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# Anti-fungal activities of selected tropical plants from Bali Island

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**Abstract**

Methanolic extracts of 14 tropical plants belong to different families inhabiting in Bali Island, Indonesia were tested for their antifungal activities against *Fusarium oxysporum* f.sp. *capsici* the cause of Fusarium wilt disease in paprika pepper. All tested plants showed obvious antifungal activity against four isolates of *Fusarium oxysporum* f.sp. *capsici*, namely isolates LS05, LS14 (isolated from paprika plants grown in the plastic house in Lombok Island), BS01 and BS07 (isolated from paprika plant grown under open field condition in Bali Island Indonesia). Five plant species namely *Albizia saman F.Muell*, *Piper betle L.*, *Syzygium aromaticum* (L.) Merrill & Perry, *Sphaeranthus indicus* L. and *Alpinia galangal* L. exhibited strong antifungal activities. The minimum inhibitory concentration (MIC) of the tested plants varied from 2 mg/ml to 13 mg/ml, however, the extract of *Albizia saman* showed the lowest MIC (2 mg/ml) against all tested isolates.

**Keywords**: tropical plants; antifungal activity; *Fusarium* wilt disease

## Introduction

Paprika pepper (*Capsicum annuum* L.) is important vegetable crops for Indonesia since it is contains good nutritive value such as protein, fat, carbohydrates, minerals and vitamins (Cahyono, 2003; Pijoto, 2003). Market demand for this vegetable is tend to increase year by year, both for domestic consumption and export particularly to United State of America, Germany, Sweden, French, Switzerland, Spain, Italy, Belgium, Japan, Singapore and Malaysia (Cahyono, 2003). The main problem for paprika production is the *Fusarium* wilt disease caused by pathogenic fungus, *Fusarium oxysporum* f.sp. *capsici* (Prajnanta, 1998). The disease may occur in the plants of various development stages. The symptom of the disease appears as dwarf of stalk and the plants continue to be wilt and finally die. The disease incidences vary from 15% to 40% and on some cases may cause the total yield losses.

Synthetic fungicide that contains copper and sulfur is commonly applied to control the disease; however, the use of this synthetic fungicide could not control the disease approp-

 

riately. In addition, the residue of these synthetic chemicals may be influence the quality of the paprika fruits, particularly when it is exported to the foreign countries. In response to this problem, it is necessary to find out alternatives measures to control the *Fusarium* wilt disease, particularly by utilizing the plant extracts from the higher plants.

The higher plants can produce a great diversity of substances that have antimicrobial activity (Doenum *et al*., 1993; Lis-Balchin *et al*., 1996; Nakamura *et al*., 1996). In Indonesia, several plants have traditionally been used as medicines to care several human diseases (Reijntjes *et al*., 1999; Wijayakusuma *et al*., 1998; Dalimartha, 1999). Several other plants have also been studied for their ability to produce antifungal substances and have been proven to be good sources of botanical pesticides (Kardinan, 1999; Widyastuti, 1996). In this study, several plants of Bali were tested for their antifungal activity against *F. oxysporum* f.sp. *capsici*. The purpose of this study was to determine the antifungal potential of extracts of 14 plant species collected from Bali Island against *F. oxysporum* f.sp *capsici*, the cause of *Fusarium* wilt disease in Paprika.

## Materials and Methods

### *Plant materials and Extraction*

Plant parts (leaf, fruit, flower, seed or rhizome) of fourteen species of tropical plant those traditionally used as spices or medicines were collected during the dry season (June-September) 2010 in Bali Island, Indonesia. The plants were kindly identified by Dr. Ngakan Putu Oka, Laboratory of Plant Taxonomy and Ecology, Hasanuddin University, Indonesia. The plant samples were air-dried in shade under room temperature and grounded into the powder. Extraction was done using methanol (PA grade) by soaking the powdered leaves for 48 h in the dark under room temperature. The filtrate was obtained through filtration using Whatman No.1 filter paper. The residues were extracted again with the same procedure. The filtrates were combined and then evaporated in a rotary evaporator (Iwaki, Tokyo Japan) to separate the methanol and the crude extracts. The crude extracts were used for antifungal test against *Fusarium oxysporum* f.sp*. capsici.*

### *Fungal isolates*

Four isolates of *Fusarium oxysporum* f.sp. *capsici* were kindly provided by the Laboratory of Plant Pathology, Faculty of Agriculture Udayana University, Indonesia, namely isolates LS05 and LS14 (isolated from paprika pepper grown in the plastic house in Lombok Island); isolates BS01 and BS07 (isolated from paprika pepper grown under open field condition in Bali Island). All isolates were pathogenic strains against paprika pepper. The isolates were maintained on slant medium of malt extract agar (MEA).

### *Antifungal activity*

Antifungal activity was determined by the well diffusion method. Petri dish containing 20 ml of MEA medium was seeded with 0.2 ml of conidial suspension (2 x $10^6$ conidia/ml). A well (6 mm in diameter) was made on agar using a cork borer. Into the well,

 

50 µl plant extract (20 mg/ml) was added and incubated at 27°C for 48 h. The antifungal activity was determined based on the diameter of the inhibition zone formed around the well.

### *Determination of Minimum Inhibitory Concentration*

Minimum inhibitory concentration (MIC) was determined by dilution method using serially diluted plant extracts at concentration 0.0-20 mg/ml. Petri dishes containing 20 ml of MEA medium were seeded with 0.2 ml of conidial suspension (2 x $10^6$ conidia/ml). Wells were made on agar using a cork borer. Into the wells, 50 µl plant extracts (0.0-50 mg/ml) was added and incubated at 27°C for 48 h. Three Petri plates were prepared for each plant extract. The lowest concentration of the extract that produced visible inhibition zone around the well was regarded as MIC.

## Results and Discussion

Several works have done by other researchers on the antifungal potential of tropical plants. Some of them showed significant antifungal activity against various plant pathogenic fungi (Bandara *et al*., 1989; Kardinan, 1999; Widyastuti, 1996; Suprapta and Ohsawa, 2007; Suprapta *et al*., 2005; Suprapta *et al*., 2001) under *in vitro* condition. Other workers have proven that application of plant extract formulation could suppress the fungal diseases under field condition (Arya *et al*., 2001; Suprapta *et al*., 2002; Suprapta and Khalimi, 2009). In this study, the antifungal activities of extracts of 14 plant species collected from Bali Island, Indonesia (table 1) were evaluated for their antifungal activities by the well diffusion method as shown in table 2. All test plants exhibited antifungal activity against all test isolates of *Fusarium oxysporum* f.sp. *capsici*. However, three plant species namely *Albizia saman*, *Syzygium aromaticum* and *Piper betle* showed strong antifungal activity against all tested isolates of *Fusarium oxysporum* f.sp. *capsici*. The highest antifungal activity was obtained by *Albizia saman* against isolate LS05 with diameter of inhibition zone 24 mm. There was a slight vari-

Table 1. Tropical medicinal plants that showed antifungal activity against *Fusarium oxysporum* f.sp. *capsici*

| No | Local name | Scientific name | Part used | Traditional use |
|---|---|---|---|---|
| 1 | Buah Naga | *Hylocereus undatus* | Fruit | Reduces blood pressure |
| 2 | Daun Salam | *Syzygium polyantahum* | Leaf | Spices |
| 3 | Kedondong | *Spondias dulcis* | Leaf | Spices |
| 4 | Bunga Terompet | *Allamanda cathartica* | Leaf | Improves blood circulation |
| 5 | Dringo | *Acorus calamus* | Rhizome | Spices |
| 6 | Sembung Delan | *Sphaeranthus indicus* | Leaf | Anti inflammation |
| 7 | Akasia | *Acacia melanoxilon* | Leaf | Reduces fever |
| 8 | Daun Suar | *Albizia saman* | Leaf | Skin care |
| 9 | Teratai | *Nelumbo nucifera* | Seed | Reduces enteritis |
| 10 | Lidah Buaya | *Aloe vera* | Leaf | Skin care |
| 11 | Sirih | *Piper betle* | Leaf | Tooth care |
| 12 | Cengkeh | *Syzygium aromaticum* | Flower (bud) | Spices |
| 13 | Cemcem | *Spondias piñata* | Leaf | Spices |
| 14 | Lengkuas | *Alpinia galanga* | Rhizome | Spices |

 

Table 2. Antifungal activity of the methanolic extracts of tropical plants against different isolates of *Fusarium oxysporum* f.sp. *capsici*

| No. | Test plants | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|---|
| | | LS05* | LS14* | BS01** | BS05** |
| 1 | *Hylocereus undatus* | 09 | 09 | 10 | 09 |
| 2 | *Syzygium polyantahum* | 12 | 11 | 09 | 10 |
| 3 | *Spondias dulcis* | 09 | 10 | 10 | 11 |
| 4 | *Allamanda cathartica* | 13 | 10 | 10 | 12 |
| 5 | *Acorus calamus* | 09 | 07 | 09 | 09 |
| 6 | *Sphaeranthus indicus* | 17 | 14 | 16 | 15 |
| 7 | *Acacia melanoxilon* | 13 | 10 | 12 | 12 |
| 8 | *Albizia saman* | 24 | 23 | 21 | 23 |
| 9 | *Nelumbo nucifera* | 09 | 10 | 09 | 11 |
| 10 | *Aloe vera* | 09 | 08 | 09 | 10 |
| 11 | *Piper betle* | 21 | 19 | 19 | 20 |
| 12 | *Syzygium aromaticum* | 22 | 21 | 21 | 21 |
| 13 | *Spondias piñata* | 10 | 09 | 09 | 09 |
| 14 | *Alpinia galanga* | 14 | 15 | 12 | 13 |

*Isolated from paprika plants grown in the plastic house, in Lombok Island, Indonesia.
** Isolated from paprika plants grown under open field condition in Bali Island, Indonesia.

ation in the response of different isolates of *Fusarium oxysporum* f.sp. *capsici* against the treatment of the same plant extract. No report available on the antifungal activity of *Albizia saman*, particularly against pathogenic fungus, *Fusarium oxysporum* f.sp. *capsici*.

Zain *et al*. (2012) reported seven plant species those exhibited antifungal activities against filamentous fungi namely *Tamarix aphylla*, *Conyza dioscoridis*, *Diplotaxis acris*, *Euphorbia cuneata*, *Origanum syriacum*, *Cynanchum acutum,* and *Solenostemma argel.* Metanolic extract of *Piper betle* leaf has been reported to posses antifungal activity against *Fusarium oxysporum* f.sp. *vanilla*, the cause of stem rot disease on vanilla (Suprapta and Ohsawa, 2007). Several plant extracts have been tested for antifungal activities and used to control plant fungal diseases in the field, such as leaf extract of *Pometia pinnata*, effectively suppressed the late blight disease on potato caused by *Phytophthora infestans* (Suprapta *et al*., 2002), leaves extracts of Piper betle and Carica papaya effectively suppressed the development of banana wilt disease caused by *Fusarium oxysporum* f.sp. *cubense* (Arya *et al*., 2001). An extract formulation containing the mixture of leaves extracts of *Eugenia aromatica* and *Piper betle* effectively suppressed the stem rot disease on vanilla seedlings caused by *Fusarium oxysporum* f.sp. *vanillae* (Suprapta and Khalimi, 2009).

The plant extracts showed obvious variation in the minimum inhibitory concentration (MIC) against *Fusarium oxysporum* f.sp. *capsici*. However, there was no significant difference of MIC among four tested isolates as shown in table 3. Four plant species namely *Albizia saman*, *Piper betle*, *Syzygium aromaticum* and *Alpinia galanga* showed relatively low MIC values (lower than 5 mg/ml). However, the lowest MIC value ( 2 mg/ml) was obtained by the extract of *Albizia saman* against all four tested isolates. Study done by Zain *et al*. (2012) showed that methanolic extract of *Tamaric aphylla* exhibited strong antifungal activity against three filamentous fungi, i.e. *chrysogenum*, with MIC as low as 2 mg/ml. Jayawijaya (2003) showed that the leaf extract of *Piper betle* exhibited a strong antifungal activity agai-

 

Table 3. Minimum inhibitory concentration (MIC) of the methanolic extracts of tropical plants against different isolates of *Fusarium oxysporum* f.sp. *capsici.*

| No. | Test plants | MIC (mg/ml) | | | |
|---|---|---|---|---|---|
| | | LS05* | LS14* | BS01** | BS05** |
| 1 | *Hylocereus undatus* | 12 | 12 | 11 | 12 |
| 2 | *Syzygium polyantahum* | 10 | 11 | 10 | 10 |
| 3 | *Spondias dulcis* | 09 | 10 | 10 | 08 |
| 4 | *Allamanda cathartica* | 10 | 11 | 11 | 11 |
| 5 | *Acorus calamus* | 13 | 12 | 11 | 11 |
| 6 | *Sphaeranthus indicus* | 03 | 04 | 04 | 04 |
| 7 | *Acacia melanoxilon* | 04 | 05 | 05 | 05 |
| 8 | *Albizia saman* | 02 | 02 | 02 | 02 |
| 9 | *Nelumbo nucifera* | 12 | 12 | 10 | 12 |
| 10 | *Aloe vera* | 11 | 13 | 12 | 12 |
| 11 | *Piper betle* | 03 | 03 | 03 | 03 |
| 12 | *Syzygium aromaticum* | 03 | 03 | 03 | 02 |
| 13 | *Spondias piñata* | 05 | 05 | 04 | 05 |
| 14 | *Alpinia galanga* | 03 | 03 | 03 | 04 |

nst *Fusarium oxysporum* f.sp. *vanilla* with MIC by 3 mg/ml on Malt extract agar medium.

This study concluded that all 14 plant species of tropical plants inhibiting in Bali Island, Indonesia showed obvious antifungal activities against *Fusarium oxysporum* f.sp. *capsici*, the cause of Fusarium wilt disease in paprika. This study enriched the reference of tropical plants which may be used to control plant fungal diseases. Since only about 10% of the tropical plants have been investigated for their antimicrobial activities, there are still many other plants that can be used as the sources of antifungal substances. Formulation and delivery methods are the factors that may affecting the effectiveness and consistency of natural product derived from plants. These aspects should be developed based on both laboratory and field experiments.

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**Conflict of interest**

The Authors have declared that there is no conflict of competing interest.

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