

# ANTIBACTERIAL AND ANTIBIOFILM POTENTIAL OF ETHANOLIC EXTRACT FROM BINTARO FLOWER (*Cerbera odollam*) AGAINST *Staphylococcus aureus* ATCC 6538

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## INTRODUCTION

*Staphylococcus aureus* are examples of Gram-positive bacteria that can cause infection. Treatment of infection with antibiotic synthesis can bring its own problems, namely the resistance of bacteria to antibiotics. Resistant bacteria is a bacterial pathogen that is capable of forming biofilms on living beings. Traditional medicine can be another alternative in the treatment of infections. Natural compounds that have the potential as an antibacterial commonly contain steroids, tannins, polyphenols, flavonoids<sup>1</sup>, alkaloids, saponins<sup>2</sup>.

Some studies related to the use as an antibacterial Bintaro explained that the mangrove plant that belongs to the family *Apocynaceae* is grown widely in the southern coast of East Asia and the Indian Ocean has antibacterial activity<sup>3</sup>.

This study was conducted to determine the antibacterial activity and antibiofilm of ethanolic extract from *Cerbera odollam* flower against *Staphylococcus aureus*.



Figure 1. *Cerbera odollam* flower

## RESEARCH METHODS

### Tools and materials

Micropipette, microplate reader (ThermoFisher Scientific, USA), UV light, Chambers, Laminar Air Flow (LAF) (Type V-130, Indonesia), incubator (Mettler and Binder, Germany), oven (Mettler, Germany), autoclave (All America Model 25x, USA), microscope (Olympus, Germany), vortex (Labinco, Netherlands)

### Material

The plant material used in the study is the flower of the plant bintaro (*Cerbera odollam*) obtained from the area of West Surabaya (East Java), 96% ethanol, DMSO (Merck Schuchardt, Germany), TSB / Trypticase Soy Broth (Merck, Germany), microplate, distilled water, the wells, a solution of 1% crystal violet.

### Antibacterial Activity Test by Well Diffusion Method

A 1.5 ml Suspension of *Staphylococcus aureus* (~  $1.5 \times 10^8$  CFU / ml) was inoculated in 15 mL media MHA 50 ° C. Pre-incubation carried out for 1.5-2 hours at 37 ° C. The concentration of 10%; 20% and 30% flower extract as much as 100 µL bintaro put in wells and the comparison of 100 µL were put into wells used as a positive control. Media incubated for 24 h at 37 ° C. Diameter growth inhibition were measured with calipers.

### Biofilm Formation Inhibition Activity Test

U-bottom microplate 96 wells each filled with extracts (with the highest concentration of 30% and dilution rows) of 100 µL, the suspension of *Staphylococcus aureus* (~  $1.5 \times 10^8$  CFU / mL) as much as 20 µL in each of the wells, and 100 µL Tryptic Soy Broth. As a positive control comparator used the comparison of 100 µL. Incubated for 48 h at 37 ° C (in humid conditions before entering the incubator). After incubation, the contents of the wells was removed and rinsed 3 times with running water and dry in an incubator at 37 ° C for 15 minutes. Each of these wells are given a 1% crystal violet 200 µL for 15 minutes at room temperature which is useful for coloring biofilm formed. Microplate rinsed with running water 2 times. Each of these wells are given a 96% ethanol 200 µL for 15 minutes which is used to dissolve biofilm formed rings. Microplate reader and dishake entered for 30 seconds, as well as analysis on  $\lambda$  595 nm. The results are calculated % inhibition<sup>4</sup>.

% Inhibition biofilm formation =

$$\left\{ \frac{100\% - \frac{OD \text{ extract} - OD \text{ control extract}}{OD \text{ blanko (+)} - OD \text{ blanko (-)}}}{x100\%} \right\}$$

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

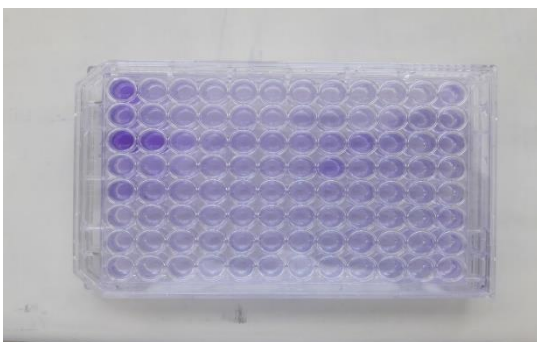
Data which collected from antibacterial activity test and *Staphylococcus aureus* antibiofilm are quantitative data in the form of zone of inhibition (ZI) and optical density. The results of the testing data of antibacterial activity is ZI. The highest ZI shows the most active antibacterial activity.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

RESULTS AND DISCUSSION

**Table 1. Test the antibacterial activity of ethanol extracts of flowers bintaro against *Staphylococcus aureus* with a diffusion method.**

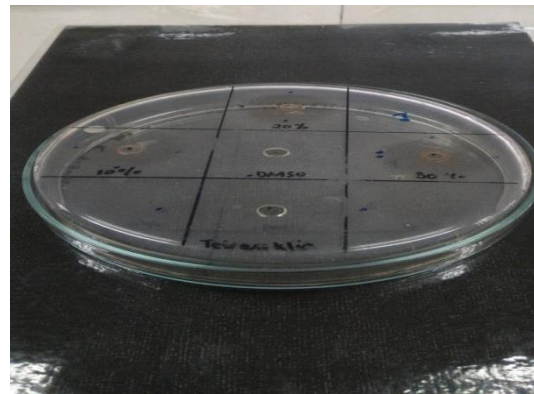
No	Concentration (% b/v)	Zone of Inhibition (ZI) (mm)
1	10 %	30,53 mm
2	20 %	32.36 mm
3	30 %	33.68 mm

Antibacterial activity of ethanol extracts of bintaro flower using well diffusion method. This method is a diffusion method most appropriate for testing substances for their antimicrobial suspended particulates in the antimicrobial agent that does not interfere with the antimicrobial agent diffusion in the media. Result of antibacterial activity test showed ZI values at concentrations of 10% already have antibacterial activity of 30.53 mm and



**Figure 3. Microtitre plate assay of biofilm for Bintaro flower ethanolic extract**

increase in concentration also increased the zone of inhibition *Staphylococcus aureus* (Table 1).



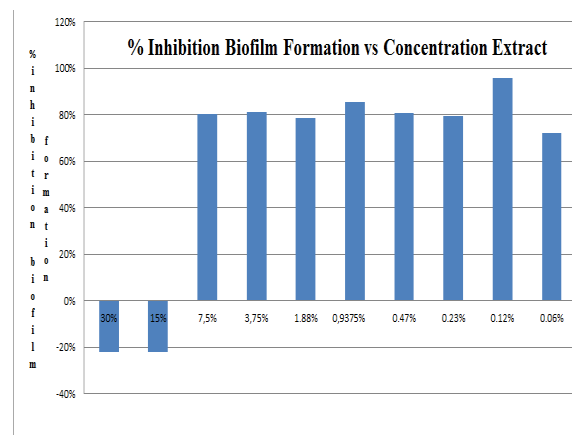
**Figure 2. Zone of inhibition *Staphylococcus aureus* by well diffusion for Antibacterial activity test**

Biofilm Formation Inhibition Activity Test

The results obtained show the greatest biofilm formation inhibition activity at concentration of 0.12% by inhibiting biofilm of 95.694% (Table 2).

**Table 2. Test antibiofilm activity of ethanolic extracts of flowers bintaro against *Staphylococcus aureus*.**

No	Concentration (%)	% Inhibition biofilm formation
1	30 %	-21.91 %
2	15 %	-21,94 %
3	7.5 %	80.234 %
4	3.75 %	81.24 %
5	1.88 %	78.467 %
6	0.9375 %	85.584 %
7	0.4687 %	80.633 %
8	0.23 %	79.682 %
9	0.12 %	95.694 %
10	0.06 %	72.1 %



**Figure 4. % Inhibition biofilm formation vs Concentration extract**

CONCLUSION

The ethanol extract of flowers bintaro have antibacterial activity against *Staphylococcus aureus* is at a concentration of 10% already have a zone of inhibition of 30.53 mm. And ethanol extracts of

flowers bintaro also have antibiofilm activity with the highest percentage of 95.694% in inhibiting the growth of biofilm.

#### REFERENCES

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