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Synthetic C-Methoxyphenyl Calix [4] Resorcinarene and Its Antioxidant Activity

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ABSTRACTS

C-methoxy calix[4]resorcinarene macromolecule is one of calysarene group compounds.The calysarene compounds have been reported to have any beneficial functions in various applications in medical field, such as an adsorbent for sunscreen and heavy metal, anti-HIV and HCV, and anti tumour. The C-methoxyphenyl calix[4]resorcinarene (CMPCR) is one of the C-methoxy calix [4] resorcinarene derivatives, in which researches in application of C-methoxy calix[4]resorcinarene itself in the medical field is rarely conducted. In present study, the researcher attempted to synthesize the CMPCR compound and followed by charaterizations of the synthetic compound resulted using infrared spectrophotometer instrument, proton nuclear magnetic resonance (H-NMR), and carbon-nuclear magnetic resonance (C-NMR) methods to ensure that the product is CMPCR compound. The yielded compound was further tested to recognize its antioxidant activity. The antioxidant testing required is in line with the increases of diseases caused by over concentration of the free radicals existing in biocellular system. The CMPCR synthesis was performed by condensation and antioxidant activity was tested using 1,1-diphenyl-2- picrylhydrazyl (DPPH) methods. Results showed that the reaction product was white precipitate with 390 ^{o}C melting point and the reaction yield was 97.05%. The antioxidant activity showed that its IC_{50} value equal to 79 ppm. Comparing to the vitamin C which has IC50 equal to 20.96 ppm, the CMPCR compound-the synthetic product, has level qualification of antioxidant activity equal to strong (IC50 <100).

Keywords: activity, antioxidant, calix[4]resorcinarene, characterization

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1. Introduction

Currently, study on antioxidant beneficial functions continouesly receives serious attentions of researchers both in natural and pharmaceutical chemical fields. In fact, those attentions couldnot be separated from the increasing number of diseases caused by the endogen and/or exogen free radi-cal, the oxidants. It is known that hydroxyl radicals ($^{\bullet}$OH) is very dangerous free radical in biocelular system contributing the developments of various generative diseases (Khodr and Hallil, 2001; Lee et al., 2008; Yohanes et al., 2011).

Free radicals are atoms or molecules that contain one or more unpaired electrons and they exhibit high reactivity. For being stable, the free radical compounds should uptake electrons from other molecules then lead to abnormalities and initiate chain reactions which can damage the healthy tissues. Some of the free radicals are useful for removal of pathogenous microorganisms (exogen oxidants), but when their presences in over concentration in biocellular system, they are predominantly thought to involving in developments of various degenerative disease such as liver disease, cancer, premature aging, and Alzheimer's (Hernani and Rahardjo, 2005). The antioxidants are required to reduce the negative effect of the oxidants.

Antioxidants are energitically substances in low levels that can inhibit the oxidation process. In protecting the body from free radical attacks, antioxidant substances serve to stabilize free radicals by supplementing electron deficiencies from free radicals thereby inhibiting chain reaction (Windono et al., 2001; Buang et al., 2017). One of the organic compounds that are being discussed today is calixarene macromolecule. Calixarene has been widely used in many ways in medical field as sunscreen and heavy metal absorbent (Budiana, 2015), anti-HIV and HCV (Tsou et al., 2010) and anti-tumor (Kamada et al., 2010). Macromolecules, discovered to have some biomedical applications, behave like drugs, and have shown great potential in drug delivery systems. Among the synthetic macromolecules such as crown ether, cyclodextrin, and calixarenes with their derivatives are of great significance. In addition to their unique physicochemical properties, they also serve as hosts that can encapsulate many types of guests (Shinkai, 1995).

Although calixarene macromolecule has known to have various health beneficiaries, the study on the use of calixarene as an antioxidant are rarely conducted. A group of calixarene, synthesized from resorcinol derivative compound has reported to have the ability as antioxidant, is calix [4] resorcinarene (Sayekti et al., 2016). This calix [4] resorcinarene compound is cyclic oligomers containing aromatic rings of the resorcinol

linked by methylene bridges and having a geometric cavity (Utomo, 2011). Calix [4] resorcinarene compound which is synthesized from the basic material of resorcinol and 4-methoxy benzaldehyde (*p*-anisaldehyde) with HCl as catalyst is C-methoxyphenyl calix [4] resorcinarene. These compounds have electron-donor groups such as hydroxyl groups that potentially have activity as antioxidants. The structure of C-methoxyphenyl calix [4] resorcinarene is reported in Fig. 1.

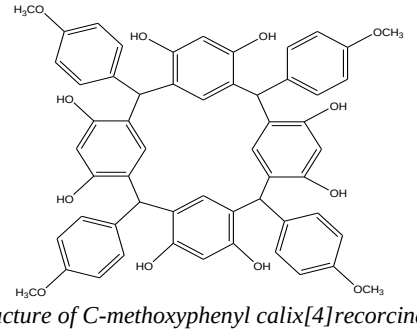

Fig. 1. Structure of C-methoxyphenyl calix[4]recorcinarene

The preliminary study performed to evaluate the antioxidant activity of calix [4] resorcinarene compound was done by Handayani et al. (2016) in formed of the C-2-hydroxiphenyl calix [4] resorcinarene which reported that it has powerful antioxidants with IC_{50} value of 77.43 ppm. Comparing to the IC50 of vitamin C (20.96 ppm), the antioxidant activity of the calix [4] resorcinarene compound was far below IC_{50} of the vitamin C. This result leads the author/researcher to change the structure of calixarene which made by Handayani et al (2016) with the expectation that the new structure would have stronger antioxidant activity. The structure of C-Hydroxyphenyl calix [4] resorcinarene is reported in Fig. 2.

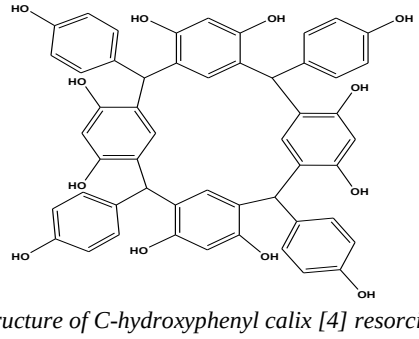

Fig. 2. The structure of C-hydroxyphenyl calix [4] resorcinarene

One of the ways to study the antioxidant activity of compounds is by using the DPPH method which is a simple, quick, easy, accurate, and reliable method of colorimetry to estimate the antiradical activity (Koleva et al., 2001). DPPH is an unstable free radical compound having an unpaired free electron, capable of resonance and can bind to other compounds to achieve stable structures. The purpose of this method is to know the equivalent concentration parameters to give 50% effect of antioxidant activity (IC) (Molyneux, 2004). The DPPH free radical dampening method is based on the reduction of a DPPH free radical in methanol solution by inhibition of free radicals. When the purple DPPH solution meets the electron donor, DPPH will be reduced and caused the purple color faded and replaced by the yellow color of the picryl group (Prayoga, 2013). These changes can be measured by a spectrophotometer at a maximum wavelength and plotted against concentrations (Reynertson, 2007). The qualifications of the standard strength of antioxidant activity is shown in Table 1.

Table 1. Standard strength of antioxidant based on the $^{*}IC_{50}$

No.	Value of IC_{50}	Antioxidant activity
1.	< 50 ppm	Very Strong
2.	51 – 100 ppm	Strong
3.	101 – 150 ppm	Medium
4.	151 – 200 ppm	Weak

**Source: (Zuhra et al., 2008)*

2. Materials and Methods

2.1. Materials and Instrument

Materials are used by researcher to synthesize CMPCR consist of recorcinol, p-methoxy benzaldehyde, chloride acid (HCl). Materials used in antioxidant testing such as CMPCR, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, DMSO, water and aluminum foil. Instruments used in this research are a set of reflux devices, separating funnel, electric heater Cordtrol II Z10,769-7, magnetic stirrer, shaking waterbath, rotary evaporator Buchii, desicators, drying lamp, pH meter, test tube (pyrex), flask (5 ml, 10 ml and 100 ml) beaker (pyrex), digital analytical scales. Instruments used in the antioxidant activity test were UV-VIS Spectrophotometer, Cuvet, and Incubator.

2.2. Procedures

2.2.1. Synthesis of CMPCR

Weighed 1.60 grams of resorcinol (0.015 mol) dissolved in 50 mL of 95% ethanol. Into the mixture, 2.04 grams (0.015 mol) of p-methoxy benzaldehyde was added. After being soluble, 1 mL of concentrated HCl were added drop by drop. Then, the mixture stirred and refluxed for 24 hours and the precipitate will form quickly. After the reaction time, the mixture cooled in an ice bath and filtered. The precipitate washed by ethanol and water (1:1) until neutral and dried. After that, determined the melting point of reaction product and characterized by using infra red and proton NMR spectrometer.

2.2.2. Antioxidant activity testing of the CMPCR

2.2.2.1. Preparation of standard curve

In the early stages of the test, a calibration curve for the DPPH solution was prepared. A total of 10 mg of DPPH was inserted into 100 mL flask and dissolved with methanol up to the boundary marker. DPPH solution was made to have a concentration of 100 ppm then diluted into variation of concentration: 5, 10, 15, 20 and 25 ppm. Then the λ maximum was detected using 15 ppm of the DPPH solution at 400 – 700 nm wavelengths. The maximum λ was used to measured absorbance of other concentrations.

2.2.2.2. Preparation of control solution

DPPH solution that would be used as control solution and reactant of the sample were in concentration of 15 ppm, at the center of the curve.

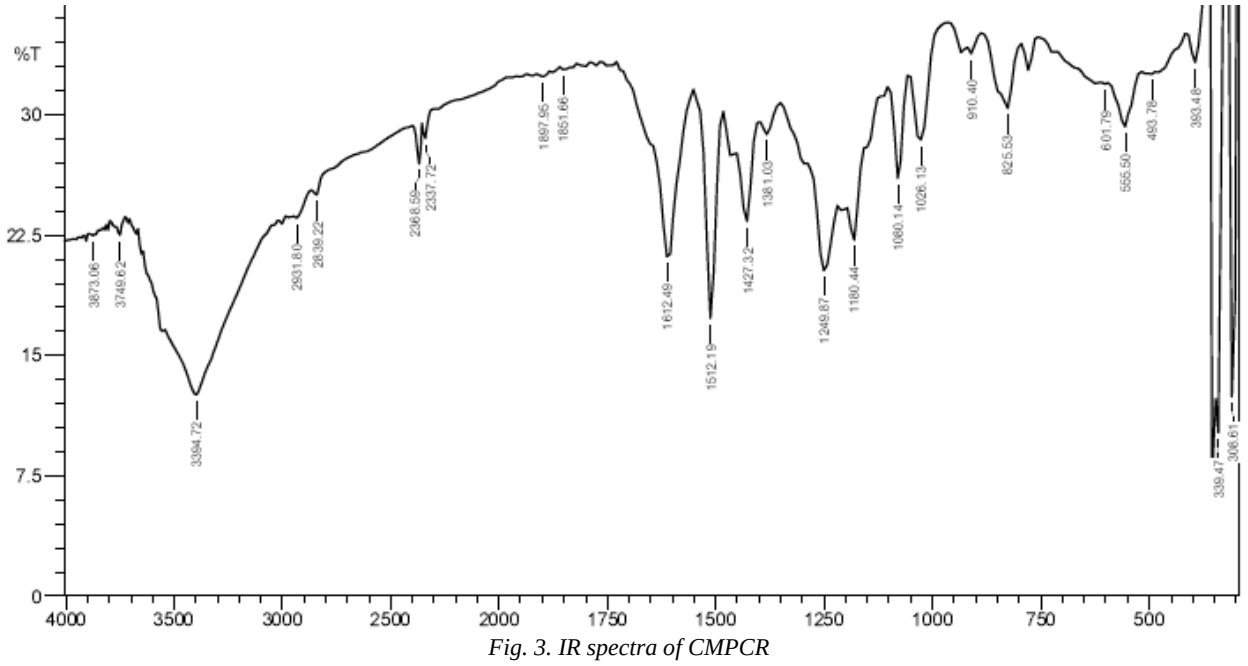

Fig. 3. IR spectra of CMPCR

2.2.2.3. Sample (CMPCR) preparation

Weighed 5 mg of sample dropped by 2-3 drops of DMSO, then dissolved by 100 mL of methanol up to the boundary marker to make 50 ppm of solution. The solution diluted to vary the concentrations as 15, 20, and 25 ppm.

2.2.2.4. Tested the antioxidant activity of the CMPCR sample

Added 3 mL of each concentration into test tube and 2 mL of 15 ppm DPPH solution into the same test tube then the mixture was incubated for 30 minutes at room temperature (37°C). The incubated mixture poured into the cuvette then measured the absorbance by UV-Vis spectrophotometer at wave length of 517 nm.

2.2.2.4. Data Processing Techniques

The formula to measure the capacity of antioxidant from absorbance of each test solution is (Musialik et al., 2005):

$$\textbf{\% Antioxidant activity} = \frac{A_{control} - A_{sample}}{A_{control}} \textbf{ x 100\%} \quad \text{(Eq. 1)}$$

where :

$A_{control}$ = control absorbance (DPPH + Ethanol), A_{sample}= sample absorbance (CMPCR + DPPH + Ethanol)

Furthermore the calculation results are entered into the regression equation with the concentration of the solution as the abscis (X axis) and the value of % antioxidant activity (% inhibition) as its ordinate (Y axis). Then the curve converted into linear regression to obtain the equation of: ***y = a + bx,*** where*:* y = dependent variable (antioxidant activity), x = independent variable (concentration of the test solution), a = intercept, and b = coefficient of regression.

The IC_{50} value as parameter of antioxidant activity was calculated from regression equation obtained by entering value of 50 % at y to know the value of effective concentration. IC_{50} value of test solution and comparative solution were determined from the equations obtained by each curve (Quey et al., 2014). IC_{50} is a number that shows the concentration of sample (ppm) which is able to inhibit oxidation process by 50%. The smaller IC_{50} number of a solution means that the solution has a high antioxidant activity (Koleva et al., 2001; Musialik et al., 2005).

3. Results and Discussion

3.1. Result of the CMPCR synthesis

Result of CMPCR showed that the product was a solid light yellow with 390 °C melting point and there were 97.05 % of reaction yield. The characterization by using infra red spectrophotometer (IR) as showed in Fig. 3 implied that there was a high peak at wave number of 3394.92 cm^{-1} which caused by the existence of –OH group. Twins peak at the wave number of 1500 and 1600 cm^{-1} caused by the absorbance of C=C aromatic group and absorbance at wavelength of 1427 caused by the existence of C-H calix[4]resorcinarene bridge. This wave number (1425 cm^{-1}) showed the formation of CMCPR.

Characterization by using ^{1}H-NMR Spectrophotometer showed that all protons are on the appropriate chemical shift (Fig. 4). Proton of methoxy group (-OCH_3) were discovered at 3.6 ppm (A). Proton of –OH (B) and –C-H (C) bridge of calix [4] resorcinarene groups appeared at 5.6 ppm. Proton of aromatic appeared at chemical shift of 6.1 ppm (D) and 6.2 ppm (E). Meanwhile, proton of aromatic group binding into the bridge of calix [4] resorcinarene appeared at chemical shif of 6.5 ppm (F) and 6.7 ppm (G).

The mechanism of CMPCR formation was estimated started from the protonation of aldehyde *p*-methoxy benzaldehyde group making C atom positively charged. Thereafter, the C positive charge attacked by the double bonds of resorsinol to form dimers, trimers, and cyclization as shown in Fig. 5. Using ^{13}C-NMR indicated that the signal was very low, it probable was caused by the weakness solubility of the CMPCR in DMSO.

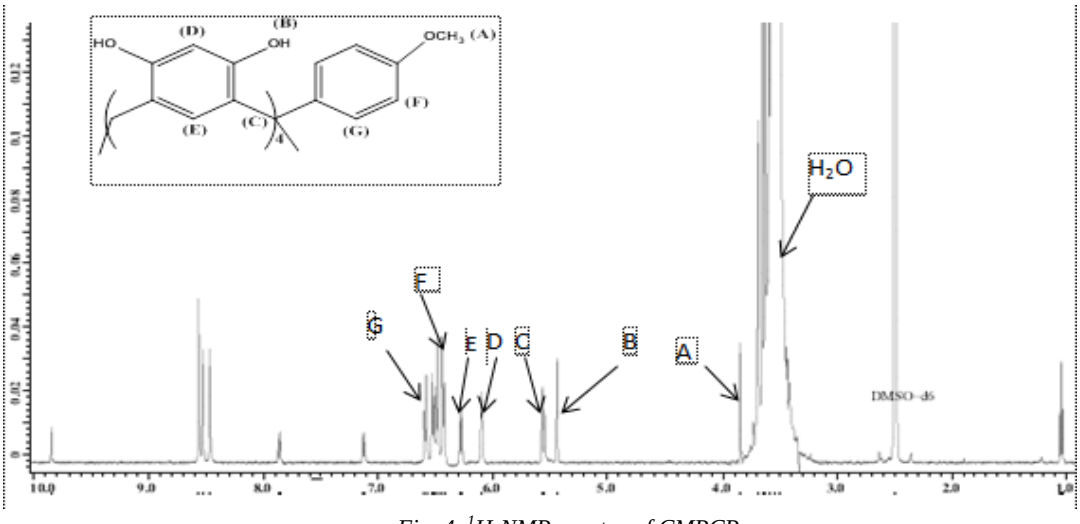

Fig. 4. ^{1}H-NMR spectra of CMPCR

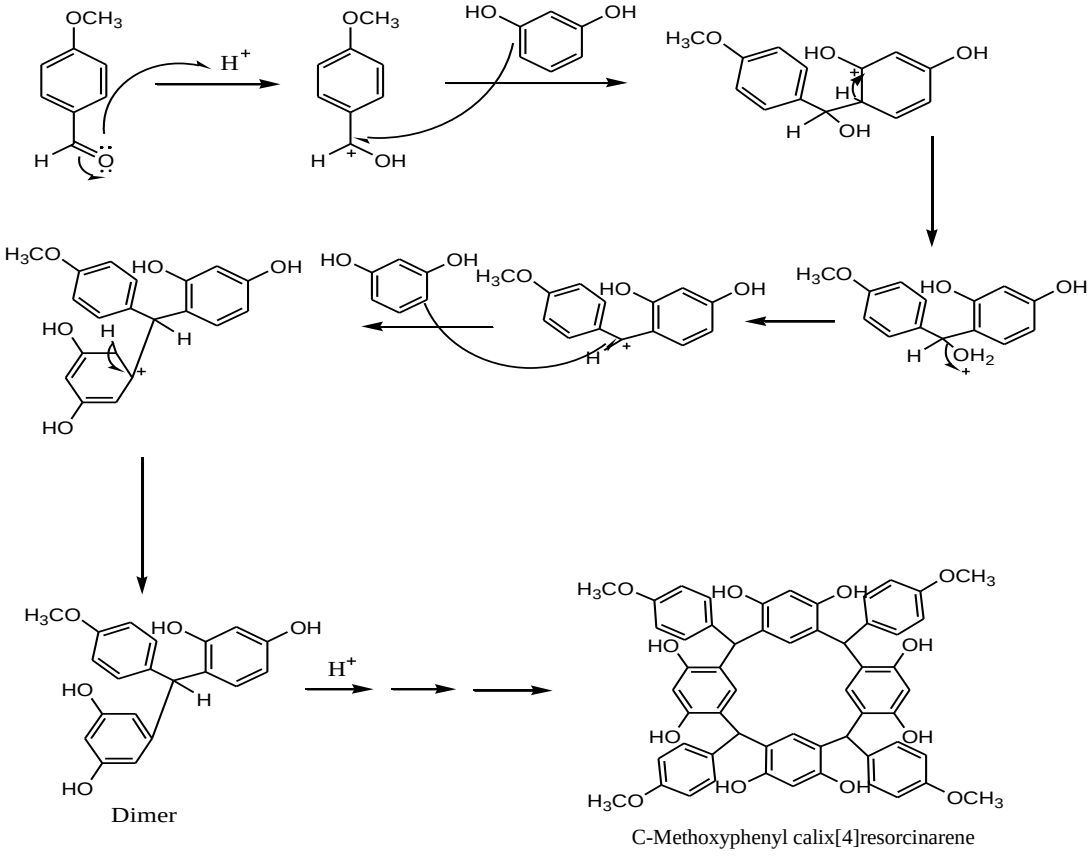

Fig. 5. Mechanism of CMPCR synthesis

3.2 The CMPCR's antioxidant activity

The method used in the test of antioxidant activity in this research was DPPH method. Testing of antioxidant activity with DPPH method is a very simple method, easy, requires few samples and the results are more accurate than other methods (Koleva et al., 2001). The steps of this test consist of: making DPPH calibration curve and control DPPH solution, measuring the DPPH control absorbance and the DPPH absorbance after the added antioxidant. Determination of the % antioxidant activity, equivalent with an inhibition (Eq. 1), was then followed by calculation of the IC_{50} value of CMPCR. The results of the determining calibration curve shows that DPPH was stable and has excellent linearity (Fig.6).

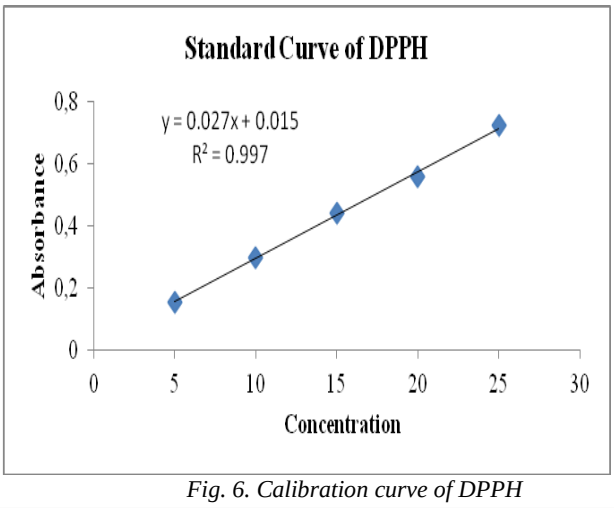

Fig. 6. Calibration curve of DPPH

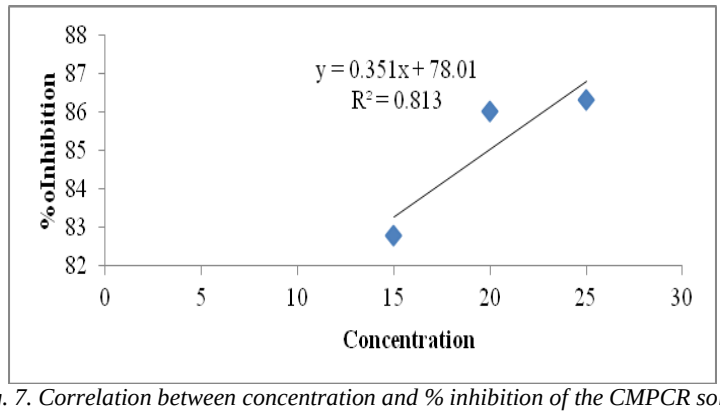

Fig. 7. Correlation between concentration and % inhibition of the CMPCR solution

Table 2. Result of % inhibition and IC_{50} measurements

Concentration (ppm)*	λ max (nm)	Abs of sample	Abs blank	% Inhibition (%)	Linear Regression	IC_{50} (ppm)
15		0.075 ± 0.0014		82.804 ± 0.0011		
20	516	0.061 ± 0.0014	0.435 ± 0.000	86.022 ± 0.0011	Y= 78.1 + 0.351X	79.00
25		0.060 ± 0.0014		86.321± 0.0012		

*n = 3

The calibration curve shows that the DPPH condition was very stable and there was a relationship between concentration and absorbance, so it was feasible to be used in antioxidant activity testing. The DPPH solution used as the control and reagent solution in this study was a DPPH solution of 15 ppm (taken from the dilution in making the calibration curve). Concentration 15 ppm was used as control solution because it was in the middle of the curve so that the level of uncertainty (error) was very small.

The main solution of CMPCR was prepared at 50 ppm concentration, then diluted into several concentrations of 15, 20, and 25 ppms. These solutions were diluted in a 10 mL measuring flask. These various concen-trations of solution would be reacted with DPPH free radical compound and measured by absorbance using a UV-Vis spectrophotometer at a maximum wavelength of 516 nm.

The absorbance measurement of C-methoxyphenyl calix [4] resorsinarene solution was performed to determine the ability of CMPCR sample in reducing free radical compounds. From the result of sample absorbance measurement, inhibition from each concentration of 15, 20, and 25 ppms were calculated by using Eq.1 and obtained the value of inhibition % : 82.80, 86.02, and 86.32. The correlation curve between concentration and % inhibition shows the linear value of the regression, which was Y = 78.01x + 0.351 with R_2 value 0.813 which meant that the 81.3% of the inhibition was influenced by the concentration (Fig. 7), while the rest was influenced by other factors such as by the temperature, light, and others.

Based on that linear regression equation, the value of 50% plotted to y-Cartesian ordinate to calculate the value of x absis indicating the value of IC_{50} (Inhibition Concentration). IC50 value obtained from the calculation was 79 ppm. This means that 50% free radicals can be reduced by 79 ppm of CMPCR. In testing of antioxidant activity usually uses the comparison as a positive control. In this study, the comparison used was vitamin C. Vitamin C is a natural and pure antioxidant that is classified as a very powerful antioxidants. The IC50 of the vitamin C used in this study was taken from the literature (Widono et al., 2001). IC_{50} value of vitamin C has IC_{50} value equal to 21.09 ppm (IC50 <50 ppm) classified as a very powerful antioxidant; hence the CMPCR belong to a strong antioxidant category. The completed antioxidant activity measurements of the CMPCR synthetic compound are shown on Table 2.

4. Conclusion

C-Methoxyphenylcalix[4]recorcinanere (CMPCR) was successfully prepared and the high yield was 97.05%. The results of antioxidant activity test showed that CMPCR had antioxidant activity in strong category which was 79 ppm but the value still lower than the Vitamin C antioxidant activity which had a very strong category (IC50 = 20.95 ppm).

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Conflict of interest: Non declare