The effect of *Hyptis suaveolens* (L.) Poit extract on the growth of *Sclerotium rolfsii* with in-vitro

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**Abstract.** *Sclerotium rolfsii* is a fungus that has a wide range of host and high pathogenicity that can cause stem rot, root rot, and wilt in plants. Diseases caused by *S. rolfsii* can be solved by using synthetic or chemical fungicides. The use of synthetic or chemical fungicides on an ongoing basis can have a negative impact on human health. Natural pesticides derived from plant leaf extracts are explored to treat plant diseases. One of them is *Hyptis suaveolens* (L.) Poit. The compounds contained in this plant are alkaloids, flavonoids, tannins, and suaveolic acid. This study aims to examine the effect of *H. suaveolens* (L.) Poit. extract and the most effective concentration in inhibiting the growth of *S. rolfsii*. *H. Suaveolens* (L.) Poit. leaf extract was prepared by maceration method using 96% ethanol. The leaf extract solution was evaporated using a vacuum rotary evaporator. The concentration used is 0% (control), 5%, 10%, 15%, and 20% with 4 repetitions. The data obtained were analyzed using one way ANOVA and Duncan test. The results showed that *H. suaveolens* (L.) Poit. leaf extract could inhibit the growth of *S. rolfsii* and the most effective concentration was at concentration 15% with 58% inhibition percentage.

1. **Introduction**

*Sclerotium rolfsii* is a soil-borne pathogenic fungus that usually occurs in tropical, subtropical and other warm temperature regions in the world which causes root rot, stem rot, and withering to more than 500 species of plants, including almost all agricultural crops (Aycock, 1996 in Yaqub and Shahzad, 2005). *S. rolfsii* is also known as the fungus that causes seedling fall (*damping off*) by attacking the base of the stem. *S. rolfsii* is easily recognized by seeing the presence of white mycelium and in further attacks there will be sclerotia. Diseases caused by this fungus occur throughout the world and affect plants at all stages of growth, including seeds, mature plants and crops (Agrios, 2005).

In humid environment, *S. rolfsii* forms thin mycelium, which is white and like feathers at the base of the stem as well as the surrounding surface. Soil with this mycelium will later form many small and round grains with a slippery surface. These small grains are white in the beginning, then turn in to brown to dark brown. This grain is called sclerotia. Sclerotia acts as a fungus defense tool because it has properties that are very resistant to the environment that do not support. (Agrios, 2005).

Sclerotia in the soil can survive for up to 2-3 years depending on the availability of organic matter (Agrios, 2005). *S. rolfsii* has a wide host range and high levels of pathogenicity (Yaqub and Shahzad, 2005). The production of sclerotia is very large and their ability to survive in the soil for several years as well as the growth rate of abundant fungi make it a facultative parasite (Punja, 1985). In the inner
layer of sclerotia there are bubbles which are food reserves. The inside of the old sclerotia contains sugar, amino acids, fatty acids, and fats, while the walls contain sugar, chitin, laminarin, \( \beta 1-3 \) glucoside. The surface of the sclerotium can release exudates in the form of ion bonds, proteins, carbohydrates, endopoligalakturonase enzymes, and oxalic acid (Sumartini, 2012). The outer skin has thick walls and contains melanin (Elad, et al., 1984).

Symptoms of \( S. \) rolfsii attack on plants begins from infecting the roots or stems of plants which is close to the ground. Furthermore, it will infect the roots or stems which cause nutrient and water transport clogged so that the plants wither (Xie and Vallad, 2016). The entry of pathogens into plant tissues can destroy plant tissue by secreting oxalic acid and pectinase enzymes before penetration into the host tissue. If the tissue has been damaged by this pathogenic infection, the transportation of food from the soil will be disrupted until finally the plant withers and causes decay in the plant (Punja, 1985). The pathogen then spreads to all parts of the plant and causes decay. The soil around the affected plants consists of white mycelium and sclerotia (Pratama, et al., 2013).

Disease caused by \( S. \) rolfsii is overcome by using pesticides, namely synthetic or chemical fungicides. Continuous use of synthetic or chemical fungicides in agricultural activities can have a negative impact on human health because there will be pesticide residues in the food products (Sofia, 2001). The fact is that all this time, generally people are not aware of the symptoms of pesticide poisoning due to non-specific symptoms such as dizziness, nausea, vomiting, fever, and others. But chronically can cause serious diseases such as cancer (Raini, 2007). Natural pesticides derived from plant leaf extract are now widely explored to treat diseases in plants. This is expected to reduce the impact of chemical pesticides on the environment and health. One of them is \( H. \) suaveolens (L.) Poit.

\( H. \) suaveolens (L.) Poit belongs to the family Lamiaeceae in the form of terma with crossed / opposite leaves and has no foliage and has essential oil glands that provide a pleasant odor (Tjitrosoepomo, 1993). The compounds they contain in addition to essential oils are alkaloids (14.32%), flavonoids (12.54%), tannins (0.52%), phenols (0.05%), and saponins (0.30%) (Edeoga et al., 2006). In addition to these compounds, this plant also contains suaveolic acid which is phytotoxic (Prawatsri, et. al., 2013).

Flavonoids have benefit as antifungi (Chusnie and Lamb 2005). Flavonoids are the largest group of phenols resulted from plants that have antimicrobial properties against fungi. The mechanism of flavonoids action as antifungal works by damaging the permeability of cell wall membranes and extracellular proteins of the \( C. \) albicans (Permatasari, et al., 2016). In addition to flavonoids, alkaloids are also compounds that have antimicrobial activity. Alkaloid compounds from meniran plants (\( P. \) niruri) are able to inhibit the growth of \( S. \) aureus and \( C. \) albicans (Mangunwardoyo, et al., 2009). According to Retnowati (2011), alkaloids will damage the microbial cell wall and affect the inhibition of microbial growth. In general, the work of a chemical as an antimicrobial agent can result in changes that lead to damage to the inhibition of the growth of microbial cells. Tannin is also thought to have effectiveness in inhibiting growth or killing fungi. In addition, tannin also has antioxidant and antisepetic activities (Sulistyawati and Mulyati, 2009). Meanwhile, suaveolic acid is a toxic phytotoxin that inhibits growth (Prawatsri, et. al., 2013).

The purpose of this study is to see the effect of \( H. \) suaveolens (L.) Poit leaf extract and to determine the most effective concentration in inhibiting the growth of \( S. \) rolfsii.

2. Methods

The study was conducted from February to April 2018 at the Microbiology Laboratory and Integrated Research Laboratory, Faculty of Mathematics and Natural Sciences, Padang State University.

The tools used in this study include test tubes, test tube racks, measuring cups, cup cups, Erlenmeyer, petri dishes, drop pipettes, electric stoves, autoclave, scalpel, digital scales, vortex, vacuum rotary evaporator, stirring rod, lamp spirits, calipers, ovens, tweezers, blenders and stationery. While the ingredients used are leaves of \( H. \) suaveolens (L.) Poit. Cultures of \( S. \) rolfsii, ethanol
96%, Potato Dextrosa Agar (PDA) medium, 70% alcohol, sterile aquades, aluminum foil, gauze, cotton, plastic, tissue, newspaper and label paper.

Fresh leaves of the *H. suaveolens* plant are cleaned with aquadest, finely chopped and then dried air, after that the leaves are mashed using a blender and then put in an opaque bottle of 300 grams then inundated with 96% ethanol until the entire sample is submerged.

The container is tightly closed and placed in a light protected area and left for 5 x 24 hours, then filtered using filter paper. The extract of *H. suaveolens* obtained was purified by the evaporation process using vacuum rotary evaporator to obtain thick extracts (Renisheya et.al., 2012). Furthermore, the pure extract obtained was diluted according to the treatment of 5%, 10%, 15%, and 20%.

The test was carried out by taking 2 mL of *H. suaveolens* leaf extract from each treatment was added to 8 mL PDA in the test tube, homogenizing it using vortex, then pouring it into a petri dish, then let it freeze completely. For positive control, 10 mL PDA media were not added with *H. suaveolens* extract.

The *S. rolfsii* that has grown (3 days old) then inoculated on PDA medium which was added with *H. suaveolens* leaf extract according to the treatment. The size of the fungus colonies taken is approximately 0.5 cm x 0.5 cm (length x width) taken by using a scalpel, then placed in the middle of the petri dish containing a mixture of medium with *H. suaveolens* leaf extract, the culture was placed at a temperature space.

Observations were made by looking at the growth of *S. rolfsii* colonies by measuring colony diameter 24 hours after incubation on days 2 to 4. The data analyzed is data on the 4th day.

Furthermore, the calculation of the percentage inhibition of growth of each concentration was carried out using a formula

\[ P = \frac{D_1 - D_2}{D_1} \times 100\% \]  

\( P \) = Percentage of inhibition  
\( D_1 \) = Average mushroom diameter in the control (mm)  
\( D_2 \) = Average mushroom diameter in each treatment (mm)  
(Achmad and Suryana, 2009).

The data obtained were analyzed by variance (ANOVA) and if there were differences, it would be continued by Duncan's new Multiple Range Test (DNMRT) at a significant level of 5%. Data on *S. rolfsii* colonies and percentage inhibition were presented in table form.

### 3. Result

Based on the research that has been done, it can be seen that the extract of *H. suaveolens* (L.) Poit leaves can inhibit the growth of *S. rolfsii*. The results of analysis of variance in each treatment of inhibition of *S. rolfsii* colony diameter shows a significantly different effect between control and each treatment. The diameter of *S. rolfsii* colonies with the treatment of *H. suaveolens* (L.) Poit extract in various concentrations can be seen in Table 1.

| Treatment | Average of colony diameter (cm) |
|-----------|---------------------------------|
| D (20%)   | 0,00 a                          |
| C (15%)   | 3,73 b                          |
| B (10%)   | 4,13 c                          |
| A (5%)    | 6,52 d                          |
| control   | 9,00 e                          |

Description: The numbers followed by the same letter indicate a real difference about 5% in Duncan's advanced test (DNMRT).
In Table 1 it can be seen that the diameter of \( S. \) rolfsii colonies in the control was significantly different from the colony diameter in treatments A, B, C, and D. Treatment A was significantly different from treatment B, C and D. Treatment B was significantly different from treatment C and D. Treatment C is significantly different from treatment D.

The diameter of \( S. \) rolfsii colonies at the concentration of 5% extract showed a difference when compared to the controls. This shows that at a concentration of 5%, the chemical compounds contained in the extract of \( H. \) suaveolens (L.) leaf Poit have been active to inhibit the growth of the fungus.

D treatment (20%) was able to kill \( S. \) rolfsii, shown in Figure 1, the fungus did not grow on the medium. This concentration was indeed effective in inhibiting the growth of the fungus. However, in the principle of control it is not recommended to kill but only to suppress growth.

Fungal death at this concentration was caused by the concentration of 20% extract chemical compounds higher than the concentration of other treatments. The content of the extract of \( H. \) suaveolens (L.) Poit as reported by Nantitanon (2007) that the content of the compounds in the extract has the potential as an antifungal and and antibacterial. The contents of \( H. \) suaveolens (L.) Poit include alkaloids (14.32%), flavonoids (12.54%), tannins (0.520%), phenols (0.050%), and saponins (0.300%) (Edeoga et al., 2006).

The percentage of inhibition of \( S. \) rolfsii growth can be seen in Table 2.

| Treatment | Inhibition Percentage |
|-----------|-----------------------|
| control + | -                     |
| A (5%)    | 27%                   |
| B (10%)   | 53%                   |
| C (15%)   | 56%                   |
| D (20%)   | 100%                  |

In Table 2, it can be seen that the difference in percentage of inhibition of \( S. \) rolfsii colonies depends on the concentration of extract given. Treatments A, B and C have inhibition percentages, namely 27%, 53% and 56%. Whereas in treatment D, \( S. \) rolfsii experienced death. Although the percentage of fungal inhibition at a concentration of 20% is very high, it is not recommended to use the concentration, because it can kill the fungus. On the principle of control, it only suppresses the growth of pathogens. So that the most effective concentration in inhibiting the growth of \( S. \) rolfsii is at a concentration of 15% which shows an inhibitory percentage of 56%.

The leaves extract content of \( H. \) suaveolens (L.) Poit includes alkaloids (14.32%), flavonoids (12.54%), tannins (0.520%), phenols (0.050%), and saponins (0.300%). These compounds have been shown to inhibit fungal growth because they have antifungal properties. Flavonoids as antifungals have been proven by Permatasari et al. (2016) in her research. The results of the study indicate that flavonoids were able to damage the permeability of cell wall membranes and extracellular proteins of \( C. \) albicans.

Other chemical compounds in the extract of \( H. \) suaveolens (L.) Poit leaves within alkaloids, flavonoids, tannins and phenol are suaveolic acids. This suaveolic acid is phytotoxic. Islam et al., (2014) conducted a study using the chemical substance \( H. \) suaveolens (L.) Poit which were suaveolic acid on several plants. The results of his research stated that suaveolic acid was able to inhibit the growth of roots and shoots of the test plants. Prawatsri et al., (2013) conducted a study by isolating diterpene in \( H. \) suaveolens (L.) Poit, one of them included suaveolic acid.
Figure 1. Growth of S. rolfssii colonies with treatment in some concentrations of H. suaveolens (L.) Poit. extract.

4. Conclusion

Here are the conclusions obtained from research data: 1). H. suaveolens (L.) Poit leaf extract can inhibit the growth of S. rolfssii. 2). Concentration of H. suaveolens (L.) Poit leaf extract which is the most effective in inhibiting S. rolfssii fungus growth is recommended at a concentration of 15% with 56% inhibition percentage.

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