

# Assisted ultrasonic wave of vanillin derivatives synthesis and antioxidant activity using DPPH method

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## Abstract

In recent years, the need for compounds with antioxidant activities has expanded. Generally, the natures of these compounds involve the presence of conjugated double bonds, phenolic groups, and resonance effects on the structure. One of the compounds with the phenolic group is vanillin. It can be modified into the derivative of 1,8-dioxo-octahydroxantene compound due to its aldehyde content. Meanwhile, 1,8-dioxo-octahydroxantene compound has two 2- cyclohexenone rings bound in the pyran ring and one phenolic group, expected to carry more excellent antioxidant activities than vanillin. The 9-(4-hydroxy-3-methoxyphenyl)-3,4,5,6,7,9- hexahydro -1*H*-xantene-1,8(2*H*)-dione (Compound 1) was synthesized from 1,3- cyclohexanedione and vanillin through Knoevenagel's condensation reaction aided with an acid catalyst with a single reaction phase. Besides, in this study, green chemistry was adopted using environmentally friendly catalysts from lime juice and ultrasonic wave. The synthesized compounds' structure was confirmed through the spectrophotometer IR, GC-MS, and <sup>1</sup>H-NMR spectrometer, while its antioxidant activity was tested using the DPPH method. The reaction occurred with and without lime juice catalyst, producing yields of 70.58% and 6.65%, respectively. The results of the antioxidant activity test suggested that Compound 1 carried substantially robust antioxidant activities with IC<sub>50</sub> of 0.99 ppm.

**Keywords:** 1,8-dioxo-octahydroxantene; lime juice catalyst; ultrasonic wave; antioxidant

## 1. Introduction

The current demand for compounds with pharmacological properties, mainly antioxidant, has escalated. For being easily oxidized, antioxidants can be oxidized by the free radical and prevents other molecular damages in the cells caused by free radicals oxidation. Besides, they complement the electron deficiency in the free radicals and produce single electron antioxidant intermediates. However, those intermediates are stabilized by resonance, which hinders the chain reaction and oxidative stress. Oxidative stress represents an imparity between the number of free radicals and natural antioxidants produced by the living body [1,2]. The excessive amount of free radicals within a living body may generate various degenerative diseases as they contain a number of unstable unpaired electrons so that they attempt to gain electrons from their surroundings [3]. Therefore, living creatures need external antioxidants to outgrow the disproportionate free radicals.

Commonly, compounds with antioxidant activities have conjugated double bonds, phenolic groups, and resonance effects on structure [4]. Vanillin or 4-hydroxy-3-methoxy benzaldehyde (C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>) is one of the compounds with

antioxidant activities. It is one of the food additives providing unique vanilla fragrances. This compound is generated from vanilla (*Vanilla planifolia*) isolation that can be easily found in Indonesia [5]. Vanillin only has phenolic groups, so that it requires a modification to enhance its antioxidant activity by adding conjugated double bond chromophores. Additionally, it can be transformed into a 1,8-dioxo-octahydroxantene derivative compound as it has aldehyde groups. The 1,8-dioxo-octahydroxantene compound has two 2- cyclohexenone rings bound in the pyran ring and one phenolic group predicted to have a better antioxidant activity than vanillin. This study investigated the antioxidant activity of 1,8-dioxo-octahydroxantene derivative compound synthesized from vanillin and 1,3- cyclohexanedione,9-(4-hydroxy-3-methoxyphenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanten-1,8(2*H*)-dion, which is referred as Compound 1.

Research carried out by Zukic *et al.* [6] mentioned that the 1,8-dioxo-octahydroxantene derivatives substituted with hydroxyl groups in the ortho position and methoxy group in the meta position toward the benzene ring had an antioxidant activity with 62.83% inhibition and EC<sub>50</sub> of 0.13 mM. Another study conducted by Retnosari *et al.* [7] identified that 1,8-dioxo-octahydroxantene derivatives substituted with hydroxyl groups in the para position toward the benzene ring had an antioxidant activity with IC<sub>50</sub> of < 2 ppm. It indicated that the 1,8-dioxo-octahydroxantene derivatives had the robust

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antioxidant activities. Compound 1 has hydroxy groups in the para position and methoxy groups in the meta position toward the benzene ring. Therefore, the addition of methoxy group is expected to be capable of accelerating its antioxidant activity. Since the antioxidant activity of Compound 1 has never been reported, this study aims to investigate its possible antioxidant activity.

Compound 1 can be synthesized through Knoevenagel's condensation reaction between 1,3-cyclohexanedione and vanillin (4-hydroxy-3-methoxybenzaldehyde) using an acid catalyst [8]. The acid catalyst protonates the carbonyl in the vanillin, easily inducing the nucleophile to infiltrate during the condensation. Also, the acid catalyst facilitates the pyran ring cyclization process [9].

A number of methods have been developed to synthesize Compound 1, such as the reflux method with a stirrer and alumina-sulfuric acid catalyst [9], reflux with  $\text{NbCl}_5$  catalyst [11], and heating with  $\text{VOSO}_4$  catalyst [12]. Those three catalysts have been reported to synthesize Compound 1 efficiently, but they presented a complicated catalyst preparation. Therefore, an alternative catalyst from easily obtained natural ingredients with a simple preparation process is required.

Lime and lemon juice have been confirmed to be the natural catalysts that effectively synthesize the 1,8-dioxo-octahydroxantene derivative through reflux method at  $78^\circ\text{C}$  and  $90^\circ\text{C}$  temperature for four hours [13]. That method has been reported to produce a relatively high yield, but the synthesis process required a high temperature and an extended period. Therefore, the ultrasonic wave has been selected as an alternative for a more efficient synthesis process, as proven in the study carried out by Dadhania *et al.* [14] and Mulakaya *et al.* [15]. The ultrasonic wave presents some advantages, such as high efficiency, produced minimum waste, and required low energy. Therefore, this study synthesized Compound 1 using lime juice catalyst and ultrasonic wave to identify its antioxidant activity.

## 2. Materials and Methods

### 2.1. Equipment and apparatus

The equipment used in this study included 50 ml Erlenmeyer, 10 ml measuring cup, volumetric flask (10 ml and 50 ml), vials, watch glass, thermometer, dropping pipette, object-glass, glass funnel, spatula, hotplate, vortex, analytical balance (Shimadzu with 0.001 g accuracy), oven, centrifuge, measuring pipette, a set of ultrasonic tools, a set of distillation apparatus, a set of melting point instruments Fischer Scientific Melting Point Apparatus 2555 (No. Series: 1695080502282), GC-MS (Agilent 19091S-433, Jakarta Forensic Laboratory), FTIR (Shimadzu, IR Prestige21, Central Laboratory of Universitas Negeri Malang),  $^1\text{H-NMR}$  (Agilent 500 MHz with DD2 console system, Laboratory of Institut Teknologi Bandung) and a set of UV-VIS spectrophotometer (Pharmaspec UV-1700 Shimadzu, Laboratory of Universitas Negeri Malang).

### 2.2. Materials

The materials used in this study included 1,3-

cyclohexanedione (Sigma Aldrich), vanillin (Sigma Aldrich), lemon juice, vitamin C (C IPI), DPPH 40 ppm solution, technical ethanol, chloroform, DMSO, methanol p.a., aquadest, filtering paper, and wrap. Meanwhile, the lime was obtained from a plantation in Punten Village Batu Indonesia.

### 2.3. Lime juice production

It began by slicing and squeezing the limes. Then, the lime juice was centrifuged for 15 minutes before separating the filtrate through decantation. The obtained filtrate was used as the catalyst in this study then.

### 2.4. Synthesis of compound 1

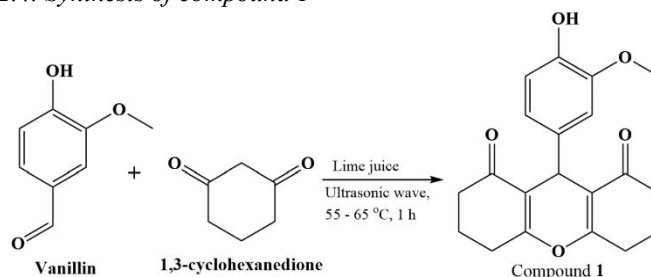


Fig. 1. Synthesis reaction

A 0.038 gram of vanillin and 0.056 gram of 1,3-cyclohexanedione were placed in Erlenmeyer. This continued by adding and stirring a 10 ml lime juice filtrate to the Erlenmeyer as shown in Figure 1. Here, the Erlenmeyer was closed using a wrap. The solution was later vibrated in the ultrasonic bath at  $55\text{--}65^\circ\text{C}$  temperature for 60 minutes. The solution within the Erlenmeyer was left all night. Subsequently, the reaction result was filtered to detach the filtrate from the residue, while the obtained residue was dried and recrystallized until obtaining the pure compound. The similar procedures were repeated without any catalysts. The developed product's yield was estimated using Formula 1.

$$\% \text{ Yield} = \frac{\text{synthesized results mass}}{\text{theoretical mass}} \times 100 \% \quad (1)$$

### 2.5. Characterization of compound 1

This started by investigating the pure synthesized compound's shape and color through a visual observation. Meanwhile, the melting point was determined by placing a small amount of sample on the object-glass. The object-glass was then placed at the Fischer Scientific melting point tool. The temperature was set, and the tool was turned on. The temperatures from the sample were entirely noted started from melting until being melted.

### 2.6. GC-MS analysis

A 5 mg of the residue from the synthesized product before the purification process was placed in the Eppendorf and dissolved using 1 ml DMSO p.a. until. The sample was then analysed using GC-MS Agilent 19091S-433UI instruments with a capillary column (HP-5MS 5% phenyl methyl siloxane, 30 m, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness, 1 mL/min initial flow). Meanwhile, Helium was used as the carrier gas. The initial and final temperatures were  $80^\circ\text{C}$  and  $325^\circ\text{C}$ ,

sequentially.

### 2.7. FT-IR analysis

A 1 mg of purely synthesized compound was mixed with dried potassium bromide (KBr) with a 1:10 ratio. It was then pressed using a hydraulic pressure vacuum pump until forming the KBr chip or film with certain thickness. The chip then was placed into the FT-IR spectrophotometer (Shimadzu IR Prestige 21).

### 2.8. <sup>1</sup>H-NMR analysis

A 10 mg of the pure synthesized result was dissolved into DMSO- *d*<sub>6</sub> solvent and tested using <sup>1</sup>H-NMR 500 MHz spectrometer (Agilent 500 MHz with DD2 control system). The <sup>1</sup>H-NMR spectrum showed the number of neighboring protons and the ratio of its protons following a particular chemical shift signal (ppm).

### 2.9. Antioxidant activity

The antioxidant activity of Compound 1 was investigated using the DPPH method [16]. A 0.001 gram of residue was placed into the 10 ml volumetric flask and dissolved into chloroform until the border mark. Then, it continued with the dilution of 1 ml of the solution with 10 ml chloroform to produce a solution with a ten ppm concentration. 0.5 ml, 1 ml, 1.5 ml, and 2 ml of the ten ppm solution were blended with 10 ml chloroform, generating Compound 1 with 0.5, 1, 1.5, and 2 ppm concentration. DPPH was prepared with methanol as a solvent. 1 ml from each sample was mixed with 2 ml of DPPH 40 ppm reagent. The solution was vortexed and incubated for 30 minutes. Its absorbance was measured at 517 nm wavenumber using a UV-Vis spectrometer (Pharmaspec UV-1700 Shimadzu). The mensuration was carried out triple using 2 ml of DPPH 40 ppm added with 1 ml of chloroform as the negative control. Besides, this study also measured the antioxidant activity of vitamin C with 2, 4, 6, 8, and 10 ppm as the positive control. Meanwhile, the antioxidant activity of vanillin was investigated using the same procedure used to estimate the antioxidant activity of vitamin C. The vanillin's antioxidant activity investigation was to compare the antioxidant activity between vanillin and Compound 1. The obtained absorbance value from each measurement was used to estimate the inhibition percentage of each compound, calculated using Formula 2.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \% \quad (2)$$

## 3. Results and Discussion

The results of 1,3-cyclohexanedione and vanillin reaction using ultrasonic wave at 55–65°C temperature for 60 minutes with and without lime juice catalyst were in the form of white powder. The powder was later analyzed using GC-MS to identify the components within the product obtained. The GC chromatogram from the 1,3-cyclohexanedione and vanillin reaction using lime juice catalyst showed a single peak at 15.909 minutes retention time with 100% area. This peak was

presumed to be Compound 1 with 100% area, as presented in Figure 2a. It was also emphasized by the appearance of a peak at the mass spectrum of *m/z* 340.2, the molecular ion weight [M]<sup>+</sup> of the target compound. Besides, the presence of fragment *m/z* 325 and *m/z* 309 as the release of methyl and methoxy groups also sustained the presumption.

The results of GC-MS analysis suggested that the water solvent with no catalyst synthesis produced four peaks of the reaction product, as presented in Figure 2b. The first peak with 3.864 retention time and 35.98% area was assumed to be the remaining unreacted 1,3-cyclohexanedione compound, as a peak with an *m/z* value of 112 appeared at the mass spectrum corresponding to the molecular weight of 1,3-cyclohexanedione. The second peak with a retention time of 6.305 minutes and an area of 2.05% was inferred as the unreacted vanillin compound, as proven from the presence of a peak with 152 *m/z* value, consistent with vanillin's molecular mass.

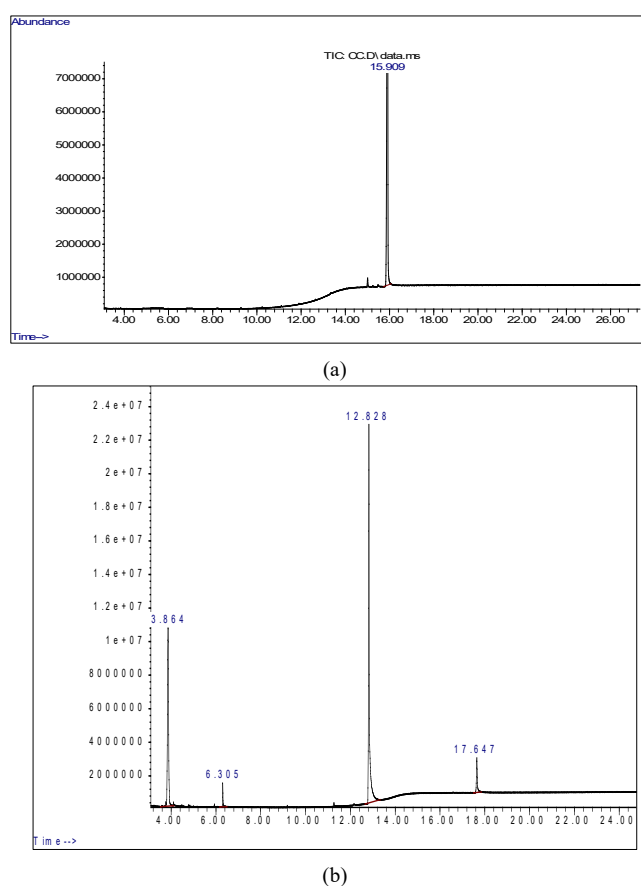


Fig. 2. GC (Gas Chromatography) Chromatogram of (a) Synthesized Product with Lime Juice Catalyst, (b) Synthesized Product without Catalyst

The 151 *m/z* and 123 *m/z* fragments due to the discharge of 1 H and the aldehyde group (-CHO) also strengthened that presumption. Meanwhile, the third peak with 12.828 minutes retention time and 56.71% area was assumed to be Compound 2 (2-(4-hydroxy-3 methoxybenzylidene) cyclohexane-1,3-dione), resulted from the first Knoevenagel's condensation between 1,3-cyclohexanedione and vanillin, as a peak with *m/z* 246 value appeared along with the presence of *m/z* 231 and *m/z* 215 fragments caused by the discharge of methyl and methoxy groups. The fourth peak was observed to have 17.647 minutes retention time, and 5.28% area was expected to be Compound

1. Table 1 presents the summary of GC-MS analysis results of the synthesized product with no catalyst.

Table 1. GC-MS Analysis of synthesized compounds without catalyst

Peak	Retention Time (minute)	Area (%)	Compound's Name
1	3,864	36,96	1,3-cyclohexanedione
2	6,305	2,05	Vanillin
3	12,828	56,71	Compound 2
4	17,647	5,28	Compound 1

As shown from the GC-MS analysis results, Compound 1 could be formed even using water solvent, but it produced low yield. Table 2 shows the yields of the reaction using lime juice catalyst without catalyst. Compound 2 was the primary product from the non-catalyst reaction that became the intermediate of Compound 1 formation. Therefore, water acted not only as a solvent but also as the catalyst in the synthesis process using ultrasonic wave [17]. Water has a role in the formation of the hydrogen bonds that are formed from a reaction between the carbonyl group from 1,3-cyclohexanedione and the oxygen atom in the water, along with the reaction between oxygen from vanillin's carbonyl group and hydrogen atom in the water. That hydrogen bond enolized the 1,3-cyclohexanedione, increasing its nucleophilic character due to the activated methylene carbon from 1,3-cyclohexanedione. Meanwhile, the hydrogen bond between water and vanillin activated vanillin's carbonyl group that enhanced its electrophilic character.

Table 2. Synthesis Results

Catalyst	55-65 °C (60 minutes)	
	Mass	%Yield
With no catalyst	0,107 gram	6,65%
Lime juice	0,060 gram	70,58%

The addition of lime juice catalyst on the 1,3-cyclohexanedione and vanillin reaction using ultrasonic waves significantly increased the obtained yield, compared to the non-catalyst synthesis result, as shown in Table 1. Consequently, the acid catalyst expedited the Compound 1 reaction. In addition, lime contains several organic acids, such as citric acid, lactic acid, malic acid, tartaric acid, ascorbic acid, and oxalic acid [18].

The adoption of the ultrasonic wave also brought some effects on the obtained Compound 1's yield. A research conducted by Napoleon [8] identified that the synthesis of 1,8-dioxo-octahydroxantene derivative compounds using the reflux method for three hours using lime juice catalyst produced 60% yields. However, the reflux method required a long period and a high temperature. Meanwhile, this study has successfully generated 70.58% yield using ultrasonic waves at 55-65°C temperature for 60 minutes. Therefore, the ultrasonic wave has been confirmed to be more effective and efficient in synthesizing Compound 1. Also, the ultrasonic wave has been confirmed to be an alternative energy for organic reactions that require heating, such as cycloaddition, nucleophilic addition, condensation, oxidation [19], and transesterification reactions [20]. In the ultrasonic wave, sound energy concentration occurs, inducing the formation of unstable bubbles that grow bigger in a short time until the maximum limit and burst, creating considerable energy, high pressure, and local

temperature increase. Thus, it exceeds its activation energy [21,22]. Hotspot represents the local area with high temperature and pressure, which becomes the chemical and physical effect sources caused by acoustic cavitation [23]. Therefore, ultrasonic wave offers a number of benefits, including low temperature, short period, and minimum amount of energy [24].

The GC-MS analysis results showed that Compound 1 has been composed using lime juice catalyst and no catalyst. Consequently, a purification process for Compound 1 was carried out through recrystallization using ethanol. The purification result was in the form of white powder with a melting point at 226-228°C temperature. This result was linear with the previous study carried out by Ghatak *et al.* [12]. The purified product was analyzed using an infrared spectrophotometer <sup>1</sup>H-NMR spectrometer.

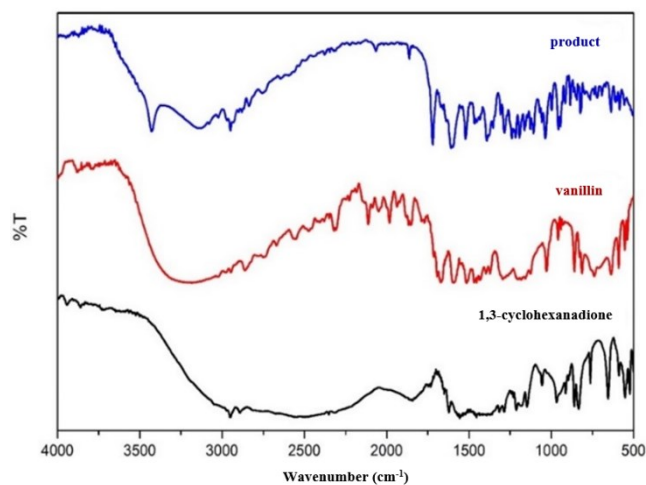


Fig. 3. The FTIR Spectrum of the Product and Reactant

The synthesized product's structure identification was completed through FTIR analysis to identify the compound's function group. The IR spectrum of Compound 1 presented six particular absorption bands, as shown in Figure 3. The first band had a strong and wide intensity at 3313.71 cm<sup>-1</sup> wave number, indicating the O-H bond stretching vibration. The second absorption band showed medium and sharp intensity at 2953.02 cm<sup>-1</sup> wavenumber, representing the aliphatic C-H bond stretching vibration. Meanwhile, the third absorption band showed robust and sharp intensity at 1662.21 cm<sup>-1</sup> wavenumber, signifying the C=O bond stretching vibration. The fourth band showed strong and sharp intensity at a wavenumber of 1620.21 cm<sup>-1</sup>, suggesting C=C aliphatic bond stretching vibration. The fifth band had robust and sharp intensity at a wavenumber of 1512.19 cm<sup>-1</sup>, indicating the C=C aromatic bond stretching vibration existence. Lastly, the sixth absorption band had strong and sharp intensity at 1274.95 cm<sup>-1</sup> wavenumber, signifying the C-O stretching vibration. The IR analysis results are in accordance with the study conducted by Dos Santos *et al.* [11]. The formation of the product was also indicated by the disappearance of the signal at wave number 1714 cm<sup>-1</sup> in the IR spectra of the product, representing the carbonyl aldehyde signal on vanillin. Also, the appearance of a signal at wave number 1662.61 cm<sup>-1</sup> represented a carbonyl ketone signal, followed by the appearance of a signal at wave number 1620.21 cm<sup>-1</sup>, which represented a C=C aliphatic bond. The appearance of a signal at wave number 3313.71 cm<sup>-1</sup> representing the -OH bond in the IR spectrum of the product,

also distinguished it from the IR spectrum of 1,3-cyclohexanadion.

Furthermore, the results of the  $^1\text{H-NMR}$  analysis identified ten primary signals at the H-NMR spectrum. The signal (2H, *m*) was observed at 1.82 ppm and 1.93 ppm chemical shift, constituting the methylene group in the cyclohexene ring (H-17 and H-20). The signal (4H, *m*) is at 2.24 ppm and 2.54 ppm chemical shift, represented by H-16, H-18, H-20, and H-21. The primary features of 1,8-dioxo-octahydroxantene's H-NMR spectrum were the presence of (1H, *s*) signal at 4.5 ppm chemical shift, and the methine group proton at pyran ring (H-9). Meanwhile, the proton from benzene ring (H-1, H-2, and H-4) was exhibited by signal (1H, *s*), signal (1H, *dd*), and signal (1H, *d*) at 6.73, 6.49, and 6.58 ppm chemical shift, respectively, while the proton from methoxy group (H-8) was shown by the signal (3H, *s*) at 3.70 ppm chemical shift. Simultaneously, the proton from the hydroxyl group (H-7) was presented by signal (1H, *s*) at 8.71 ppm chemical shift. The multiple signals that appeared at  $\delta$  2.24 ppm and  $\delta$  2.54 ppm should have been materialized as triple signals due to the presence of two neighboring hydrogen. Similarly, the multiple signals at  $\delta$  1.82 ppm and  $\delta$  1.93 ppm should have been emerged as quintet because of the presence of four adjacent hydrogen. It may occur due to the low resolution of the  $^1\text{H-NMR}$  spectrophotometer so that the proton signal cannot experience splitting.

The mensuration carried out through integration technique has observed seven signals' intensity of 2.3, 2.1, 4.3, 4.3, 3.2, 1.1, 1.1, 1.0, 1.0, and 0.9. The signal intensity's simplification results in the hydrogen ratio of 2: 2: 3: 4: 4: 1: 1: 1: 1: 1. It illustrated 20 hydrogen atoms, consisting of four CH groups, six  $\text{CH}_2$  groups, one OH group, and one  $\text{OCH}_3$  group. Therefore, the hydrogen ratio corresponded to the H number of the proton group in the targeted compounds' structure. The interpretation of the  $^1\text{H-NMR}$  spectrum for the synthesized product was correlated with previous studies, as summarized in Table 3 and shown in Figure 4 for corresponding proton numbering in the compound.

Table 3.  $^1\text{H-NMR}$  of Synthesized Product Interpretation and Previous Study's Interpretation

Group	The Result of $^1\text{H-NMR}$ (500 MHz; DMSO- <i>d</i> 6) research		[25] $^1\text{H-NMR}$ (400 MHz; DMSO- <i>d</i> 6)	
	$\delta$ (ppm)	Multiplicity	$\delta$ (ppm)	Multiplicity
H-17	1.82-	2H, <i>m</i>	1.80-	2H, <i>m</i>
	1.88		1.91	
H-20	1.93-	2H, <i>m</i>	1.92-	2H, <i>m</i>
	1.97		2.10	
H-18 dan H-19	2.24-	4H, <i>m</i>	2.28-	4H, <i>m</i>
	2.33		2.31	
H-16 dan H-21	2.54-	4H, <i>m</i>	2.50-	4H, <i>m</i>
	2.68		2.71	
H-8	3.70	3H, <i>s</i>	3.71	3H, <i>s</i>
H-9	4.50	1H, <i>s</i>	4.51	1H, <i>s</i>
H-2	6.49	1H, <i>dd</i>	6.50-	1H, <i>m</i>
			6.53	
H-4	6.58	1H, <i>d</i>	6.59-	1H, <i>m</i>
			6.61	
H-1	6.73	1H, <i>s</i>	6.74-	1H, <i>m</i>
			6.75	
H-7	8.71	1H, <i>s</i>	8.71	1H, <i>s</i>

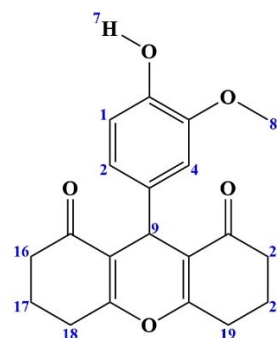


Fig. 4. Compound 1 proton numbering

The antioxidant activity test was carried out through DPPH free radicals scavenging using UV Vis spectrophotometer for the absorbance measurement. This method was selected for its modesty, convenience, speed, and low cost. Once the DPPH free radical reacted with the compound that could supply hydrogen, the DPPH molecules were reduced into non-radicals (DPPH<sub>2</sub>). The interaction between antioxidant and DPPH<sub>2</sub> could be in hydrogen donor or electron transfer [5]. The reaction was indicated by the fading of its purple color and the reduction of its absorbance value. After 30 minutes, the absorbance of Compound 1 solution, negative control, and positive control were measured using UV Vis spectrophotometer at 517 nm wavenumber. The 30 minutes incubation time was selected to ensure the occurrence of the reaction. The antioxidant compound's inhibition percentage was obtained from a calculation using Formula 2.

The chloroform solution was divided into the positive and negative control used to investigate the unreduced DPPH radical absorbance by Compound 1. A higher absorbance difference between the control and tested solution at various concentrations represented a more significant inhibition percentage. The  $\text{IC}_{50}$  value was a number signifying the tested sample's concentration (ppm) and its ability to hinder 50% of the oxidation process. A lower  $\text{IC}_{50}$  value illustrated the compound's higher antioxidant activity. This value was determined by drawing an inhibition percentage chart toward the vitamin C, vanillin, and Compound 1 concentration. Then, the linear regression equation of the graph was attained by substituting the y with 50. The results showed that Compound 1  $\text{IC}_{50}$  value was 0.99 ppm, as presented in Figure 5. It indicated that Compound 1 had an excellent ability in obstructing DPPH in a minimum concentration. Comparatively, the  $\text{IC}_{50}$  value of the compound's initial material (vanillin) was 10.06 ppm, as presented in Figure 6. Meanwhile, the positive control (vitamin C) attains  $\text{IC}_{50}$  of 6 ppm, as shown in Figure 7. Therefore, Compound 1 had exceptional antioxidant activity from the increased antioxidant activity between vanillin, classified as a phenolic compound, and Compound 1, the 1,8-dioxo-octahydroxantene derivative compound.

The antioxidant activity of Compound 1 in this study was found greater than the antioxidant activity of 1,8-dioxo-octahydroxantene derivative compound substituted with hydroxyl and methoxyl groups, previously reported by Zukic et al. [6], and the 1,8-dioxo-octahydroxantene derivative compound substituted with hydroxyl group revealed in the study by our previous publication [7,13]. The study carried out by Nirwani et al. [26] presented that the hydroxyl group

could hinder the free radicals reaction continuity through the hydrogen radical donation to the free radicals by hydroxyl group homolytic reaction producing more stable and less reactive free radicals. Similarly, the same reaction also occurred in the methoxy groups, as the electron booster, increasing the compound's antioxidant activity. The position differences between hydroxyl and methoxyl groups toward the benzene ring have brought some substantial effects on the antioxidant activity. This difference may be induced by the distinctive stability of the formed radicals.

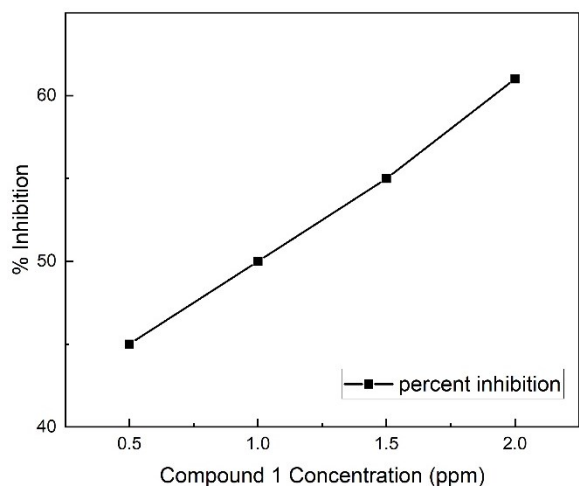


Fig. 5. Inhibition Percentage toward Compound 1 Concentration Variation

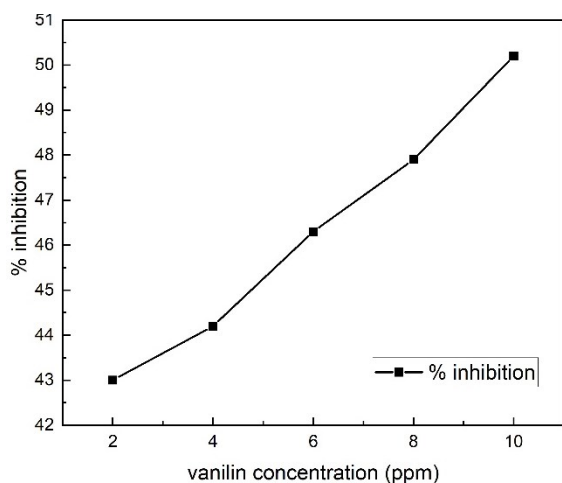


Fig. 6. Inhibition Percentage toward Vanillin Concentration Variation

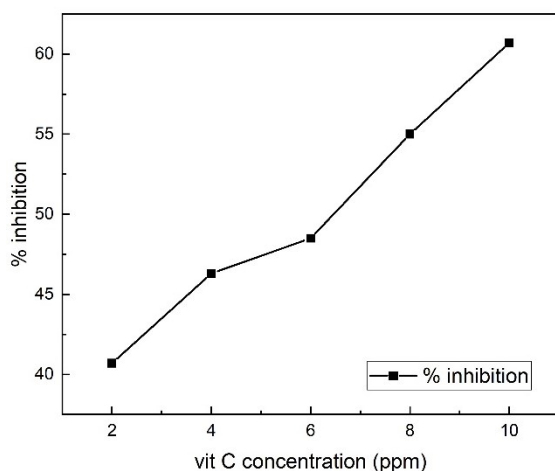


Fig. 7. Inhibition Percentage toward Vitamin C Concentration Variation

## 4. Conclusion

Compound 1 has been successfully synthesized from vanillin and 1,3-cyclohexanedione with and with no catalyst at 55–65° C temperature for 60 minutes. The yields of synthesized products with and without lime catalysts were 70.58% and 6.65%, respectively. It confirmed ultrasonic wave efficiency in synthesizing Compound 1 using lime juice catalyst since it required a lower temperature and shorter period. Besides, Compound 1 has been verified to carry an excellent antioxidant activity with an IC<sub>50</sub> value of 0.99 ppm. Its antioxidant activity was found greater than the antioxidant activity of vanillin and vitamin C. Therefore, Compound 1 had great potential as the new source of antioxidants. However, further in vivo study on antioxidant activity and cytotoxicity test of Compound 1 is required.

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