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EFFECTIVENESS OF BINTARO (*Cerberra odollam* Gaertn.) LEAF ETHANOLIC EXTRACT AGAINST *Staphylococcus aureus* IN-VITRO BIOFILM FORMATION

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INTRODUCTION

Biofilm is a structural form of a group of microorganisms protected by matrik extracellular called *Extracellular Polymeric Substance* (EPS), where EPS is a product produced by these microorganisms itself and as a shelter from the bad influence in environment. The structure of these matrix is strings which cross each other which can be adhesive for biofilm[4)].

Biofilm from *Staphylococcus aureus* seen in chronicle infection cases as ulcer on diabetics foot, *venous statis ulcer* and *pressure sores*. Patients with infection wound with chronicle ulcer in vein feet found positive culture of *Staphylococcus aureus* as much as 88-93,3% from a number of infection found.

Figure 1. *Cerberra odollam* Gaertn.

Bintaro can be used as an analgesic, anticonvulsant, kardiotonik and antihypertensive [2)].

The aim of this study is to test the effectiveness of the ethanol extracts of bintaro leaves (*Cerberra odollam* Gaertn.) towards the *Staphylococcus aureus* formation by in vitro.

MATERIALS

Bintaro leaves (*Cerberra odollam* Gaertn.), *Staphylococcus aureus* culture ATCC 6538, petri dish, ose wire , micro pipet, autoclave (All American Model No 25x, USA), incubator (Memmert and Binder, Germany), *Laminar Air Flow* (LAF) (Type V-130, Indonesia), *microplate*, *microplate reader* (Thermofisher Scientific, America), *vacuum rotary evaporator* (BÜCHI, Germany), weight analytic (Sartorius TE 214 S, Germany), oven (Memmert, Germany).

METHOD

Antibacterial Activity Test on Ethanol Extract of Bintaro Leaves (*Cerberra odollam* Gaertn.) by Well Difussion Method

The total of bacteria suspension 0,15 mL *Staphylococcus aureus* with the number of $1,5x10^8$ CFU/ml (0,5 *McFarland* I bacteria pouring into sterilized petri dish. Then 15 ml media MHA (*Mueller Hinton Agar*) at 50°C temperature then added to the petri dish and shake twistedly so the bacteria suspension of *Staphylococcus aureus* mixed well and allowed to condense. And then do the incubation for 1,5-2 hour by put it in incubator with 37°C temperature. After pra incubation is done, made the media well by using perforator ± 6 mm diameter with the same hole range.

20µL ethanol extract of bintaro leaves (*Cerberra odollam* Gaertn.) various concentration, the positive antibiotic control solution of tetracyclin HCl and negative control of DMSO put into sumuran hole ± 6 mm diameter using micropipet and incubated in incubator for 24 hours with 37°C temperature. After the incubation period, then count the amount of zone of inhibition (ZI) by using calipers. The testing has done three times replication. Zone of inhibition (ZI) shows the amount of the antibacterial activities from that compounds test (Brooks, Butel dan Morse, 2007).

The Antibiofilm Effect Testing on Ethanol Extract of Bintaro Leaves Towards *Staphylococcus aureus* Biofilm Formation

The inhibition of biofilm formation tested by *in vitro* using *Microtiter Plate Biofilm Assay* method[5)]. 100 µL solution test from each concentration, 100 µL TSB media, 10 µL bacteria

suspension put into *microplate,* closed and incubated for 24 hours with 37°C temperature. After the incubation process, the contents of *microplate* were taken and washed with water. *Microplate* was given 200 µL of 1% crystal violet solution then incubated for 15 minutes at room temperature. Then the dye washed with clean water and left dry at room temperature. After *microplate* dry, 200 µL ethanol 96% put into *microplate* and incubated for 15 minutes at room temperature. *Microplate* measured by using *microplate reader* in 595 nm optical density (Bjarnsholt *et al*, 2011).

The results are calculated %inhibition biofilm[6].

$$\% \text{ Inhibition biofilm formation} = \left\{ 100\% - \frac{\text{OD extract} - \text{OD control extract}}{\text{OD blanko (+)} - \text{OD blanko (-)}} \times 100\% \right\}$$

Information:

- OD : Optical density
- OD extract : extract + media TSB + *S.aureus*
- OD control extract :extract + media TSB
- OD blanko negatif : media TSB
- OD blanko positif :media TSB + *S. aureus*

Data Analysis

Data which collected from antibacterial activity test and *Staphylococcus aureus* antibiofilm are quantitative data in the form of zone of inhibition (ZI) and optical density. The results of the testing data of antibacterial activity is ZI. The highest ZI shows the most active antibacterial activity. The results of the testing data of antibiofilm activity is % biofilm inhibition where the greater % biofilm inhibition means the greater its antibiofilm activity.

RESULT AND DISCUSSION

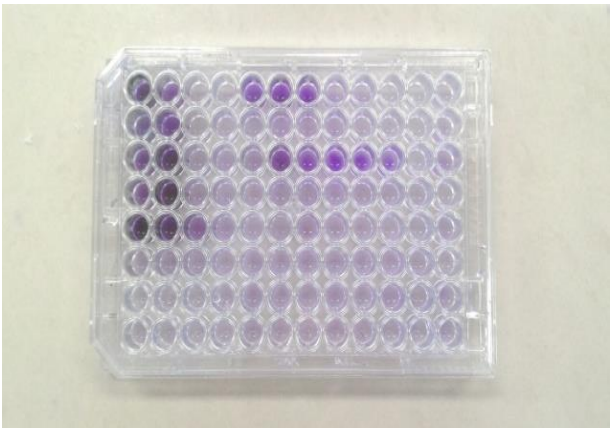
Result

Table 1. Antibacterial Activity Test Result by Well Diffusion Method

Extract Concentration (ppm)	Zone of Inhibition (ZI)
100000	18.76 mm
200000	28.56 mm
300000	28.29 mm

Table 2. Antibiofilm Activity Test Results

Concentration (ppm)	% Inhibition biofilm formation
75000	80,82431398
37500	71,93615393
18750	87,29638133
9375	91,56007434
4687,5	93,48420247
2343,75	92,62053132
1171,875	87,5259648
585,9375	73,0403411
292,96875	90,69640319



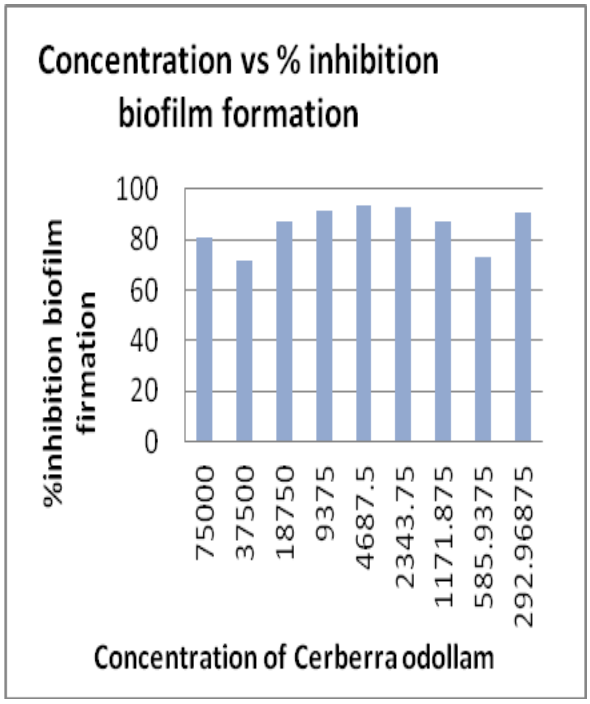


Figure 3. Graphic of Microtitre plate assay of biofilm for Bintaro leaf ethanolic extract

Table 3. The Extract Standardization

Parameters	Test Result
Organoleptic	Colour : Green dark Smell : Spesific Texture : Smooth
Phytochemical Screening	Flavonoid (+) Saponin (+) Alkaloid (-) Tannin (+) Quinone (-) Sterol or terpenes (-)
Total Ashes Contents	3.39%
Acid Insoluble Ashes Contents	0.67%
Water Contents	9.96%

DISCUSSION

Ethanol extract of bintaro leaves standardized nonspecifically with water content test, total ashes content test, and acid insoluble ashes content test. The water content in ethanol extract of bintaro leaves test result is 9.96 %. The total ashes content is 3.39%. While acid insoluble contents on extract is 0,67%.

The determination of antibacterial power in ethanol extract of bintaro leaves using well difussion method. This method is the most appropriate difussion method to test antimicrobial substances wether its homogeneous suspension and is non homogeneous due to the presence of suspended particulate in the antimicrobial substances which do not interfere with antimicrobial substances diffusion in the media. The diffusion test result can be seen from its Zone of Inhibition (ZI) which is generated from 300000 ppm, 200000 ppm and 100000 extract concentration which each ZI is 18.76 mm, 28.56 mm dan 28.29 mm. Meanwhile ZI from Tetracyclin HCl 500 ppm comparison antibiotic is 21.64 mm. This means ethanol extract of bintaro leaves have the antibacterial activity.

In Vitro Biofilm Culture with Static Microtiter Plate Assays Method

This method designed to measures the microbial ability that they may adhere to the abiotic surface within incubation about 1-2 hours. As an early attachment initiation to the surface which already can be assessed, while for incubation periods longer than 20 hours makes it possible to measure the formation of biofilm (Bjarnsholt *et al*, 2011). The obtained result is % of biofilm resistance. The results of the testing data of antibiofilm activity is % biofilm resistance where the greater % of biofilm resistance means the greater its antibiofilm activity. The result of % biofilm resistance is 4687.5 ppm.

CONCLUSION

1. Ethanol extract of bintaro leaves contained secondary metabolite category which are flavonoid, saponin, tannin.
2. Ethanol extract of bintaro leaves have antibacterial activity
3. Ethanol extract with 4687.5 ppm concentration have the greatest antibiofilm activity.

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