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Potential of Hexadecanoic Acid as Antimicrobials in Bacteria and Fungi that Cause Decay in Mustard Greens *Brassica juncea* L.

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Abstract

Mustard greens *Brassica juncea* L. one of vegetable that is very easily damaged by microorganisms known as soft rot disease. This causes a decrease in the quality of green mustard so that it cannot last long. A study entitled "The Potential of Hexadecanoic Acid Compounds as Antimicrobials in Bacteria and Fungi that Cause Decay in Mustard Greens *Brassica juncea* L.". This research aims to specify effect of hexadecanoic acid compounds in inhibiting the growth of bacteria and fungi that cause decay in mustard greens. An inhibition test was carried out on *Xanthomonas campestris* bacteria and *Fusarium oxysporum* fungus using 10%, 20%, and 40% hexadecanoic acid test compounds. The results obtained showed that 10%, 20%, and 40% hexadecanoic acid extracts were able to inhibit the growth of *Xanthomonas campestris* bacteria and the fungus *Fusarium oxysporum*. Hexadecanoic acid compounds are bacteriostatic in *Xanthomonas campestris* and fungi *Fusarium oxysporum* are fungistatic.

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Introduction

Indonesia is an agrarian country, because of that agriculture have an crucial role within the standard the overall wheels of the country's economy. This can be proven by the large number of people who work in agriculture as a source of livelihood which is also supported by suitable natural conditions that allow residents to plant throughout the year (Suratman, 2018).

Green mustard *Brassica juncea* L. is a vegetable commodity that is in great demand by the people of Indonesia because it has commercial value and good prospects from various aspects. In addition, mustard has a high demand and is always increasing along with the increasing population in Indonesia and increasing public awareness of the importance of nutritional needs (Sarif, et al, 2015).

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But in its cultivation it often has drawbacks because it is easy for green mustard to be attacked by diseases caused by bacteria and fungi that cause decay to occur in mustard greens so that the quality of mustard greens decreases and the selling price of mustard greens decreases. Some of the causes of decay in green mustard are *Xanthomonas campestris* bacteria, and there is also *Fusarium oxysporum* fungus which can be some of the rots in mustard greens (Semangun, 2004). Therefore, natural preservatives are needed to inhibit the bacteria and fungi can cause decay in mustard greens.

One of the natural preservatives that can be used to inhibit bacteria and fungi that cause decay in green mustard is hexadecanoic acid which is an isolate from the hydroid *Aglaophenia cupressina* Lamoureoux. by Johannes, 2009. Hexadecanoic acid is a derivative of carboxylic acid which has antibacterial and antifungal properties, so it is hoped that this study can extend the shelf life of mustard greens.

Materials and Methods

Materials

The materials used in this study included hexadecanoic acid compounds, *Xanthomonas campestris* bacteria culture (InaCC B1449), *Fusarium oxysporum* fungus culture (InaCC F641), Potato Dextrose Agar (PDA) medium, label paper, Nutrient Agar (NA) medium, sterile distilled water, 70% alcohol, physiological NaCl 0.9%, aluminum foil, cotton, cotton swab, Ciprofloxacin, and Ketoconazole.

Methods

This study used the well diffusion method to test the activity of the hexadecanoic acid compound by looking at the inhibition zone formed around the well. Place 5 containers on nutrient agar medium and potato dextrose agar medium in each petri dish. The media containing the suspension of bacteria and fungi pour into each petri dish and are allowed to solidify. The reservoir is removed to form a well for the test solution (Pangemanan, et al, 2016).

Hexadecanoate extract with 3 different concentrations (10%, 20%, 40%), positive control (Ciprofloxacin) for antibacterial test, positive control (Ketoconazole) for antifungal test, and negative control (aquades) was dripped as much as 50 l on each Each different well was carried out in duplicate and then incubated in an incubator at 37°C for 24 hours for bacteria and 72 hours for fungi. Diameter of the inhibition zone formed was observed and measured using a caliper (Pangemanan, et al, 2016).

Results and Discussion

Hexadecanoic Acid Activity Test Against *Xanthomonas campestris* Bacteria

The results of the observation of the inhibition zones formed at extract concentrations of 10%, 20%, and 40% on *Xanthomonas campestris* bacteria after incubation 24 hours and 48 hours can be seen in Figure 1 below.

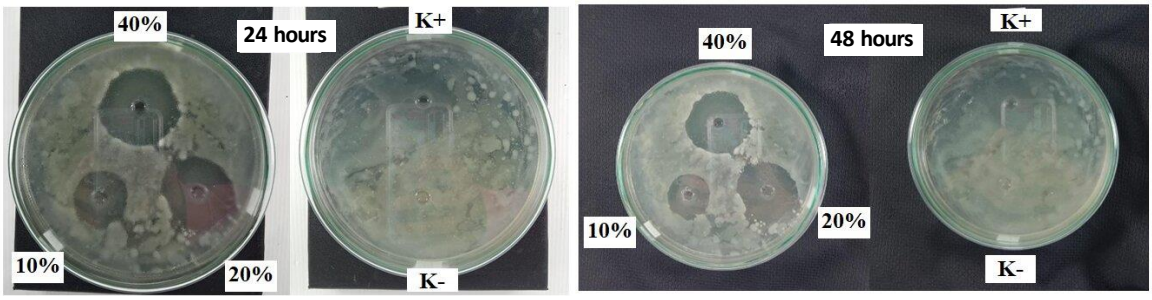

Figure 1. The results of the inhibition test of 10%, 20% and 40% hexadecanoic acid extract and control against *Xanthomonas campestris* after incubation for 24 and 48 hours.

Table 1. Average diameter of inhibition zone of hexadecanoic acid extract with concentrations of 10%, 20%, 40%, and control against *Xanthomonas campestris* bacteria after 24 hours and 48 hours incubation periods.

Concentrations	Average diameter of inhibition zone (mm) *Xanthomonas campestris*	
	24 hours	48 hours
40% extract	48,22	47,87
20% extract	43,35	43,15
10% extract	32,37	32,04
+ Control	37,4	34,3
- Control	0	0

Based on Table 1, the measurement results of the hexadecanoic acid extract with concentrations of 10%, 20%, and 40% showed a clear zone around the well in the *Xanthomonas campestris* bacterial culture. The administration of 10%, 20% and 40% hexadecanoic acid extract and positive control (Ciprofloxacin) in each well showed a clear zone which was the inhibition zone of each treatment. The measurement results above show a decrease in the diameter of the inhibition zone after observations were made at incubation times of 24 hours and 48 hours. This indicated that the hexadecanoic acid extract was bacteriostatic against *Xanthomonas campestris* bacteria. According to Sinurat, et al, (2019), Bacteriostatic means a substance can inhibit the growth of bacteria which is characterized decrease in the area of the inhibition zone which is directly proportional to the increase in the incubation period. When the administration of antimicrobial compounds is stopped, microbial growth will increase again because the compounds given cannot kill but only inhibit microbial growth.

It could be seen that the inhibition zone formed will have a larger diameter when given hexadecanoic acid extract with a higher concentration. According to Cappucino (1978), the difference in the size of the large or small diameter of the inhibition formed is influenced by the growth rate of the microbe, the sensitivity of the microbe to the active substance, the ability of the active ingredient to diffuse in the medium, and the viscosity of the medium used. The cause of the inhibition by antimicrobial substances is due to interference with cell membranes from microbes, inhibition of enzyme work, disruption of protein and nucleic acid synthesis, or inhibition of cell wall synthesis (Pelczar dan Chan, 1998). There is a reaction between the hydroxyl group of lipopolysaccharide which is a constituent of the cell wall with hexadecanoic acid, causing changes in the structure of the lipopolysaccharide membrane from the cell wall to being asymmetrical. This causes the cell

to become lysed or damaged due to disruption of the balance of the lipid membrane structure so that it will disrupt the integrity of the bacterial cell membrane (Sjafaraenan, et al, 2021).

Based on the observations, it can be seen that the 40% concentration has the largest inhibition area among the other concentrations used, this indicates that the hexadecanoic acid extract has antibacterial activity. Of the three concentrations, 10%, 20%, and 40% were categorized as having very strong inhibitory power because they showed an inhibitory power of more than 20 mm, which means they are classified as very strong (Davis dan Stout,1971 in Palupi dan Nugraha, 2014).

In this study, Ciprofloxacin was used as a positive control in the inhibition test of *Xanthomonas campestris* bacteria. Ciprofloxacin was used as a comparison of the effects of drugs, antimicrobials, standard with hexadecanoic acid extract test solution. Ciprofloxacin is a fluoroquinolone antibiotic that has the ability to inhibit bacterial DNA synthesis so that it becomes anti-microbial. Ciprofloxacin is also an antibacterial that is can against Gram-positive and Gram-negative bacteria, therefore Ciprofloxacin is often used as a treatment for several infections caused by bacteria (Castro, *et al*, 2013).

Ciprofloxacin was used as a positive control because Ciprofloxacin has an antibacterial against *Xanthomonas campestris*. The data obtained above shows a reduction in the area of the inhibition area which indicates that Ciprofloxacin is bacteriostatic against *Xanthomonas campestris* bacteria. Similar results were reported by Rojas, et al. (2019) which stated that Ciprofloxacin was bacteriostatic against *Xanthomonas campestris* which was characterized by a reduction in the area of the inhibition area.

Hexadecanoic Acid Activity Test Against *Fusarium oxysporum* Fungus

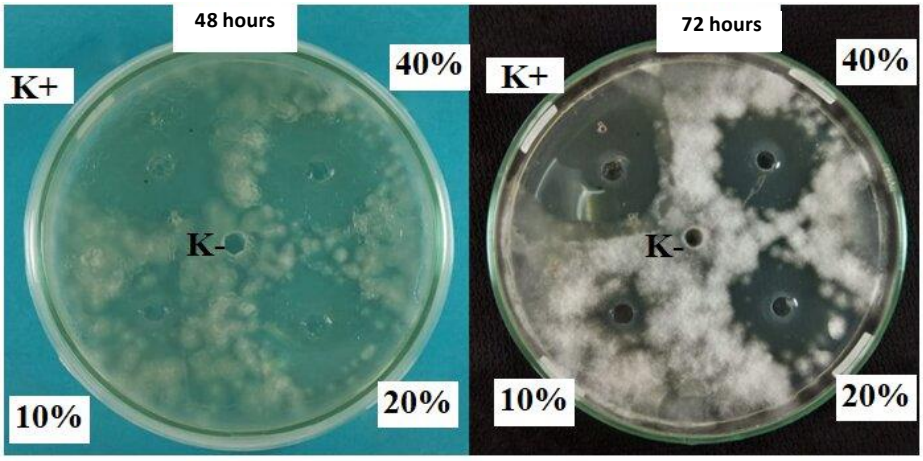

Figure 2. Resultsuof the inhibition test of hexadecanoic aciduextract at concentrationsuof 10%, 20% and 40% and control of the fungus *Fusarium oxysporum* after incubation for 48 and 72 hours.

Table 2. Average diameter of inhibition zone of hexadecanoic acid extract with concentrations of 10%, 20%, 40%, and control against *Fusarium oxysporum* fungus after 48 hours and 72 hours incubation periods.

Concentrations	Average diameter of inhibition zone (mm)	
	Fusarium oxysporum	
	48 hours	72 hours
40% extract	22,6	21,45
20% extract	19,25	18,4
10% extract	11,7	10,34
+ Control	35,4	34,65
- Control	0	0

Based on Table 2, it can be seen that there were clear zones formed in the 10%, 20%, 40% hexadecanoic acid extract and in the positive control (Ketoconazole). The hexadecanoic acid extract could inhibit the fungus *Fusarium oxysporum* which was seen from the inhibition zone that appeared around the well. , it can be seen that the inhibition area formed at a concentration of 40% had the largest area of the inhibition zone compared to other extract treatments. After observing the incubation time of 48 hours and 72 hours, there was a decrease in the area of inhibition because the hexadecanoic acid extract was fungistatic. According to Jana, et al (2020) in Sjafaraenan (2021), an antimicrobial substance is fungistatic if there is no increase in the area of the inhibition zone after incubation and the second observation is because the antimicrobial antimicrobial substances are not able to kill microbial growth.

Based this results above, the concentration of 40% has the largest inhibition among other concentrations. The area of the inhibition zone at a concentration of 40% is included in the very strong group because it has an inhibitory zone area of more than 20 mm. Meanwhile, at concentrations of 20% and 10%, it is included in the strong group because it has an area between 10-20 mm according to Davis and Stout (1971) in Palupi and Nugraha (2014).

Hexadecanoic acid has the capability to inhibit fungal accretionby forming complex compounds when it will bind to the active groups of fungal cell walls. Fungal cells have chitin compounds in their cell walls. Although there is a reaction between hexadecanoic acid and cell wall active groups, this reaction will only react with the outer ring structure (CO2-OH) and cannot damage the main structure of chitin in fungal cell walls. This causes the lack of this reaction affects the integrity of the fungal cell wall so that the hexadecanoic acid compound only inhibits or does not kill fungal cells. (Johannes, 2013).

In wells with positive control, Ketoconazole had an antifungal effect. The data obtained above showed a reduction in the inhibition area which indicated that Ketoconazole was fungistatic against the fungus *Fusarium oxysporum*. Ketoconazole was used as a positive control because Ketoconazole is a synthetic broad-spectrum antifungal drug that belongs to the imidazole group. Imidazoles and triazoles are from the azole group which are synthetic compounds. Ketoconazole was the first oral azole to be used clinically from several other drugs (Lely, et al, 2017).

In addition to the negative control, in this study aquadest was used as negative control which was also used as a solvent for the hexadecanoic acid extract. The purpose of using distilled water as a negative control is to prove that the solvent used does not affect

the antimicrobial test results of the compounds to be tested. Table 1 and table 2 state that distilled water did not show antibacterial and antifungal activity which was indicated by the absence of an inhibition area formed around the well. Thus it can be believed that the use of distilled water as a solvent does not affect the antimicrobial test results of the extract.

Conclusions

Extracts of hexadecanoic acid compounds have an effect on inhibiting bacteria and fungi that cause decay in mustard *Brassica juncea* L. based on inhibitory and bacteriostatic tests on *Xanthomonas campestris* and fungistatic on *Fusarium oxysporum*.

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