



## The effectiveness of basil extract on the mildew saprolegnia within in vitro



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### ABSTRACT

Fish disease is a serious problem faced in the aquaculture business. One of the diseases that attack cultured fish is saprolegniasis caused by the fungus *Saprolegnia sp.* The use of chemical drugs tends to be environmentally unfriendly and has other negative effects on these fish and humans. For this reason, a safer alternative treatment is needed to control fish disease caused by the fungus *Saprolegnia sp.* One of the plants that have the potential to treat disease caused by the fungus *Saprolegnia sp.* is basil (*Ocimum basilicum* L). This research aims to determine the effectiveness of basil extract against the fungus *Saprolegnia sp.* in vitro. This research was conducted from May to June 2019. The research method used was a completely randomized design (CRD) with 5 treatment levels and 3 replications including 0 ppm (control), 20 ppm, 40 ppm, 60 ppm, and 80 ppm treatments. The test parameter observed was the inhibition zone diameter. In this study, 200 grams of basil extract were produced with 16.67% yield, the conclusion is that basil extract with a concentration of 60 ppm has the largest inhibition zone diameter of 22 mm in inhibiting the growth of the fungus *Saprolegnia sp.* in vitro compared to other ethanol extract concentrations of basil.

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### INTRODUCTION

The indicator of success in fish farming is the good health condition of the fish. If the health of the fish decreases, which is supported by poor environmental conditions, it will result in the emergence of disease in cultivated fish, which can cause losses to the cultivation business. One of the diseases in cultured fish is a mycotic disease that occurs due to fungal attack, the source of which can be derived from *Saprolegnia sp.* which causes saprolegniasis in cultured fish.

According to Setyawati (2011), marked saprolegniasis disease from the appearance of a mass of hyphae like cotton, white to gray/brown on the body of the infected fish. This opinion is also

strengthened by Juniati, Amir & Mukhlis (2015), which states that fish infected with saprolegniasis are characterized by the presence of lumps on the body surface of the fish resembling cotton which causes damage to the skin and fins due to cell necrosis by penetration of hyphae in the epidermis and dermis.

Prevention and treatment efforts that are commonly carried out on fish affected by mycotic diseases are using chemical drugs such as malachite green, formalin, hydrogen peroxida, and so on, but the use of chemicals tends to be not environmentally friendly and some are carcinogenic (Kusdarwati, Murtinintias & Meles, 2013). Besides, it is feared that the use of chemical drugs will have a further negative impact on organisms. This is by the opinion of Sudarno, Sabrina & Suprpto, (2014), which states that the use of chemicals causes negative effects, namely the resistance of microorganisms, the dangers they cause to the surrounding environment, the fish concerned, and the humans who consume them.

To overcome this problem, a safer alternative treatment is needed to control the disease caused by the fungus *Saprolegnia sp.* Alternatives that can be used include using plants that contain antifungal substances. Besides, these plants are also easy to obtain and easy to use for the prevention and handling of diseases in fish. One of the plants that have the potential to treat disease caused by the fungus *Saprolegnia sp.* is basil (*Ocimum basilicum* L). According to Kurniasih (2014), basil (*Ocimum basilicum* L) is a plant belonging to the Lamiaceae family/family, with the characteristics of a woody trunk, including a shrub with a height of 30-150 cm, a rectangular stem, a grooved stem surface, and hair, branched and colored. Green has white flowers and the aroma of this plant is very distinctive. The results of the basil leaf phytochemical screening test conducted by Martiningsih & Suryanti (2017) using the water distillation method to produce Basil leaf essential oil contains flavonoids, saponins, terpenoids, and tannins. Meanwhile, in research by Kumalasari & Andiarna (2020) using the maceration method, it was found that 96% ethanol extract of basil leaves contained flavonoids, alkaloids, saponins, and tannins. Furthermore, Hasan, Raharjo & Ariyani (2016) added that the basil leaves contain substances as antibacterial and antifungal. Besides, the orientin and vicenin compounds in basil can protect the body's cell structure, while cineole, myrcene, and eugenol function as natural antibiotics and anti-inflammatory.

The implementation of research related to the performance of basil leaf extract (*Ocimum basilicum* L) has been reported by several researchers, including research conducted by Hidayati & Bahar (2018) who concluded that the ethanol extract of basil leaves (*Ocimum basilicum* L.) had the effect of inhibiting the growth of *Staphylococcus epidermidis* bacteria with an effective dose of 850 mg/mL, while the research conducted by Berlian, Aini & Lestari (2016) The result showed that the antifungal activity of basil leaf extract (*Ocimum americanum* L.) to inhibit the growth of *Fusarium oxysporum* Schlecht. is at a concentration of 10% (w/v). Further research conducted by Trenesia & Ramadhian (2019), obtained the results of antibacterial activity in the ethanol extract of basil leaves against the growth of *S. aureus* and *S. Typhi* bacteria with the highest inhibition zone in *S. aureus*, namely at a concentration of 100% with an inhibition zone of 21.75 mm and *S. Typhi* at a concentration was 80% with an inhibition zone was 6.25 mm.

From this description, the authors feel the need to do research using 96% ethanol extract in basil leaves which aims to determine the effectiveness of basil leaf extract against the fungus *Saprolegnia sp.* in vitro as the basis for developing the potential of basil extract as an antifungal for treating disease in cultured fish.

## RESEARCH METHODS

### Research Design

This research was conducted from May to June 2019. Basil leaf extraction activities were carried out at the process engineering laboratory, chemical industrial products, department of



chemical engineering, Sriwijaya University. The antifungal effectiveness test of *Saprolegnia sp* was carried out at the Mycology Laboratory, Health Analyst Department, Health Polytechnic of the Ministry of Health, Palembang.

This research method was carried out using a completely randomized design (CRD) with 5 treatments and 3 replications which refers to the research of Hasan, et al. (2016). The treatments used are presented in Table I.

**Table I.** Treatment of basil extract on *Saprolegnia sp*

Treatment	Concentration
P0	Basil extract with a concentration of 0 ppm
P1	Basil extract with a concentration of 20 ppm
P2	Basil extract with a concentration of 40 ppm
P3	Basil extract with a concentration of 60 ppm
P4	Basil extract concentration of 80 ppm

### Instruments

The tools used in this research are knife, analytical scale, glass jar, measuring cup, funnel, blender, Erlenmeyer, vortex, aluminum foil, rotary evaporator, spatula, Petri dishes, rubber band, dropper pipette, test tube, test tube rack, hot plate, sieve, beaker glass, cotton buds, autoclave, laminar airflow, refrigerator/fridge, sprayer, bunsen fire, ose needles, tweezers, magnetic stirrer, tissue, cotton, disc paper, micropipettes, calipers, incubators, water bath, vial bottles, TLC plates, digital cameras, and stationery, while the materials used in this study were 12 kg basil leaves, fungal isolates *Saprolegnia sp*, fish feed, 96% ethanol as much as 5 liters, SDA, and alcohol.

Observation of the fungal inhibition zone was carried out by measuring the diameter (minimum diameter and maximum diameter) of the clear zone around the disc paper on the petri dish. The response to microbial growth inhibition refers to Morales, Sierra, Parades, Loyola, Gallardo & Borquez, (2003), which is very strong antifungal potential if the barrier area is > 20 mm in diameter, strong antifungal potential if the barrier area is 10-20 mm in diameter, has moderate antifungal potential if the barrier area is 5-10 mm in diameter and has weak antifungal potential if the barrier area is < 5 mm.

### Procedures

#### I. Making Basil Extraction (*Ocimum sanctum* L)

12 kg of fresh basil washed clean then cut into small pieces and then dried in indirect sunlight for 7 days until dry and weighed, then 1.2 kg of dry basil is obtained, dried basil then mashed using a blender until obtained. dry powder (simplicia) of basil. The simplicia of basil is extracted by immersion (maceration) using 5 liters of ethanol 96% as much as 5 liters. Simplicia basil used as much as 1.2 kg then put into 6 beaker glass to do soaking, then add 96% ethanol which has been distilled until submerged, then close the beaker glass tightly and store for 3 days in a dark place and protected from light. After 3 days the solution was filtered with a Whatman filter vacuum pump No. 42. Then the last step was to do the evaporation using a Rotary Evaporation tool. Then prepare a complete set of vacuum distillation then add the basil maserate from half the capacity of the distillation flask (can be added). Heat a rice cooker that has been filled with water then let the vacuum distillation process run then stop the distillation if there is no more ethanol dripping so that you get a thick basil extract. The thick evaporated extract is stored in the freezer (Widyastuti, 2012). The stages of making a complete basil leaf extract are presented in Figure 1.

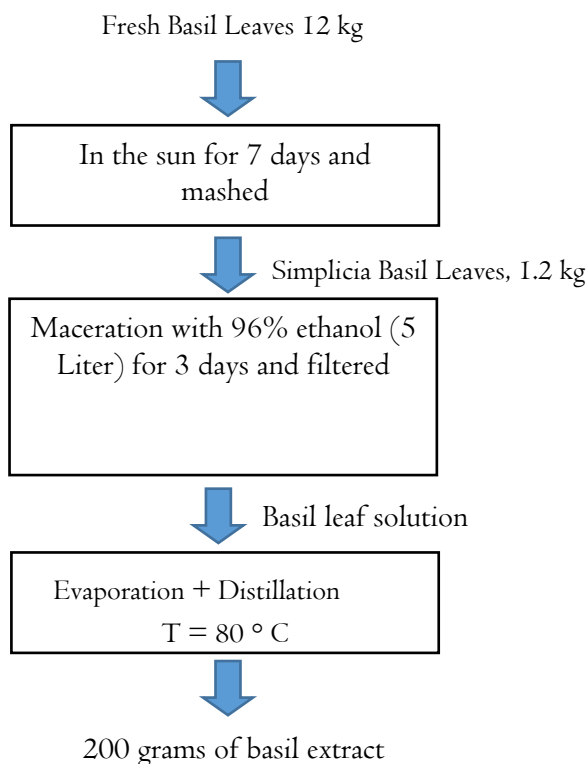


Figure I. Basil extraction process flow

## 2. Antifungal Effectiveness Test

### a. Preparing Media

Making dextrose sabouraud media so that (+) refers to Tanjung (2013), namely weighing the sabouraud dextrose agar as much as 65 grams (composition: 10 grams of mycological peptone, 40 grams of dextrose, 15 grams of agar), then dissolved in 1 liter of aqua dest. Sterilize into the autoclave for 15 minutes with a temperature of  $\pm 121^{\circ}\text{C}$ , then cooled until lukewarm. Add 1 gram of chloramphenicol antibiotic, stir until homogeneous then pour into a petri dish as much as 15-20 cc. Let it cool and freeze.

### b. Mushroom Rejuvenation

Rejuvenation of *Saprolegnia sp* as much as 1 ose and inoculated into SDA oblique aseptically by placing an oce needle containing the fungus on the base of the agar slope and pulling it with a zigzag motion, then incubating using an incubator at a temperature of  $30^{\circ}\text{C}$  for 24 hours (Lay, 1994).

### c. Antifungal Sensitivity Test

The antifungal sensitivity test of basil extract on the fungus *Saprolegnia sp* is to determine the level of sensitivity or sensitivity of mushrooms to basil extract using various concentrations, namely 20 ppm, 40 ppm, 60 ppm, and 80 ppm. and repeated 3 times ignoring the research (Lingga, Rustikawati & Burwono, 2012). The sensitivity test was carried out using the diffusion method so that 0.1 ml of the mushroom suspension was spread over each solidified SDA using L glass. Disc paper with a diameter of 6 mm was dripped with 20  $\mu\text{l}$  with various concentrations using a micropipette. After that, it is placed on the agar medium which has been spread by the fungus *Saprolegnia sp* and labeled. Furthermore, the agar medium was incubated for 24 hours at a temperature of  $30^{\circ}\text{C}$ .

Observed and recorded by measuring the clear zone around the disc paper using a ruler and calculating the average value of the diameter of the formed inhibition zone. The antifungal sensitivity test was stated to be active by looking at the inhibition zone in the clear area around the disc paper which indicated a growth inhibitor.

### Data Analysis

The research data were analyzed using analysis of variance (ANOVA). If the analysis of variance (ANOVA) results show the value of  $F_{count} > F_{table}$  on  $\alpha = 0.05$  and  $0.01$  then it will be further tested. For the KK value (Coefficient of Diversity) 1-5%, it will be further tested using BNJ (Honest Real Difference), if the KK value is 5-10% then it will be further tested using BNT (Least Real Difference) and if the KK value is  $> 10\%$  then it will be further tested using Duncan (Hanafiah, 2010), while the data on the results of the effectiveness test were discussed descriptively.

### RESULTS

The total amount of basil extract using 96% ethanol with the maceration method yields 200 grams of basil extract with a yield of 16.67%. The resulting basil extract is served in Figure 2.



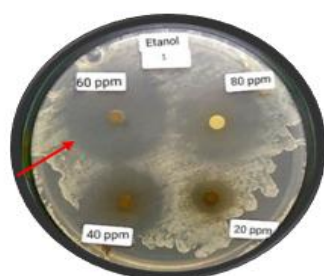
Figure 2. Basil extract (*Ocimum sanctum* L)

The results of testing the effectiveness of basil extract against *Saprolegnia sp.* were obtained by measuring the resulting inhibition zone diameter as presented in Table 2 and Figure 3.

Table 2. Inhibition zone diameter of basil extract against *Saprolegnia sp.*

Treatment	The diameter of the inhibition zone of basil extract (mm)	Inhibition Ability
P0 (control)	0 <sup>a</sup>	-
P1 (20 ppm)	11.33 <sup>b</sup>	Strong
P2 (40 ppm)	18.30 <sup>d</sup>	Strong
P3 (60 ppm)	22 <sup>c</sup>	Very strong
P4 (80 ppm)	15 <sup>c</sup>	Strong

(Morales, et al, 2003)



The diameter of the clear inhibition zone on the test

Figure 3. Inhibition zone diameter of basil extract against *Saprolegnia sp.*

## DISCUSSION

Based on Table 1 above, it was found that 20 ppm treatment resulted in an inhibition zone diameter of 11.33 mm, 40 ppm treatment resulted in an inhibition zone diameter of 18.33 mm, 60 ppm treatment showed an inhibition zone diameter of 22 mm and treatment of 80 ppm resulted in an inhibition zone diameter of 15 mm. The concentration of basil extract in each treatment resulted in a different diameter of the inhibition zone. This is by the opinion of Martiningsih (2017) which states that the diameter of the inhibition zone formed in each treatment is different, this is because the growth of fungal isolates in the media is influenced by several factors, including the concentration of antimicrobial substances, the number of microorganisms, the presence of organic matter, temperature, degree of acidity. (pH) and species of organisms.

Refers to Figure 2, it can be seen that the diameter of the inhibition zone is a clear area around the Paper Disc that is not overgrown with fungus. Based on Duncan's continued test, the concentration of basil extract was significantly different at concentrations of 0 ppm and 20 ppm with different notations, namely a and b, this indicates that the concentration of the basil extract had a different effect on the growth of the fungus *Saprolegnia sp.* It is suspected that the lower the concentration of basil extract, the smaller the diameter of the clear zone formed around the disc paper. This is by the opinion of Fadilah (2018), which states that the smaller the concentration of the extract, the less the amount of active substance dissolved in the extract so that the ability to inhibit fungal growth is lower. Conversely, the higher the concentration, the more active substances that function as anti-fungi, so that the ability to inhibit fungal growth is greater.

The concentrations of 40 ppm and 60 ppm in Duncan's continued test showed significantly different results because they had different notations, namely d, and e. The concentration of 60 ppm resulted in the highest clear zone diameter of 22 mm compared to the concentration of 40 ppm which was 18.33 mm, it is suspected that the increase in the concentration of basil extract was due to the content of alkaloids, tannins, saponins, flavonoids and phenols which functioned. as an antifungal so that it can increase its ability to inhibit fungal growth (antifungal). This is by the opinion of Morales et al. (2003) which states that, if the diameter of the resistance area is 20 mm or more, it means that it has a very strong anti-fungal activity. While the concentration of 80 ppm experienced a decrease in the inhibition zone diameter in the fungus *Saprolegnia sp.* This is presumably because the basil extract was too thick so it was difficult to diffuse maximally in the fungus *Saprolegnia sp.*

Waluyo (2008) said that the diffusion method is often used to determine the sensitivity of fungi. The principle of this method is the inhibition of the growth of microorganisms in the presence of an inhibition zone in the form of a clear area around the paper disc containing an antifungal agent. The diameter of the zone of growth inhibition indicates the sensitivity of the fungus to antifungal substances, then it is said that the wider the diameter of the inhibition zone formed, the more sensitive the fungus is. Other than that, Dani, Nurtjahja & Zuhra (2015) also added that too high a concentration of the extract could result in saturation, causing the active compounds contained to be unable to dissolve completely.

## CONCLUSION

Based on the results of the research that has been done, it is concluded that the extract of basil (*Ocimum sanctum* L) is proven to inhibit the growth of the fungus *Saprolegnia sp.* in vitro. The ethanol extract of basil with a concentration of 60 ppm had the largest inhibition zone diameter of 22 mm in inhibiting the growth of the fungus *Saprolegnia sp.* in vitro compared to the concentrations of other ethanol extracts of basil. The limitation of this study is that the application of basil leaf extracts to *Saprolegnia sp.* Was carried out in a laboratory. For this reason, it is

necessary to carry out further research with the direct application of basil leaf extract to various types of cultivated fish commodities.

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