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EFFECT OF CYNAMMYLDEHYDE FROM CINNAMON EXTRACT AS A NATURAL PRESERVATIVE ALTERNATIVE TO THE GROWTH OF *Staphylococcus aureus* BACTERIA

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ABSTRACT

Food is one of the best media for the microorganism to live and grow. Therefore, food is often broken because it has been contaminated by the microorganism. In industry country, approximately 30% of population infected by food borne disease. Food borne disease is caused of phatogen bacteria food borne. *Staphylococcus aureus* is a kind of bacteria that can make food rotten and also it is a phatogen bacteria cause food born disease, no forming spora, positive gram bacteria and the food substance which is contaminated by *Staphylococcus aureus* will cause poisoned because of enterotoxin which is heat resisting. Essential oil is antimicrobial and anti bacterial that the most effective, it can inhibit the growing of microba and bacteria. One of the example of essential oil is Cinnamon.sp oil. Cinnamon oil is antimicroba agent for bacteri and fungi because it contain cynammyldehyde and cynammyl alcohol and also eugenol. The aim of this study is to understand the antimicrobacterial potential of cynammyldehyde from cinnamon extract to *Staphylococcus aureus*. This study is laboratory experimental research. Essential oil from Cinnamon by destilation, then redistilation was done to get cynammyldehyde from cinnamon. Cynammyldehyde was tested to *Staphylococcus aureus*. Test method was done as dilution in the form. From this result, it show that cynammyldehede from cinnamon extract has ability in inhibit the *Staphylococcus aureus* growth. We can conclude that Cynammaldehyde from cinnamon extract has antibacterial effect especially for positive gram bacteria that is *Staphylococcus aureus*. The optimum inhibiting effort is 0.09%.

Key words: Cinnamon, Cynammyldehyde, Antibacterial, *Staphylococcus aureus*

INTRODUCTION

Food is one of the medium for bacteria growth so it can break due to microorganism contamination. Microorganism can breaks components in the food into simpler compounds. It will changes, decomposition both nutrition and organoleptic.¹

More than two million people dead because of *food borne disease*. *Food borne disease* caused by pathogenic bacteria of *food borne*. So, it need aan alternative method which eliminate pathogenic bacteria of *food borne disease*.¹ *Staphylococcus aureus* is the kind of decaying food bacteria which pathogenic bacteria of *food borne disease*, not producing spore, Gram positive bacteria and contaminant from it can be toxic because of enterotoksin.²

Preservation food is one of the ways to prevent food which contaminated. One of the kind of preservation food

is using synthetic materials likes boraks.³ Boraks is used by people but it has toxicity which danger if consume for along day. Recently, formalin and boraks are agent which have high reactivity so they can reacts with macromolekul on body system. Consuming formalin continuously can effet cancer. Preservation substances which can use is antimicroba and antibacterial substances.^{4,5}

Essential oil is the effective antimicroba and antibacterial which can inhibit bacteri and microba growth. One of the kind of essential oil is cinnamon oil. Cinnamon oil is antimicroba to bacteria and fungi,⁶ because they have cynammyldehyde, cynammyl alcohol and eugenol,⁷ so cinnamon oil can inhibit pathogenic food borne bacteria growth.⁸ In industrial country find about 30% population suspect food borne disease. So it need a new method to decrease and eliminate pathogenic bacteria cause of food borne disease.¹

Laboratory experiment needed to determine the concentration of cynammyldehyde can optimally inhibit *Staphylococcus aureus* bacteria growth. The researches want cynammyldehyde of cinnamon extract can use as antibacterial to keep food quality and it can realize to society. Natural preservation of cynammyldehyde is safe to consume if in appropriate dose.

METHOD AND MATERIALS

Materials

This experiment is laboratory experimental to prove the ability antibacterial cynammyldehyde of cinnamon extract to standard laboratory bacteria such as *Staphylococcus aureus*. Using laboratory tools such as micropipette, petridisc, test tube, test tube rack, spectrophotometer, incubator, brender, and standard oase. The materials are Brain Hearth Infusion, Muller Hinton, aquades steril, Sinamat aldehyd, and DMSO.

Bacterial Test

Bacterial test is standard bacteri which sensitive to standard therapy. Bacteria found in microbiologi laboratory Medicine Faculty Airlangga University.

Producing Extract

The material is cynammyldehyde of cinnamon extract. Firstly, determine cinnamon which has thickness about 1,5 mm, long about 1 m and good smell if it broken. After that, wash and dry to produce extract. Producing extract in Research Institute for Industrial Research and Standards Surabaya. Do steam destillation process to get essential oil from cinamon. The cinnamon size is reduced about ± 2 cm by 5 kg, and cinnamon was processed with a tool distiller so it can result essential oil about 5 ml. Next Essential Oil is the next process is Redestilation Oil Bath Process to separate the content of eugenol and cynammyldehyde contained in the essential oil. Bath Oil Redestilation Process is performed to obtain names of cynammyldehyde of 3 ml. The oil lab tested to know the size of the content of cynammyldehyde. In the oil we found water content of 0.03%, cinnamic names of aldehydes 72.86% 18.78% and eugenol. This will be the basis of dilution of cynammyldehyde, which will be tested to *Staphylococcus aureus*.

Preparation of Bacteria Test

Bacteria prepared by creating suspense in accordance with the methods of microbiology laboratory. Bacteria grown in BHI liquid medium, then turbidity adjusted to Mc Farland turbidity standard $0.5 (1 \times 10^8 \text{ CFU / ml})$ and then diluted to concentrations of bacteria $1 \times 10^6 \text{ CFU/ml}$.

Dilution Test Materials

Then performed a serial dilution: 0.18%, 0.14%, 0.10%, 0.06%, 0.02% and then added bacterial suspension with an equal volume of 1 ml so that the concentration is

half that of the original, which is 0.09%, 0.07%, 0.05%, 0.03 %, and 0.01%.

Determining the Activity Test Solution

Concentrations that have been given the suspense of bacteria were incubated for 24 hours at 37°C. Furthermore, all media were incubated for 24 hours grown on Muller Hinton for 24 hours at a 37° C to determine the number of colonies that are still growing. And media that have been incubated the absorbance values read using a spectrophotometer at a wavelength of 600nm to determine the percentage of inhibition, respectively - each concentration. Using the formula:⁹

$$\% \text{ inhibition} = [(abs \text{ control} - abs \text{ sample}) / abs \text{ control}] \times 100\%$$

Then count of the colony. Each bacterial test was done 5 times. The independent variables in this study were from the names of cynammyldehyde from cinnamon extract that had been serially diluted in several concentrations. Meanwhile, as the dependent variable is the presence of bacterial growth. Data analysis was performed by descriptive statistical One Way ANOVA after the data obtained from 5 times repetition of the *Staphylococcus aureus* bacteria (gram positive).

RESULTS AND DISCUSSION

Results and Characterization of Materials

Experiments with 5 times the repetition in the concentration of 0.01%, 0.03%, 0.05%, 0.07%, 0.09% obtained by the addition of 0.18%, 0.14%, 0.10% 0.06% 0.02% with each of the levels provided and 1 ml of 1×10^6 of *Staphylococcus aureus* bacteria cfu/ml. After incubated for 24 hours and counting the number of bacteria with the spectrophotometer giving the following results.

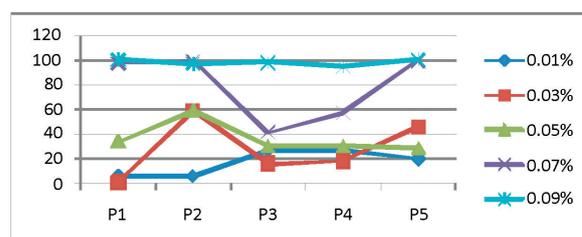


Figure 1. Graph of percentage inhibition

Then, to know the number of bacteria that live at each concentration in every experiment performed with bacterial cultures growing on Muller Hinton solid medium. One plate media in each experiment were divided into 4 sections, and each part drops 50 μl droplets of liquid medium with concentration of 0.01%, 0.03%, 0.05%, 0.07%, and 0.09%. After planting, each plate were incubated in an incubator for

24 hours to determine colony growth on each plate section. These calculations show the following results.

Table 1. Colony count results

Concentration of cynammyldehyde	Number of Research				
	1	2	3	4	5
Control +	∞	∞	∞	∞	∞
0.01 %	∞	∞	∞	∞	∞
0.03 %	∞	∞	∞	∞	∞
0.05 %	∞	∞	∞	∞	∞
0.07 %	6	15	8	18	27
0.09 %	4	8	5	10	0

Statistical analysis using one-way annova produces data that has been attached. In descriptive tests 0.01% concentration, the average value is 17.04%, the minimum value is 5.2%, and 27% for the maximum value. For concentration of 0.03%, the average value is 28.12%, 1.7% is the minimum value and the maximum value is 58.8%. For concentration of 0.05%, the average value is 36.56%, 29% is the minimum value, and the maximum value is 59%. For concentration of 0.07%, the average value is 78.8%, 41% is the minimum value, and the maximum value is 99.4%. For concentrations of 0.09% has an average rating of 98.0%, 95% is the minimum value, and the maximum value is 100%. It can be concluded that the highest inhibition at a concentration of 0 : 09% and the lowest at 0.01% concentration.

In the test for homogeneity of variances obtained value of significance of the 0.00 ($0.00 < 0.05$ (α)). This results indicate that there are differences of the variance of the inhibition for each concentration. In Annova test showed that the value of F test is 18.945 and P value is $0.00 < 0.05$ (α), it shows that H1 is accepted which means that there is an average difference of inhibition for each concentration. In the post hoc test, it prove that there is a difference between the concentration of 0.01% with concentration of 0.07%, and 0.09%. For the concentration of 0.03%, there is a difference with the concentration of 0.07%, and 0.09%. At a concentration of 0.05%, there is a difference with the concentration of 0.09%.

The activity of Cynammyldehyde against *Staphylococcus aureus*

The results showed that cynammyldehyde from extracts of cinnamon can inhibit the growth of *Staphylococcus aureus*. This would have been due to a chemical compound as cynammyldehyde, eugenol, and alcohol in the extract of cynammon, especially the compound of cynammyldehyde. That compounds as the active ingredient, which can inhibit growth of *Staphylococcus aureus*. It inhibited the growth of bacteria or bacterial death by an antibacterial agent can be caused by inhibition of the synthesis of cell walls, the inhibition of the cell membrane

function, inhibition of protein synthesis, or inhibition of the synthesis of nucleic acids.¹⁰

Cynammyldehyde from cinnamon extract has the potential to inhibit cell wall synthesis. This is based on the content of cynammyldehyde that is aldehyde compounds.¹¹ Potential cynammyldehyde from cinnamon extract inhibits *Staphylococcus aureus* by cell wall protein agglomerate, so that the cell wall can not functionate anymore. *Staphylococcus aureus* is a gram-positive bacteria. The cell wall of Gram-positive bacteria consist of a very thick peptidoglycan that provides rigidity to maintain the integrity of the cell. Bacterial cell wall assembly process begins with the formation of peptide chains that will form the cross bridge peptide chains that incorporate glican chains from peptidoglycan to the another chain leading to complete cell wall assembly. If there is damage to the cell walls or any obstacles in its formation can occur in bacterial cell lytic which makes the bacteria lost the ability to form colonies, and it will cause bacterial cell death.

In *Staphylococcus aureus*, the delivery of antimicrobial can inhibit cell wall assembly and cause generate merger glican chain is not connected to cross the cell wall peptidoglycan, being weak structures and cause death of bacteria. Any compound that blocks any step in the synthesis of peptidoglycan will cause bacterial cell wall is weakened and cell lysis.¹⁰ Bacterial cell lysis does not work anymore because the cell wall that maintains shape and protects the bacteria that have a high osmotic pressure. *Staphylococcus aureus* is a gram-positive bacteria that have an osmotic pressure in 3–5 times larger than gram-negative bacteria, making them more susceptible to lysis.¹⁰ Without a cell wall, bacteria can not survive against outside influence and soon die.¹²

Therefore, the lysis of bacteria suspected of interference or inhibition of cell wall Assembly and lysis of the cell wall can explain the bacteriostatic effect of cynammyldehyde of extract of cinnamon. The use of the concentration of cynammyldehyde of different extracts of cinnamon to give different levels of influence in the growth of *Staphylococcus aureus*. At a concentration of 0.07% and 0.09% there are colonies of bacteria which grow, but less in number in comparison with the cultivated in a concentration of 0.01%, 0.03%, 0.05% and the positive control group. Bacterial growth was really inhibited at the concentrations of extract of 0.07% and 0.09%. All indicated that higher concentrations of extract of cinnamon the growth of the bacteria *Staphylococcus aureus* increasingly hampered because the active ingredient in the test solution.

Therefore, this study found that treatment with the potential to inhibit the growth of the bacteria *Staphylococcus aureus* is the initial concentration of 0.07%. In other words, the lowest concentration to inhibit the total growth of *Staphylococcus aureus* is a 0.07%, and the optimal concentrations have the potential to inhibit the growth of the bacteria *Staphylococcus aureus* is 0.09%.

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