In vitro test for inhibition of betel (*Piper betle* L.) and tembelekan (*Lantana camara*) extracts to anthracnose disease (*Colletotrichum acutatum*) in cayenne chili (*Capsicum frutescens*)

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**Abstract:** The continuous and excessive use of fungicides will disrupt the balance of the environment and is directly harmful to the health of consumers. The use of plants as botanical pesticides is an ecologically safe control method that has begun to be developed. The purpose of this study was to determine the effectiveness of betel and tembelekan extracts and the effect of the concentrations used in suppressing the growth of *Colletotrichum acutatum* fungi. Extraction was carried out using the maceration method, then the extract was concentrated using a rotary vacuum evaporator at a temperature of 40°C repeatedly until a concentrated solution was obtained. In the test of using treatments with several concentrations, that is K0:0%, K1:0.5%, K2:2%, K3:3.5% and K4:5%. Tests were carried out by growing *C. acutatum* mycelium on PDA media that had been mixed with plant extract solutions according to the treatment concentration. *Colletotrichum acutatum* mycelium was taken by cutting the PDA which was overgrown with a pure culture of *C. acutatum* with a media cutter using a cork borer. The results showed that the higher the concentration of the extract given would reduce the colony diameter of *Colletotrichum acutatum* and increase the percentage of inhibition. Betel extract at a concentration of 5% had the highest average percentage of inhibition compared to the tembelekan extract, namely 72.45%.

1. **Introduction**

Anthracnose in chilies is the most common disease and almost always occurs in every chili planting area. This disease can result in a decrease in the yield of up to 50 percent more [1]. *Colletotrichum* species that cause anthracnose disease are characterized by symptoms to wit, the fruit rot with sunburn black rot, because it is full of black hair (setae) [2]. The growth of *C. capsici* can be detrimental to the yield of red chilies during post-harvest, so an effective and safe post-harvest control action is needed to reduce post-harvest yield losses [3, 4]. Farmers still use chemical fungicides to control these pathogenic fungi. The continuous and excessive use of fungicides will disrupt the environmental balance and be harmful to the health of consumers [5]. Various control techniques are used to control anthracnose, one of the
safe and environmentally friendly alternatives to anthracnose disease control is by using various plants to become vegetable fungicides, one of them is the betel plant [5, 6]. Another component contained in betel leaf, namely acetyl eugenol, the OH group of eugenol can form hydrogen bonds with the active conditions of the target enzyme and increase the activity of the enzyme by denaturing the enzymes responsible for the secretion of toxins [7]. *L. camara* contains flavonoids, terpenoids, alkaloids, essential oils, and compounds such as phytosterols, saponins and tannins [8].

2. Methods

2.1. Preparation of Potato Dextrose Agar (PDA) as a culture medium for isolates

PDA media was made by peeled 200 g of potatoes, cut into small cubes, boiled until boiling. Potato extract was put into an erlenmeyer containing 20 grams of granulated sugar and 17 grams of agar and then homogenized using a stirring rod. Erlenmeyer’s mouth was tightly closed using aluminum foil and wrapping, then the media was sterilized in an autoclave for approximately one hour at a temperature of 121 °C. Once warm, 1-2 capsules of chloramphenicol were added to the media and then poured into a petri dish.

2.2. Preparation of Colletotrichum acutatum isolate

The inoculum was obtained from the field, isolated and identified. The isolates would be augmented on Potato Dextrose agar (PDA) media.

2.3. The process of making plant extracts

The materials to be processed into plant extracts are selected from healthy plants. Standard betel leaf and *L. camara* leaves were chosen, that was leaves that had fully developed, the leaves look clean and did not show symptoms of a disease. A total of 500 grams of plant leaves were washed underwater flows, then dried and mashed in a blender. The blended ingredients were put into a glass jar and put in a little bit of ethanol solvent until they were completely immersed in a ratio of 1:4 (w/v) while stirring with a glass stirring rod. Soaking time was 3 x 24 hours. After that, the extraction solution was filtered with filter paper and the results are put into a closed plastic bottle. The extracts of *Piper betle* L and *L. camara* obtained were concentrated using a rotary vacuum evaporator at a temperature of 40°C repeatedly until a concentrated solution (stock solution) was obtained.

2.4. In-vitro test for growth inhibition of *C. acutatum*

The test was carried out by growing *C. acutatum* mycelium on PDA media which had been mixed with a solution of plant extract according to the treatment concentration. The liquid PDA that has been mixed with the extract is poured into a petri dish and left to stand until the medium solidifies. *C. acutatum* mycelium was taken by cutting the PDA that was covered with pure *C. acutatum* cultures using a 8 mm diameter cork borer. Fungal mycelium was inoculated on PDA which had been mixed with plant solution in the center of the petri dish, then incubated by inserting the petri dish into an incubator at room temperature and observed every day until the control treatment (media without extract) was full of pathogenic mycelium *C. acutatum*.

3. Results and discussion

3.1. Results

Observation of the inhibition of several types of plant extracts against the pathogenic fungi *C.acutatum* which was carried out for 7 days of observation obtained the results of the analysis of variance in table 1.
After carrying out the *C. acutatum* mycelium growth test against several types of plant extracts with different concentrations then the inhibition percentage was calculated, so the results of variance were obtained as shown in table 2.

**Table 1.** The average growth rate of *C. acutatum* in *Piper betle* L and *Lantana camara* plant extracts.

| Treatment                  | Inhibition of Day |
|---------------------------|-------------------|
| Factor A                  | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
| *Piper betle L.*          |      |      |      |      |      |      |      |
| Control (0%)              | 0.4b | 1.48d| 2.99c| 4.15d| 5.28d| 6.71d| 7.99d|
| 0.5%                      | 0.34b| 1.38c| 2.71e| 4.09d| 5.23d| 6.48c| 7.84d|
| 2.0%                      | 0.00a| 0.98bc|2.15bc| 3.58b| 4.88d| 5.73c| 6.64bc|
| 3.5%                      | 0.00a| 0.59ab|1.21ab| 2.09ab|2.66ab| 3.19ab| 3.50ab|
| 5.0%                      | 0.00a| 0.13a | 0.29a | 0.61a | 1.15a | 1.71a | 2.23a |
| *L. camara*               |      |      |      |      |      |      |      |
| Control (0%)              | 0.50b| 1.63d | 2.71c| 3.96d | 4.91d | 6.86d | 8.16d |
| 0.5%                      | 0.39b| 1.35c | 2.66c | 3.80d | 4.94d | 6.88d | 7.95d |
| 2.0%                      | 0.16ab|1.44c | 2.50c | 4.63c | 4.63c | 6.61d | 7.74c |
| 3.5%                      | 0.00a| 0.78ab|2.26bc| 3.23bc|4.45bc| 5.68cd| 7.30c |
| 5.0%                      | 0.00a| 0.10a | 1.95ab| 2.46bc|2.95b | 4.06b | 5.06b |

Note: The numbers followed by the same letter do not show any significant differences based on the Tukey test results at the 0.05 degree

**Table 2.** Average percentage inhibition of *C. acutatum* in *Piper betle* L and *Lantana camara* extracts.

| Treatment                  | Percentage of inhibition by day |
|---------------------------|--------------------------------|
| Factor A                  | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
| *Piper betle L.*          |      |      |      |      |      |      |      |
| Control (0%)              | 0.00c | 0.00d | 0.00c | 0.00d | 0.00d | 0.00c | 0.00c |
| 0.5%                      | 15.28bc| 6.57d | 8.98c | 1.50d | 0.94d | 3.52c | 2.95c |
| 2.0%                      | 100.00a|32.98bc| 27.88bc|13.83b | 7.53c | 14.68b| 17.78bc|
| 3.5%                      | 100.00a|58.51ab|59.65b |49.72ab|49.59ab|52.52a |56.67ab|
| 5.0%                      | 100.00a|92.19a |90.87a |85.36a |78.31a |74.49a |72.45a |
| *L. camara*               |      |      |      |      |      |      |      |
| Control (0%)              | 0.00c | 0.00d | 0.00c | 0.00d | 0.00d | 0.00c | 0.00c |
| 0.5%                      | 20.59b| 17.41b |2.26c | 11.88c |2.63d | 0.43c | 2.60c |
| 2.0%                      | 75.00a| 11.94c | 7.80c | 4.01c | 8.67c | 4.24c | 5.20b |
| 3.5%                      | 100.00a|52.80ab|16.57bc|25.51b |12.13b |17.81b |10.56b |
| 5.0%                      | 100.00a|94.01a |28.03bc|43.02ab|41.83ab|41.17ab|37.97bc|

Note: The numbers followed by the same letter do not show any significant differences based on the Tukey test results at the 0.05 level
The results of the study on the percentage inhibition of *C. acutatum* against *Piper betle* L. and *L. camara* extracts showed that each type of extract had different inhibitions (figure 1). The concentration level of plant extracts also showed effectiveness in inhibiting *C. acutatum* colonies on PDA media which was observed for 7 days of observation. The concentrations tested were 0.5%, 2%, 3.5%, and 5% then compared with the control (0%) without mixing plant extracts. Betel extract at a concentration of 5% and 3.5% had the highest percentage levels.

3.2. General discussion
Secondary metabolite compounds vary widely in number and type from each plant [8]. Plant extracts that have secondary compounds have been investigated for their potential to control phytopathogens and some have been shown to have anti-microbial properties that affect fungal growth both in a manner in vitro and in vivo [9, 10]. The concentration of plant extracts affects the effectiveness of vegetable fungicides. The concentration of the extract is related to the number of active compounds contained in the extract that can inhibit the growth of pathogens, to wit the higher the concentration of the extract will decrease prolong the incubation period, decrease the percentage of attack intensity of *Colletotrichum* sp and reduce the shrinkage of chilies [5]. Figure 1 shows a significant effect on the two types of extracts tested. The type of extract and concentration is very influential in suppressing the growth of the pathogen *Colletotrichum acutatum*. the higher the concentration of the extract, the higher the percentage of inhibition and the growth of mycelium on PDA media is slower due to an inhibition zone which makes *C. acutatum* pathogens difficult to grow due to exposure to active compounds contained in the extract. Betel leaf has anti-fungal, anti aflatoxigenic, and antioxidant properties with essential oil components, namely eugenol 63.39%, acetyl eugenol 14.05% [11]. 13% phenolic compounds that can inhibit pathogen growth [12]. Phenol acts as a poison for microbes by inhibiting the activity of enzymes and the mechanism of saponins and flavonoids in disrupting fungal cell membranes, that is by forming complexes with extracellular proteins, damaging membranes and cell walls [13].

![Figure 1](image-url)
4. Conclusion

Extracts of *Piper betle* L and *L. camara* have the potential to be used as vegetable pesticides. Betel extract at a concentration of 5% was the best in suppressing the growth of *C. acutatum* with an inhibition percentage of 72.45%

References

[1] Nurhayati 2011 The growth of *Colletotrichum capsici* causes anthracnose of chilies in various media containing plant extracts *J. Res.* 3 32-35

[2] Maja M P, Valentina S, Jerneja J, Vlasta C, Robert V, Alenka M and Vranci S 2013 Phenolic compounds as defence response of pepper fruits to *Colletotrichum coccodes* *Physiol. Mol. Plant Pathol.* 84 138-145

[3] Siswadi 2007 Post-harvest handling of fruits and vegetables *Inovasi Pertanian* pp 68-71

[4] Silva D D, Groenewald J Z, Crous P W, Ades P K, Nasruddin A Mongkolporn O and P W J Taylor 2019 Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia *IMA Fungus* 10 1-32

[5] Syabana M A, Andree, Saylendra and Deri R 2015 Antifungal activity of fragrant lemongrass leaf extract (*Cymbopogon nardus* L) against *Colletotrichum* sp. which causes anthracnose disease in chilies (*Capsicum annuum* L) in-vitro and in-vivo *J. Agrologia* 4 21-27

[6] Aulifa D L, Aryantha I N and Sukrasno 2014 In vivo and in vitro antifungal activity of citronella leaves extract (*Cymbopogon nardus* L.) against *Colletotrichum* sp caused anthracnose disease on chili *J. Bionatural Ilmu-Ilmu Hayati dan Fisik* 16 12-18

[7] Trisnawati D 2016 The benefits of *Piper betle* extract as inhibitor of antracnose (*Colletotrichum acutatum*) on chili during storage (Bogor: Institut Pertanian Bogor) p 1-50

[8] Dalimartha S 2008 *Atlas of Indonesian Medicinal Plants* Jilid 1 (Jakarta: Trubus Agriwidya)

[9] Srangarlin J, Kuhn O J, Assi L and Schwan-Estrada K R F 2011 *Science Agains Microbial Phatogens; Communicating Current Research and Technological Advences* vol 2, ed Mendez-Vilas A (Badajoz: Formatex) pp 1033-1042

[10] Srangarlin J, Kuhn O J and Schwan-Estrada K R F 2008 control of plant diseases by plant extract *Revisao Anual de patologia de Plants* 16 265-304

[11] Bhanu P, Ravindra S, Priyanka S and Ashok K 2010 Efficacy of chemically characterized *Piper betle* essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity *Int. J. Food Microbiol.* 142 114-119

[12] Nisa G K, Nugroho W A and Hendriawan Y 2014 Ekstraksi daun sirih merah (*Piper Crocatum*) dengan metode Microwave Assisted Extraction (Mae) *J. Bioproses Komoditas Tropis* 2 72-78

[13] Pepeljnjak S, Kalodera Z and Zovko M 2005 Antimicrobial activity of flavonoids from *Pelargonium radula* (Cav.) L’Hérét *J. Acta Pharm.* 55 431–435