

# Preparation and Antibacterial Activity of Inclusion Complex Sulfonatocalix[4]arene-Xanthone against *Escherichia Coli*

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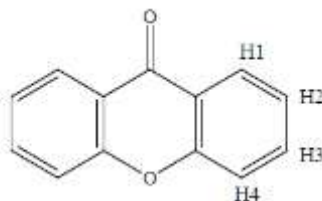
**Abstract.** The preparation of inclusion complex sulfonatocalix[4]arene (SC[4]A) with xanthone and measurement of antibacterial activity against *Escherichia coli* have been performed. Analysis of the structure of inclusion complex between SC[4]A and xanthone was investigated by using Fourier Transform Infrared Spectroscopy (FT-IR) and H Nuclear Magnetic Resonance (H-NMR). The solubility of the product in water was examined. Surface area and porous determination were analyzed by Surface Area Analyzer (SAA). Antibacterial activity test was performed for 0, 3, 6, 9, 12, and 24 hours. The results showed that antibacterial activity of the complex of xanthone SC[4]An against *Escherichia coli* was higher than that of xanthone it self.

## 1. Introduction

Infectious diseases have been known since ancient times. Infection is a major cause of disease in the world, especially in tropical regions like Indonesia because tropical regions have a high humidity that the microbes can flourish. Infectious diseases of the respiratory tract and digestive tract disease is an infection that most often occurs. Infections of the digestive tract caused by bacteria such as *E. coli*. Excess *E.coli* in the human gut are pathogenic that can cause diseases in the digestive tract, can also cause diarrhea, leading to severe intestinal bleeding [1]. Therefore, required an antibacterial compound that can inhibit the proliferation of the bacteria.

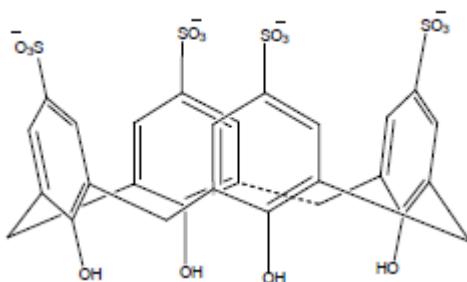
Indonesian people already know and use plants to treat a wide range of infections caused by microbes. Quite many types of plants are used as medicinal plants, one plant as a medicine is the skin of the mangosteen fruit (*Garcinia mangostana* L.). The skin of the mangosteen fruit contains flavonoids, xanthone and derivatives, as well as tannin [2]. According to research [3] and [4], xanthone is the most important chemical content contained in the skin of the mangosteen fruit. Xanthone (figure 1) has antioxidant activity [5], anti-inflammatory and hypo-allergenic [6], antifungal [7], anticancer [8] and antibacterial [9]. Xanthone has a low solubility in water and therefore its solubility in water should be improved to enhance its antibacterial activities.





**Figure 1.** Chemical structures of xanthone

Sulfonatocalix[4]arene/SC[4]A were widely used in the pharmaceutical field because it is harmless and has good water solubility (up to 0.1 M) in aqueous media [10]. It was interesting for the inclusion complex of xanthone with SC[4]A to increase solubility xanthone. The Chemical structures of SC[4]A can be seen in figure 2. Synthesis of *p*-sulfonatocalix[n]arene ( $n = 4,6,8$ ) can only be done by two methods, e.i sulfonation of *p*-Hydroxycalix[n]arene [11] and ipso-sulfonation method of *p*-tert-butylcalix[n]arene. Ipso-sulfonation method simpler than the method proposed by Shinkai but the reaction is often disrupted by the substitution incomplete and this method can only be used to get an instance of calix[8]arene because it has a low solubility [12].



**Figure 2.** Chemical structures of SC[4]A

In this paper, the preparation of inclusion complex of xanthone with SC[4]A was discussed and the antibacterial activity of the inclusion complex SC[4]A-xanthone and xanthone against bacteria *E. coli* were examined.

## 2. Material and methods

### 2.1. Tools and chemicals

UV-Vis Spectrophotometer Double Beam (Perkin Elmer Lambda 25), infrared spectrometry (FTIR, Shimadzu, IR Prestige-21), nuclear magnetic resonance ( $^1\text{H}$ -NMR, Agilent NMR 400 MHz), Surface Area Analyzer (SAA, Nova 1200e). Bacterial cultures used was *E. coli* ATCC 25922. Bacterial media used was Luria Bertani Agar, Miller (Merck) and Luria Bertani Broth, Miller (Merck).

### 2.2. Methods

#### 2.2.1. Preparation of inclusion complex SC[4]A-xanthone

SC[4]A was synthesized according to the literature procedures [13]. Inclusion complex SC[4]A-xanthone at 1:1 M ratio were obtained by mixing SC[4]A and xanthone in a solution mixture of methanol and water ( $v:v = 1:9$ ) and stirred for 48 hours at room temperature [10]. The mixture was evaporated to remove methanol and water, then dried in vacuum to obtain SC[4]A-xanthone complex. Complex SC[4]A-xanthone was analyzed using FTIR,  $^1\text{H}$  NMR and SAA.

#### 2.2.2. Solubility Test

Inclusion complex SC[4]A-xanthone (50 mg) was dissolved into 5 mL of water and then was stirred for 1 hour at room temperature. The insoluble complex was separated by filtration and the filtrate was evaporated to dryness and the residue was weighed. The same treatment was also carried out on the xanthone, C[4]A, and SC[4]A.

### 2.2.3. Antibacterial test

LB Broth (1.25 g) was dissolved into 50 mL of distilled water, then sterilized by autoclaving at 121 °C for 15 minutes. A total of 1 ose bacteria were grown in LB Broth by incubating at 37°C for 24 hours at 150 rpm [14]. Xanthone (0.1 g) was dissolved in distilled water and added of LB Broth 2.5% (w/v), and then sterilized by autoclaving at 121 °C for 15 minutes. Formed mixture was cooled and added 0.5 mL of the bacterial inoculum 1%. Samples were incubated at 37°C with agitation of 150 rpm (Lahmer, *et. al.*, 2012). Antibacterial activity was investigated by measuring absorbance sample solution for 0, 3, 6, 9, 12, and 24 hours using UV-Vis spectrophotometer at  $\lambda_{\max}$  600 nm. The control is a solution of bacteria in the media. The inclusion complex SC[4]A-xanthone was also examined for antibacterial activity test using the above procedures. The % Inhibition of antibacterial activity was calculated by the Equation (1):

$$\% \text{ Inhibition} = [(At - Ao) - (Bt - Bo) / (At - Ao)] \times 100\% \quad \dots\dots\dots(1)$$

Ao = OD control at 0 hours At

= OD control at t hours

Bo = OD sample at 0 hours Bt

= OD sample at t hours

## 3. Results and discussion

Evaluation of the complex

The reaction between SC[4]A and xanthone resulted in a solid brownish white, has a melting point of > 350 °C and has a yield of 84.075%. Table 1 shows a summary of wave number (cm<sup>-1</sup>) and the corresponding functional groups of C[4]A, SC[4]A, and SC[4]A-xanthone obtained from FT-IR spectra.

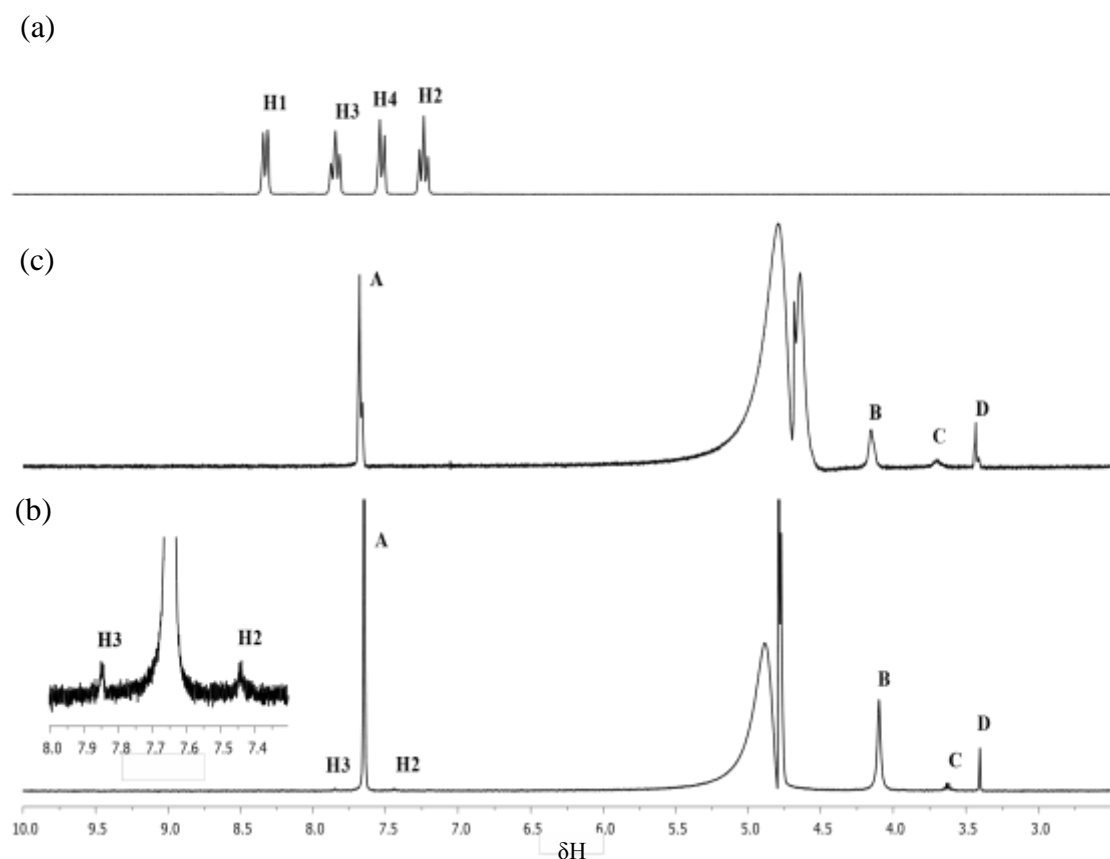
**Table 1.** The wave number (cm<sup>-1</sup>) of functional groups for C[4]A, SC[4]A, and SC[4]A-xanthone

Functional groups	Wave number (cm <sup>-1</sup> )		
	C[4]A	SC[4]A	SC[4]A-xanthone
OH	3149	3417	3443
C-H <i>Stretch</i>	2868, 2933	2871, 2959	3016
C=C <i>Aromatic</i>	1465, 1593	1487, 1618	1459, 1607
Sulfonate (SO <sub>3</sub> <sup>-</sup> )	-	1049, 1122, 1202	1047, 1121, 1208
C-O-C <i>stretch</i>	-	-	1331, 1346
C=O	-	-	1656

The FTIR spectra of SC[4]A showed a new peak which appears at 1049, 1122, and 1202 cm<sup>-1</sup> from sulfonate (SO<sub>3</sub>) [15]. In the spectra of SC[4]A-xanthone appears the new groups are carbonyl groups indicated that the vibration C=O stretch at 1656 cm<sup>-1</sup> which is the characteristic absorption for carbonyl on xanthone [16] and 1331 and 1346 cm<sup>-1</sup> (COC stretch) where both is the group of

xanthone.

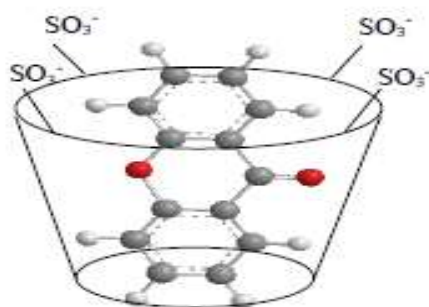
The  $^1\text{H}$  NMR measurement of the xanthone, SC[4]A, and complex SC[4]A-xanthone were employed to obtain the interactions between xanthone with SC[4]A.  $^1\text{H}$  NMR spectrum SC[4]A in figure 3 showed the presence of four peaks of protons that peak A is  $\delta\text{H}$  7.67 ppm, peak B at  $\delta\text{H}$  4.16 ppm, peak C at  $\delta\text{H}$  3.71 ppm and peak D at  $\delta\text{H}$  3.43 ppm.  $^1\text{H}$  NMR spectra were obtained suspected that peak A is a proton from the group on the benzene ring ( $\alpha\text{-H}$ ), and peak B, C, D is a proton from the group  $\text{CH}_2$  ( $\beta\text{-H}$ ). Integration of peak A is 1.00 and peak B, C and D when the integration is summed were obtained 1.02 that the ratio is 1:1. The conformation of SC[4]A affects the results of  $^1\text{H}$  NMR spectra were obtained. It is suspected that the most likely conformation of SC[4]A is cone and cone partial. The  $^1\text{H}$  NMR spectrum of SC[4]A-xanthone also showed the four protons that peak A is  $\delta\text{H}$  7.65 ppm, peak B at  $\delta\text{H}$  4.10 ppm, peak C at  $\delta\text{H}$  3.63 ppm and peak D at  $\delta\text{H}$  3.40 ppm. The fourth peak is one proton signal of a benzene ring (peak A) and three proton signal of  $\text{CH}_2$  (peak B, C and D). The  $^1\text{H}$  NMR spectrum of both compounds SC[4]A and SC[4]A-xanthone seems like there is no difference peaks are visible, but when done magnification  $^1\text{H}$  NMR spectrum SC[4]A-xanthone at the chemical shift  $\delta\text{H}$  7-8 ppm it will show two small peaks that peak H2 appeared at 7.45 ppm and peak H3 at chemical shift of 7.85 ppm. Peak H2 and H3 in  $^1\text{H}$  NMR spectrum of SC[4]A-xanthone is proton of CH aromatic in xanthone.



**Figure 3.** The  $^1\text{H}$  NMR spectrum of (a) xanthone ( $\text{DMSO-}d_6$ ), (b) SC[4]A, (c) SC[4]A-xanthone ( $\text{D}_2\text{O}$ )

The invisibility peak of proton from xanthone at the  $^1\text{H}$  NMR spectrum of SC[4]A-xanthone is possible because xanthone is small in width  $2.506 \text{ \AA}$  have entered entirely into the cavity of SC[4]A which has a cavity size  $8.941 \text{ \AA}$  so that the protons signal of xanthone hindered by proton signal of

SC[4]A surrounding it. Therefore, proton signals of xanthone were not visible. The occurred shift in the chemical shift spectrum  $^1\text{H}$  NMR SC[4]A-xanthone toward smaller chemical shift also indicates the presence of a new incoming substituent. Width and length of xanthone and SC[4]A-xanthone were measured using Avogadro program. The  $^1\text{H}$  NMR spectrum and the results of the application of the Avogadro Program showed that the xanthone inclusive in the cavity of SC[4]A (figure 4).



**Figure 4.** Possible binding mode of SC[4]A-xanthone complex

The surface area and total pore volume of the inclusion complex SC[4]A-xanthone were smaller than the SC[4]A (table 2). This suggests that xanthone entered the cavity of SC[4]A, leading to decreasing of surface area and pore volume. The results showed the average pore size SC[4]A-xanthone was greater than that of the SC[4]A. However, if the xanthone into the cavity of SC[4]A then it should cavity of the SC[4]A smaller and showed the pore size of the SC[4]A-xanthone smaller than the SC[4]A, but the results are the opposite. This could be due xanthone that enter into the cavity of the SC[4]A porous rammed outskirts of SC[4]A so that the periphery pore dissolved and were obtained the larger pore sizes.

**Table 2.** Comparison of Surface Characteristics SC[4]A and SC[4]A-xanthone

Characterization	SC[4]A	SC[4]A-xanthone
Surface area ( $\text{m}^2/\text{g}$ )	2.429	1.394
Total pore volume ( $\text{cc/g}$ )	0.006729	0.005642
Average pore size (nm)	5.541	8.097

#### Solubility test

Water solubility of C[4]A was the amount of 0.4 mg/mL and xanthone solubility in water was the amount of 1.3 mg/mL. The solubility of SC[4]A and SC[4]A-xanthone were 8.35 and 6.35 mg/mL, respectively. This indicates that solubility test of inclusion complex SC[4]A-xanthone in water is good.

#### Antibacterial test

The amount of the antibacterial activity shown by the percentage of inhibition of bacteria. Value bacterial inhibition against *E. coli* is showed in table 3. Amoxicillin was used as positive control.

**Table 3.** Percent inhibition of xanthone and SC[4]A-xanthone against *E. coli*

Sample (25 mg)	Percent inhibition (%)				
	3 hours	6 hours	9 hours	12 hours	24 hours
xanthone	21.99	34.43	45.54	51.48	29.35
SC[4]A-xanthone	101.31	170.86	192.33	215.43	139.07
Amoxicillin	94.59	86.61	84.73	90.80	86.33

The results showed that the antibacterial activity of inclusion complex SC[4]A-xanthone and xanthone have the most optimum incubation time for 12 hours. Amoxicillin as positive controls has optimum incubation time at 3 hours. Percent inhibition of xanthone has lower than SC[4]A-xanthone because xanthone was insoluble in water solvent thereby reducing its ability to slow down the cell replication in bacteria. Inclusion complex SC[4]A-xanthone has the ability to slow down the cell replication in bacteria better than xanthone because xanthone complexed with the SC[4] A. An increased solubility in water. Increased water solubility which occurred at a drug compound (in this case xanthone) is possible due to the interaction between the nonpolar part of xanthone with a cavity nonpolar of SC[4]A [12].

#### 4. Conclusion

Preparation of inclusion complex SC[4]A-xanthone was resulted in a solid brownish white, has a melting point of  $> 350^{\circ}\text{C}$ , and has a yield of 84.075%. The SC[4]A-xanthone was characterized by using FTIR,  $^1\text{H}$  NMR, and SAA. Water solubility experiments show that SC[4]A are able to solubilise xanthone. The antibacterial activity of inclusion complex SC[4]A-xanthone against *E. coli* shows the percent inhibition better when compared with xanthone at optimum incubation time 12 hours. It shows indicate that the complexation of xanthone and SC[4]A can increase the antibacterial activity of xanthone.

#### Acknowledgements

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