume of the extract that was added into Petri dish was adjusted according to the concentration tested. Five Petri dishes were prepared for each concentration. The Petri dishes were shaken gently to allow the extract to distribute evenly in PDA medium. A mycelia plug (5 mm diam.) of *F. oxysporum* f.sp. *lycopersici* taken from the edge of a 5-day old culture was put in the center of PDA. The cultures were incubated for seven days in the dark under room temperature. The diameter of fungal colony was measured every day (Astiti and Suprpta, 2012). The inhibitory activity to the radial growth was determined according to the following formula:

$$IR (\%) = \frac{DC-DT}{DC} \times 100$$

Where

IR= inhibitory activity against radial growth in percent.

DC = diameter of fungal colony without extract treatment (control).

DT = diameter of fungal colony treated with extract.

Test for inhibitory activity against biomass formation

Potato dextrose broth (PDB) medium (approx. 95 ml each) was placed in 200-ml Erlenmeyer flasks and various concentrations of extract of *C. burmanni* viz. 0% (control), 1.0%, 1.25%, 1.5%, 1.75%, and 2.0% (w/v) were added into the flasks. The PDB medium was inoculated with spore's suspension of *F. oxysporum* f.sp. *lycopersici* (10^5 spores/ml). The final volume of culture was adjusted to 100 ml by adding PDB medium. Five flasks were prepared for each concentration. The cultures were incubated in the dark under room temperature ($28 \pm 2^\circ\text{C}$) for 7 days. The biomass was harvested through centrifugation at 5,000 rpm for 5 minutes. The biomass was taken and placed on glass filter paper and dried in an oven at 60°C until constant weight. The inhibitory activity to the biomass formation was calculated according to the formula developed by Astiti and Suprapta (2012), as follows:

$$IB (\%) = \frac{WC - WT}{WC} \times 100$$

Where

IB = inhibitory activity to the fungal biomass in percent.

WC = dry weight of biomass on control

WT = dry weight of biomass treated with extract

2.4. Test for inhibitory activity against spore's formation

Spores of *F. oxysporum* f.sp. *lycopersici* was harvested from cultures maintained on slant PDA using fine brush and sterile distilled water. The suspension was sieved through No.2 Whatman filter paper to separate the spores and mycelia or hyphae. Potato dextrose broth medium (10 ml) was put into test tubes with various concentrations of extract of *C. burmanni* viz. 0% (control), 1.0%, 1.25%, 1.5%, 1.75%, and 2.0% (w/v). Five tubes were prepared for each concentration. The cultures were incubated in the dark under room temperature ($28 \pm 2^\circ\text{C}$) for five days. The number of spores was counted using hemocytometer under light microscope. The inhibitory activity on spore's formation was calculated according to the following formula (Astiti and Suprapta, 2012).

$$IS (\%) = \frac{dc - dt}{dc} \times 100$$

Where

IS = inhibitory activity against spore's formation

dc = spore's density on control

dt = spore's density with extract treatment

3. Results and Discussion

The crude extract of *C. burmanni* obviously inhibited the growth of *F. oxysporum* f.sp. *lycopersici* on PDA medium indicated by the formation of inhibition zone around the diffusion wells as shown in Figure 1. The average diameter of inhibition zone was 30 mm. According to the criteria developed by Ardyansyah (2005) this inhibitory activity is categorized as very strong.

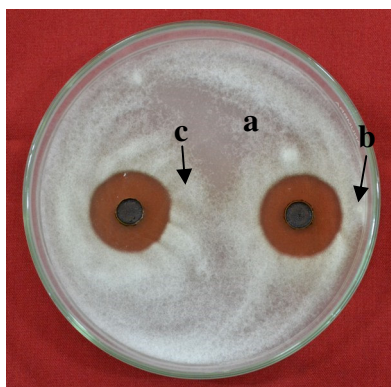


Figure 1. Inhibitory activity of the crude extract of *C. burmanni* against *F. oxysporum* f.sp. *lycopersici* on PDA medium. a. *F. oxysporum* f.sp. *lycopersici*, b. Diffusion well, c. Inhibition zone.

The minimum inhibitory concentration (MIC) of the crude extract of *C. burmanni* was 1% (w/v). The radial growth of *F. oxysporum* f.sp. *lycopersici* on PDA indicated by the diameter of colony was significantly ($P < 0.05$) suppressed by the treatment with crude extract of *C. burmanni* at concentration ranged from 1 to 2% (Table 1). Inhibitory activity resulted from 1% extract treatment was 41.66%, while no fungal growth (inhibitory activity = 100%) was observed on treatment with extract concentration 2%.

Table 1. Inhibitory activity of the leaf extract of *C. burmanni* against diameter of colony of *F. oxysporum* f.sp. *lycopersici* on PDA medium

Extract concentration (% w/v)	Diameter of fungal colony (mm)	Inhibitory activity (%)
0 (Control)	90a*	-
1.00	52.5b	41.66
1.25	19.8c	78.11
1.50	10.5d	88.33
1.75	8e	91.11
2.00	0f	100

*Means followed by the same letters are not significantly different according to the *Duncan Multiple range test* at 5% level.

Treatment with the crude extract of *C. burmanni* at concentration between 1 to 2% (w/v) significantly ($P < 0.05$) inhibited the biomass formation of *F. oxysporum* f.sp. *lycopersici* on potato-dextrose (PD) broth medium (Table 2).

Table 2. Inhibitory activity of the leaf extract of *C. burmanni* against the formation of biomass of *F. oxysporum* f.sp. *lycopersici* on PD broth medium

Extract concentration (%)	Dry weight of biomass (mg/100 ml)	Inhibitory activity (%)
0 (Control)	217.5a*	-
1	122.5b	43.68
1.25	100.0c	54.02
1.50	85.0d	60.92
1.75	72.5e	66.67
2	57.5f	73.56

*Means followed by the same letter are not significantly different according to the *Duncan Multiple range test* at 5% level.

Spore formation of *F. oxysporum* f.sp. *lycopersici* was significantly ($P < 0.05$) suppressed by the treatment with the leaf extract of *C. burmanni* at concentrations ranged from 1 to 2% (Table 3). The treatment with extract concentration at 1% (w/v) resulted in inhibitory activity on spore formation by 48.43% when

compared to control, while treatment with extract concentration 2% (w/v) reduced the spore formation by 93.71%.

Table 3. Inhibitory activity of the leaf extract of *C. burmanni* against the spores formation of *F. oxysporum* f.sp. *lycopersici* on PD broth medium

Extract concentration (%)	Spore density ($\times 10^4 \text{ ml}^{-1}$)	Inhibitory activity (%)
0 (Control)	79.5a*	-
1.00	41.0b	48.43
1.25	32.8c	58.81
1.50	21.0d	73.58
1.75	12.0e	84.91
2.00	5.0f	93.71

*Means followed by the same letters are not significantly different according to Duncan Multiple range test at 5% level

Several studies have been done and showed that extracts of several tropical plants possess antifungal activities against plant pathogens (Suprapta and Khalimi, 2012; Suprapta *et al.*, 2008; Suprapta *et al.*, 2005; Astiti and Suprapta, 2012). Suprapta and Khalimi (2012) proved that 14 tropical plants obviously inhibited the growth of *Fusarium oxysporum* f.sp. *capsici*, the cause of *Fusarium* wilt disease on paprika. Among them, five plants species *viz.* *Albizia saman*, *Piper betle*, *Syzygium aromaticum*, *Sphaeranthus indicus*, and *Alpinia galanga* showed minimum inhibitory concentrations less than 0.5% (w/v). The rest of nine plant species *viz.* *Hylocereus undatus*, *Syzygium polyanthum*, *Spondias dulcis*, *Allamanda cathartica*, *Acorus calamus*, *Acacia melanoxylon*, *Nelumbo nucifera*, *Aloe vera*, dan *Spondias pinata* showed the inhibitory concentrations ranged between 0.5 to 1.4% (w/v).

Five plant species have been tested for their antifungal activity against *Phytophthora palmivora* the cause of black pod disease on cocoa. The results revealed that the bud extract of *Eugenia aromatica*, the leaves extracts of *Piper betle*, *Pometia pinnata*, *Sphaeranthus indicus*, and rhizomal extract of *Alpinia galanga* effectively inhibited the growth of *P. palmivora* at concentration ranged from 0.05 to 0.3% (w/v) (Suprapta *et al.*, 2008). The leaves extracts of *Piper betle* and rhizome extract of *Alpinia galanga* could be used to control the wilt disease on banana caused by *Fusarium oxysporum* f.sp. *cubense* (Suprapta *et al.*, 2005).

Astiti and Suprapta (2012) reported that the leaf extract of *Tectona grandis* significantly inhibited the radial growth, biomass formation and spore formation of *Arthrinium phaeospermum* (Corda) M.B. Ellis, a fungus that cause wood decay on *Albizia falcataria* (L.) Fosberg. Treatment with the leaf extract at concentration 0.5% inhibited the radial growth of this fungus by 81.4%. Similar work was also reported by Rai (2012) the leaf extract of *Piper majusculum* Blume effectively suppressed the radial growth of *Fusarium oxysporum* f.sp. *vanilla* on PDA medium. No fungal growth was observed when it was treated with the leaf extract at concentration of 0.35% (w/v).

Methanol leaves extracts of *Eugenia polyantha* and *Cytrus hirtix* effectively suppressed the growth of *Fusarium oxysporum* under *in vitro* test (Noveriza and Miftakhurohmah, 2010). Essential oils from *Cinnamomum burmanni*, *Cimbopogon nardus*, and *Citrus grandis* obviously suppressed the development of mycelia of *Colletotrichum* sp. the cause of anthracnose disease on banana. The essential oil of *C. burmanni* showed the highest inhibitory activity (65 to 72%), followed by *C. nardus* (62 to 64%) and *C. grandis* (14 to 19%) (Istianto and Eliza, 2009). Other work also showed that the leaf extract of *Azadirachta indica* containing flavonoid, tannin and saponin showed antifungal activity against *Candida albicans* (Puspitasari *et al.*, 2009). Flavonoids, tannin, and saponin are thought to be responsible for the antifungal activity against *C. albicans*.

Based on our phytochemical analysis indicated that the leaf extract of *C. burmanni* containing steroid, flavonoid, phenolate and tannin. These compounds probably related to the antifungal activity resulted from the leaf extract of *C. burmanni* against *F. oxysporum* f.sp. *lycopersici*. Which of these compounds is the main compound for antifungal activity against *F. oxysporum* f.sp. *lycopersici* is still unclear, and need further study.

4. Conclusion

The leaf extract of *C. burmanni* using acetone as solvent, effectively suppressed the radial growth, biomass formation and spore's formation of *F. oxysporum* f.sp. *lycopersici*, the cause of *Fusarium* wilt disease on tomato with minimum inhibitory concentration (MIC) 1% (w/v). The leaf extract of *C. burmanni* containing steroid, flavonoid, phenolate and tannin. These substances probably responsible for the antifungal activity against *F. oxysporum* f.sp. *lycopersici*. Further study is still necessary to be done in order to identify the main substance that responsible for the antifungal activity as well as the effectiveness of the leaf extract to control *Fusarium* wilt disease on tomato plant.

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