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# BIOACTIVITY TEST OF COMPOUND (Hexa-Tetra Contana) FROM SPONGE (*Callyspongia pseudoreticulata*) AS ANTIBACTERIAL OF WITHERED DISEASE ON POTATO PLANT (*Ralstonia solanacearum*)

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## ABSTRACT

Sponge is one of the marine organisms of coral reefs that is able to produce secondary metabolite compounds with varieties of bioactivity and structural variations; one of its biological activities is as an antimicrobial. This study is aiming to test the bioactivity of *hexa-tetracontane* compounds that have been isolated from sponge of *Callyspongia pseudoreticulata*. An antibacterial activity test was performed on the *Ralstonia solanacearum* bacteria found in potato by using agar diffusion method with the use of filter paper discs. The result of bioactivity of *hexa-tetracontane* compounds with varied concentrations; 400mg/mL, 500 mg/mL, 600mg/mL with incubation periode of 1 – 7 days, showing that the diameter of the inhibition zone is at the peak of the bacterial growth under the concentration of 400 mg/ml and incubation period of 7 days and the diameter of inhibition zone is *21,33* mm with great difference compared to the other concentrations.

**KEYWORDS:** ***Bioactivity, Hexa-tetra contane, Abtrivacterial, Callyspongia Pseudoreticulata, Ralstonia solanacearum.***

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## INTRODUCTION

*Callyspongia pseudoreticulata* is one of sponge that is easy to find in Indonesian ocean. Sponge is one of sea biota that contains many secondary metabolite compounds. The isolate of the sponge was reported has antimicrobial activity and antiparasite and some of its secondary metabolite compounds group successfully identified like terpenoid, steroid, and hydrocarbon.[1-2] There are not many researches about the exploration of secondary metabolite compounds of *Callyspongia pseudoreticulata*, especially the determination of the structure as the important step in developing the biogenetic pathway analysis as the guidance of compounds synthesis in industry. [3-6] Therefore, we need a further research in order to identify the secondary metabolite molecule structure and bioactivity characteristic that can be used in any sides, especially the prevention of the plant cultivation disorder that appears in sponge *Callyspongia pseudoreticulata* in order to obtain the optimum data related to the previous research. [7-8] That research is about the advantages and identifying the secondary metabolite compound group that exists in that sponge and also can be used for the food plant. One of the common disease that suffering the plant is bacteria *Ralstonia solanacearum*, mainly for the potato. Potato is an essential horticultural commodity in Indonesia that becomes an alternative food currently due to its potential as carbohydrate source with high protein. It encourages the food diversification program and also be as the industry raw materials. This plant is becoming one of the commodities that obtain the development priority because the need of potato tends to increase in line with the population escalation, income improvement, and the developing of food cause. That condition causes the expansion of potato farming, also the increasing of high-quality potato seeds demand.[9] Two sorts of products that show the tendency to be more popular in the society consumption pattern are french fries and potato chips. The main obstacle to potato production in the tropical country including Indonesia is the existence of any dangerous diseases. This disease inflicts the significant damage to the decreasing of the crops and the limitation of good quality potato seeds by the farmer. Bacterial withered disease by *Ralstonia solanacearum* is able to give huge disadvantages potentially because it tend to reduce the quality and the quantity or even eliminate the plant.[9] Generally, the plant that suffered the withered disease is those more than 6 weeks old. The plant become withered firstly that started to form the bud into the bottom part, so the mucous vessels will be seen The diseased potato tubers' color will change into brown and will also be smelly. One of the management methods is the good seeds choice, plant rotation, favorable water and air regulation around the plant. But the potent product that can restrain the disease has not found yet until today. Thus further research is very necessary to take, especially in order to know the *Callyspongia pseudoreticulata* sponge bioactivity toward the *Ralsitonia solanacearum* bacteria. One of the compounds that has been successfully isolated from *Callyspongia pseudoreticulata* sponge is *hexa-tetrcontane* as shown in Figure 1. The compound is solid white crystalline-shaped with melting point of 79-80℃ with high enough toxicity towards *Artemia salina* with $LC_{50}$ 61,5 µg/ml. [9]

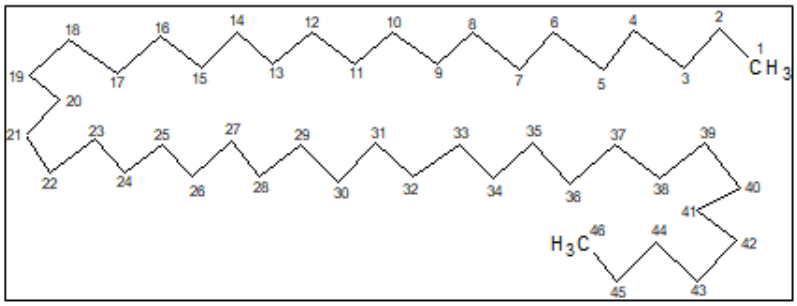


**Figure 1**
***Hexa-tetra contana compound structure.***

The purpose of the research is to know the *Hexa-tetracontane* concentration's influence towards the inhibitory effect of *Ralstonia solanacearum* grows for the potato plant.

## MATERIALS AND METHODS

***Media Making***
20 grams of Nutrient Agar (NA) medium powder and 1 liter of aquades was heated inside an Erlenmeyer flask until it's completely dissolved. After that, the cylindrical neck was covered with a gauze and was sterilized inside an autoclave with the temperature of 121 ℃ for 15 minutes.

***Culture of Microorganism Test***
Culture of *Ralsitonia solanacearum* that is used as bacterial test is collected from the Laboratory of Microbiology of Agriculture Faculty Hasanuddin University. The bacteria culture is rejuvenated by transferring bacteria with inoculation loop from the stock culture to the NA medium and is incubated at the temperature of 37 ℃ for 18 – 24 hours.

***Preparation of Sample Solution***
The concentrations of hexa-tetracontane sample solution that is used to test the activity of the antibacterial are 400 µg/ml, 500 µg/ml dan 600 µg/ml, which is made by weighing as much as 5 milligrams of hexa-tetracontane and dissolving it into 0.5 mL methanol.

***Bacterial Activivity Test***
The culture of *Ralsitonia solanacearum* was suspended

into sterile physiological saline, then each culture was dropped onto solid NA media surface in a sterile petri dish as they were diffused into the whole media, and was kept for 15 minutes. Having it done, 3 filter paper discs were dyed into sample solution of *hexa-tetracontane* for each concentrations; which are 400, 500, and 600 μg/ml, and another two discs were dyed into methanol (negative control) and tetracycline (positive control) simultaneously and was dried at room temperature for 10 minutes. The dried filter paper discs were put on the surface of the test media and were incubated at 37℃ for 24 hours. The antibacterial activity can be seen by measuring the diameter of the growth inhibition zone (clear zone) around the filter paper disc.

## RESULTS AND DISCUSSION

The result of antibacterial activity and the diameter measurement of inhibition zone of *hexa-tetracontane* with varied concentrations; 400 mg/ml, 500 mg/mL dan 600 mg/ml, with incubation period of 1 – 7 days, can be seen at Table 1.

**Table 1**
***Average of inhibition zone diameter of hexa-tetracontane towards the growth of Ralstonia solanacearum***

| Concentration (mg/mL) | Incubation period (per day) and average of inhibition zone diameter (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 400 | 9.66 | 11.33 | 13.33 | 15.33 | 17.33 | 19.33 | 21.33 |
| 500 | 13.33 | 11.33 | 13.66 | 15.66 | 18.88 | 20.33 | 21.00 |
| 600 | 7.66 | 9.00 | 10.33 | 11.66 | 13.33 | 14.33 | 15.33 |

***a- Diameter of inhibition zone (mm); b- 1 – 7 incubation period shown in days***

According to the result of bioactivity test of *hexa-tetracontane* as seen in Table 1, showing that at the concentration of 400 mg/ml and incubation period of 7 days, the hexa-tetracontane reached the highest inhibition zone diameter towards the bacterial growth of *Ralstonia solanacearum* for 21,33 mm. Whereas the lowest inhibitory capacity is at 600 mg/ml and incubation period of 1 day for 7,66 mm. If the data above is shifted into diagrams, these are the following profiles of the inhibition zones towards the growth of the used bacteria as the effect of addition of several concentrations of *hexa-tetracontane* (Figure 2).

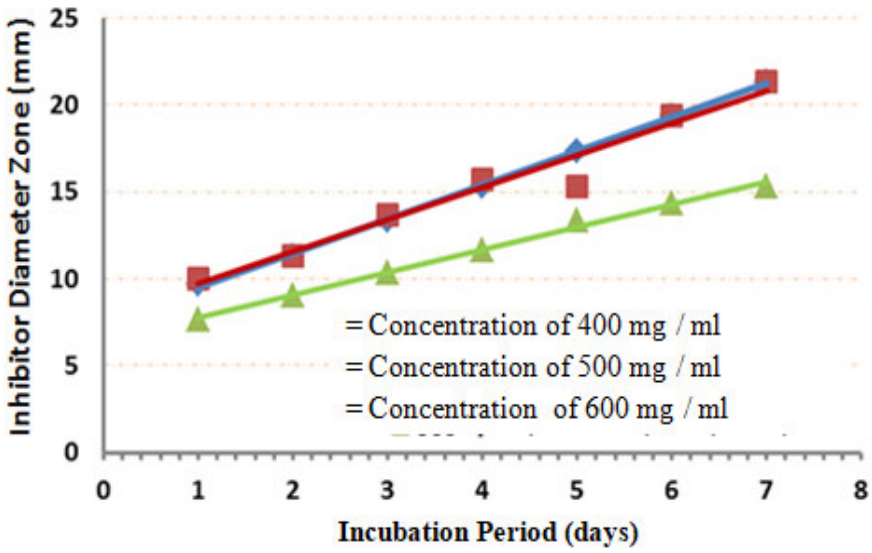


**Figure 2.**
***Diagram of the diameter of inhibition zone profile towards the growth of Ralstonia solanacearum bacteria***

The result of regression analysis in Figure 2 shows that the incubation time on day 1 until day 7 has a linier positive correlation with diameter of inhibition zone of the test bacteria. The longer the incubation period, the bigger the diameter of inhibition zone become and follow linier equations, $y = 1{,}9643x + 7{,}5238$; $R^2 = 0.9994$ for concentration 400 mg / ml, $y = 1.8452x + 7.8571$; $R^2 = 0.9604$ for concentration 500 mg / ml, $y = 1.3095x + 6.4286$; $R^2 = 0.9957$ for concentration 600 mg / ml. Based on the criteria of the capacity of an antibacterial activity, the hexa-tetracontane constituents are included in the strong category to inhibit the growth of *Ralstonia solanacearum* bacteria with inhibition zone diameter of > 20 mm. [12]. The positive result that is shown towards the bacteria is indicating that the compound has a wide spectrum for the bacteria itself. Generally the groups of gram-positive bacteria are more sensitive to compounds that have antimicrobial activity compared to the gram-

negative bacteria. This sensitivity difference of gram-positive and gram-negative bacteria is due to the fact that there is a difference between structures of each of the bacteria's cell walls. Positive-Gram bacteria have one layer cell wall structure containing peptidoglycan, a thin laments of teapixic acid and theuric acid. While Gram-negative bacteria have layer outside the cell wall containing 5 -10% of peptidoglycan, It also consists of proteins, lipopolysaccharides and lipoproteins. Gram-negative bacteria have two layers of lipid (lipid bilayer) called the lipopolysaccharide layer (LPS). In addition, the gram-negative bacteria's peptidoglycan walls are quite solid and compact and have the capability of "efflux-pump mechanism" by removing unnecessary compounds in the bacterial cellular biotransformation process, thus inhibiting the process of internalizing the compounds to affect the cellular mechanisms of bacteria. Therefore, antimicrobial substances are easier to penetrate into Gram-positive bacterial cells than Gram negative bacteria. [13-14]

## CONCLUSION

According to the research result of *Callyspongia pseudoreticulata*, we can conclude that *Hexa-tetra contana* give diameter of inhibition zone to the bacteria *Ralstonia solanacearum* in potato plant. The compound with concentration 400 mg/ml show the best diameter of inhibition zone, 21,33 mm, and significantly different with other concentration.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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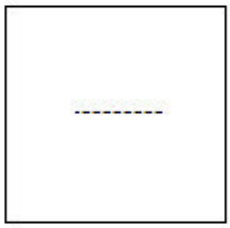


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