Ethyl-2-(3,5-Dihydroxyfenol): Phloroglucinol derivatives as potential anticancer material

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Abstract. Ethyl-2-(3,5-dihydroxyfenol) based phloroglucinol compounds have been synthesized. Ethyl-2-(3,5-dihidroksifenol) were synthesized by reacting phloroglucinol with ethyl 2-chloro acetate in excess. Phloroglucinol reaction using 2-chloro ethyl acetate was carried out under reflux for 24 hours at a temperature of 56 °C. The reaction products were identified by a thin layer chromatography and were characterized by melting point test. Analysis of the structure of the products was obtained by FTIR spectrophotometer, 1H-NMR, and 13C-NMR. The result of the reaction between phloroglucinol and 2-chloro ethyl acetate was brownish black solid, and has a melting point of 191 °C. Based on the structural analysis by FTIR, 1H-NMR and 13C-NMR, the reaction product was a mixture of compounds which is ethyl 2- (3,5-dihidroksifenol) acetate, ethyl-2-(2,4,6-trioxocyclohexyl) acetate, and the rest of phloroglucinol which can not react.

1. Introduction
Phloroglucinol (1,3,5-trihidroxybenzene) and its derivatives have been used extensively in some commercial products. Phloroglucinol also widely used as a raw material or intermediate in the synthesis of medicinal substances, microbicides or in organic synthesis. Phloroglucinol also used as a dye for samples containing tannins on the analysis with a microscope and is also used as a dye in the leather industry, textile, and hair dyes. This compound is also used in the manufacture of explosives were stable against temperature and impacts, such as 1,3,4-triamino-2,4,6-trinitrobenzena [1].

Research on the synthesis of phloroglucinol compounds has been carried out by reacting with other compounds to form phloroglucinol derivatives to improve its bioactivity. The synthesis of a polyphenols based phloroglucinol aims to improve the bioactivity of phloroglucinol. It can be used as medicine because it was proven to prevent gastric ulcer in mice by decreasing oxidative stress in the stomach tissue. This study carried out the reaction between phloroglucinol reagent and ethyl-2-chloro acetate to form the derivatives of phloroglucinol [2].

Phloroglucinol compounds can be obtained from the isolation and synthesis. In order to enhance its bioactivity as a drug, the compounds can be prepared by derivatization of phloroglucinol. Phloroglucinol compounds have been produced through chemical synthesis. Phloroglucinol compound synthesis was done by modifying and adding a new group to improve its bioactivity as medicinal compounds. The
modifications of phloroglucinol compounds can be carried out by reacting it with an acyl phloroglucinol, phloroglucinol terpenes, glycosides phloroglucinol, halogen phloroglucinol, dimer phloroglucinol, trimer phloroglucinol, phlorotannin, and cyclic polyketides [3].

Phloroglucinol compound has two forms or tautomers, namely as the 1,3,5-trihydroxybenzene that has characters such as phenol, and 1,3,5-sikloheksanatrion (phloroglucin) which has a character such as ketones, in which the two tautomers are in equilibrium.

In the reaction that use a phloroglucinol reagent, i.e. 2-chloro ethyl acetate polyphenols, which reacts with phloroglucinol. The functional group can be in enol and keto forms. The reaction products were then to be analyzed by FTIR spectrophotometry, 1H-NMR and 13C-NMR to study their structure. In addition, the reaction product can be characterized by melting point analysis to ensure the product. Synthesis of phloroglucinol derivative with ethyl 2-chloroacetic has not been previously reported. The 2-chloro-ethyl acetate was chosen as a chemical reagent due to its availability and also inexpensive.

2. Methodology

2.1. Tools and Materials Research
The equipment used included infra-red (FT-IR, Prestige 8201 PC, KBr Pellet), Nuclear Magnetic Resonance (1H-NMR, Agilent 400 MHz). The materials used are phloroglucinol (Sigma-Aldrich), ethyl 2-Chloro Acetate (E-Merck), calcium dichloride/CaCl₂ (E-Merck), Sodium Iodide/NaI (E-Merck), Potassium carbonate/K₂CO₃ (E-Merck), Sodium Sulfate/Na₂SO₄ (E-Merck), acetone p.An (E-Merck), chloroform (E-Merck), saturated sodium chloride, sodium hydroxide p.An (E. Merck), chloric acid p.An (E. Merck), dichloromethane (E. Merck), ethyl acetate (E. Merck), acetic acid (E. Merck), methanol (E. Merck), and distilled water.

2.2. Procedure
Into a 100 mL, three-necked round bottom flask equipped with a reflux condenser and a nitrogen pipe connector, phloroglucinol (0.25 mmol), ethyl-2-chloroacetic (0.2875 g), NaI (0.355 g) and K₂CO₃ (0.425 g) were dissolved in dry acetone (50 mL). The mixture was refluxed for 24 hours. After cooling, the excess of K₂CO₃ was filtered, and the solvent was evaporated. The residue obtained was dissolved in chloroform, and then was washed with 1 M HCl (3 times) and once with saturated NaCl. After drying with anhydrous Na₂SO₄, the solvent was evaporated, then recrystallized with a mixture of methanol-chloroform (1: 1). The resulted product was dried in a desiccator and analyzed by FT-IR, ¹H NMR, Scanning Electron Microscopy (SEM) and Surface Area Analyzer (SAA).

2.2.1. Determination of Melting Point. Test the melting point by using the tool Melting Point Apparatus SMP10 Stuart. Set the temperature observations were 0-200 °C.

2.2.2. Identification of the derived phloroglucinol compound. Phloroglucinol and phloroglucinol derivative were dissolved in the mixture of ethyl acetate: methanol at a ratio of 4:1 and then was performed in a Thin Layer Chromatography (TLC). The eluent used in the process was the mixture of dichloromethane, methanol, and acetic acid at a various ratio of 9:1:1; 6: 1:1; 1:1:1, respectively.

3. Results and Discussions
Phloroglucinol can be treated with several reagents, in order to obtain products include acyl phloroglucinol terpenes, glycosides phloroglucinol, halogen phloroglucinol, phloroglucinol dimer, trimer phloroglucinol, phlorotannin and cyclic polyketides. In this study, phloroglucinol was reacted with 2-chloro ethyl acetate.
The reaction of phloroglucinol derivative was carried out by reacting the compound with ethyl-2-chloro acetate. Phloroglucinol compounds were a white powder. Meanwhile, the ethyl-2-chloro acetate was a yellow colored liquid. The colour changed from white to brown-black. It indicates the production of a new compound. Phloroglucinol compound in the reaction process is capable of experiencing Tautomerizations. The reaction is described in Figure 1. The reaction phloroglucinol compounds can be in two different forms, which where keto and enol forms.

![Figure 1](image1)

**Figure 1.** (a) scheme of phloroglucinol reaction with 2-chloro ethyl acetate in enol form (b) scheme of phloroglucinol reaction with 2-chloro ethyl acetate in keto form

3.1. Characterization of compound derivative phloroglucinol.
Characterization of the compounds was performed with melting point test resulting the 191 °C as the melting point. Meanwhile, the melting point of phloroglucinol compound is 217 °C. The change of melting point indicates that there was a reaction occurred between phloroglucinol compound, and ethyl-2-chloro acetate to produce new compounds.

3.2. Identification of Compounds Derived phloroglucinol.
The TLC identification was conducted by dissolved the product of the reaction in the mixture of ethyl acetate: methanol (4: 1). Meanwhile, the eluent was a mixture of dichloromethane: methanol: acetic acid in the ratio 9: 1: 1; 6: 1: 1; 1: 1: 1. The result of TLC identification are described in Figure 2.

![Figure 2](image2)

**Figure 2.** The TLC result of phloroglucinol and the product of reaction of phloroglucinol with the eluent of a) dichloromethane: methanol: acetic acid (9:1:1), b) dichloromethane: methanol: acetic acid (6:1:1), c) dichloromethane: methanol: acetic acid (1:1:1).
The analysis of TLC results is listed in Table 1.

Table 1. The result of TLC analysis on phloroglucinol and the product of reaction

| Eluent                                      | Phloroglucinol | Product reaction |
|---------------------------------------------|----------------|------------------|
| dichloromethane : methanol : acetic acid (9:1:1) | 1 spot, \( R_f \) : 0.58 | 1 spot, \( R_f \) : 0.92 |
| dichloromethane : methanol : acetic acid (6:1:1) | 1 spot, \( R_f \) : 0.86 | no spot, \( R_f \) : 0 |
| dichloromethane : methanol : acetic acid (1:1:1) | 1 spot, \( R_f \) : 0.94 | 3 spots \( R_f \) : 0.81 ; \( R_f \) : 0.94 ; \( R_f \) : 1.09 |
| n-hexane : acetic acid (9:1)                | No spot        | No spot          |
| n-hexane : acetic acid (6:1)                | No spot        | No spot          |
| n-hexane : acetic acid (1:1)                | No spot        | No spot          |

Table 1 shows that the products reaction exists in three spots, it indicates that the compound is still a mixture, i.e., phloroglucinol, the residual of reaction products, and the reaction that proceeded to form the keto compounds of phloroglucinol and also in the enol form. \( R_f \) value is very characteristically for a particular compound in a particular eluent. The \( R_f \) of the product of the reaction in dichloromethane: methanol:acetic acid eluent is the highest, i.e. 0.94. It indicates low polarity. That is because the polar stationary phase. The more polar compounds will be stuck firmly in the stationary phase, resulting in a low \( R_f \) value.

3.3. Derivative Structure Analysis phloroglucinol

The FTIR spectra of the phloroglucinol compounds compared to the initial reaction is shown in Figure 3.

Figure 3. FTIR spectra of phloroglucinol and the product reaction
The FTIR spectra in Figure 3 can be presented in Table 2.

| Wave number (cm⁻¹) | Phloroglucinol | Product reaction | Functional group |
|-------------------|----------------|------------------|------------------|
| 3472.02; 3339.89; 3197.15 | 3416.08 | -OH | Phloroglucinol |
| - | 2922.28 | C-H alkane | Phloroglucinol |
| - | 2850.91 | C-H alkane | Phloroglucinol |
| 1761.08 | 1709.00 | 1,3,5substitute benzene | Phloroglucinol |
| 1616.42 | 1611.59 | C=C cyclic | Phloroglucinol |
| - | 1066.68 | aryl-O-CH₂ | Phloroglucinol |

Table 2 shows that there is a new group that emerged in reaction to the compound produced from the substitution of the CH alkane of 2-chloro ethyl acetate that reacted with phloroglucinol compounds. There are two possible products which where keto and enol forms. These products were further analyzed by ¹H-NMR and ¹³C-NMR spectroscopy.

3.3.1. Structural analysis using a spectrophotometer ¹H-NMR. The reaction between phloroglucinol and 2-chloro ethyl acetate were analyzed using ¹H-NMR spectrophotometers. The spectrum is presented in Figure 4.

![Figure 4. ¹H-NMR spectra of phloroglucinol compounds](image)

Figure 4 shows that the structure of starting compound of phloroglucinol was changed. The structural change was described in Figure 1. The compounds undergo a process of phloroglucinol tautomerizations. Tautomerizations process depends on the several factors, including temperature, solvent, and pH. Phloroglucinol compounds can undergo tautomerization in polar protic solvents such as water; a single partner will be involved in a hydrogen bonding with solvents, making them less available to form hydrogen bonds with the enol form. It shows that the protic hydrogen atoms are bonded to an electronegative atom which in this case is oxygen [4].
Figure 4 shows that the analysis in the solvent CDCl₃, phloroglucinol are dominated in keto form. It because the phloroglucinol is in the solid phase and acidic conditions. Phloroglucinol compounds as well as curcumin. Curcumin in acidic conditions, neutral (with its pH at 25 °C was set as 7.0) and also in the solid phase are dominated by keto forms. Meanwhile, in alkaline condition is dominated by enol form [5]. Aromatic compounds which are in equilibrium with significant comparisons can be reduced into alkenes at a suitable condition. The product of reaction was also analyzed by ¹H-NMR to determine the structure. Based on the results of TLC identification, the compound has three spot which indicates that the reaction product is a mixture of compounds. The ¹H-NMR spectra of compounds as produced by phloroglucinol reaction are presented in Figure 5.

![Figure 5](image)

**Figure 5.** Spectra ¹H-NMR of the product of phloroglucinol reaction

Figure 5 shows 8 signals, indicating the existence of protons with 8 kinds of different environments. The interpretation of ¹H-NMR of data on the reaction products can be seen in Table 3.

**Table 3.** Interpretation of compound based on the ¹H-NMR spectra

| Signal | Chemical shift (ppm) | Integration | Functional group          |
|--------|---------------------|-------------|---------------------------|
| 1      | 0.83-0.89           | 1.27        | Methyl (-CH₃)              |
| 2      | 1.19                | 0.25        | CH₂                       |
| 3      | 1.25                | 2.17        | CH₃                       |
| 4      | 3.36-3.44           | 0.71        | -O-CH₂                    |
| 5      | 3.52-3.56           | 0.37        | -O-CH₂$_{-}$              |
| 6      | 6.7-6.74            | 0.34        | -C-H                      |
| 7      | 7.04-7.06           | 0.66        | -C-H aromatic             |
| 8      | 10.18               | 0.38        | Hidroxy (-OH)             |

The reaction scheme of phloroglucinol compounds in Figure 5, shows that the reaction product can be in the form of keto and enol. Two kinds of reactions produced enol from phloroglucinol which was reacted
with 2-chloro ethyl acetate, or phloroglucinol in the form of keto compounds which reacted with the 2-chloro ethyl acetate compound. Integration of the reaction products showed a signal that appears on the spectra as two compounds of enol and keto form. Meanwhile, for the rest of phloroglucinol compound that was not reacted is appeared on signal number 3 with the integration of 2.17 (group H totaled 10, which belongs phloroglucinol 6H and 4H keto in the form of reaction products in the form of keto). Based on analysis of the 1H-NMR spectra in Figure 5, it is known that the product of these reactions was still a mixture of compounds.

3.3.2. The structural analysis by using $^{13}$C-NMR. The product of reaction which are in enol form of phloroglucinol can be analyzed by using $^{13}$C-NMR, and the result is in Figure 6.

![Figure 6](image)

**Figure 6.** The $^{13}$C-NMR spectra of reaction product of phloroglucinol

The interpretation of $^{13}$C-NMR spectra is presented in Table 4.

**Table 4.** The interpretation of $^{13}$C-NMR spectra

| signal | Wave number (ppm) | Functional group |
|--------|------------------|-----------------|
| 1      | 11.65            | CH$_3$          |
| 2      | 14.13            | CH$_3$          |
| 3      | 22.53            | CH$_2$          |
| 4      | 29.53            | CH$_2$          |
| 5      | 31.52            | CH-R            |
| 6      | 70.15            | -O-CH$_3$       |
| 7      | 77.18            | -O-CH$_3$       |
| 8      | 122.225          | C aromatic benzene |
| 9      | 128.2            | =C-O            |
| 10     | 128.95           | C aromatic benzene |
| 11     | 148.75           | =C-O            |
| 12     | 181.0            | -C=O            |
| 13     | 199.5            | -C=O            |
| 14     | 206.2            | -C=O            |
Table 4 shows the presence of a new compound. The results of $^{13}$C-NMR analysis shows the presence of -O-CH$_2$, CH$_3$, =CO, C aromatic benzene groups. There are many other peaks that might come from phloroglucinol tautomeric keto compounds that react with 2-chloro ethyl acetate.

Structural analysis by using $^1$H-NMR and $^{13}$C-NMR, can be confirmed with the reaction mechanism between compounds in the form of enol phloroglucinol and 2-chloro ethyl acetate. The reaction mechanism is described in Figure 7. Meanwhile, the reaction mechanism between compounds phloroglucinol in the form of keto and 2-chloro ethyl acetate is described in Figure 8.

![Figure 7. The reaction mechanism in the form of enol phloroglucinol](image_url)

![Figure 8. The reaction mechanism in the form of keto phloroglucinol](image_url)

Reactions to phloroglucinol compound is strongly influenced by the leaving group on the alkyl halide. NaI use in this reaction, will change the ethyl-2-chloroacetic into ethyl-2-iodoacetat. Species I is a stronger nucleophile than Cl, therefore I able to repel Cl. As a leaving group I will more possibly move than Cl. The Cl substitution reaction by insitu I was occured to the hydroxy group of phloroglucinol. Furthermore, phloroglucinol that has hydroxyl group (OH), with O bearing a negative charge will bind the existing C atoms in the ethyl 2-iodoacetic which has positive charge. Then the I- as a leaving group will bind the existing H atoms in the hydroxy-owned phloroglucinol cluster.

Analysis using FTIR, $^1$H-NMR and $^{13}$C-NMR shows the structure of the reaction products formed phloroglucinol. The weak peak present in the FTIR, $^1$H-NMR and $^{13}$C-NMR analysis is a carbonyl group (-C=O) of ester compound that is present in the reaction products. Results of the reaction process of phloroglucinol compound with 2-chloro ethyl acetate according to the Figure 9 (phloroglucinol early in the enol form), that shows the substitution of hydroxy group at phloroglucinol with existing ester groups.
on 2-chloro ethyl acetate and it is described in Figure 10 (phloroglucinol early in the keto form).

4. Conclusions
The reaction between phloroglucinol and 2-chloro ethyl acetate produced a solid brownish black that has a melting point of 191 °C. The analysis using FTIR, 1H-NMR, and 13C-NMR confirm that the product of reaction is a mixture of compounds, i.e. phloroglucinol, ethyl 2-(3,5- dihydroxyphenol) acetate and ethyl-2-(2,4,6-trioxocyclohexyl) acetate.

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