POTENTIAL OF MEDICINAL PLANT EXTRACTS IN INDUCING PLANT RESISTANCE ON GINGER AGAINST BACTERIAL WILT DISEASE

Potensi Ekstrak Tanaman Obat Untuk Menginduksi Ketahanan Jahe Terhadap Penyakit Layu bakteri

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ABSTRACT

Bacterial wilt caused by Ralstonia solanacearum is one of the most destructive diseases on ginger. The aim of this study was to evaluate the potential use of five different medicinal plant extracts (Acalypha indica, Andrographis paniculata, Centella asiatica, Curcuma xanthorrhiza, and Spinosa oleracea) as sources of plant resistance inducer compounds (elicitor) against bacterial wilt disease on ginger. Salicylic acid was used as a standard synthetic compound, as well as, water as a control treatment. The study was conducted at the Indonesian Spice and Medicinal Crops Research Institute, Bogor in 2010-2011. Research was conducted in Completely Randomized Design that consisted of 7 treatments, 3 replicates, and 10 plants/ replicate. Ginger seeds were planted in a mixture of soil and manure in polybags. One-month old ginger plants were sprayed or drenched with each of the medicinal plant extract before and after R. solanacearum inoculation. This experiment indicated that the medicinal plant extracts tested were effective in reducing wilt disease incidence on ginger. Their effectiveness varied depended on the plant species and the application method used. Among those five medicinal plant extracts tested, A. indica, A. paniculata, and C. xanthorrhiza were the most stable and effective. Their effectiveness were comparable with the standard compound of salicylic acid. This finding indicated that A. indica, A. paniculata, and C. xanthorrhiza were potentially used as sources of botanical elicitor compounds. The use of those medicinal plant extracts as sources of botanical elicitor, hopefully could increase ginger resistance and rhizome production, as well as reduce the use of synthetic pesticides.

Keywords: Medicinal plant extracts, elicitor compounds, induced resistance, ginger, wilt disease

ABSTRAK


Kata kunci: Ekstrak tanaman, senyawa elicitor, induksi ketahanan, jahe, penyakit layu.

INTRODUCTION

Ginger is one of most important medicinal plants in Indonesia. The plant is often attacked by Ralstonia solanacearum, the causal agent of wilt disease. So far, none of developed control strategy was effective against the disease. Therefore, the disease always become a constrain and responsible for the large decline on ginger production in Indonesia. Planting ginger resistant varieties is one of the promising control strategies to control the disease. However, there are still no ginger resistant varieties available yet in the country.

The mechanism of induced plant resistance is one of promising control strategies against plant diseases. Induced plant resistance is a response of plants against pathogen infections. The expression of this is related to the mechanisms of disease infection, plant senescence, wounded, and cool stressed. It could be expressed in flowers, fruits, and vegetative parts of plants as well. This mechanism acts as an immune system as is in human and animal (Edreva, 2004; Walters et al., 2005). The mechanism of induced plant resistance is studied by many researcher recently (Bakker et al., 2007; Edreva, 2004; Heil and Bostock, 2002; Kawamura et al., 2009; Park et al., 2007; Pavla et al., 1994; Pradanan et al., 2005; Siddiqui and Shaukat, 2004; Walters et al., 2005).
It was reported that certain plants extracts contain elicitor compounds that capable of inducing plant resistance against some plant pathogens. The successful rate of the botanical elicitor compounds in controlling plant pathogens varied between 20-89%. It depends on the plant species, physiological condition, and abiotic factors, such as, humidity and temperature (WALTER et al., 2005).

This experiment was conducted to evaluate the potential use of five different medicinal plant extracts (Acalypha indica, Andrographis paniculata, Centella asiatica, Curcuma xanthorrhiza, and Spinosa oleracea) as sources of elicitor compounds. Those medicinal plant extracts might capable of inducing ginger resistance against wilt disease caused by R. solanacearum.

MATERIALS AND METHODS

Experiment was conducted at the Indonesian Spice and Medicinal Crops Research Institute, Bogor in 2010-2011. Materials used were the extract of five different medicinal plants (A. indica, A. paniculata, C. asiatica, C. xanthorrhiza, and S. oleracea), ginger seeds (rhizomes), soil, manure, an isolate of R. solanacearum (T1060), and a Sucrosa Pepton Agar (SPA) medium.

Preparation of the medicinal plant extracts

The whole plant parts of A. indica, A. paniculata, C. asiatica, and S. oleracea were extracted and used in this experiment. While for C. xanthorrhiza only the rhizome part was used. Those medicinal plant parts were washed in running tap water, then sliced into small pieces and air dried. The dried plant parts were then powdered and extracted. The plant powders were immersed in ethanol 95% (1:10) for six hours and agitated for some times. They were then incubated at room temperature for 24 hours, percolated, and then distillated. To produce crude plant extracts.

Preparation of R. solanacearum inoculum

An isolate of R. solanacearum (T1060), originally from an infected ginger plant was grown on SPA medium. Two days-old bacterial isolate was suspended in sterile water. The bacterial suspension was adjusted to produce cell density of 10^7 cfu/ml (Optical Dencity = 0,01).

In vitro testing of antibacterial activities of the medicinal plant extracts

Those five medicinal plant extracts were diluted with ethanol 70% to produce a serial concentration of 25, 50, 75, and 100% respectively. Ethanol (70%) was also used as a standard (control). All of those medicinal plant extracts concentration were then tested for their antibacterial activity. 10 ml of R. solanacearum suspension (10^7 cfu/ml) was mixed with 90 ml of a melted SPA medium and poured in Petridishes. Sterile pepper dishes were put on the inoculated SPA medium in the petridishes. Each concentration of those plant extracts (20 ul) was dropped on each petridish plates and then incubated for 2-3 days. Antibacterial activity of those medicinal plant extracts tested was observed based on inhibition zones that produced surrounding the pepper disks.

Phytotoxicities of the medicinal plant extracts against ginger plants

One month old ginger plants grown in polybags and raised in a glass house were sprayed with each of the medicinal plant extracts (5%) every week for three months continuously. Phytotoxic effects of the medicinal plant extracts on the treated ginger plants were evaluated.

Preparation of ginger plants

Ginger seeds (rhizomes) were planted in a medium consisted of soil and organic manure (2 : 1) in polybags. One month-old ginger plants were treated with each of those medicinal plant extracts by spraying or drenching methods. Salicylic acid was also tested as a standard chemical compound (elicitor) and water was used as a control treatment.

Application of the medicinal plant extracts by spraying method

Those five medicinal plant extracts were diluted with ethanol (70%) and adjusted to a concentration of 5.0%. One month-old ginger plants were sprayed with each plant extract (5.0%) and salicylic acid (10%) every week for 3 times continuously. One week after the third application, ginger plants were inoculated with R. solanacearum (10^7 cfu, 100 ml/plants). One week after the bacterial inoculation, the ginger plants were sprayed again with each of the same plant extract or salicylic acid. Research was conducted in a Completely Randomized Design that consisted of 7 treatments, 3 replicates, and 10 plants/replicate. The treatment of medicinal plant extracts and salicylic acid was shown in Table 1.
Table 1. Treatment of medicinal plant extracts and salicylic acid compound applied by spraying and drenching methods.  
Tabel 1. Perlakuan ekstrak tanaman obat dan senyawa asam salisilat yang diaplikasikan dengan metode semprot dan siram.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Extracts concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perlakuan</td>
<td>Konsentrasi ekstrak (%)</td>
</tr>
<tr>
<td>Water (Control)</td>
<td>0</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>10</td>
</tr>
<tr>
<td>A. indica/akar kucing</td>
<td>5.0</td>
</tr>
<tr>
<td>A. paniculata/sambiloto</td>
<td>5.0</td>
</tr>
<tr>
<td>C. asiatica/pegagan</td>
<td>5.0</td>
</tr>
<tr>
<td>C. xanthorrhiza/temulawak</td>
<td>5.0</td>
</tr>
<tr>
<td>S. oleracea/bayam duri</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Application of the medicinal plant extracts by drenching method

Treatment of the medicinal plant extracts and salicylic acid compound, as well as the experiment design used in this experiment were similar with the previous experiment. However, those medicinal plant extracts and salicylic acid in this experiment were applied by drenching method.

Disease assessment

Symptom of wilt on the ginger plants was observed every week. Some of wilted ginger plants were isolated to confirm the occurrence of *R. solanacearum* in the plant tissues. Disease incidence based on the number of wilted ginger plants due to *R. solanacearum* was evaluated every week. Concentration of salicylic acid in the treated ginger plants was analyzed three months after the last treatment of the medicinal plant extracts. Disease incidence was calculated based on the number of the wilted ginger plants as a result of *R. solanacearum* infection. While effectiveness of the medicinal plant extracts were calculated as follow:

\[
E = \frac{N0 - N1}{N0} \times 100\% 
\]

Notes:

- \( E \) = Effectiveness
- \( N0 \) = Number of died ginger plants in control treatment
- \( N1 \) = Number of died ginger plants treated with each medicinal plant extract

RESULT AND DISCUSSION

Antibacterial activities of the medicinal plant extracts tested

In-vitro testing of those five medicinal plant extracts showed that they did not inhibit the growth of *R. solanacearum* on a SPA medium. No inhibition zones were noticed on the surrounding pepper disks. Thus indicated that the medicinal plant extracts tested did not exhibit antibacterial activities against *R. solanacearum*.

Phytotoxicity of the medicinal plant extracts tested

The ginger plants sprayed with those five medicinal plant extracts (5%) were healthy and their growth were not inhibited. Application of the medicinal plant extracts did not cause any plant damages. Thus, the medicinal plant extracts tested were not toxic against ginger plants.

This experiment indicated that those five medicinal plant extracts tested did not show antibacterial activities against *R. solanacearum* and did not toxic against ginger plants. Therefore, they submit the requirement to be used as botanical elicitors. According to Hammerschmidt (1999), elicitor compounds do not directly toxic against pathogens and non target organisms, as well as neither toxic to the plants and nor affects the plant growth and production.

Elicitors are signal compounds, providing information for the plant to trigger defence (Lorrain et al., 2003). They are generally less effective to control plant diseases, however, it could reduce disease development and disease intensity, as well as reduce the number and diameter of leaf spots (Edreira, 2004; Walters et al., 2005). Induced plant resistance by elicitor is systemicity, long lasting, and broad spectrum against plant pathogens, such as fungi, bacteria, and virus (Heil and Bostock, 2002; Teniente et al., 2010; Walter et al., 2005).

Effectiveness of the medicinal plant extracts applied by spraying method

Symptom of wilt was developed both either on the treated and untreated ginger plants. First symptom was observed approximately two weeks after *R. solanacearum* inoculation. The average number of wilted ginger plants varied according to the medicinal plant species. Most of the untreated ginger plants (inoculated with *R. solanacearum* and sprayed with water) were wilted and died (80%). While the average number of wilted ginger plants sprayed with those five medicinal plant extracts varied from 20 to 40% (Table 2).
Table 2. Average number of wilted ginger plants after spraying of the medicinal plant extracts (5.0%) and salicylic acid compound (10%)  
Tabel 2. Rata-rata jumlah tanaman jahe layu setelah disemprot dengan ekstrak tanaman obat (5.0%) dan senyawa asam salisilat (10%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percentage of wilted ginger plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (Control)</td>
<td>80 a</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>20 b</td>
</tr>
<tr>
<td>A. indica/akar kucing</td>
<td>20 b</td>
</tr>
<tr>
<td>A. paniculata/sambiloto</td>
<td>20 b</td>
</tr>
<tr>
<td>C. asiatica/pegagan</td>
<td>20 b</td>
</tr>
<tr>
<td>C. xanthorrhiza/temulawak</td>
<td>20 b</td>
</tr>
<tr>
<td>S. oleracea/bayam duri</td>
<td>40 b</td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same letter are not significantly different at 5% Duncan's test.
Keterangan: Angka yang dikuat oleh huruf yang sama tidak berbeda nyata pada uji Duncan's 5%

Application of A. indica, A. paniculata, C. asiatica, and C. xanthorrhiza plant extracts (5.0%) and salicylic acid (10%) could significantly reduced disease incidence by 75%. While application of S. oleracea could reduced disease incidence by 50%. This indicated that effectiveness of A. indica, A. paniculata, C. asiatica, and C. xanthorrhiza plant extracts (5.0%) were comparable with the standard elicitor compound of salicylic acid (10%). While S. oleracea was less effective than the other medicinal plant extracts (Figure 1).

Figure 1. Effectiveness of the medicinal plant extracts (5,0%) and salicylic acid compound (10%) applied by spraying method in reducing wilt disease incidence on ginger plants
Gambar 1 Efektivitas ekstrak tanaman obat (5,0%) dan senyawa asam salisilat (10%) yang diaplikasikan sengan metode siram untuk mengurangi kejadian penyakit layu pada tanaman jahe

Effectiveness of the medicinal plant extracts applied by drenching method

Wilt symptom was developed approximately two weeks after R. solanacearum inoculation both either on the treated and untreated (control) ginger plants. The average number of wilted ginger plants varied according to the medicinal plant species. All the untreated ginger plants (inoculated with R. solanacearum and drenched with water) were wilted (100%). While the average number of wilted ginger plants drenched with those five medicinal plant extracts varied from 20 to 100% (Table 3).
Table 3. Average number of wilted ginger plants after drenching of the medicinal plant extracts (5%) and salicylic acid compound (10%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percentage of the wilted ginger plants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. solanacearum + Water (Control)</td>
<td>100 a</td>
</tr>
<tr>
<td>R. solanacearum + Salicylic acid (10%)</td>
<td>20 c</td>
</tr>
<tr>
<td>R. solanacearum + A. indica (5%)</td>
<td>20 c</td>
</tr>
<tr>
<td>R. solanacearum + A. paniculata (5%)</td>
<td>20 c</td>
</tr>
<tr>
<td>R. solanacearum + C. asiatica (5%)</td>
<td>100 a</td>
</tr>
<tr>
<td>R. solanacearum + C. xanthorrhiza (5%)</td>
<td>20 c</td>
</tr>
<tr>
<td>R. solanacearum + S. oleracea (5%)</td>
<td>40 b</td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same letter are not significantly different at 5% Duncan’s test.

Application of A. indica, A. paniculata, and C. xanthorrhiza plant extracts by drenching method could significantly reduce 80% of wilt disease incidence on ginger plants. Their effectiveness were comparable with the standard compound of salicylic acid. S. oleracea was less effective compared with those three medicinal plants and salicylic acid compound. While C. asiatica was not effective in reducing disease incidence (Figure 2). This experiment indicated that A. indica, A. paniculata, and C. xanthorrhiza and S. oleracea plants extracts (5%) applied by drenching method were capable of inducing ginger resistance against R. solanacearum. Their effectiveness were similar either applied by spraying as well as drenching method. While C. asiatica that applied by spraying method was more effective than by drenching method. Thus indicated that effectiveness of this medicinal plant extract was not stable and it was affected by the application method used.

![Effectiveness of the medicinal plant extracts](image_url)

Figure 2. Effectiveness of the medicinal plant extracts (5.0%) and salicylic acid compound (10%) applied by drenching method in reducing wilt disease incidence on ginger plants

Gambar 2. Efektivitas ekstrak tanaman obat (5.0%) dan senyawa asam salisilat (10%) yang diaplikasikan dengan metode siram dalam mengurangi kejadian penyakit layu pada tanaman jahe
Application of A. indica and S. oleracea plant extracts could reduce development of rhizomes rot due to *Pythium aphanidermatum* on ginger (GOSHI and PURKAYASTHA, 2003). Application of those two plant extracts reduced the loss of rhizome weight due to this pathogen. The reduction of rhizome weight on ginger treated with those two plant extracts were 4.6 and 8.14% respectively. Those were comparable to the treatment of a standard chemical compound of jasmonic acid that used as a resistance plant inducer (8.08%) and lower than other plant extracts tested, such as *A. paniculata* (21.9%), *Curcuma longa* (24.31%), and *C. asiatica* (16.48%). While according SHI et al., (2007), application of a *Cnidii monieri* seed extract before or after infection of fungal (mold) *Sphaerotheca fuliginea* (Schlecht Pollacci) could control disease development and increase the resistance of yellow pumpkin plant.

**Salicylic acid concentration in the treated ginger plant**

Salicylic acid concentration was analyzed only in the survival ginger plants that were drenched with *A. indica*, *A. paniculata*, *C. xanthorrhiza* plant extracts (5.0%) and salicylic acid (10%), as well as in the untreated ginger plants (control). The salicylic acid concentration was analyzed three months after inoculation of *R. solanacearum* on the ginger plants. The salicylic acid concentration in the ginger plants treated with *A. indica*, *A. paniculata*, and *C. xanthorrhiza* plant extracts (5.0%) were higher than both in the untreated ginger plants and in the ginger plants treated with salicylic acid (10%) (Figure 3).

![Graph showing salicylic acid concentration in the ginger plants treated with medicinal plant extracts and salicylic acid (ppm)](image)

**Figure 3.** Salicylic acid concentration in the ginger plants treated with the medicinal plant extracts and salicylic acid (ppm)

*Gambar 3. Konsentrasii asam salisilat dalam tanaman jahe yang diaplikasi dengan ekstrak tanaman obat dan asam salisilat (ppm)*

This result indicated that concentration of salicylic acid in the ginger plants treated with *A. indica*, *A. paniculata*, and *C. xanthorrhiza* plant extracts (5.0%) were increased. Higher production of salicylic acid compound in those treated ginger plants might lead to induction plant resistance against *R. solanacearum*. According to SMITH and BOYKO (2007), salicylic acid is one of the signaling compounds that promotes the development of a long-lasting systemic acquired resistance (SAR) that leads to induced plant resistance against a large spectrum of pathogens.

The mechanism of SAR is related with increasing production of pathogenesis-related proteins (PR proteins), such as chitinase, b-1,3 glucanase, peroxidase, endoproteinase, oxalate oxidase (HEIL and BOSTOCK, 2002; VAN LOON, 2006; TENIENTE et al., 2010). Most of those proteins work through resistance signal compounds, such as, salicylic acid, jasmonic acid, and ethylene (BRUGGER et al., 2006; EDREVA, 2004; HEIL and BOSTOCK, 2002; TENIENTE, et al., 2010). Those signalling compounds are important regulators of defence-gene expression (BARI and JONES, 2009).

Plant resistance could be induced by application of biological agents, such as pathogens, i. e. *Phytophthora megasperma* f. sp. *glycinea* (OKINAKA et al., 1995), *Botrytis cinerea* (REPKA, 2001), *Pseudomonas fluorescent* (BAKKER et al., 2007; HASSAN and BUCHENAUER, 2008).
**CONCLUSION**

All those five medicinal plant extracts tested (A. indica, A. paniculata, C. xanthorrhiza, C. asiatica, and S. oleracea) contain elicitor compounds that capable of inducing ginger resistance against wilt disease caused R. solanacearum. Their effectiveness varied depended on the plant species and the application methods used. A. indica, A. paniculata, and C. xanthorrhiza were more stable and effective in reducing wilt disease incidence. While C. asiatica and S. oleracea were less effective.

The medicinal plant extracts tested did not show any antibacterial activities against R. solanacearum and did not toxic against ginger plants. Thus, they were meet the requirement to be used as sources of botanical elicitor compounds. The use of those medicinal plants extracts as botanical elicitor, hopefully could increase ginger resistance and rhizome production, as well as reduce the use of synthetic and environmentally hazardous pesticides.

**REFERENCES**


