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Antioxidant Assay of C-2-Hydroxyphenylcalix[4] Resorcinarene using DPPH Method

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Abstract: C-2-Hydroxyphenylcalix[4]resorcinarene can be synthesized via condensation of 2-hydroxybenzaldehyde and resorcinol with acid catalyst. Structural elucidation of products was performed using FT-IR spectrophotometer, GC-MS, ^{1}H NMR and ^{13}C NMR. The product of condensation was subjected to antioxidant assays using DPPH (1-1-diphenyl-2-picrylhydrazyl) method. Reaction of 2-hydroxybenzaldehyde, HCl and ethanol was performed by refluxing the mixture for 24 hours. The aromatic electrophilic substitution-cyclization 2-hydroxybenzaldehyde and resorcinol in presence of HCl gave C-2-hydroxyphenylcalix[4] resorcinarena as yellow solid in 93.14 % yield with m.p > 368.8 ^{o}C. The product was analyzed with FT-IR, ^{1}H NMR and ^{13}C NMR. It has strong antioxidant activity in DPPH methods with ES_{50} 77.4322 ppm.
Keywords : antioxidant assay, 2-hydroxyphenylcalix[4]resorcinarene, DPPH method.

1. Introduction

Excessive sun exposure may cause the process of aging. This will trigger formation of free radicals that will cause autoxidation reactions in the body, especially in the lipid layer of the cell membrane[1]. Free radicals are known to have high activities to trigger a chain reaction in the cell. Autoxidation process will continue to produce hydroperoxides which may react further to form aldehydes, ketones, alcohols and hydrocarbons[2]. This may damage cells and cause various diseases, such as inflammation, cardiovascular disease, atherosclerosis, cancer, Alzheimer's, Parkinson's, diabetes etc[1]. The radical activity can be inhibited by the action of antioxidants[3,4]. Antioxidants are chemical compounds that function as inhibitors of oxidation reaction. Antioxidants inhibit oxidation by donating one or more electrons to free radicals, so the free radicals activities can be suppressed[5]. Antioxidants also inhibit the formation of reactive oxygen species (ROS), such as superoxide, $O2^{\bullet -}$, radical hydroxyl, OH•, peroxy, ROO•, alkoxyl, RO•, and hidroperoxcyl, $HO2^{\bullet}$ [6,1].

Sunscreens which also function as antioxidants might be prepare from compounds having conjugated double bonds likes aromatic or phenolic group as active materials. Calixarenes, groups of synthetic oligomer compounds containing an aromatic ring in a cyclic series connected by methylene or methyne bridges are suitable for this purpose. The structure of calix[4] resorcinarene allows to be modified to have properties as antiradical, antioxidant as well as sunscreen agents. Hasbullah[7]. reported *p*-methoxyphenylcalix[4]resorcinarene

as an antioxidant with free radicals reduction of 67.30%. Antioxidants and antiradical activities of resorcinarene tetranitroxide are found 100 times more effective than resorcinol in reducing free radicals [8]. Synthesis of calix[4] resorcinarene and its antioxidant activities will be reported in this study.

2. Experimental

1.1 Chemicals

Chemicals used in this research were salicylaldehyde, resorcinol, hydrochloric acid (HCl 37%), ethanol, aquadest, acetone, hexane, methanol, DMSO, and DPPH ((1,1-diphenyl-2-picrilhidrazyl). All chemicals except aquadest were purchased from E. Merck. Aquadest was obtained from Laboratory of General Chemistry, UGM.

1.2 Equipments

Equipments used in this research were laboratory glassware, Büchner funnel, Buchi evaporator R-124, melting-point apparatus (Electrothermal 9100), Camac UV-Cabinet II, analytical mass balance (Mettler AT200), infra red spectrophotometer (IR, Shimadzu-Prestige 21), proton nuclear magnetic resonance spectrometer (^{1}H-NMR, JEOL JNM-MY60 and JEOL MY-500 MHz), carbon nuclear magnetic resonance spectrometer (^{13}C-NMR, JEOL MY-500 MHz), and UV-Vis spectrophotometer (Type 722).

2.3 Procedures

2.3.1 Synthesis of C-2-hydroxyphenylcalix[4]resorcinarene

Into 100 mL three-necked flask equipped with water condenser, 1.1 g (10 mmol) of resorcinol and 1.22 g (10 mmol) of salicylaldehyde were dissolved in 100 mL of ethanol. Then, concentrated hydrochloric acid (1 mL) was added into the solution. The mixture was refluxed for 24 hours and monitored by TLC (acetone:hexane = 7:1) and allowed to cool to room temperature. The solid was collected by vacuum filtration with Büchner funnel, washed using the mixture of water and ethanol (1:1) and dried. The C-hydroxyphenylcalix[4]resorcinarene was obtained orange solid in 93.14 % yield with m.p. > 368.8 ^{o}C. . IR (KBr pellet) wave number in cm^{-1} : 3479.29 (-OH), 1612.49 and 1427.2 (C=C aromatic), 1427.9 (C-H bridge and methylen), 2931.80 (Csp^{3}-H), 1426 and 1072.42 (C-OC). ^{1}H NMR (DMSO) δ in ppm from TMS: 5.8418 and 5.9492 (H bridge), 6.0595 -6.7288 ppm (Ar-H), and 8.1336-8.4124 ppm (-OH). ^{13}C NMR (DMSO) δ in ppm from TMS: 27 (-C-H bridge) and 100- 135 ppm 101.3641 (Ar-C).

2.3.2 Antioxidant test

A stock solution of DPPH was prepared by dissolving 0.4 mg of DPPH in 1L methanol and the solution was kept in the dark at 4 ^{o}C [9]. A stock solution of the C-2-hydroxyphenylcalix[4]resorcinarene was prepared at 12.5, 25, 50, 100, and 200 ppm in DMSO respectively. 500µL from the stock solution of the compound was added to the 2 mL of DPPH 0,05 mM. The mixture was shaken well and kept in dark at room temperature for 2 hour. The absorbance of the mixture was measured at 517 nm by using spectrophotometer. The percent inhibition of radical scavenging ability was calculated as :

$$\% \text{ inhibition} = \frac{(A\,DPPH - A\,sample)}{A\,DPPH} \times 100\ \%$$

Linear regresion regresion y = ax + b made concentration as absis (x axis) and % inhibition as ordinat y. The 50% inhibition (ES_{50}) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radicals activity under these conditions [10].

4. Results and Discussion

4.1. Synthesis of C-2-hydroxyphenylcalix[4]resorcinarene

2-hydroxybenzaldehyde is a benzaldehyde derivative and can be condensation with resorcinol as raw materials in the synthesized of calix[4]resorcinarene. In order to obtain it, 2-hydroxybenzaldehyde and resorcinol (1:1) was refluxed in ethanol in the presence of hydrochloric acid catalyst. The reaction was carried out for 24

hours. The reaction gave orange solid in 93.14 % yield with m.p > 368,8 ^{o}C. The compound is insoluble in water, ethanol and good soluble in DMSO. The solubility of limited in organic solvent and high of melting point are caused the more hydroxyl groups and phenyl group in C-phenylcalix[4]recorcinarene molecule.

The FT IR spectrum (Figure.1) of calix showed characteristics absorption at 3479,29 cm^{-1} indicating the presence of hydroxyl (-OH) group. The most important evidence indicated that the reaction had taken place was the disappearance of strong aldehyde carbonyl absorption of the reactant.

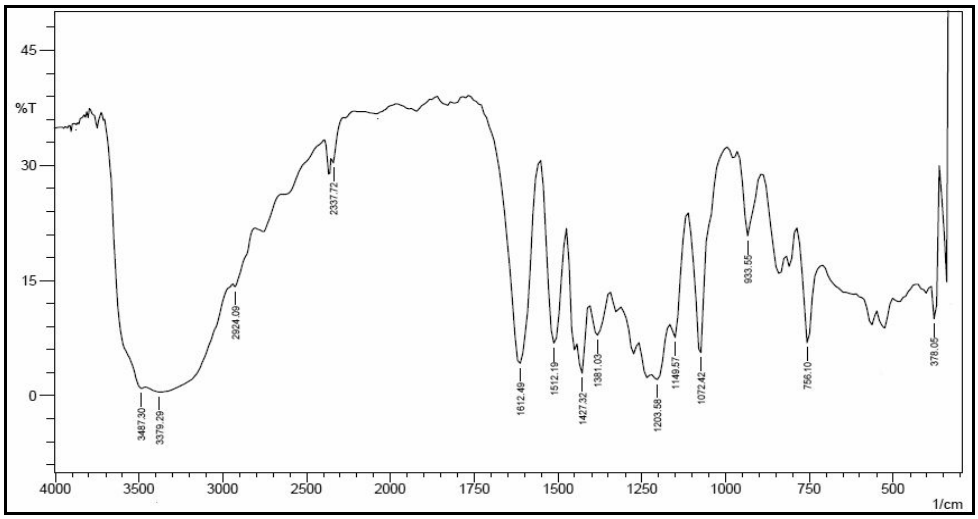


Figure 1. IR spectrum of the condensation between 2-hidroxybenzaldehyde and resorcinol

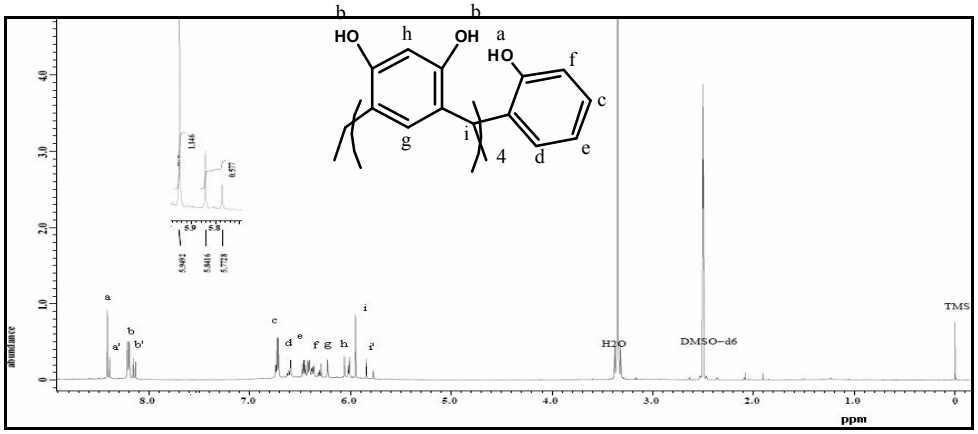


Figure 2. ^{1}H-NMR spectrum of the condensation between 2-hidroxybenzaldehyde and resorcinol

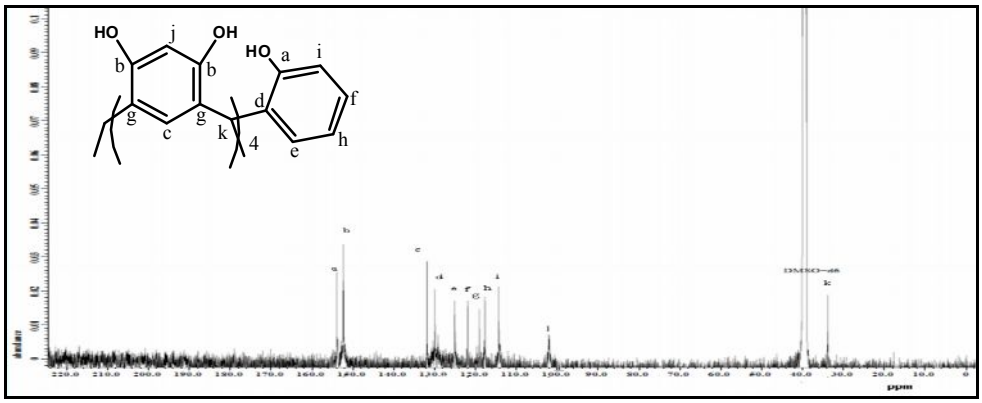
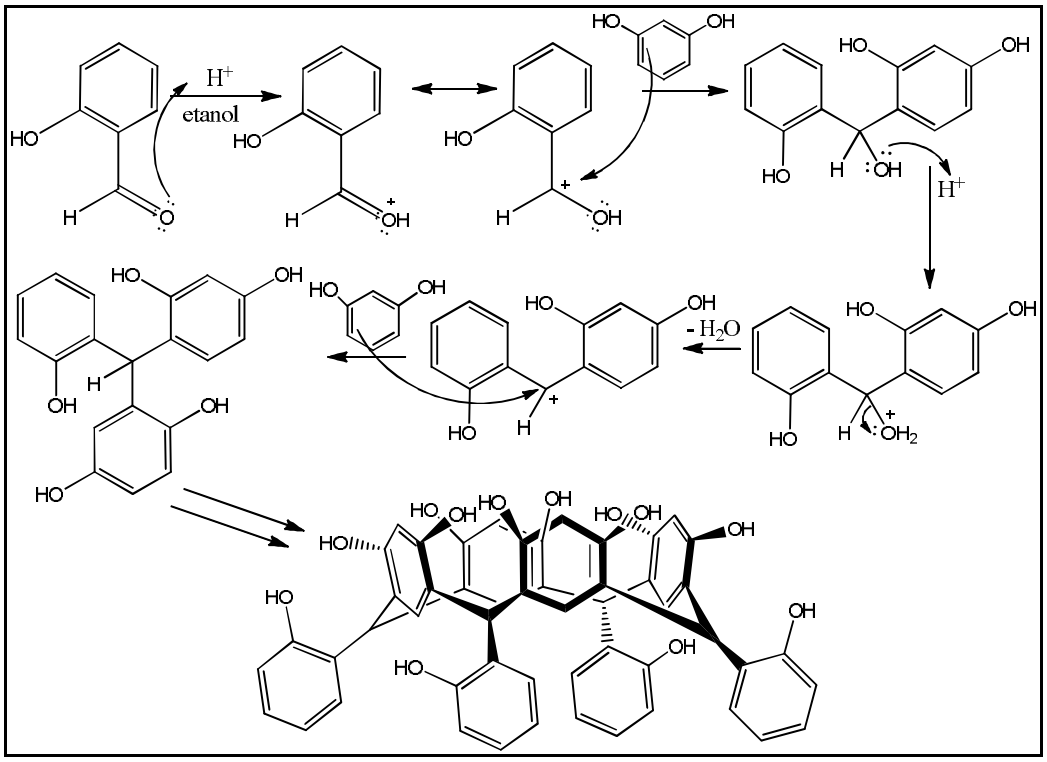


Figure 2. ^{13}C NMR spectrum product of cyclisation 2-hydroxyphenylcalix[4]resorcinarene

^{1}H NMR spectrum of the synthesized product (Figure 2) showed that were 3 proton groups with different chemical environment. They were aromatic and hydroxyl groups. First group (A) consisted of signal at δ 5.8418 and 5.9492 ppm with total integration of 4 protons belonged to proton of methyne bridge. The product condensation reaction shown a mixture more conformer calyx[4]resorcinarene in cone, partial cone or the other (12). Signal A with ratio of integration δ 5.8418 and 5.9492 ppm 1.1146 : 0.577 are shown partial cone (C_{4v}) conformer and crown (C_{2v}) with comparation: 1:2. Second group (B) consisted of signal multiplet (24 H, δ 6.0595 -6.7288 ppm) came from the resonance of aryl protons with the detail 16 protons from residue of 2-hydroxybenzaldehyde and 8 protons residue of resorcinol. The last signals (C, 8.1336-8.4124 ppm) represented 8 hydroxyl protons.



2-hydroxyphenylcaliks[4]resorcinarene C_{2h} : C_{4v} = 2 :1

Figure 3. Mechanism reaction of 2-hydroxyphenylcalix[4]resorcinarene

Based on ^{13}C NMR spectrum (Figure 3), there were several peaks representating the carbons existed on the product. Spectrum ^{13}C NMR analysis was also conducted to support the previous analyses. Peaks at 27 ppm ppm indicated methylen bridge carbons in compound. Peaks at 100 ppm shown carbons in aromatic between carbons of bonds with OH groups. Peaks at region 115- 135 ppm indicated the presence of aromatic carbons (12 C). This reaction condensation and cyclization between 2-hydroxybenzaldehyde and resorcinol yielded 2-hydroxyphenylcalix[4]resorcinarene.

4.2 Antioxidant assays of product cyclisation with DPPH methods

Antioxidant property of C-2-ethoxyphenylcalix[4]resorcinarene was calculated with decrease in absorbance 1,1-diphenyl-2-picrylhydrazyl (DPPH). Calix[4]resorcinarene can donated proton to the non radical from DPPH is highly antioxidant agent.

Percent inhibition can calculated % inhibition = $\frac{(A\ DPPH - A\ sample)}{A\ DPPH}$ x 100 %.

The electron scavenging 50 (ES_{50}) was calculated with intrapolation concentration (axis x) versus % inhibition (axis y) curve (Figure 4 and Tabel 1))represent 77.4322 ppm. 2-hydroxyphenylcalix[4]resorcinarene has a medium antioxidant property, because it can stabilize the radical DPPH to reaction with the hidroxy phenol groups produce in molecul stabilization.

Table 1. Concentration of 2-hydroxyphenylcalix[4]resorcinarene versus % inhibition

Concentration (ppm)	Absorbans	% Inhibition	Y= mx + C	ES $_{50}$ (ppm)
12.5	0.87	22.043	y = 0.3096x + 26.027	77.4322
25.0	0.822	26.344	R^2 = 0.8498	
50.0	0.516	53.763		
100.0	0.376	66.308		
200.0	0.205	81.631		
Kontrol	1.116			

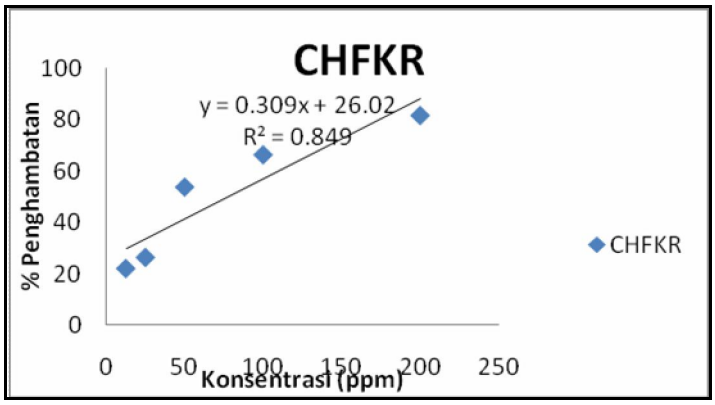


Figure 4. Curve concentration of 2-hydroxyphenylcalix[4]resorcinarene vs % inhibition

Nihlati *et al.* [11] sugested a standard activities antioxidant of degree a group of compound based activites values of that : - ES_{50} < 50 µg/mL = very strong

- ES_{50} 50- 100 µg/mL = strong
- ES_{50} 101- 150 µg/mL = moderate
- ES_{50} > 150 µg/mL = weak

Conclusion

2-Hydroxyphenilcalix[4]resorcinarene was synthesize in a good yield (93.14 %) and displayed strong antioxidant activities (ES $_{50}$ 77.4322 ppm) with DPPH methods.

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