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Research Article

Exploration and Investigation of Antifungal Activity of Plant Leaf Extracts on Growth of *Scletorium rolfsii* Sacc.

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Leaf extract,
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Abstract: Botanical fungicides are fungicides derived from plants that produce chemical compounds that potentially inhibit microbial growth. These fungicides are safe because to its not harmful to humans and the environment. In the present study, the plant materials used often compete with plant materials used for food and medicine such as galangal rhizomes and betel leaves. Therefore, it is necessary to explore materials derived from plants that have not been widely utilized. So the research was conducted to determine the effect of leaf extracts from several plants on the growth of *Sclerotium rolfsii* Sacc. the fungus that causes wilt disease in plants and determines the level of antifungal activity. This research was conducted using a completely randomized design (CRD). The leaf extracts used were from the plants *Muntingia calabura*, *Terminalia cattapa, Syzygium oleina, Morinda citrifolia, Dimocarpus longan,* and *Artocarpus altilis* with concentrations of 10%, 20%, 30%, 40%, and 0% as control. The data obtained were analyzed using variance analysis (ANOVA) with Duncan's New Multiple Range Test (DNMRT). The results showed that all treatments used could inhibit the growth of *S. rolfsii* Sacc because they were significantly different from the control. It was determined that antifungal activity in leaf extracts of *M. calabura*, *T. cattapa*, *S. oleina*, and *D. longan* was very strong, and also *A. altilis* had a strong antifungal activity, while *M. citifolia* had a moderate antifungal activity.

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1. Introduction

The *Sclerotium rolfsii* Sacc. is a soil-borne fungus that causes stem rot disease, consequently, the plants wilt and die (Sektiono et al., 2019). It also causes the dumping of disease and is easily recognized by the presence of white mycelium on the infected plant parts and in subsequent attacks will form sclerotia. Globally, this disease affects plants at all stages of growth, including seeds, and mature plants (Agrios, 2005).

The stem rot disease begins with the yellowing at the base and is accompanied by the emergence of mycelium. On the underside of the leaves and near the plant, sclerotia were found and the leaves close to the soil surface will experience chlorosis and change into brown, thereby causing plants to wither,

rot, and die (Kator et al., 2015; Martinius et al., 2019). This fungus has a wide host range, which makes it difficult to control (Semangun, 2004), and can survive in the soil in the form of sclerotia for several years (Punja, 1985). According to Agrios (2005), sclerotia can last for 2-3 years depending on the availability of organic matter.

S. rolfsii Sacc. attack on plants begins by infecting the roots or stems of plants close to the soil surface. Furthermore, it will infect the roots or stems, causing nutrient and water transportation to be blocked (Xie and Vallad, 2016). Early symptoms begin with yellowing of the stem base, accompanied by the emergence of mycelium. Sclerotia are found on the underside of leaves and near the plant. Leaves that are close to the soil surface will experience chlorosis and turn brownish. Blockage of nutrient and water transportation causes plants to wilt, rot, and eventually die (Martinius et al., 2019). *S. rolfsi* can produce oxalic acid (Monazzah, et al., 2018) which is directly toxic to plant tissues (Kyoung, 2008).

Control of plant diseases is generally achieved through the use of pesticides. Pesticides are chemicals used in agriculture to protect plants by destroying unwanted organisms. Such as insecticides, rodenticides, herbicides, fungicides, and others. However, these chemicals can cause a large number of negative impacts on health and the environment because they can cause toxic effects or poisoning due to excessive exposure to chemicals or doses (Alewu and Nosiri, 2011; Stamati, et al., 2016). According to Mesnage and Seralini (2018), the human population exposed to pesticides is currently in increasing numbers due to continuous use.

Pesticides used to control plant diseases caused by fungi are fungicides. Due to the negative impact caused by fungicides, it is necessary to find alternative sources of new pesticides, where the use of these pesticides is expected to be efficient, safe, and selective against target pests and pathogens (Shukla, et al., 2012). One of them is biological control such as the use of botanical fungicides. Botanical fungicides are fungicides obtained from plant organ extracts, such as from tubers, roots, stems, or leaves (Husein and El-Anssary, 2018). Mazid et al., (2011), explained that plants can produce chemical compounds or secondary metabolites that can protect themselves from pathogen attacks because these compounds are antibiotics, antifungal, and antiviral. These secondary metabolite compounds have a broad spectrum such as terpenes, phenols, flavonoids, tannins, alkaloids, saponins, and others. Each plant chemical derivative can be utilized based on differences in the content of its biological properties (Dubey, et al., 2008; Shukla et al., 2012). Secondary metabolite compounds can be utilized as botanical fungicides. Botanical fungicides have advantages, including being environmentally friendly, easily degraded, and abundant local resources so that they are easily available (Dalimunthe and Rachmawan, 2017).

Some plants have been utilized as botanical fungicides, such as extracts from garlic, betel and cloves (Prasetyorini, 2020), lemongrass (Martinius et al., 2019), basil (Nugroho et al., 2019) and some seaweed plants (Mabrouki, 2020; Inci et al., 2021; Ozakin et al., 2021) have been discovered to inhibit the growth of the fungus *S. rolfsii* Sacc. However, the plants used as botanical fungicides are plant organs that are widely used as raw materials for medicines and food flavorings. So that if used as a botanical fungicide for plant disease control, it will certainly compete in the supply of raw materials; as a result, the price becomes expensive and will make it difficult for farmers. The existence of different interests is one of the challenges in biological control. Based on this, it is necessary to explore plant organs such as leaves, which are not widely used by humans and have the potential to be applied as botanical fungicides. So a study was conducted on several plant leaf extracts to see their effect on inhibiting the growth of *S. rolfsii* Sacc. and its antifungal activity.

2. Material and Methods

2.1. Plant material

The choice of plant leaves is a plant that grows many leaves and the leaves are not widely used by people like for food or medicine. There are six plants, namely *Muntingia calabura (kersen), Terminalia cattapa (ketaping), Syzygium oleina* (Redbud), *Morinda citrifolia* (noni), *Dimocarpus longan* (longan), and *Artocarpus altilis* (breadfruit).

2.2. Methods

This study was carried out in January-April 2022 at the Research Laboratory at the Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University. The experiment was conducted using a Completely Randomized Design (CRD). CRD is one where the treatments are assigned completely at random so that each experimental unit has the same chance of receiving any one treatment. Each of these plant extracts was distinguished by concentrations of 10%, 20%, 30%, and 40%. The choice of concentration is based on previous research, in which a concentration of 20% of *Hyptis suaveolens* leaf extract was able to inhibit the growth of S. *rolsii* Sacc. Then, in this study, the concentration was reduced to 10% and increased to 30% and 40% (Chatri et al., 2019). The negative control was the treatment without leaf extract and the positive control was the treatment with chemical fungicides (Antracol 70 WP), intending to see if the leaf extract gives the same effect as chemical fungicides. The positive control concentration used was the lowest concentration of leaf extract (10%).

2.3. Preparation media incubation

Approximately 7.8 g PDA media was put into a 250 mL Erlenmeyer and dissolved to 200 mL of distilled water, then heated using a hot plate until boiling and homogeneous. After homogeneous, it was left until the temperature of the solution decreased, and then the Erlenmeyer was closed with a cotton plug, aluminum foil, and plastic wrap. Then the PDA media was sterilized in an autoclave at 121°C at a pressure of 15 psi for 15 minutes. After that, the PDA media was poured into petri dishes and allowed to solidify.

2.4. Preparation of leaf extract

Fresh leaves of the six plants were rinsed with distilled water, then finely chopped, and then dried, after that, the leaves were pulverized using a blender, then put into an opaque bottle of 300 grams, and soaked with 96% ethanol. The container was tightly closed and placed in a place protected from the light and left for 5x24 hours, then filtered using filter paper. The leaf extract solution obtained was purified by the evaporation process using a vacuum rotary evaporator to obtain a thick extract (Renisheya et al., 2012). Furthermore, the pure extract obtained was diluted according to the treatment, namely 10%, 20%, 30% and 40%. For a concentration of 10%, 1 gram of leaf extract was taken and then added with distilled water to a volume of 10 mL, and so on.

2.5. Leaf extract essay

Leaf extract testing was carried out by taking 2 mL of extract from each treatment and then adding it to 8 mL of PDA in a test tube, homogenizing it using a vortex, then it was poured into a Petri dish, after which it was allowed to freeze perfectly. For the control, 10 mL of PDA medium that was not added with leaf extract was used. *S. rolfsii* Sacc. that has been grown (3 days of age), inoculated on PDA medium that has been added with leaf extract according to the treatment. The size of the fungal colony taken was approximately 0.5 cm x 0.5 cm (length x width) taken using a scalpel, then placed in the center of a petri dish that contained a mixture of medium with leaf extract, and then incubated at room temperature.

2.6. Measurement of *S. rolfsii* growth

Fungal growth was done by measuring the diameter of S. *rolfsii* Sacc. (cm) on day 2 to day 5 after incubation. The data analyzed was the data on day 5. Measurement of the diameter of fungal colonies is done by making a horizontal and vertical line on the surface of the Petri dish. The cutting point of both lines was right in the center of the fungal colony that grew as shown in Figure 8.

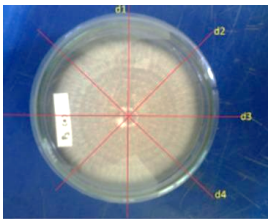

Figure 1. Diameter measurement of fungal colonies (d = diameter).

2.7. Assessment antifungal activity

To determine the antifungal activity of the leaf extract, the percentage of inhibition of *S. rolfsii* Sacc. growth was calculated by the formula (Ouoba, et al., 2018):

$$P = \frac{D1 - D2}{D1} \times 100\% \quad (1)$$

Description:
P = Percentage of inhibition
D1 = Average diameter of fungus in negative control (cm)
D2 = Average diameter of fungus in each treatment (cm)

Based on the percentage of growth inhibition of *S. rolfsii* Sacc. the criteria for antifungal activity were determined as shown in Table 1.

Table 1. Antifungal activity (Mori, et al., 1997)

Inhibition Percentage	Activity Level
P≥75	Very strong
75≤P<50	Strong
50≤P<25	Moderate
25≤P<0	Weak
0	Inactive

2.8. Data analysis

Colony diameter data were examined using Analysis of Variance (ANOVA) and was continued with Duncan's New Multiple Range Test (DNMRT) at α=0.05. The antifungal activity data were analyzed descriptively.

3. Results and Discussion

3.1 Growth of *S. rolfsii* Sacc.

The results of the investigation on the effect of several leaf extracts on *S. rolfsii* Sacc. are shown in Table 2. According to Table 2, it was discovered that at the lowest concentration of 10%, several leaf extracts such as *M. calabura, D. longan, S. oleina,* and *A. altilis*. effectively inhibit the growth of *S. rolfsii* Sacc. The results of statistical analysis showed that the diameter of the *S. rolfsii* Sacc. colonies was significantly different from the control (-). This is because the compounds contained in the secondary metabolites of these plants can act as antifungals. Tiwari et al. (2009) stated that the ability of compounds contained in plants as antimicrobials depends on the concentration and chemical structure of the active components such as saponins, flavonoids, thiosulfinates, glucosinolates, phenols, and organic acids. However, the main components in plants that are active as antimicrobials are phenolic

compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, and isoflavonoids. Leaf extracts of *M. calabura*, *D. longan*, *S. oleina*, and *A. altilis* contain saponins and flavonoid compounds. In this study, the leaf extracts of other plants also contained these two compounds, but in small concentrations. Therefore, it has not been able to inhibit the growth of *S. rolfsii* Sacc. According to Cushnie et al. (2005), saponins and flavonoid compounds can be found in fruit, seeds, stems, flowers, and leaves.

Table 2. Colony diameter (cm) of *S. rolfsii* Sacc. by treatment of several plant leaf extracts at different concentrations

Leaf Extract Concentration	***M. calabura***	***T. cattapa***	***D. longan***	***S. oleina***	***M. citrifolia***	***A. altilis***
control (-)	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]	9.30 [a]
10	7.68 [b]	8.95 [a]	4.94 [b]	7.11 [b]	9.00 [a]	5.53 [b]
20	7.24 [b]	8.40 [a]	4.75 [b]	5.74 [c]	3.10 [b]	4.71 [c]
30	4.38 [c]	5.59 [b]	2.03 [c]	3.75 [d]	7.74 [c]	3.73 [c]
40	0.50 [d]	0.50 [c]	1.89 [c]	1.41 [e]	6.63 [d]	3.45 [c]
control (+)	--	--	--	--	--	--

Remarks: The number followed by the same letter is not significantly different in each treatment based on the Duncan test (α=0.05).

The diameters of *S. rolfsii* Sacc. colonies at a concentration of 40% (the highest concentration) are shown in Figure 1. It was discovered that the largest colonies of 6,63 cm were treated with *M. citrifolia* leaf extract and the smallest, namely 0.5 cm was treated with *M. calabura* and *T. cattapa*. Based on the analysis, all treatments with leaf extracts showed significant differences from the control. This proves that the leaf extracts of the tested plants can inhibit the growth of *S. rolfsii* Sacc. All leaf extracts have shown the ability to inhibit the growth of *S. rolfsii*. Even in the leaf extracts of *M.calabura* and *T.cattapa* with a concentration of 40%, the growth of *S.rolfsii* colonies did not exist at all until the end of observation (day 5), as can be seen in Figure 2. This inhibitory ability is due to the presence of active compounds contained in the leaves of these plants, such as alkaloids, phenols, flavonoids, saponins, tannins, steroids, and triterpenoids.

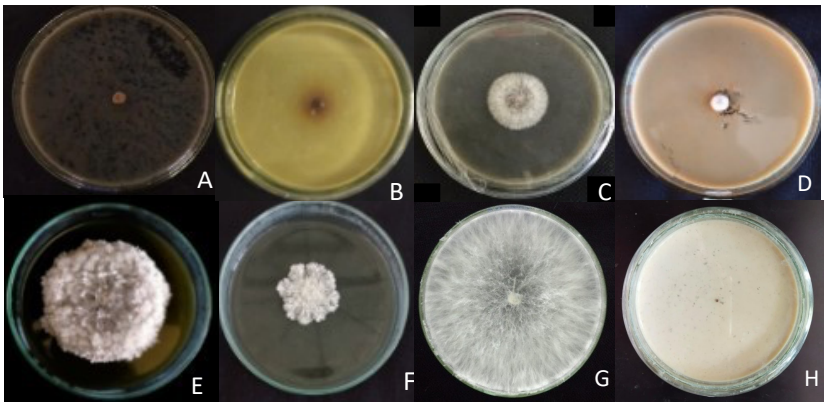

Figure 2. Diameter of *S. rolfsii* Sacc colonies at a concentration of 40% with the treatment of several plant leaf extracts. A. *M. calabura,* B. *T. cattapa,* C. *D. longan,* D. *S. oleina,* E. *M. citrifolia,*, F. *A. altilis,* G. control (-) and H. control (+).

Alkaloids are one of the secondary metabolites found in plants, which can be found in leaves, twigs, seeds, and bark. In general, alkaloids are often used in medicine. Alkaloids interfere with fungal growth by entering the cell wall and preventing DNA replication so that the formation of DNA and RNA will be disrupted (Aniszewski, 2007). Alkaloid compounds prevent replication of nucleic acid biosynthesis in fungi so that fungi cannot develop (Adegoke and Adebayo-Tayo, 2009). Alkaloid compounds also bind strongly to ergosterol to form holes or channels, causing the cell membrane to leak

and lose some intra-cellular materials such as electrolytes (especially potassium) and small molecules. This results in permanent damage to the cell and cell death in fungi (Mycek et al., 2001; Setiabudy and Bahry, 2007).

The phenolic compounds in flavonoids can denature cell proteins and shrink cell walls, causing fungal lysis, and disrupting growth, and death (Cowan, 1999). The mechanism of flavonoids inhibits fungal growth by disrupting cell membrane permeability. This can change organic components and interfere with nutrient transport, thereby creating a toxic effect on fungi (Komala and Siwi, 2019). Saponins can also function as antifungals, leading to the leakage of proteins and enzymes from the cell (Rijayanti, 2014). This leakage occurs because the saponins damage the permeability of the cell membrane by lowering the surface tension of the fungal cell wall. Furthermore, saponins will diffuse through the cytoplasmic membrane which destabilizes the membrane and the cytoplasm exits the cell leading to cell death (Sudarmi, 2017).

The mechanism of action of tannins as antifungals inhibits the synthesis of chitin which is used for the formation of cell walls in fungi and damages cell membranes, therefore, disrupting fungal growth (Watson and Preedy, 2007). Tannins can also cause cells to lyse because they target cell wall polypeptides and inhibit formation (Sapara, 2016). The imperfect formation makes the cells unable to withstand osmotic or physical pressure, thereby causing death (Rijayanti, 2014). Furthermore, tannins also inactivate fungal cell adhesins and enzymes as well as interfere with protein transport within cells (Egra, 2019). Triterpenoids inhibit fungal growth through the cytoplasm or interfere with the growth and development of fungal spores (Lutfiyanti, 2012).). *T.cattapa* has been shown to inhibit the growth of the fungus *Pyricularia grisea* (Zuraidah and Wahyuni, 2019) because the plant contains flavonoid, alkaloid, steroid, saponin, and tannin compounds (Salimi et al., 2022).

Testing of leaf extracts as antifungal was rarely carried out. Testing of leaf extracts in inhibiting bacterial growth has been carried out, such as *M. calabura* against *Escherichia* coli (Handoko et al., 2019) and *Porphyromonas gingivalis* (Muflikhah et al., 2017). Ethanol extract from *T. catappa* leaves can inhibit the growth of *Aeromonas hydrophila* bacteria (Purba et al., 2020). *D.longan* can inhibit the growth of *Staphylococcus aureus*, *Salmonella typhii,* and *Vibro mimicus* bacteria (Ripa et al., 2010), The results of research from Haryati et al., (2016) show that 96% ethanol extract of *S.oleina* leaves has antibacterial activity against *Staphylococcus aureus* bacteria and *Escherichia coli* bacteria.

The growths of *S. rolfsii* Sacc. colonies treated with several leaf extracts with different concentrations were observed until the 5th day after incubation, as shown in Figure 3-8. The growth of *S. rolfsii* Sacc. colonies still showed an increase in colony diameter until the last day of observation, except for the treatment of *M. calabura, T. cattapa*, and *S. oleina* leaf extracts. The colony diameter still increased because not all components of the active compounds in the leaf extract inhibited the growth of the fungus. The growth of *S. rolfsii* Sacc. colony diameter occurred very quickly in the 0% treatment or without treatment with leaf extract. This shows that the active compounds contained in the leaf extract affect the growth of fungi.

Positive control tests using chemical fungicides showed that *S. rolfsii* could not grow even at low concentrations such as the leaf extract (10%). *S. rolfsii* Sacc. mycelium grown on PDA media mixed with chemical fungicides initially formed sclerotia, but then the sclerotia died. This shows that chemical fungicides have very high toxicity, so they can kill microorganisms. In the principle of plant disease control, plant pest organisms are not eradicated to extinction, but only controlled until they do not interfere with plants which results in reduced production. The extinction of an organism or microorganism will result in a reduction in the diversity of living things. According to Mesnage and Seralini (2018), the use of pesticides can kill insects or fungi and have adverse long-term effects on agricultural systems due to a lack of biodiversity. Then, in this case, it is better to use botanical fungicides for plant disease control.

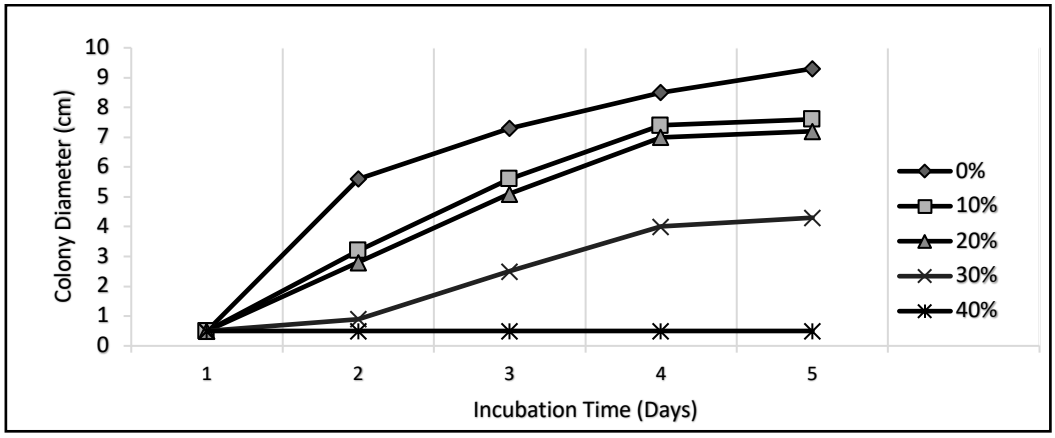

Figure 3. Growth of *S. rolfsii* Sacc with *M. carabola* leaf extract at different concentrations.

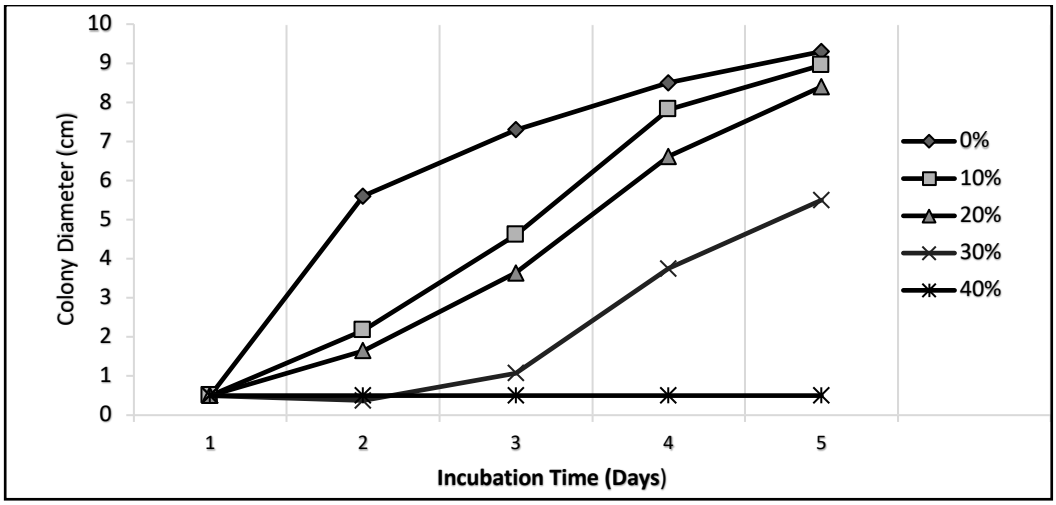

Figure 4. Growth of *S. rolfsii* Sacc with *T. cattapa* leaf extract at different concentrations.

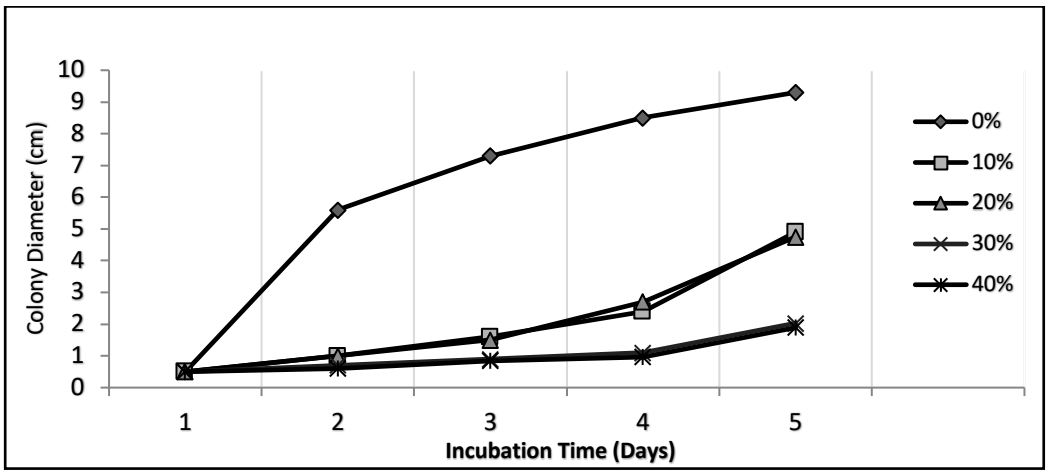

Figure 5. Growth of *S. rolfsii* Sacc with *D. longan* leaf extract at different concentrations.

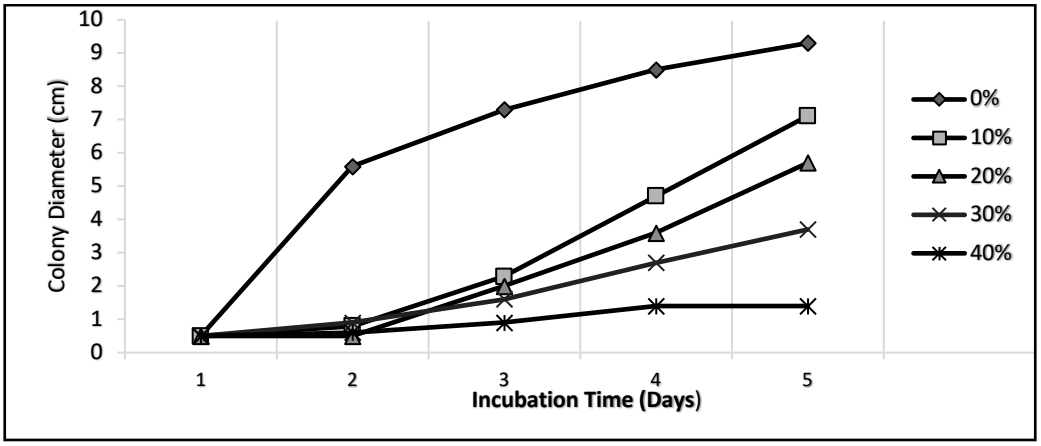

Figure 6. Growth of *S. rolfsii* Sacc with *S. oleina* leaf extract at different concentrations.

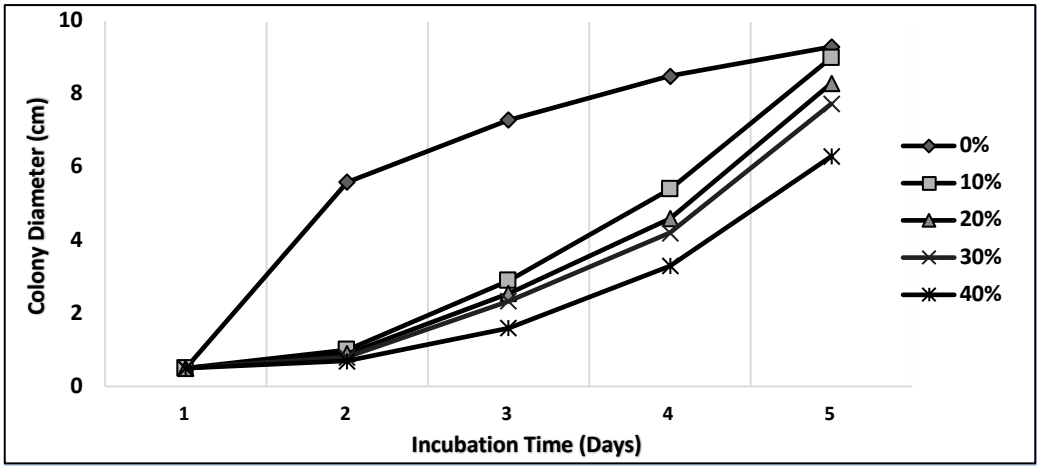

Figure 7. Growth of *S. rolfsii* Sacc with *M. citrifolia* leaf extract at different concentrations.

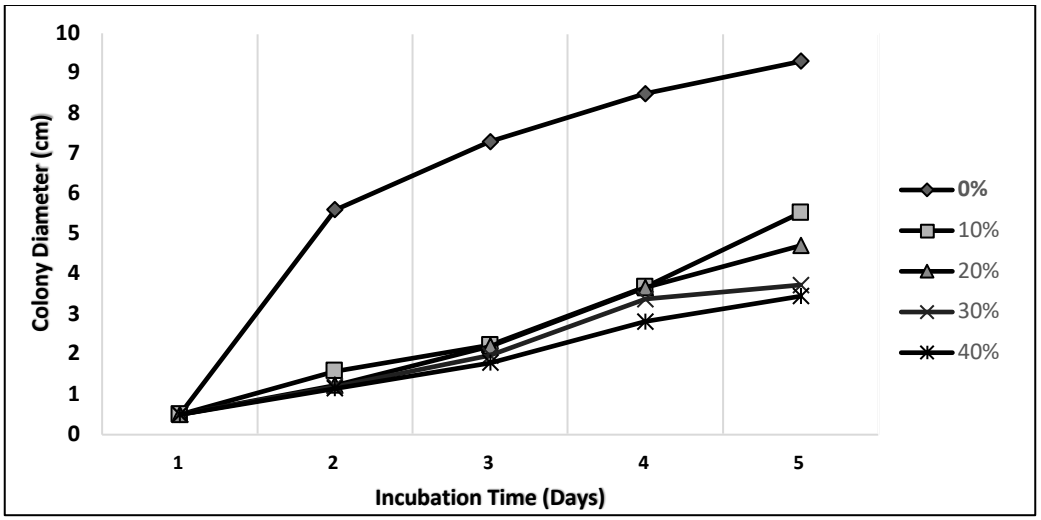

Figure 8. Growth of *S. rolfsii* Sacc with *A. altilis l*eaf extract at different concentrations.

3.2. Antifungal activity

The level of antifungal activity of leaf extracts can be known based on the percentage of growth inhibition (Table 3). It can be seen that some antifungal activities are the same and some are different in each plant extract treatment with different concentrations. At a concentration of 40%, treatment with *M. citrifolia* leaf extract showed a moderate level of antifungal activity (50%≤PP<25%), although the

highest concentration and the results of statistical analysis have shown a significant difference with the control on colony diameter. The treatment of *M. calabura*, *T. cattapa*, *D. longan,* and *S. oleina* leaf extracts at a concentration of 40% has a very strong antifungal activity because it is more than 75%. Treatment of *A. altilis* leaf extract with strong antifungal activity level. The occurrence of different levels of antifungal activity shows that the concentration of leaf extracts influences the growth of *S. rolfsii* Sacc. Because the difference in concentration will cause differences in the levels of active compound components or secondary metabolite compounds contained in plant leaf extracts. The same thing happened in the treatment of extracts of several Leguminosae plants, the difference in extract concentration gave a significant difference to the biomass of *S. rolfsii* Sacc mycelium (Sana et al., 2016). From the results of Chatri et al. (2022), the treatment of *Melastoma malabatrichum* L. leaf extract against *S. rolfsii* Sacc showed weak antifungal activity, even at 40% extract concentration. This indicates that the levels of antifungal substances in these plants are low. However, the treatment of *Hyptis suaveolens* L. leaf extract, at a concentration of 20% already showed a percentage of 100% growth inhibition with very strong antifungal activity (Chatri et al., 2019). This shows that the concentration of plant leaf extracts also affects anti-fungal activity. The higher the concentration of leaf extract used, the higher the content of active compounds that act as antifungal so that the antifungal activity will be greater. Conversely, the smaller the concentration of leaf extract, the less the content of active compounds that act as antifungals so antifungal activity will also be smaller. This is in line with Pelczar (1998) which states that increasing the concentration of an antimicrobial substance is proportional to its activity.

Table 3. Percentage growth inhibition of S. rofsii Sacc and antifungal level of leaf extracts of some plants

Plants	Leaf Extract Concentration (%)	Growth Inhibition Percentage	Antifungal Level
M. calabura	10	15.00	Weak
	20	15.20	Weak
	30	49.10	Moderate
	40	94.03	Very strong
T. cattapa	10	16.41	Weak
	20	27.80	Moderate
	30	61.70	Strong
	40	92.90	Very strong
D. longan	10	47.16	Moderate
	20	49.19	Moderate
	30	78.28	Very strong
	40	79.78	Very strong
S. oleina	10	22.80	Weak
	20	37.78	Moderate
	30	59.28	Strong
	40	84.69	Very strong
M. citrifolia	10	2.57	Weak
	20	10.93	Weak
	30	17.04	Weak
	40	32.15	Moderate
A. altilis	10	44.74	Moderate
	20	49.97	Moderate
	30	57.80	Strong
	40	60.69	Strong

Conclusion

According to the results of the research, all leaf extracts used can inhibit the growth of *S. rolfsii* Sacc. colonies. Because of the results of the statistical analysis, all treatments were significantly different from the control at the end of the observation. However, antifungal activity can be different even at the same concentration of different plant extracts. Antifungal activity in leaf extracts of *M. calabura, T.*

cattapa, *S. oleina,* and *D. longan* is very strong, *A. altilis* has strong antifungal activity, whereas *M. citifolia* has moderate antifungal activity. The results of this study are still on a laboratory scale (in vitro). To prove the real results, it is necessary to test it directly on plants (in vivo) with the right method. The results of the study can be used to control plant diseases at a lower price and do not cause negative effects, both on humans and the environment**,** as with chemical pesticides that have high toxicity**.**

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