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PENGARUH EKSTRAK DAUN SIRIH TERHADAP PERTUMBUHAN *Colletotrichum capsici* PADA BUAH CABAI MERAH

THE EFFECT OF BETEL LEAF EXTRACT ON THE GROWTH OF Colletotrichum capsici IN RED CHILI

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Abstrak

Tanaman cabai merupakan jenis sayuran penting yang banyak dibutuhkan oleh masyarakat. Namun, berdasarkan data Badan Pusat Statistik menunjukkan bahwa produksi cabai mengalami penurunan yang salah satunya disebabkan oleh *Colletotrichum capsici*. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian ekstrak daun sirih terhadap pertumbuhan *C. capsici.* Penelitian ini dilakukan menggunakan Rancangan Acak Lengkap (RAL) dengan 3 kali pengulangan. Proses ekstraksi daun sirih dilakukan dengan metode maserasi dengan pelarut etanol 96% dan uji antifungi dilakukan dengan metode difusi Kirby-Bauer. Konsentrasi ekstrak daun sirih yang digunakan adalah 5%, 10%, 15%, 20%, dan kontrol negatif menggunakan dimetil sulfoksida (DMSO). Data yang diperoleh dianalisis secara statistik menggunakan uji *One-Way Anova* dan uji lanjut *Least Significant Difference* (LSD). Hasil penelitian ini menunjukkan bahwa konsentrasi ekstrak daun sirih 20% menghasilkan zona hambat terbesar, yaitu 0,84 mm. Berdasarkan hasil pengujian disimpulkan bahwa ekstrak daun sirih memiliki aktivitas antifungi dalam menekan pertumbuhan *C. capsici*.

Kata kunci*:* Antifungi; *Colletotrichum capsici*; Tanaman cabai

Abstract

Chili plants are important vegetables normally used by the community. However, based on the data of Badan Pusat Statistik, chili production is decreasing because of many factors, including Colletotrichum capsici pathogen. This study was aimed to determine the effect of betel leaf extract on the growth of C. capsici. This research was conducted using a completely randomized design with 3 replications. The extraction process was carried out by the maceration method with 96% ethanol solvent and the antifungal test was performed by the Kirby-Bauer diffusion method. The concentration of betel leaf extract used was 5%, 10%, 15%, 20%, and dimethyl sulfoxide (*DMSO*) *was used as the negative control. The data obtained were analyzed statistically using the One-Way Anova test and the Least Significant Difference (LSD) posthoc test. The results of this study showed that the concentration of 20% betel leaf extract produced the largest inhibition zone of 0.84 mm. Based on the test results, it is concluded that betel leaf extract had antifungal activity in suppressing the growth of C. capsici.*

Keywords*: Antifungal; Chili Plants; Colletotrichum capsici*

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INTRODUCTION

Red pepper (*Capsicum annuum* L.) is one of important horticultural vegetables with high economic value (Devi et al., 2021). This plant is commonly used as a kitchen spice by household as well as restaurant and hotel industry. Red chilies are cultivated in all regions of Indonesia, including the province of South Sulawesi which contributed to most of national chili production. Chili production in South Sulawesi Province in 2018 reached 26,943 tons, yet it decreased to 21,055 tons in 2019, and 17,549 tons in 2020 (Badan Pusat Statistik, 2020). Based on these data, there was annual decrease of chili production in South Sulawesi Province. The decreasing number of chili plant production is influenced by various factors, including pathogenic fungal infection that causes anthracnose disease.

Anthracnose is a disease caused by the fungus *Colletotrichum truncatum* (formerly called as *C. capsici* (Than et al., 2008; Damm, Woudenberg, Cannon, & Crous, 2009; Ranathunge & Sandani, 2016) which attacks chili plants. This disease could lead to harmful effect on plants because it attacks the fruit of chili plants at various stages of development, such as newly formed fruit or ready-to-harvest fruit. As reported by Hidayat, Sulastrini, Kusandriani, and Permadi (2004), *C. truncatum* was found to have the ability to infect 20 strains of fruit chili chilies, both young (green) and ready-to-harvest (red) chili fruit. Moreover, Prathibha, Rao, Ramesh, and Nanda (2013) also observed that anthracnose caused a decrease in the percentage of phenol, capsaicin, and oleoresin content. To say, anthracnose disease control is necessarily done since this disease could decrease the quantity and quality of chili.

Anthracnose can be controlled chemically (Shao, Zeng, Tang, Zhou, & Li, 2019) or biologically (Montiel et al., 2018; Nagaraju, Sriram, & Achur, 2020). Chemical control has often been carried out by farmers using fungicides, however, the continuous use of fungicides can affect natural organisms or microorganisms in soil, thus reducing soil fertility (Meena et al., 2020). Bacmaga, Wyszkowska, and Kucharski (2016) confirmed that the use of a dose of the fungicide Falcon 460 could inhibit the activity of dehydrogenase, catalase, urease, and acid alkaline phosphatase enzymes. These enzymes are commonly found in natural soil microorganisms, thus they become parameters of toxicity of fungicides in soil. In addition, continuous exposure to chemicals will lead to resistance *of C. truncatum*, hence antrhacnose disease cannot be controlled. Therefore, biological control is necessary. Biological recognition is possibly done through the use of active compounds in plants, such as betel leaf.

Betel leaf has been widely studied as an antimicrobial because it contains secondary metabolites. Sarma et al. (2018) reported that betel leaf extract concentrations of 5, 10, 25, and 50 g/mL showed antimicrobial activity against *Bacillus subtilis, Escherichia coli,* and *Aspergillus niger bacteria.* Lubis, Marlisa, and Wahyuni (2020) also found that betel leaf extract concentrations of 2% and 3% showed antimicrobial activity against *Staphylococcus aureus* bacteria. According to Sivareddy et al. (2019), ethyl acetate and ethanol extract of betel leaf had antimicrobial activity against the fungus *Candidi albican*. Singburaudom (2015) reported that the crude ethanol extract of betel leaf showed antimicrobial activity against 7 types of plant pathogenic fungi. Based on several research reports above, the purpose of this study was to determine the effect of betel leaf extract on the growth of *C. truncatum* on red chili plants.

MATERIALS AND METHODS

This research was conducted at the Pharmacy Microbiology Laboratory and the Biological Pharmacy Laboratory, Faculty of Health Sciences, Alauddin State Islamic University, Makassar. The tools used in this study were petri dishes, test tubes, measuring pipettes, loops, vials, micropipettes, caliper, autoclave, laminar air flow, rotavapor, water bath and vortex. The materials used in this study were betel leaf (Piper betle), sterile distilled water, pure culture of *Colletotrichum truncatum*, 96% ethanol, Potato Dextrose Agar (PDA) medium, dimethyl sulfoxide (DMSO), and paper disc.

Procedure of Leaf Extract Preparation

Betel leaves of 750 g (± 165 leaves) were dried using an oven at a temperature of 40 ° C

for 1 day (Hoque et al., 2011). The dried betel leaves were extracted through maceration method using 96% ethanol as solvent. The maceration process was started by squeezing dry betel leaves, putting them in a maceration vessel, and soaking them with 96% ethanol liquid to further covered and stored them for 2 days. Later, the sample was filtered and the residues were put back into the maceration vessel for the next maceration process. The maceration process was carried out 4 times. The results obtained from filtering were combined and evaporated in a rotator at a temperature of 40 °C and further placed in a *water bath* to obtain a thick extract (Azwanida, 2015; Madhumita, Guha, & Nag, 2020).

Procedure of Leaf Extract Concentration

The suspension concentration of betel leaf extract used in this study was 5%, 10%, 15%, and 20%. Approximately 20,000 mg of betel leaf extract was added with 0.4 mL of DMSO and 9.6 mL of sterile distilled water to obtain a concentration of 20%. A total of 7.5 mL of 20% extract concentration was added with 2.5 mL of sterile distilled water to obtain a concentration of 15%. A total of 6.7 mL of 15% extract concentration was added with 3.3 mL of sterile distilled water to obtain a concentration of 10%. At last, a total of 5 mL of 10% extract concentration was added with 5 mL of sterile distilled water to obtain a 5% concentration.

Inhibitory Test of Betel Leaf Extract on the Growth of *Colletotrichum truncatum*

The inhibition test of betel leaf extract on the growth of *C. truncatum was* carried out using the Kirby-Bauer diffusion method with 3 replications (Babiah, Preeti, Upreti, & John, 2014). The culture of *C. truncatum was* diluted in 3 mL of NaCl, mixed with Potato Dextrose Agar (PDA) media, poured into petri dishes, and allowed to solidify. Each concentration of betel leaf extract was pipetted onto paper disks. Next, the paper disk was placed on the surface of the PDA media. The PDA media were incubated for 3 days at room temperature (25–27 ° C). The inhibitory activity of betel leaf extract on the growth of *C. truncatum* was seen in the presence of a clear zone (inhibitory) which was calculated by the average diameter formula as shown in Figure 1. Average diameter (mm) = ((A-0,4) + (B-0,4) + (C-0,4) + (D-0,4))/ 4.

Data Analysis

This research was conducted using a completely randomized design with three replications. The data obtained were analyzed statistically with the *One-Way Anova* test and the *Least Significant Difference* (LSD) post-hoc test with a 95% confidence level using the *Statistical Product and Service Solutions* (SPSS) program.

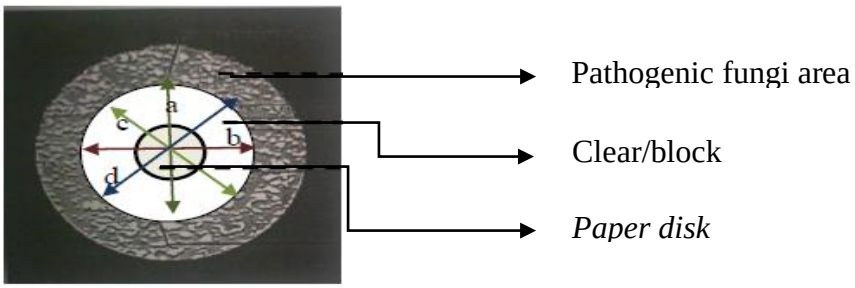

Figure 1. Calculation of clear zone concentration of betel leaf extract on the growth of *Colletotrichum truncatum*

RESULTS

The use of betel leaf extract concentration showed growth inhibition against *Colletotrichum truncatum*. This was indicated by the presence of the zone of inhibition (clear) around the *paper disk* previously treated with 5%, 10%, 15%, and 20% betel leaf extract. Each concentration of betel leaf extract produced different diameters of the clear zone (Table 1). As presented in Table 1, greater concentration of betel leaf extract used will result in greater diameter of the clear zone.

The effects of various concentrations of betel leaf extract on the growth of *C. truncatum* were tested statistically using *One-*

Way ANOVA (Table 2). Based on Table 2, it is known that the $_{calculated}$F value= 16,779 with a significance value of less than 0.05, that is 0.001, indicating that the concentration of betel leaf extract affected the average growth diameter of the tested *C. truncatum*.

Table 1. Diameter of inhibition (clear) zone of different concentrations of betel leaf extract on the growth of *Colletotrichum truncatum*

Treatment	Diameter of clear zone (mm)			Average (mm)
	I	II	III	
Negative control 1 (DMSO)	0	0	0	0
Negative control 2 (DMSO)	0	0	0	0
Negative control 3 (DMSO)	0	0	0	0
Negative control 4 (DMSO)	0	0	0	0
20%	1.05	1.05	0.42	0.84
15%	0.48	0.48	0.22	0.39
10%	0.22	0.22	0	0.14
5%	0	0	0	0

Table 2. The results of the *one-way ANOVA* test for the effect of various concentrations of betel leaf extract on the growth of *Colletotrichum truncatum*

	Sum of squares	df	Mean square	F_{count}	Sig
Beetween group	13,583	4	3,396	16,779	0,001
Within group	1,417	7	0,202		
Total	15,000	11			

Each concentration of betel leaf extract was further tested statistically by the *Least Significant Difference* (LSD) test with a 95% confidence level to determine the level of significance. Based on Table 3, it is known that the 20% concentration is very significant to the 5% concentration, while the other concentrations show a significant category.

Table 3. LSD test results for the effect of the concentration of betel leaf extract on the growth of *Colletotrichum truncatum*

Concentration	Concentration	Mean difference	Std. error	Sig.	Conclusion
	15%	,4500*	,16729	,027	Significant
20%	10%	,6917*	,16729	,003	Significant
	5%	,8417*	,16729	,001	Very significant
	20%	-,4500*	,16729	,027	Significant
15%	10%	,2417	,16729	,187	Significant
	5%	,3917*	,16729	,047	Significant
	20%	-,6917*	,16729	,003	Significant
10%	15%	-,2417	,16729	,187	Significant
	5%	,1500	,16729	,396	Significant
	20%	-,8417*	,16729	,001	Very significant
5%	15%	-,3917*	,16729	,047	Significant
	10%	-,1500	,16729	,396	Significant

Information:
20%, 15%, 10%, and 5%= concentration of betel leaf extract
* = significant (0,02–0,049)
** = very significant (0,00–0,01)

DISCUSSION

This study examined the effect of betel leaf extract on the growth of *Colletotrichum truncatum* on red chili plants. The betel leaf extract is expected to suppress the growth of *C. truncatum*. Table 1 shows the data of inhibition

zone of various concentrations of betel leaf extract on the growth of *C. truncatum.* Negative control 1–4 (DMSO) did not produce the zone of inhibition, indicating that DMSO, as an extract solvent, is neither microbiocidal nor microbiostatic on the growth of *C. truncatum.* Microbiocidal is the effect of a treatment that can kill bacteria, while microbiostatic is the effect of a treatment that can inhibit the growth of bacteria. The microbiostatic effect was shown by the treatment of red betel leaf extract.

The treatment of red betel leaf extract with 4 different concentrations showed different microbiostatic effects as confirmed by the data in Table 2 which shows that the significance value of 0.001 is lower than 0.005. This indicates that the betel leaf extract has antifungal activity with ability to inhibit the growth of *C. truncatum.* The effect of microbiostatic is indicated by the presence of the zone of inhibition which was formed due to the activity of antifungal compounds contained in the betel leaf extract to further form a clear zone around the growth of *C. truncatum.*

Different betel leaf extract concentration was found to produce different diameter of inhibition zone. The result of LSD test in Table 3 shows that the treatment between the concentrations of betel leaf extract of of 20%, 15%, 10%, and 5% obtained a very significant and significant value. The concentration of betel leaf extract of 20% was very significant to betel leaf extract concentration of 5%. This means that the concentration of betel leaf extract of 20% had such effect that was significantly different from the concentration of betel leaf extract of 5%. Significant value between the betel leaf extract concentrations of 20%, 15%, and 10% was observed, indicating that the three treatments showed no significant effect.

The largest diameter of the zone of inhibition (0.84 mm) was produced by the highest concentration of betel leaf extract of 20%. Based on Table 1, it is known that the diameter of the observed inhibition zone is smaller along with the decreasing concentration of the betel leaf extract used. This suggests that greater concentration of betel leaf extract is proportional to the larger diameter of inhibition zone produced. This finding is in line with the research of Flores et al. (2016) which reported that the higher the concentration of *Bixa orellana* (achiote) extract, the larger the inhibition zone for the suppression of the growth of *Streptococcus mutans* and *S. sanguinis.* Masri et al. (2021) reported that the concentration of black cumin seed extract of 1% and 3% showed a resistant reaction to *Mycobacterium tuberculosis* strain H37RV and MDR-TB, while the concentration of black cumin seed extract of 5% and 10% pointed out a sensitive reaction. This indicates that higher concentration of black cumin seed extract was able to suppress the growth of *M. tuberculosis* strain H37RV and MDR-TB. Based on several research reports above, higher concentration of an extract will lead to larger zone of inhibition In conclusion, the higher the concentration of betel leaf extract, the greater the effect in suppressing the growth of *C. truncatum.* Furthermore, higher concentration of betel leaf extract was also found to be directly proportional to higher number of active substances contained in the betel leaf extract.

A total of 69.46% hydroxychavicol content, 24% 4-chromanol content, and 4.86% eugenol were found in betel leaf extracted using ethanol as a solvent. These three compounds were observed to have antioxidant, anti-inflammatory, antibacterial, and antifungal activities (Muruganandam, Krishna, Reddy & Nirmala 2017). Phytochemical compounds, such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids and steroids, hydroxychavicol, and eugenol were reported to be present in betel leaf extract. Betel leaf extract was also reported to have antibacterial activity against 9 bacterial species (Syahidah et al., 2017). Hydroxychavicol compounds extracted from betel leaf showed antifungal activity against *Candida albicans, C. glabrata,* and *Aspergillus* sp. In addition, hydroxychavicol compounds were also reported to damage the cell membrane of *C. albicans* as indicated by the absorption of propidium iodide on the cell membrane of *C. albicans.* Propidium iodide is a fluorescent nucleic acid dye which generally cannot enter the undamaged *C. albicans* cell membrane (Ali et al., 2010). Based on several research reports above, the active compounds contained in betel

leaf extract are most likely antifungals, hence they were able to suppress the growth of *C. truncatum*.

CONCLUSION AND SUGGESTION

Based on this study, the concentration of 20% betel leaf extract resulted in the best inhibition of *Colletotrichum truncatum* growth compared to other concentrations with an average diameter of inhibition zone of 0.84 mm. Therefore, the use of betel leaf extract at concentration above 5% is recommended for best results.

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