Journal of
Food and Pharmaceutical Sciences

Original Article

DPPH Scavenging activity, Reducing Power, and Metal Chelating Capacity of Compound 1-(2,5-dihydroxyphenyl)-3-pyridine-2-yl-propenone

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Received: 16 March 2023; Revised: 28 November 2023; Accepted: 8 December 2023; Published: 21 December 2023

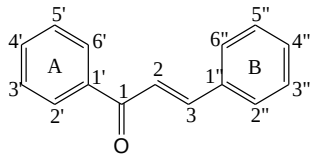
Abstract: The high amount of free radicals in the body could stimulate oxidative stress which has been implicated in several diseases such as cancer, inflammation, aging, and cardiovascular diseases. The presence of antioxidants can diminish the reactivity of free radicals. However, the antioxidant defense system in the body may be insufficient thus intake of dietary antioxidants is recommended. Natural flavonoid such as chalcone has been known to exert several biological activities, especially antioxidant activity. Previous study proved that replacement of benzene ring into pyridine ring could improve the antioxidant activities. A pyridine-based chalcone, compound 1-(2,5-dihydroxyphenyl)-3-pyridine-2-yl-propenone (AEW-1) was successfully synthesized using microwave radiation and several studies reported its biological activities such as antiinflammation, anticancer, and antibacterial activities. Those activities are thought to be related to antioxidant mechanisms. Therefore, this study aimed to evaluate its antioxidant activity. Methods: The antioxidant activities were performed with three different methods: DPPH scavenging activity, ferric reducing power, and metal chelating capacity. Results: AEW-1 showed DPPH scavenging activity, ferric reducing power, and metal chelating capacity with IC_{50s} values of 4,471 ± 0,052 µg/mL; 156.56 ± 4.42µg/mL; and 6,273 ± 0,025 µg/mL, respectively. Conclusions: Our results found that AEW-1 had quite potent antioxidant activity.

Keywords: 1-(2,5-dihydroxyphenyl)-3-pyridine-2-yl-propenone, chalcone, antioxidant activities

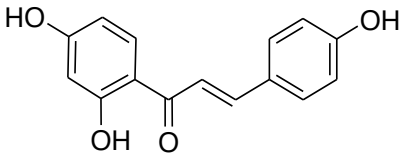
1. INTRODUCTION

Atoms or molecules that carry unpaired electrons, which are known as free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulphur species (RSS), can cause the destabilization of other molecules resulting in more and more reactive free radicals [1]–[3]. The imbalance between the generation and elimination of free radicals induces 'oxidative stress' and thus triggering the development of various illness such as cancer, inflammation, aging, cardiovascular, and neurodegenerative diseases [3],[4]. The excessive free radical in the body can be prevented or reduced by the presence of antioxidants. In addition, it is also necessary to intake dietary antioxidants to support the antioxidant defense system in the body [1],[5].

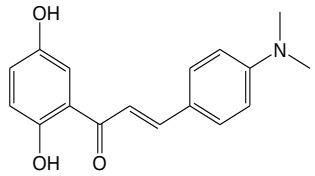
Natural products such as flavonoids have been known to exhibit antioxidant activity [2],[3]. A chalcone (Figure 1), a compound belonging to the flavonoids is characterized by typical scaffold of 1,3-diaryl-2-propen-1-one, in which two aromatic rings are connected by three carbon atoms [6],[7]. These compounds are found in all parts of the plants such as fruits, leaves, roots, and seeds and they possess a broad variety of biological activities such as antibacterial, antioxidant, anti-inflammatory, anticancer, and neuroprotective effects [2],[7]. Single chalcone exerts several types of biological activities. For an instance, isoliquiritigenin (Figure 1) exhibited antioxidant, anticancer, and anti-inflammatory activities [7]. Narsinghani et al. synthesized five hydroxychalcone derivatives and reported one derivate, 2',5'-dihydroxy-4-dimethylaminochalcone (Figure 1) was found to be the best chalcone derivate in DPPH radical scavenging, reducing power, and iron chelating activities[8]. The DPPH radical scavenging activity of 3,4,2',4'-tetrahydroxychalcone, known as butein (Figure 1) was found to be higher than Trolox (vitamin E), with reduction ratios of 7.87 and 0.32, respectively [9].



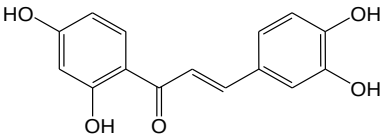
Chalcone



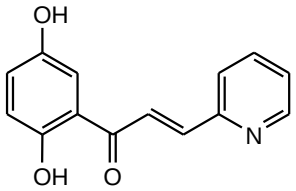
Isoliquiritigenin



2',5'-dihydroxy-4-dimethylaminochalcone



Butein



1-(2,5-dihydroxyphenyl)-3-pyridine-2-yl-propenone (AEW-1)

Figure 1. The structures of chalcone compounds

Wibowo *et al* successfully applied microwave radiation to synthesize a pyridine-based chalcone, namely 1-(2,5-dihydroxyphenyl)-3-pyridine-2-yl-propenone or AEW-1 (Figure 1) from pyridine-2-carbaldehyde and 2,5-dihydroxyacetophenonein alkaline medium using NaOH and K_2CO_3 [10], [11]. This chalcone derivate is unique since the ring B was replaced with a pyridine ring. Several studies were conducted to explore its biological activities included anti-inflammatory, anticancer, and antibacterial activities [11]–[13]. Those activities are thought to be related to the antioxidant mechanism. As we know that antioxidant reduces the excessive free radicals such as ROS, RNS, etc [1], [5]. Those radicals affect the immune system by increasing the proinflammatory

differentiation and secretion. In chronic condition, it could promote the inflammation-driven cancer. In addition, free radicals damage the DNA and modulate some signalling pathway thus it leads to cancer progression [14]. Antioxidant could act as antibacterial by disrupting membrane permeability, inhibition of cell wall formation, and inhibition of nucleic acid synthesis [15]. Mojarrab et al synthesized and tested the antioxidant activities of pyridine-based chalcones. Out of ten, three compounds exhibited higher metal chelating activity than quercetin [16]. Hence, the purpose of this study was to evaluate the antioxidant activities of AEW-1 through various methods: DPPH radical scavenging activity, ferric reducing power, and metal chelating capacity.

2. MATERIALS AND METHODS

The synthetic compound, AEW-1 was prepared according to Wibowo *et al*. (2021) [11]. The reagents used to examine the antioxidant activities were 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma), potassium ferricyanide ($K_3Fe(CN)_6$ (Merck), iron (II) chloride (FeCl2) (Merck), iron (III) chloride (FeCl3) (Merck), ferrozine (SIGMA), methanol (Merck) and ethanol (Merck), phosphate buffer, trichloroacetic acid, and distilled water. Quercetin (SIGMA) was selected as a reference compound in DPPH radical scavenging activity and ferric reducing power while EDTA (Merck) was selected in metal chelating capacity. All the chemicals listed above and others used in the experiment were of analytical grade. All of the antioxidant activities were measured using HITACHI U-2900 spectrophotometer.

2.1 DPPH radical scavenging assay

This assay was determined based on the scavenging of DPPH radicals [5], [17]. One mL of 0.1 mM DPPH solution in methanol was added to 4 mL of sample in various concentrations. The mixtures were kept for 30 min in the dark to complete the reaction. After half an hour, the absorbance was measured at 517 nm and the radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(Ac - As)}{Ac} x100\ \%$$

2.2 Ferric reducing antioxidant power (FRAP)

FRAP assay was carried out to measure the ability of a compound in reducing $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$ [5]. Two and a half mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of $K_3Fe[(CN)_6]_3$ (1% w/v) were added to 1.0 mL of the water-based solutions with various concentrations of the sample. Those mixtures were incubated at 50°C for 20 min followed by the addition of 2.5 mL trichloroacetic acid (10% w/v). The mixtures were centrifuged at 3000 rpm for 10 minutes then the upper layer (2.5 mL) was taken and mixed with 2.5 mL of distilled water. The addition of 0.5 mL $FeCl_3$ (0.1%, w/v) to the reduced product leads to the production of Perl's Prussian Blue complex, ($Fe_4[Fe(CN\text{-})_6]_3$) which could be measured at 700 nm [2].

$$\text{Ferric reducing power (\%)} = \frac{(As - Ac)}{As}\ x100\ \%$$

2.3 Metal chelating assay

The presence of chelating compounds can reduce the intensity of the color of the Fe^{2+}-ferrozin complex [5]. In this assay, 0.1 mL of sample in various concentrations was added to 0.5 mL of $FeCl_2$ solution (0.2 mM), followed by the addition of 0.2 mL of ferrozine (5 mM). The mixture was incubated for 10 minutes at room temperature then the absorbance was measured at 562 nm. The inhibition the formation of the Fe2+-ferrozine complex can be calculated using the equation as given below:

$$\text{Metal chelating capacity (\%)} = \frac{(Ac - As)}{Ac} x100\ \%$$

Where: Ac (absorbance control) is the absorbance value of the solution without the sample and As (absorbance sample) is the absorbance value of the sample solution. All data were analyzed and a linear regression equation between the concentrations and the antioxidant activity (%) was made to calculate the IC_{50} value.

3. RESULTS AND DISCUSSION

3.1 DPPH radical scavenging activity

DPPH radical scavenging assay is the most widely used method for assessing antioxidant activity [18]. The radical scavenging ability of antioxidant indicates through their interaction with the stable free radical DPPH[8]. This scavenging assay is based on the electron donation of antioxidants to neutralize DPPH radical [18]. It can be seen (Table 1) that AEW-1 showed DPPH scavenging activity and the interaction with DPPH was found to be concentration dependent (Figure 2A). Interestingly, its activity (IC_{50} 4.47 ± 0.05 μg/mL) was comparable with quercetin (IC_{50} 4.31 ± 0.07 μg/mL).

Table 1. The DPPH scavenging activity of quercetin and AEW-1

Concentration	Quercetin (%)				AEW-1 (%)			
(μg/mL)	1	2	3	$\bar{x}$ ± SE	1	2	3	$\bar{x}$ ± SE
1.4	12.67	17.60	18.00	16.09 ± 1.71	9.07	16.13	12.67	12.62 ± 2.04
2.8	30.00	28.40	30.00	29.47 ± 0.53	28.67	28.80	27.47	28.31 ± 0.42
4.2	45.60	46.67	47.20	46.49 ± 0.47	43.60	46.53	44.67	44.93 ± 0.86
5.6	65.73	66.80	70.93	67.82 ± 1.59	63.87	63.60	63.87	63.78 ± 0.09
7.0	84.00	86.00	80.40	83.47 ± 1.64	81.33	82.00	82.40	81.91 ± 0.31

3.2 Ferric reducing power

Fe (III) reducing power assay is frequently applied as an indicator of electron-donating capacity. In this assay the antioxidant reduce Fe^{3+} to Fe^{2+} by donating an electron thus Prussian blue will be produced as the end product [8]. There are two mechanisms for how Prussian blue is yielded. Antioxidants reduce Fe^{3+} to Fe^{2+}, which interacts with ferricyanide to produce Prussian blue or vice versa it reduces the ferricyanide to ferrocyanide, which interacts with free Fe^{3+} thus Prussian blue appears [18]. Our result showed that the ferric reducing ability of AEW-1 (IC_{50} 156.56 ± 4.42 μg/mL)

was higher than quercetin (IC_{50} 168.48 ± 1.23 µg/mL) and this ability also grew with the rise in their concentrations (Table 2 and Figure 2B).

Table 2. Reducing power of quercetin and AEW-1

Concentration	Quercetin (%)				AEW-1 (%)			
(µg/mL)	1	2	3	$\bar{x}$ ± SE	1	2	3	$\bar{x}$ ± SE
100	35.49	38.01	38.89	37.46 ± 1.02	42.04	35.32	37.24	38.20 ± 2.00
200	46.94	58.33	56.56	53.94 ± 3.54	56.63	66.30	59.21	60.71 ± 2.89
300	66.29	64.37	65.78	65.48 ± 0.57	65.01	62.16	65.71	64.30 ± 1.09
400	71.22	70.90	70.69	70.94 ± 0.15	71.65	74.15	70.66	72.16 ± 1.04
500	74.03	73.20	72.98	73.40 ± 0.32	75.15	75.46	76.96	75.86 ± 0.56

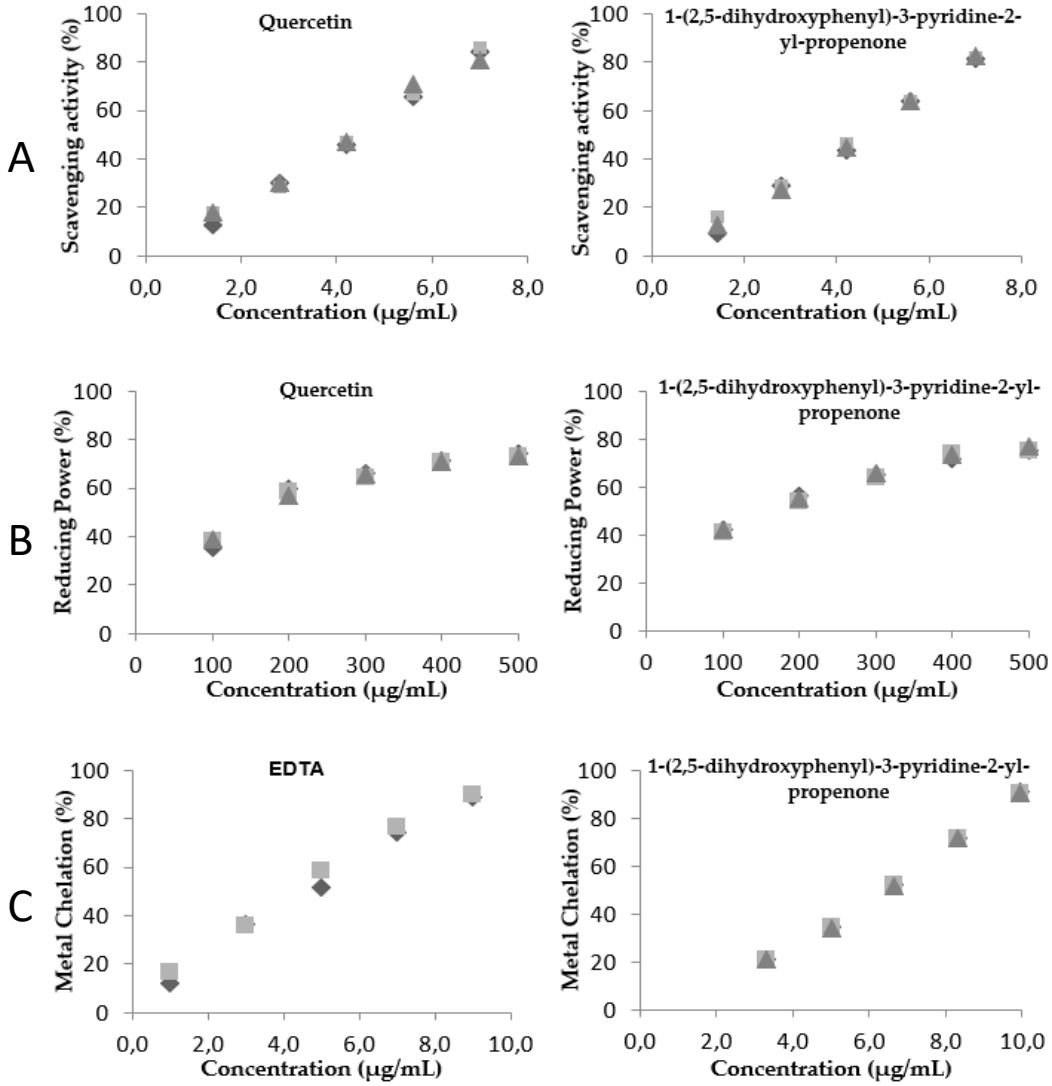


Figure 2. The antioxidant activities of AEW-1, quercetin, and EDTA in DPPH assay (A), FRAP assay (B), and metal chelating assay (C). All three compounds exhibited the antioxidant activity in a concentration-dependent manner.

3.3. Metal chelating capacity

Iron is an essential mineral because it is needed in some functional physiology such as respiration, enzyme activation, and oxygen transport[8], [18]. However, iron is known as the most reactive pro-oxidant and catalyzes oxidative changes in several cellular component, mainly the lipid [8]. An antioxidant is identified as metal chelator if there is a complex formation between the antioxidant and the metal. Based on the results, AEW-1 could chelate the metal (Fe^{2+}) in a concentration-dependent manner (Figure 2C). The chelation capacity of this compound and the reference compound, EDTA were provided in Table 3. EDTA is selected since it is often applied in pharmaceutical product analysis as the standard metal chelator [18]. EDTA had an IC_{50} of 4.567 ± 0.16 µg/mL while AEW-1 had lower activity with an IC_{50} value of 6.273 ± 0.03 µg/mL.

Table 3. Metal chelating activity of EDTA and AEW-1

Concentration	EDTA (%)				Concentration	AEW-1 (%)			
(µg/mL)	1	2	3	$\bar{x}$ ± SE	(µg/mL)	1	2	3	$\bar{x}$ ± SE
1.4	12.32	13.04	16.79	14.05 ± 1.39	3.3	21.25	21.43	21.07	21.25 ± 0.10
2.8	36.25	35.71	36.07	36.01 ± 0.16	5.0	34.82	34.46	33.93	34.40 ± 0.26
4.2	51.61	56.25	58.75	55.54 ± 2.09	6.6	52.32	52.14	51.79	52.08 ± 0.16
5.6	74.46	73.04	76.61	74.70 ± 1.04	8.3	71.79	71.61	71.43	71.61 ± 0.10
7.0	88.93	92.32	89.82	90.36 ± 1.02	9.9	91.07	90.36	90.18	90.54 ± 0.27

Three different methods were performed to assess the antioxidant activity of AEW-1, viz. DPPH scavenging activity, ferric reducing power, and metal chelating capacity. In general, there are two mechanisms how antioxidants can reduce free radical activity, namely HAT (Hydrogen Atom Transfer) and ET (Electron Transfer) [2], [8]. Two methods, DPPH and FRAP apply ET-based mechanism [18]. As a reference, quercetin (Figure 3) was chosen since it has been known as one of the best flavonoids which exhibits a broad spectrum of biological activities, especially its strong antioxidant activity [2].

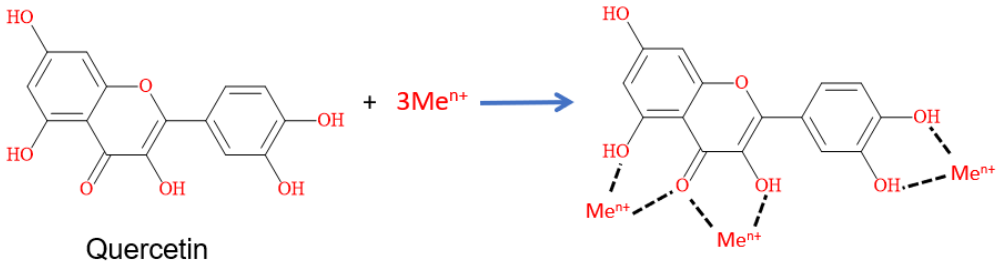


Figure 3. Metal chelating activity of quercetin

The strong antioxidant properties of quercetin are mainly due to the phenol groups which can act as radical scavengers, reducing agents, hydrogen donors, and even as metal chelators [3]. The position of OH groups on the ring determines whether the antioxidant is active or inactive. Instead of meta-position, ortho- or para-position contributes more to the antioxidant activity since the electron density increase and the oxygen-hydrogen bond energy is lower on those position [2], [6].

The metal chelating capacity of phenolic antioxidant strongly depends on the arrangement of carbonyl and hydroxyls groups. In the case of quercetin, the binding sites for metals was shown in Figure 3.

Table 4. Summary of antioxidant activities

Compound	Antioxidant Activity (IC_{50} in μg/mL)		
	DPPH radical scavenging	Ferric reducing power	Metal chelating capacity
AEW-1	4.47 ± 0.05	156.56 ± 4.42	6.27 ± 0.02
Quercetin	4.31 ± 0.07	168.48 ± 1.23	-
EDTA	-	-	4.57 ± 0.16

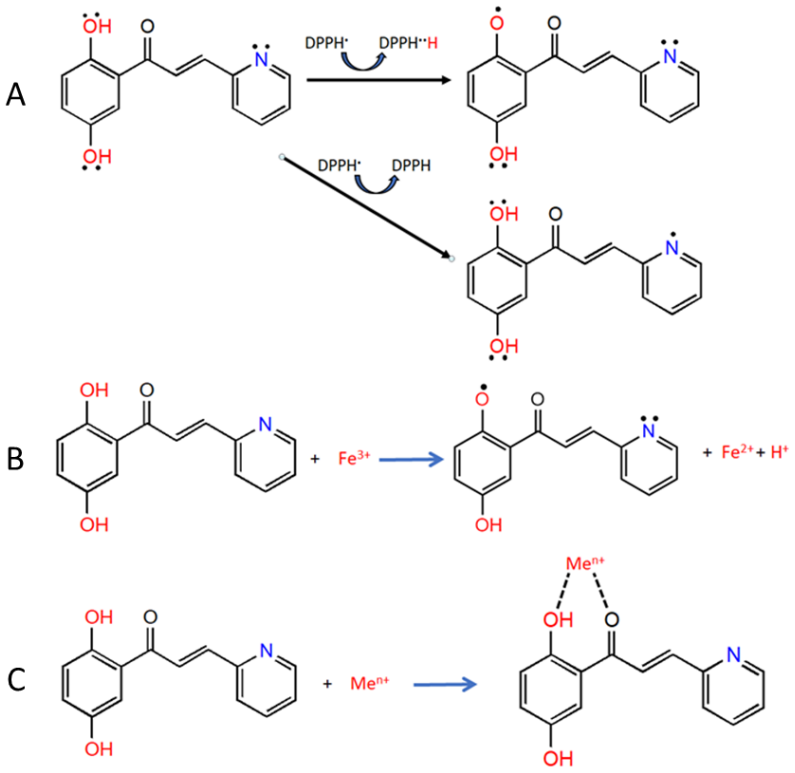


Figure 4. The antioxidant mechanisms of AEW-1: DPPH scavenging activity (A), ferric reducing power (B), and metal chelating capacity (C)

Based on the antioxidant activity results (Table 4), AEW-1 showed quite potent activity. Interestingly, its DPPH radical scavenging activity was comparable to quercetin. The OH groups and α,β-unsaturated carbonyl that present in the structure must be responsible for the antioxidant activity. As the scavenger (Figure 4A), the OH groups transfer one electron to the free radical. Subsequently, the unpaired electron delocalizes at the ring lead to the production of a stable phenoxy radical (Figure 5A). Not only occurs in the phenol ring but the delocalization of the unpaired electron also occurs in α,β-unsaturated carbonyl (Figure 5B) thus this group might enhance the activity [6]. Due to the α,β-unsaturated moiety, the hydrogen attached in Cβ could be easily abstracted by free radical which is shown in the Figure 5B. In addition, the presence of nitrogen in the pyridine ring is thought to have a similar behaviour to the phenol group (Figure 4A). The pyridine ring could

undergo the electron delocalization. Hatanaka et al synthesized α-pyridoin derivatives and their investigation revealed that together with enediol group, the pyridine ring played important roles in antioxidant activities by delocalizing the radical electron[19].

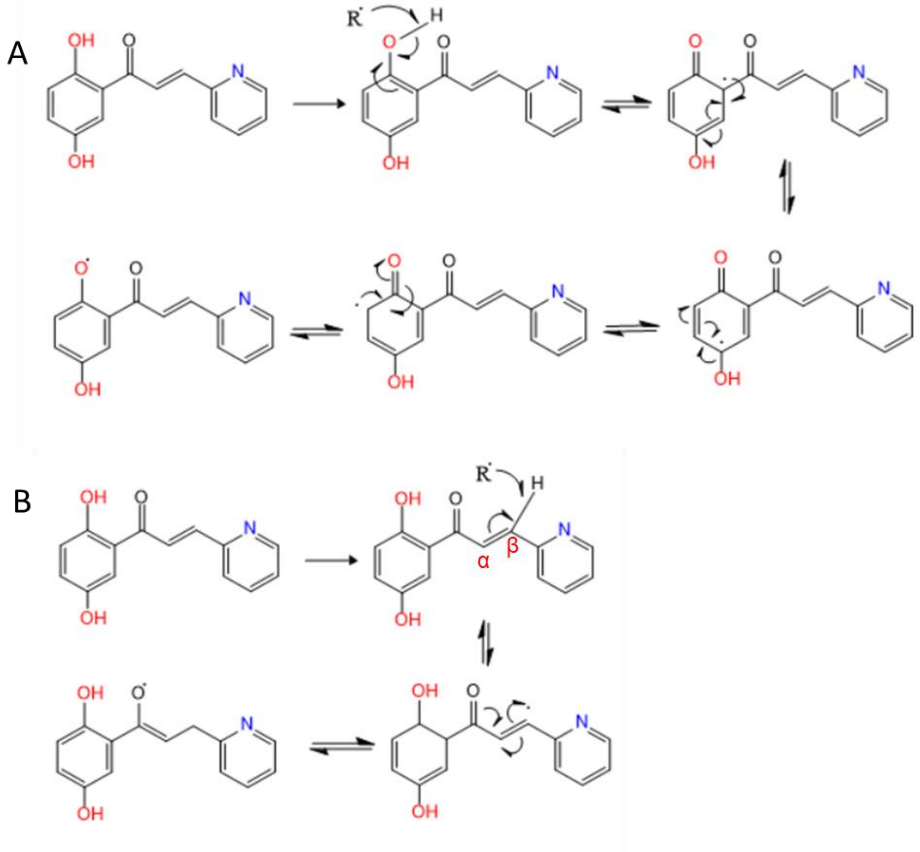


Figure 5. Delocalization of radical electron in phenol group (A) and α,β-unsaturated carbonyl (B)

Antioxidant plays a role as a reductant or reducing agent by donating electron[2]. The reducing power of AEW-1 (Figure 4B) had a similar manner with DPPH scavenging, in which those OH groups as well as nitrogen atom act as electron donors. Besides DPPH scavenging and reducing power, our compound was able to bind the metal (Fe^{2+}) with a higher affinity than ferrozine thus it interfered with the production of Fe-ferrozine complex. As depicted in Figure 4C, AEW-1 may chelate the metal in a similar way to quercetin (Figure 3). The metal chelating capacity appeared to be present due to the configuration OH group and a carbonyl group.

4. CONCLUSION

AEW-1 showed quite potent antioxidant activities: DPPH scavenging activity, reducing power, and metal chelating capacity. It is concluded that there are three parts on the AEW-1's structure that responsible for those activities: the OH groups, α,β-unsaturated carbonyl, and the pyridine ring. The arrangement of those three groups determines the antioxidant potency of AEW-1.

Funding: This research was funded by Faculty of Pharmacy, Universitas Gadjah Mada

Acknowledgments: -

Conflicts of interest: The authors declare no conflict of interest.

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