

## ANTIBACTERIAL EFFECT OF CURCUMINOID SILICA NANOPARTICLE TO ESCHERICHIA COLI BACTERIA

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### ABSTRACT

**Background:** Escherichia coli is one of the main bacteria that cause urinary tract infection. In the past few years, Escherichia coli's susceptibility to antibiotic has been decreased and causing antibiotic resistance. Furthermore, Curcuma longa plant has been used daily by the population as a cooking spice, and evidently has an antibacterial effect on some bacteria such as Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli. Curcumin as the active ingredients of Curcuma longa has a low water solubility and poor absorption so that silica nanoparticle usage will help the solubility and absorption problem of Curcumin.

**Aim:** This study aimed to know the antibacterial effect of Curcuminoid silica nanoparticles to Escherichia coli.

**Methods:** This research is an in vitro experimental study and checking by using a microdilution method and observed with a spectrophotometer.

**Results:** There is 60,6% inhibition obtained at 1000 µg/mL. MIC and MBC were not obtained at 62,5 µg/mL to 32000 µg/mL.

**Conclusion:** Curcuminoid silica nanoparticles has no antibacterial effect against Escherichia coli.

**Keywords:** Curcuminoid, Silica nanoparticles, antibacterial, Escherichia coli

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## INTRODUCTION

*Escherichia coli* becomes the main bacteria that causing either upper or lower urinary tract infection.<sup>(1)</sup> *Escherichia coli* is a normal flora from the intestine and can be pathogenic when the host cell changed or exposed to another area of the body.<sup>(2,3)</sup> In the last few years, *Escherichia coli* simultaneously decreased its susceptibility to Cefotaxime and Ciprofloxacin from 62,5% in 2006 to 48,7% in 2012.<sup>(4)</sup> *Escherichia coli* is increasing in resistance case to Ciprofloxacin from 0,5% to 15,3%, Trimethoprim increasing from 13,3% to 46%, and Nitrofurantoin from 0% to 5,6% from 2008 to 2014 and resistance to Ampicillin and Trimethoprim-Sulfamethoxazole.<sup>(5,6)</sup> *Escherichia coli* caused the greatest susceptibility decreasing to Ciprofloxacin comparing to another antibiotics.<sup>(7)</sup>

*Curcuma longa* is one of *Zingiberaceae* family that can be used for medicine. *Curcuma longa* grows in the subtropical area to the tropical area.<sup>(8)</sup> Besides used for cooking spices, *Curcuma longa* also used for traditional medicine in Asian countries like India, Bangladesh, and Pakistan.<sup>(9)</sup> *Curcuma longa* benefits for pulmonary diseases, gastrointestinal diseases, and integument diseases.<sup>(10)</sup> A study reports that *Curcuma longa* extract has *Curcumin* as the active ingredient that

has an antibacterial effect against *Staphylococcus aureus*, anti-inflammation, anti-cancer, and antioxidant, but *Curcumin* is a non-water-soluble, has low absorbance, rapid metabolism, and rapid systemic elimination.<sup>(11,12)</sup>

Nanoparticle as drug delivery is a formulation of particle that disperses in nanometer size; study reports the effective size is under 100 nm.<sup>(13)</sup> Another study reports *Curcuma longa* nanoparticle extract has solubility 1936 times bigger than *Curcuma longa* extract only.<sup>(14)</sup> *Curcuma longa* extracts active ingredient like *Curcumin* has more benefits if used with nanoparticle.<sup>(14)</sup> The study aimed to know the effect of *Curcumin* that can be used to treat *Escherichia coli* infection. The researcher wanted to know the MIC and MBC of *Curcuminoid* Silica Nanoparticle to *Escherichia coli*.

## METHODS

This study is an experimental study with a non-equivalent control group design. This study held at *Balai Besar Laboratorium Kesehatan Surabaya*, Microbiology laboratory facility of Medical Faculty Widya Mandala Catholic University Surabaya, and Research laboratory facility of Pharmacy Faculty Widya Mandala Catholic University Surabaya. The sample that used in this

study is *Escherichia coli* ATCC 25922 from *Balai Besar Laboratorium Kesehatan Surabaya*. Research is divided into two parts. In the first part, a concentration that has been used is 62,5 – 1000 µg/mL and used four control groups:

K1 = Mueller Hinton Broth.

K2<sub>a-e</sub> = Mueller Hinton Broth + *Curcuminoid Silica Nanoparticle* 62,5 – 1000 µg/mL

K3 = Mueller Hinton Broth + *Escherichia coli* + tween 20

K4 = Mueller Hinton Broth + *Escherichia coli* + Ciprofloxacin

In the second part, concentration that has been used is 2000 – 32000 µg/mL with the same control groups. This study used two intervention groups:

P0 = Mueller Hinton Broth + *Escherichia coli*

P1-P5 = Mueller Hinton Broth + *Escherichia coli* + *Curcuminoid Silica Nanoparticle* 62,5 – 1000 µg/mL

Second part is used the same intervention groups but with different concentration:

P0 = Mueller Hinton Broth + *Escherichia coli*

P1-P5 = Mueller Hinton Broth + *Escherichia coli* + *Curcuminoid Silica Nanoparticle* 2000 - 32000 µg/mL.

	1	2	3	4	5	6	7	8	9	10	11	12
A	P <sub>0</sub>		P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>		P <sub>5</sub>	
B	P <sub>0</sub>		P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>		P <sub>5</sub>	
C	P <sub>0</sub>		P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>		P <sub>5</sub>	
D	P <sub>0</sub>		P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>		P <sub>5</sub>	
E	P <sub>0</sub>		P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>		P <sub>5</sub>	
F												
G	K <sub>1</sub>		K <sub>2a</sub>	K <sub>2b</sub>	K <sub>2c</sub>	K <sub>2d</sub>	K <sub>2e</sub>		K <sub>3</sub>		K <sub>4</sub>	
H												

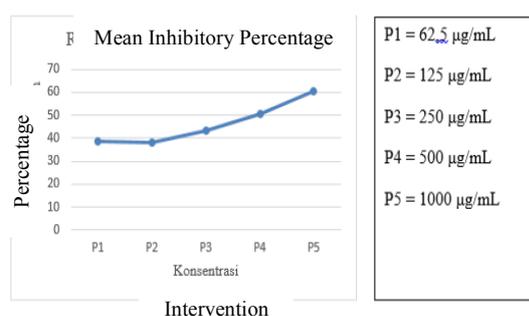
**Picture 1.** Working map of 96-well microplate

### *Curcuminoid Silica Nanoparticle* preparation

*Curcuminoid Silica Nanoparticle* in this study was made using absorption technique. 50 mg of *Curcuminoid* powder mix with 200 mg of *Silica Nanoparticle* and mix it in 20 ml ethanol. Mixture then put in a container. Container then put in a bath sonicator for 2 minutes sonication process. Ethanol then evaporated using rotary evaporator at 55°C. The final form is *Curcuminoid Silica Nanoparticle* powder.<sup>(15)</sup>

*Curcuminoid Silica Nanoparticle* extract that made first is the highest concentration first 1000 µg/mL. To make 1000 µg/mL we had to made threefold concentration that is 3000 µg/mL because it is easier to made the dilution and as a correction when we dilute the bacteria to make suspension in NaCl 0,9%. This dilution method is also used for the second part of this research. After we made the threefold concentration, we made the mixture from 100 µl Mueller Hinton Broth

and 100  $\mu\text{L}$  *Curcuminoid Silica Nanoparticle*. This mixture then put in a test tube that has been filled with 100  $\mu\text{L}$  Mueller Hinton Broth. This step was repeated to the lowest concentration; then we put the bacteria in same size 50  $\mu\text{L}$ .



**Picture 2.** Inhibitory percentage

### Bacteria test preparation

Bacteria that have been identified then made for suspension to use in microdilution process. This bacteria suspension made by mix the bacteria with NaCl 0,9%. Turbidity is adjusted with 0,5 McFarland standard. 0,5 McFarland standard is made by mixing 0,05 mL BaCl<sub>2</sub> 1% with 9,95mL H<sub>2</sub>SO<sub>4</sub> 1%.

## RESULT

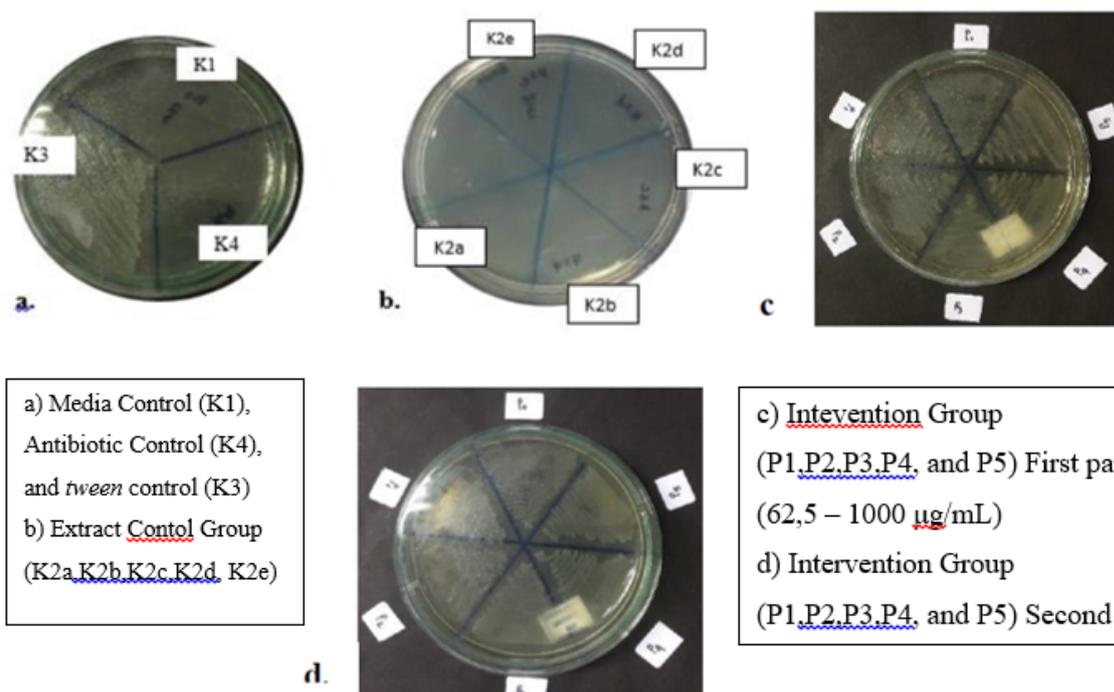
**Table 1. Optical Density**

P0 ( 0 $\mu\text{g/mL}$ )	0.724 $\pm$ 0.242
P1 (62,5 $\mu\text{g/mL}$ )	0.368 $\pm$ 0.007
P2 (125 $\mu\text{g/mL}$ )	0.370 $\pm$ 0.010
P3 (250 $\mu\text{g/mL}$ )	0.339 $\pm$ 0.007
P4 ( 500 $\mu\text{g/mL}$ )	0.296 $\pm$ 0.016
P5 (1000 $\mu\text{g/mL}$ )	0.244 $\pm$ 0.066

Optical Density was determined using spectrophotometer shows that there was declining Optical Density between each concentration in the first part of this research. Second part of this research did not measure the Optical Density but using inoculation in Mueller Hinton Agar. Picture 2 shows there is increasing inhibitory percentage from first intervention to the fifth intervention. First and second intervention has a mean inhibitory percentage of 38%, third intervention has 43,2%, fourth intervention has 50,2%. The fifth intervention is the highest percentage of 60,6%.

Inoculation of control groups in Mueller Hinton Agar have been done after Optical Density determined. Picture 3a in K4 part shows there's no bacteria grown in antibiotic control; it shows there was no contamination when inoculation process. Picture 3a in part K1 shows there was no contamination in media Mueller Hinton Agar. Picture 3b shows there was no bacteria growth meaning there was no contamination in the extract. Inoculation result shows in intervention 1 to intervention 5, bacteria still grow in Mueller Hinton Agar (Picture 3c). On the second part, intervention 1 to intervention 5 (2000 – 32000  $\mu\text{g/mL}$ ) also shows the same result; there was growth of the

bacteria in Mueller Hinton Agar (Picture 3d).



## DISCUSSION

Several things are suspected to affect MIC and MBC result including bacteria factor and *Curcuminoid Silica Nanoparticle* extract, such as:

### *Escherichia coli* factor

The first factor from the bacteria is Outer Membrane of *Escherichia coli* which function of this two layer of lipopolysaccharides is as protection membrane. Cell wall of Gram Negative's peptidoglycan is thinner than the Gram Positive's peptidoglycan, but this outer membrane feature of Gram-negative

bacteria helps protection function of the Gram Negative bacteria.<sup>(16)</sup> It caused by porin in outer membrane which function is to protect the bacteria against hydrophobic structure like *Curcuminoid Silica Nanoparticle* extract. Porin plays role to help diffusion of only hydrophilic structure because most of nutrition for bacteria is on hydrophilic structure, so porin will rule out hydrophobic structure<sup>(17)</sup>, and make the extract cannot work properly. Different with Gram-Positive bacteria, *Curcumin's* MIC on Gram Negative bacteria is greater<sup>(12)</sup>, it is caused by the difference in bacteria structure and morphology

between Gram Positive and Gram Negative bacteria, shows the difference in MIC. Second factor caused by *Escherichia coli* bacteria is flagella function that has the ability to do chemotaxis to avoid chemical material that harm the bacteria. It is started by chemical receptor response in outer cell then transmitted to the flagellar motor causing bacteria movement using its flagella avoiding the 'harmful' chemical substance that already detected<sup>(18)</sup>, so antibacterial effect of *Curcuminoid Silica Nanoparticle* is not powerful enough.

#### ***Curcuminoid Silica Nanoparticle* factor**

Factor from the *Curcuminoid Silica Nanoparticle* is because there is a difference in extract making procedure from the previous study. The previous research is using wet-milling technique, so *Curcumin* size is smaller than this research, which is 2-40 nm only<sup>(12)</sup>, while in this study using 100 nm. Another study used aqueous extract of *Curcuma longa* determined MIC at 4 mg/mL<sup>(19)</sup> because the technique that they used was different with this study.

*Curcuminoid* extract storage also affects the extract. This is because *Curcumin* structure has photosensitivity so extract storage is also important<sup>(20)</sup>. This research was already stored the extract in aluminum foil wrapped glass bottle to avoid light exposure. Besides that, when

we worked also using a biosafety cabinet with lamp turned off. Light exposure factor is still can affect this research when we did weight measurement on the extract.

Another factor is heating exposure when extract making process. Mixing extract with the nanoparticle is happened at 55°C<sup>(21)</sup>. This is because thermolabile character of the extract when expose to pH=7, but more stable in pH=3<sup>(21)</sup>, so it can affect the extract by reduce *Curcumin* retention, while *Escherichia coli* bacteria produce acidic metabolism waste<sup>(2)</sup>.

This study using nanoparticle characterization from the previous study because of device limitation so that effectiveness of nanoparticle cannot be analyzed. According to the previous study, silica nanoparticle size is 100 nm using TEM microscope, pore size is 10 nm, FTIR analysis shows peak at 3100 cm<sup>-1</sup>, it means there is hydrogen bond between silica nanoparticle and *Curcumin*<sup>(15)</sup>. In vivo bioavailability in mice is showing that silica nanoparticle has higher bioavailability (0,0291 µg/mL) than *Curcumin* without silica nanoparticle (0,0105 µg/mL). In vitro release profile while using silica nanoparticle is also better than without nanoparticle. Cumulative release rate in *Curcumin silica nanoparticle* 12% and reaching plateau in 48 hours, while *Curcumin* only has 4%

release rate and reaching plateau in 5 hours<sup>22</sup>.

## CONCLUSION

*Curcuminoid silica nanoparticle* extract has no antibacterial effect to *Escherichia coli* bacteria within concentration 62,5 - 32000 µg/mL.

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