Antifungal activity of leaf extract of Mansoa alliacea against Colletotrichum acutatum the cause of anthracnose disease on chili pepper

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Abstract. Antifungal activity of the leaf extract of Mansoa alliacea against Colletotrichum acutatum the cause of anthracnose disease on chili pepper was be done. The antifungal activity was determined based on the effects of plant extract to the fungal growth, spore’s formation, spore’s germination and total biomass. Results of this study showed that the crude extract of Mansoa alliacea obviously inhibited the growth of Colletotrichum acutatum on PDA with MIC by 0.7% (w/v). Treatment with leaf extract of Mansoa alliacea significantly \((P<0.05)\) inhibited the fungal growth, spore’s formation, spore’s germination and fungal biomass of Colletotrichum acutatum. The inhibitory activity for the colony radial growth, spore’s formation, spore’s germination, and fungal biomass were respectively ranged from 29.72 to 81.11%, 63.18 to 100%, 61.68 to 100% and 39.53 to 91.86%. This result suggested that the leaf extract of Mansoa alliacea contains the antifungal substances that responsible for the antifungal activity such as phenol and alkaloid, so this extract can be considered as one of alternative measures to control the anthracnose disease on chili pepper.

1. Introduction

Anthracnose disease on chili pepper is the most common disease and always occurs in area of chili pepper. Anthracnose diseases other than resulting the decrease of yield its also damage the aesthetic value of chili pepper. Reduction of yield due to anthracnose disease on chili pepper can reach 50% or more [1]. Anthracnose disease caused by Colletotrichum spp. in Indonesia, the most of anthracnose pathogens attacking on chili pepper by Colletotrichum capsici and Colletotrichum gloeosporioide [2]. While anthracnose disease on chili pepper in Bali mostly caused by Colletotrichum acutatum [3][4].

Control of anthracnose diseases is still based on the use of synthetic fungicides. The continuous use of synthetic fungicides can cause pathogenic resistance, pollute the environment and be harmful to consumers. Pollution by pesticides is not only in the agricultural environment but can also endanger human and animal life where pesticide residues accumulate in agricultural products and in waters [5]. Based on this, it is necessary to research for alternatives to controlling anthracnose diseases on chili pepper by utilizing plants that have the potential as botanical fungicides which are not harmful to consumers or the environment. Many plants that have the potential as botanical pesticides have the potential to control pests and diseases in plants [6]. However, before being used to control pests and diseases in plants, plant extracts (botanical pesticides) must be tested in vitro to determine their effectiveness to be effectively used in vivo in the field [7].
Mansoa alliacea is an ornamental plant from the Bignoniaceae tribe originating from South America and has been widely grown in Indonesia as an ornamental plant. Ethanol extract of Mansoa hymenaea leaves can inhibit the growth of Candida albican and *Trichophyton rubrum* [8]. Mansoa alliacea leaf extract affects to embryo formation and can inhibit tumor cell growth in mice [9]. Based on the background, it is necessary to conduct further study to find out of botanical fungicides to control anthracnose disease on chili pepper.

2. Materials and Methods

2.1. Rejuvenation of *Colletotrichum acutatum*

*Colletotrichum acutatum* used in this study was derived from pure culture of *C. acutatum* stored in the Biochemistry Laboratory, Department of Biology, Faculty of Natural Sciences, Udayana University. Its the results of isolation from chili pepper which showed symptoms of anthracnose disease taken from chili pepper owned by farmers in the village Pancasari Buleleng. Before being used in subsequent studies, the pure culture of *C. acutatum* was first rejuvenated by inoculation on healthy chili pepper then re-isolating *C. acutatum* from chili pepper which showed anthracnose symptoms after inoculation. The pure culture of *C. acutatum* from the isolated result is stored in PDA media and used for further research.

2.2. Extraction of *Mansoa alliacea* leaves

Extraction is done by chopping of *M. alliacea* leaves into smaller parts, then drying them at room temperature, and after drying, the leaves are made into powder by blending. *M. alliacea* leaf powder (100 grams) was then macerated with 1,000 ml of methanol PA (Pro Analysis) for 72 hours in a dark place and room temperature. Filtrate is obtained by filtering through 4 layers of gauze and Whatman filter paper. The filtrate obtained is then evaporated using a vaccum rotary evaporator (Iwaki, Japan) at a temperature of 40 °C so that crude extract is used to be used for further testing.

2.3. Activity test of *M. alliacea* leaf extract against *C. acutatum*

The activity test of Mansoa alliacea leaf extract on the growth of *Colletotrichum acutatum* causing anthracnose disease on chili pepper (*Capsicum annuum*) was carried out by diffusion well method. The activity of *M. alliacea* leaf extract against *C. acutatum* was measured by measuring the inhibitory zone (clear zone) formed around the diffusion well.

2.3. Minimum Inhibitory Concentration (MIC) Test

MIC test is used to determine the minimum concentration of crude extracts of Mansoa alliacea leaves that can cause barriers (minimum inhibitory concentration, MIC) to the growth of fungus *Colletotrichum acutatum* on PDA media. Based on the results of the MIC test, the concentration of *M. alliacea* leaf extract can be determined as a concentration of treatment in subsequent studies.

2.4. Effectiveness extract of *M. alliacea* leaves on *C. acutatum*

The effectiveness test was carried out to find out the effective concentration of Mansoa alliacea leaf extract which could inhibit the growth of *Colletotrichum acutatum*. The effectiveness test of *M. alliacea* leaf extract on the growth of *C. acutatum* was done by measuring the inhibitory of *M. alliacea* leaf extract on each extract treatment (1%, 2%, 3%, 4% and 5%) against the growth of *C. acutatum* compared to controls (0%). The growth of *C. acutatum* fungi measured included: diameter of fungal colonies, density of fungal spores, germination of fungal spores and fungal biomass.

3. Results and Discussions

3.1. Isolates of *Colletotrichum acutatum*

*Colletotrichum acutatum* can associate on chili pepper, especially in the fruit that can cause anthracnose symptoms. To ensure that *C. acutatum* pure isolates were obtained by doing Koch Postulate test on chili pepper fruits that showed symptoms of anthracnose disease. To restore the ability of a pathogenic fungus to infect its host, pure isolates of fungi that have long been stored on PDA media in the laboratory need to be inoculated back into the host and re-isolated before being used in subsequent tests (Figure 1).
3.2. Crude extract of Mansoa alliacea
Mansoa alliacea leaves used in this study are leaves that have grown and developed optimally (Figure 2), because the alkaloids and phenols have been formed as active compounds in these leaves. If the leaves used are too young or too old, the presence of secondary metabolites in the leaves has not been formed or secondary metabolites have turned into other compounds such as being abscisic acid compounds that function in the leaf aging process.

3.3. Activity test of M. alliacea leaf extract against C. acutatum
The results of antifungal activity test of Mansoa alliacea leaf extract against Colletotrichum acutatum cause anthracnose disease on chili pepper by diffusion well method, showed that Mansoa alliacea leaf
extract could inhibit the growth of C. acutatum with inhibition zone diameter of \(28 \pm 47\) mm. The barrier zone formed \(\geq 20\) mm means the ability to inhibition is very strong, if the zones are between 10-20 mm the ability to inhibition is strong, if the zones are between 5-10 mm the ability to inhibition is moderate and if the zones are \(\leq 5\) mm the ability to inhibition is less or weak \(\leq 10\). Based on the result at figure 3, showed that the extract of Mansoa alliacea leaves can inhibit the growth of C. acutatum very strong. The ability of M. alliacea leaf extract to inhibit the growth of C. acutatum because in the extract of M. alliacea there are active compounds (alkaloids and phenols) that can inhibit the growth of C.acutatum.

\[\text{Figure 3. Treatment of leaf extracts of M. alliacea against C. acutatum with diffusion well method on PDA media, the control zone is not formed (a); Treatment of inhibitory zone (b) is formed; diffusion well (1) and inhibition zone (2)}\]

### 3.4. Minimum Inhibitory Concentration (MIC) Test

The minimum concentration that can cause inhibition (MIC) to the growth of Colletotrichum acutatum on PDA was 0,7% with a diameter of inhibition zone of 5.25 mm on the third day of incubation as presented in Table 1.

#### Table 1. Data testing MIC extract of Mansoa alliacea leaves against Colletotrichum acutatum on PDA media

| No. | Extract concentration (%) | Inhibition zone (mm) |
|-----|---------------------------|----------------------|
| 1.  | 1                         | 8,12                 |
| 2.  | 0,9                       | 7,25                 |
| 3.  | 0,8                       | 6,47                 |
| 4.  | 0,7*                      | 5,25                 |
| 5.  | 0,6                       | 0                    |
| 6.  | 0,5                       | 0                    |
| 7.  | 0,4                       | 0                    |
| 8.  | 0,3                       | 0                    |
| 9.  | 0,2                       | 0                    |
| 10. | 0,1                       | 0                    |

* MIC = Minimum Inhibitory Concentration

Base on the data show that extract MICs are varied, the smaller the MIC value of an extract indicate the higher antifungal activity or vice versa \(\leq 11\).
3.5. Effectiveness extract of *M. alliacea* leaves on *C. acutatum*

The treatment of Mansoa alliacea leaf extract significantly (P <0.05) inhibited the growth of C. acutatum fungi colonies on PDA media, mold spore formation, germination of fungal spores and biomass of C. acutatum fungi on PDB media as presented in Table 2.

### Table 2 The inhibitory of Mansoa alliacea leaf extract against Colletotrichum acutatum

| Treatment of extract (%) | Diameter of colony (mm) | Spore formation (spore/ml x 10^5) | Spore germination (spore/ml x 10^5) | Biomass of fungi (g/100 ml) |
|--------------------------|-------------------------|-----------------------------------|-------------------------------------|-----------------------------|
| 0                        | 90.00a*                 | 11.38a*                           | 6.76a*                              | 0.86a*                      |
| 1                        | 63.25b                  | 4.19b                             | 2.59b                               | 0.52b                       |
| 2                        | 55.00c                  | 2.77c                             | 1.66c                               | 0.41c                       |
| 3                        | 47.75d                  | 0.91d                             | 0.56d                               | 0.33d                       |
| 4                        | 38.50e                  | 0.33e                             | 0.09e                               | 0.12e                       |
| 5                        | 17.00f                  | 0f                                | 0e                                  | 0.07f                       |

* = Values followed by different letters in the same column mean significantly different (P <0.05) based on DMRT test at the level of 5%.

*Mansoa alliacea* leaf extract contains alkaloid compounds, phenols, tannins, flavonoids, glycosides, quinones and sterols [12]. Leaf extracts of *M. alliacea* had antimicrobial activity against several types of fungi [13] and *Mansoa hymenaea* leaf extract can inhibit the growth of *Candida albicans*, *Microsporum gypseum*, *Trichophyllum mentagrophytes* and *Tricophyton rubrum* in vitro [14]. Sulfur compounds, especially alliin and various allyl sulfides contained in the leaf extract of *M. alliacea*, are reported to reduce cholesterol [15]. Meanwhile ethyl acetate extract of *M. alliacea* leaves has insecticidal activity against *Spodoptera litura* and *Helicovera amigera* pests [16].

The existence of inhibition of fungal growth of *Colletotrichum acutatum* by crude extracts of Mansoa alliacea leaves can be caused due to the presence of active compounds contained in the leaves of *Mansoa alliacea* which are antifungal and antimicrobial. The mechanism of antimicrobial substances in killing or inhibiting microbial growth is by: (1) damaging the microbial cell wall, resulting in lysis or inhibiting cell wall formation in cells that are growing, (2) changing the permeability of cell membranes causing leakage of cytoplasm and the nutrients contained therein, (3) cause cell denaturation, and (4) inhibit the action of enzymes in the cell.

### 4. Conclusions

The crude extract of *Mansoa alliacea* leaves can inhibit the growth of *Colletotrichum acutatum fungi* with inhibitory power of 28 ± 47 mm. MIC value of Mansoa alliacea leaf extract on Colletotrichum acutatum fungi growth was 0.7% with inhibitory power of 5.25 mm. The concentration of Mansoa alliacea leaf extract was 4% most effective in inhibiting fungal growth of Colletotrichum acutatum in vitro.

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Acknowledgement

My thanks go to the Director General of Higher Education and Chancellor of Udayana University for the assistance of research funds through the PDUPT (Higher Education Basic Research) Fund and the Head of the Biology Laboratory of the Program Study of Biology, Faculty of Natural Sciences, Udayana University for the facilities provided during the research.