Synthesis of Hydroxyl Radical Scavengers from Benzalacetone and its Derivatives

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Abstract: *Synthesis of hydroxyl radical scavengers from benzalacetone and its derivatives has been done. Benzalacetone synthesis was done by crossed aldol condensation between benzaldehyde and acetone with 1:1 mol ratio, while dibenzalacetone in 2:1 mol ratio. Benzalacetone derivatives were synthesized by replacing benzaldehyde with its derivatives, i.e. p-anisaldehyde, veratraldehyde and cinnamaldehyde. The compounds that were active as radical scavengers based on the IC_{50} value from the highest level were: dibenzalacetone, veratralacetone, dicinnamalacetone, diveratralacetone and anisalacetone.*

Keywords: benzalacetone and its derivatives, IC_{50}, hydroxyl radical scavenger

1. INTRODUCTION

In recent years, epidemiological studies show that consumption of food with high phenolic content correlates with decreasing cardiovascular diseases.[1,2] Phenolic compounds may produce their beneficial effect by scavenging free radicals. There has been much researchs which showed the implication of oxidative and free radical in the mediated reaction on the degenerative processes related to aging and other diseases.[3,4] Several methods, both *in vivo* and *in vitro*, have been developed to measure antioxidant performance. These methods focus on different mechanisms of antioxidant including scavenging of oxygen and hydroxyl radicals,[5] reduction of lipid peroxyl radical, inhibition of lipid peroxidation or chelation of metal ions. Thus, some methods that are based on the mechanisms include β-carotene bleaching method,[6] DPPH assay,[7,8] thiobarbituric acid reactive substance (TBARS) method,[9] lipid peroxidation,[10,11] and deoxyribose assay.[12] Free radical is one atom or molecule that has one or more unpaired electrons. Theoretically, free radical will be formed if a covalent bond happens to break. The compound which is scavenging hydroxyl radical can decrease deoxyribose degradation. Deoxyribose degradation will produce malonaldehyde that is identified by red color of the thiobarbituric acid (TBA) complex.[13]

Benzalacetone has a conjugated system and is expected to be easily oxidized.[14,15,16] The more the double bond, the easier it will be oxidized. Therefore, it is assumed that benzalacetone and its derivatives will show antioxidant activity. Therefore, the objectives of this study are: (1) to synthesize and characterize benzalacetone and its derivatives, (2) to develop an oxidation system using deoxyribose assay and (3) to determine the IC_{50} value of each of the antioxidant.

2. EXPERIMENTAL SECTION

2.1 Benzalacetone (1)

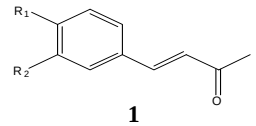
Into a solution of NaOH (0.05 mol, 2 g) in aqueous ethanol (1:1) that was prepared at ambient temperature, benzaldehyde (0.02 mol, 2.12 g) was added dropwise. After additional stirring for 10 min, acetone (0.02 mol, 1.17g) was added dropwise and stirred for 30 min. Water (20 ml) was added to the reaction mixture which was then filtered. The product was washed with water (20 ml x 3) and purified by re-crystallizing from ethanol and allowed to dry.

2.2 Dibenzalacetone (2)

Similarly prepared by changing of the molar ratio of acetone: benzaldehyde into 1:2. Similar procedure was repeated with p-anisaldehyde and veratraldehyde, respectively, replacing benzaldehyde in order to synthesize their derivatives (Fig. 1).

2.3 Cinnamalacetone and Dicinnamalacetone (3 & 4)

Synthesized with the same procedure but by using ice bath throughout the stirring. Each product was characterized and analyzed by Shimadzu FTIR 8300, Cary UV Varian 100 spectrophotometer, JEOL 60 MHz H-NMR spectrophotometer.



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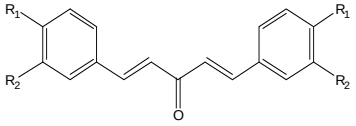

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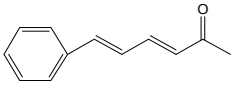

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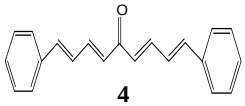

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No	R_1	R_2	Name of Compounds
1	H	H	Benzalacetone
	OCH_3	H	Anisalacetone
	OCH_3	OCH_3	Veratralacetone
2	H	H	Dibenzalacetone
	OCH_3	H	Dianisalacetone
	OCH_3	OCH_3	Diveratralacetone
3	-	-	Cinnamalacetone
4	-	-	Dicinnamalacetone

Figure 1: Structure of benzalacetone and its derivatives.

2.4 Deoxyribose Assay

The assay was performed as described by Halliwell.[12] All solutions were freshly prepared. Into a solution of 2-deoxyribose (0.2 ml 6 mM) was added ascorbic acid (0.2 ml 0.01 mM), buffer phosphate (0.2 ml) (pH 7.4), H_2O_2, (0.2 ml 0.01 mM) 0.02 ml of various concentrations of benzalacetone or its derivatives (50, 100, 250, 500 and 1000 ppm), and ferrous sulphate (0.2 ml 0.1 mM). After an incubation period of 30 min at 310 K, the extent of deoxyribose degradation was measured by the TBA reaction. 3 ml of TBA and 3 ml of TCA were added to the reaction mixture and heated for 15 min at 353 K. After the mixture being cooled, the absorbance at λ 532 nm is noted against a blank (the same solution but without sample). The percentage inhibition was calculated by the formula:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} x\ 100\%$$

The IC_{50} value represented the concentration of the compounds that caused 50% inhibition. BHT was used as a positive control.

3. RESULTS AND DISCUSSION

Synthesis of benzalacetone and its derivatives were carried out by crossed aldol condensation between acetone and benzaldehyde and its derivatives.[17,18] The structures of benzalacetone and its derivatives are shown in Figure 1.

The products of the synthesis were in the form of color powder ranging from light yellow to brownish orange. The structures were identified by IR and ^{1}H-NMR. The IR data comparison of the compounds is showed in Table 1, and the ^{1}H-NMR data shown in Table 2.

Table 1: The comparison of IR data of benzalacetone and its derivatives.

Compound	Functional group [ϒ (cm^{-1})]				
	CH aromatic	CH aliphatic	CO carbonyl	C=C aromatic	C-O ether
Benzalacetone	3060–3028	2918	1651	1602–1450	-
Anisalacetone	3060	2966; 2841	1598,9	1573; 1419,5	1251–1178,4
Veratralacetone	3030	2939; 2839	1618	1512; 1419,5	1263–1103
Cinnamalacetone	3028	2854	1604	1569; 1446	-
Dibenzalacetone	3060–3028	-	1651	1593; 1494	-
Dianisalacetone	3030	2966; 2841	1598,9	1510; 1419	1253–1178,4
Diveratralacetone	3000	2966,7; 2837,1	1620	1512; 1421	1265–1110
Dicinnamalcetone	3028	-	1602,7	1568; 1448	-

Table 2: ^{1}H-NMR data of benzalacetone and its derivatives.

Compound	Chemical shift [δ H (ppm)]					
	H aromatic	Hα	Hβ	Hγ etc	H methoxy	H methyl
Benzalacetone	7,2–7,7 (m)	7,8 (*s*)	6,9 (*s*)	-	-	1,9 (*s*)
Anisalacetone	7,6 (*d*), 7,0 (*d*)	7,8(*s*)	6,8 (*s*)	-	3,8 (*s*)	2,1 (*s*)
Veratralacetone	7–7,7 (m)	7,8(*s*)	6,8 (*s*)	-	3,9 (*s*)	1,2 (m)
Cinnamalacetone	7–7,5 (m)	6,8 (*s*)	6,7 (*s*)	6,5 (*s*)	-	-
Dibenzalacetone	7,2-7,7 (m)	7,8 (*s*)	6,9 (*s*)	-	-	-
Dianisalacetone	7,6 (*d*), 7,0 (*d*)	7,8(*s*)	5,3 (*s*)	-	3,8 (*s*)	-
Diveratralacetone	7–7,6 (m)	7,8(*s*)	6,8 (*s*)	-	3,9 (*s*)	-
Dicinnamalcetone	7–7,5 (m)	6,8 (*s*)	6,7 (*s*)	6,5 (*s*)	-	-

There is no significant difference in the IR spectra because the benzalacetone and dibenzalacetone derivatives are very similar. The only difference is that there are methyl group and C-H aliphatic bonding in benzalacetone, but there is not in dibenzalacetone.

Data of ^{1}H-NMR spectra of the compounds are similar. The only difference is the peak area integration that showed the number of protons. There is a peak at δ 1–2 ppm in the ^{1}H-NMR spectra of benzalacetone and its derivatives but none in the ^{1}H-NMR of dibenzalacetone and its derivatives.

The ^{1}H-NMR data of cinnamalacetone and dicinnamalacetone showed that there are differences in the peak area integration at δ 6.5 ppm. It explains that the compounds have Hγ of longer conjugated system.

The activity test as a hydroxyl radical scavenger was conducted *in vitro* by using Halliwell method.[12] The reaction was started by adding ferrous sulphate and H_2O_2 to produce a radical that will react with deoxyribose. The reaction was stopped by adding TBA reagent that would give a red color if the malonaldehyde was formed as the result of the reaction between the radical and deoxyribose. The absorbance of the red color was measured by using a UV spectrophotometer at the optimum wave number. The percentage (%) activity as antioxidant was calculated as the percentage of the absorbance decrease of the product of the synthesis that could prevent the degradation of the 2-deoxyribose compared to the blank. When the sample of the synthesis works well as the hydroxyl radical scavenger, then it will decrease the deoxyribose degradation so that the malonaldehyde-TBA complex will only give low intensity of red color. Thus, the more intense the red color, the less active the sample is. The graph of the antioxidant activity of the various synthesized compounds is presented in Figures 2 and 3.

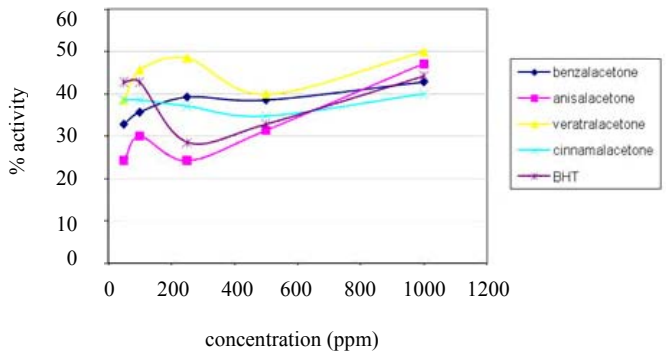

Figure 2: Antioxidant activity of benzalacetone and its derivatives.

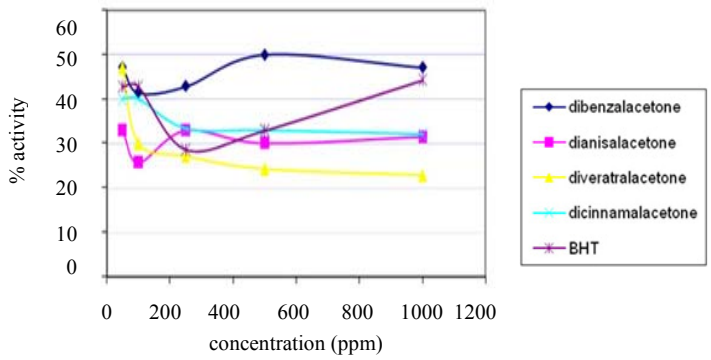

Figure 3: Antioxidant activity of dibenzalacetone and its derivatives.

From the graphs the IC_{50} values of the 8 samples by using regression linear equation are shown in Table 3. The result of the research shows that the compounds in decreasing activity as hydroxyl radical scavengers based on the IC_{50} values are veratralacetone, dicinnamalacetone, diveratralacetone and anisalacetone. No structure-activity relationship can be deduced from the above results.

4. CONCLUSION

From the above research, it is concluded that the synthesis of several compounds using 1:1 acetone:benzaldehyde and its derivatives yielded benzalacetone, anisalacetone, veratralacetone dan cinnamalacetone. Meanwhile, the synthesis using 1:2 acetone:benzaldehyde and its derivatives yielded dibenzalacetone, dianisalacetone, diveratralacetone and dicinnamalacetone.

Table 3: The results of synthesis benzalacetone and its derivatives.

Product	Color	IC_{50}	Activity
Benzalacetone	light yellow	1036,2	low active
Dibenzalacetone	light yellow	209,62	active
Anisalacetone	yellow	662,44	active
Dianisalacetone	yellow	1812,7	low active
Veratralacetone	orange	354,38	active
Diveratralacetone	orange	403,07	active
Cinnamalacetone	brownish orange	13.040	inactive
Dicinnamalacetone	brownish orange	377,20	active
BHT		198,22	active

The benzalacetone derivatives that are active as hydroxyl radical scavengers are anisalacetone and veratralacetone, while the dibenzalacetone derivatives with intense activity in scavenging the hydroxyl radical are dibenzalacetone, diveratralacetone dan dicinnamalacetone.

5. REFERENCES

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