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Research Article

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Microwave-assisted Synthesis of Dibenzalacetone Derivatives and Study of their Potential Antioxidant Activities

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ABSTRACT

A series of dibenzalacetones were synthesized by conjugating acetone with different substituted benzaldehydes. The general synthetic strategy employed for the synthesis of the compounds was based on Claisen-Smidth condensation reaction in the presence of strong base and was being irradiated by microwave. It gave good yields (88-93%), compounds (3a-c)), except (3d) only gave 11% yield. The structure of the synthesized compounds were characterized by spectral analysis. Their radical scavenging activity was evaluated through the determination of their abilities to inhibit free radicals using DPPH as a stable radical. Ascorbic acid was used as reference antioxidant compound and also the comparative study with curcumin and with the synthesized compounds were done. The compound that showed potential antioxidant activity base on IC50 value were 3d and 3b.

Keywords: Dibenzalacetone; Microwave irradiation; DPPH; Radical scavenging activity.

INTRODUCTION

Chronic diseases such as cancer, Alzheimer's and Parkinson's could be caused by oxidative stress, i.e. an imbalance condition between free radicals and antioxidant systems [1]. Reactive oxygen species (ROS) and free radicals, such as hydroxyl radicals and hydrogen peroxide, are produced in human body during normal metabolic pathways. The exposure of these radicals to exogenous stress can induce adverse effects on the normal physiological activity of cells. The body's system is equipped with antioxidant defense and enzymes which neutralize the ROS. The enhancement of ROS levels or ability of antioxidant defense system, can lead to increased oxidative stress and turn cell damage and death. Antioxidants can protect the cells from damages caused by uncontrollably produced ROS [2-4].

Curcumin, the major component of turmeric (*Curcuma longa*), has a variety of biological activities, including as antioxidant. Some researcher suggested that the antioxidant activity of curcumin is related to the phenolic- and the α,β-diketone groups. The α,β-unsaturated ketones known as benzalacetones and dibenzalacetones, as analogues of curcumin, are an interesting class of compounds for being studied and developed. Several benzalacetones have been

reported as an active antioxidant [5,6], anti-mutagenic [7], and anti-tubercular agent [8]. Due to their conjugated system, several benzalacetone analogues have been described as radical scavengers with potential antioxidant properties [9].

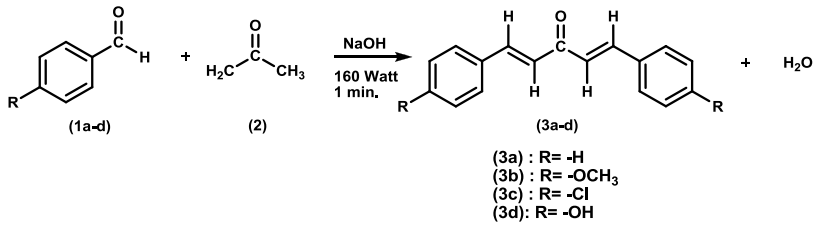
Nowadays, trend of synthetic process has been shifting from traditional concept that focused on optimum yield into eco-friendly processes that giving more attention to reaction processes. Eco-friendly reaction process is a reaction which eliminates or reduces waste, saves energy and avoids the use of toxic or hazardous compounds [10]. The advantageous of the synthesis via Microwave Assisted Organic Synthesis (MAOS) reaction are faster, cleaner, more economic, and environmental friendly than stirring method. It can also use household microwave ovens and only requires simple glassware and more pedagogic [11,12].

Based on the results of these studies, it is important to develop curcumin through modification of its structures by synthesis. In connection to our study, we would like to synthesize dibenzalacetone derivatives via Claisen-Smidth condensation using the MAOS method and studies their antioxidant activities using the stable DPPH radical.

EXPERIMENTAL SECTION

Chemistry

The general synthetic strategy employed for the preparation of the compounds under study was based on Claisen-Smidth condensation reaction. The starting benzaldehydes **(1a-d)** was treated with acetone **(2)** in the presence of 20% NaOH and was being irradiated by microwave gave respective dibenzalacetones **(3a-d)** as outlined in Scheme 1.



Scheme 1. Reaction pathway for the synthesis of dibenzalacetones (3a-d)

Material and Method

All solvents, chemicals, and reagents were obtained commercially and used without purification. The progress of the reaction and purity test of the products was performed by the TLC methods on silica gel 60 F245 plates (Merck) using hexane: ethyl acetate (4:1) as the mobile solvent; spot detection was performed with UV 254 nm. Melting points were measured with an Electro-thermal melting point apparatus without correction. Infrared (IR) spectra were recorded in KBr pellet on a FTIR spectrophotometer (Jasco FT-IR 5300). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL NMR 500 spectrometer, using TMS as internal standard. Mass spectra of the synthesized compounds were obtained using a Agilent 6980N Mass Spectrometer fitted with an Electron Impact (EI) source. UV-Visible spectrophotometer (Shimadzu 160A) was used for recording the absorbance of the test solutions. Irradiation was using a household microwave oven Sakura MW 9600, Japan.

(1E,4E)-1,5-Diphenylpenta-1,4-dien-3-one, Compound (3a)

Yellow crystal (89%), M.p. 110-111^{0}C; FTIR (KBr) cm^{-1}: 3052 (C_{sp2}-H); 1651 (C=O); 1591 and 1447 (C=C ar). ^{1}H-NMR (400MHz, $CDCl_3$, δ, ppm): 7.08 (2H,d, J=16.3 Hz, Olefinic proton) and 7.74 (2H, d, J=15.8Hz, Olefinic

proton); 7.61 (4H, dd, J=6.6 and 3.0Hz, Ar-H); 7.25-7.57 (m, 6H, Ar-H). ^{13}C-NMR (400MHz, $CDCl_3$, δ, ppm): 189.1 (C-ketone); 125.5 and 143.6 (C-olefinic); 134.8; 128.7; 129.4; and 130.6 (C-Ar). GC-MS (EI): Rt=21.91 min. (97.23%); Mass (m/z): [M]+=234.

(1E,4E)-1,5-Di(p-Methoxyphenylpenta-1,4-dien-3-one, Compound (3b)

Yellow crystal (93%), M.p. 129-130^{0}C; FTIR (KBr) cm^{-1}: 3018 (C_{sp2}-H); 2961 (C_{sp3}-H); 1654 (C=O); 1599 dan 1440 (C=C *ar*). ^{1}H-NMR (400MHz, $CDCl_3$, δ, ppm): 6.90-6.96 (2H, m); 7.69 (2H, d, *J*=15.9Hz, Olefinic proton); 7.56 (4H, d, *J*=4.1Hz, Ar-H); 6.90-6.96 (4H, m); 3.84 (s, 6H, OCH_3). ^{13}C-NMR (400MHz, $CDCl_3$, δ, ppm): 188.9 (C-ketone); 125.5 and 142.8 (C-olefinic); 127.6; 130.1; and 114.4 (C-Ar); 161.6 (C-OCH_3); 55.5 (CH_3-O). GC-MS (EI): Rt=26.91 min. (100%); Mass (m/z): $[M]^+$=294

(1E,4E)-1,5-Di(p-Chlorophenylpenta-1,4-dien-3-one, Compound (3c)

Yellow needle crystal (89%), M.p. 197-198^{0}C; FTIR (KBr) cm^{-1}: 3050 (C_{sp2}-H); 1647 (C=O); 1588 dan 1489 (C=C *ar*). ^{1}H-NMR (400MHz, aseton-d_6, δ, ppm): 7.27-7.31 (2H, m); 7.39 (2H, m, Olefinic proton); 7.75 (4H, d, *J*=9.0Hz, Ar-H); 7.45-7.48 (4H, m, Ar-H). ^{13}C-NMR (400MHz, aseton-d_6, δ, ppm): 187.8 (C-ketone); 126.2 and 141.2 (C-olefinic); 128.1 (C4,C4'); 129.1 and 130.0 (C=Ar); 134.0 (C-Cl). GC-MS (EI): Rt=25.56 min. (100%); Mass (m/z): $[M]^+$=302; [M+2]=304; [M+4]-306 (ratio 10:4:1).

(1E,4E)-1,5-Di(p-Hydroxyphenylpenta-1,4-dien-3-one, Compound (3d)

Brownish yellow crystal (11%). M.p. (decomposed). FTIR (KBr) cm^{-1}: 3021 (C_{sp2}-H); 3320 (O-H); 1656 (C=O); 1597 dan 1436 (C=C *ar*); 1248 (C-OH). ^{1}H-NMR (400MHz, CH_3OH-d4, δ, ppm): 7.04 (2H, d, *J*=16.0 Hz); 7.69 (2H, d, *J*=15.6Hz, Olefinic proton); 7.55 (4H, d, J=8.4Hz); 6.81 (4H, d, *J*=8.4Hz, Ar-H); 9.43 (s, 1H, OH). ^{13}C-NMR (400MHz, CH_3OH-d4, δ, ppm): 190.3 (C-ketone); 122.1 and 143.8 (C-olefinic); 126.3; 130.3; 115.5 (C-Ar); 160.2 (C-OH). GC-MS (EI): decomposed, giving three peaks with retention time respectively: 14.73 min (5.24%), 17.82 min (76.94%), 25.16 min (17.51%).

Biological Activity

***In vitro* DPPH assay:** The free radical scavenging activity of dibenzalacetone derivatives were measured using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). A number of methods are available for the determination of free radical scavenging activity but the assay employing stable DPPH radical has received most attention owing to its ease of use and its convenience (13). The solution of respective compounds were prepared in different concentrations (10, 25, 50, 100, 200 and 500 □M/mL) in methanol and mixed with constant volume of 0.1 mM DPPH solution. The absorbance was measured at 517 nm against methanol as blank after the incubation for 20 min. Ascorbic acid and curcumin (0.025-0.2 mM) were used for comparison. All determinations were due in triplicate. The percentage (%) radical scavenging activity was calculated by using the formula,

$$\% \text{ Inhibition} = [(A_0 - A_1)] \times 100$$

Where, A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The capability to scavenge the DPPH radical was expressed as IC_{50} (concentration of antioxidant that produces 50%

of absorbance inhibition).The percentage DPPH activity of dibenzalacetone derivatives at different concentration is depicted in Figure 1.

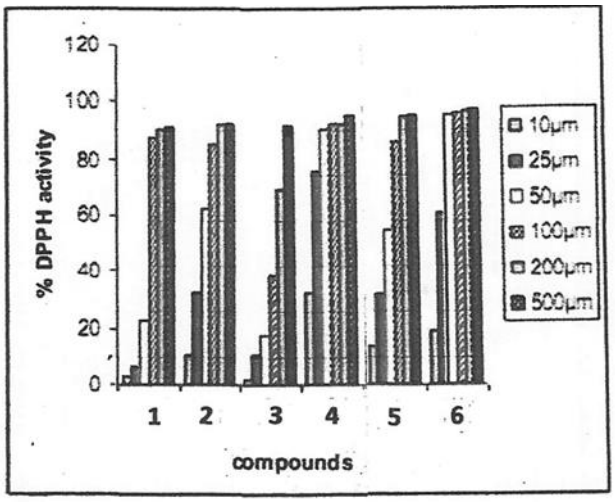


Figure 1. Percentage DPPH activity for dibenzalacetones (3a-d)

RESULTS AND DISCUSSION

We decided to investigate a microwave-assisted synthesis of dibenzalacetones to increase reaction yields, to enhance the rate of reaction, thereby reducing time and decreasing by-products formation. Microwave activation for the synthesis of dibenzalacetones has not been widely described in the literature. Kappe et al. reported the aldol condensation of p-methoxybenzaldehyde with acetone using microwave activation but could not prevent self-condensation [13,14].

In this study, the desired products were (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (=Dibenzalacetone **(3a)**); (1*E*,4*E*)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (=Dianizalacetone **(3b)**); (1*E*,4*E*)-1,5-bis(4-chlorophenyl)-penta-1,4-dien-3-one (=4,4'-Dichlorodibenzalacetone **(3c)**); and (1*E*,4*E*)-1,5-bis(4-hydroxyphenyl)penta-1,4-dien-3-one (=4,4' Dihydroxydibenzalacetone **(3d)**). These compounds were synthesized via Claisen-Smidth condensation reaction and were being irradiated by microwave (160 Watt, 1 min) using a household microwave oven. The synthesis of compounds **(3a-c)** gave good yields (88-99%), while the yield of compound **(3d)** only 11%. It was caused that the synthesis process of compound **(3d)** was employed in THF and 5molEq of NaOH. The excess of NaOH solution might be due to the Canizzaro reaction of 4-hydroxybenzaldehyde.

The IR spectra of dibenzalacetones revealed the characteristic absorption of conjugated ketone. The strong bands in the region 1656-1647 cm^{-1} were assigned to C=O stretching of the benzylidene system. In all of the compounds, the aromatic C-H stretching was found at the region 3052-3018 cm^{-1} , and two peaks of the aromatic C=C stretching were found at the region 1599-1588 cm^{-1} and 1489-1436 cm^{-1} respectively. On the other hand, in ^{1}H NMR spectra of all dibenzalacetones **(3a-d),** the aromatic protons appeared at 7.56-6.81 ppm as doublet (*J*=4.1-9.0Hz) or multiplet. The olefinic protons were appeared at 7.69-6.90 ppm as doublet (*J*=15.8-16.3Hz). Due to the value of *J* (coupling constant) we concluded that the olefinic protons confirmed the E configuration. Addition to this, in compound **(3b)** methoxy proton resonated as singlet at 3.84 ppm. In compound **(3d)** phenolic -OH proton appeared as singlet at 9.43 ppm. In ^{13}C NMR spectra of all dibenzalacetones **(3a-d),** the carbon of carbonyl group appeared at 187-190 ppm;

while the aromatic and olefinic carbons appeared at 125-143 ppm. In addition the methoxy carbon of compound **(3b)** appeared at 55 ppm. In GC analysis, compounds **(3a-c)** showed one single peak with purity 97-100%, while compound **(3d)** was decomposed during the process of GC-MS analysis. Mass spectra of compounds **(3a-c)** showed M^+ peak in agreement with their molecular formula.

In order to evaluate antioxidant properties of the synthesized compounds **(3a-d)** the effects on free radical were examined using DPPH assay according to the Blois method [13]. All the synthesized compounds scavenged DPPH radical significantly in a concentration dependent manner. IC_{50} value was calculated by linear regression algorithm and the results were compared with that of internal standard ascorbic acid (AA). The respective IC_{50} values of the synthesized compounds and standard are listed in Table 1.

Table 1. 50% inhibition of DPPH radical (IC_{50}) by dibenzalacetones

No.	Compound	IC_{50} (mMol/mL)*
1	3a	22.5 ± 0.12
2	3b	17.5 ± 1.02
3	3c	125 ± 0.43
4	3d	10 ± 0.64
5	Curcumin	12.5 ± 0.58
6	Ascorbic acid	22.5 ± 0.21

*Each value represent means ± SD (n=3).

The introduction of potent electron withdrawing group (Cl) in compound **(3c)** significantly reduced the DPPH activity, while that of electron donating groups (OCH_3, OH) in compound **(3b)** and **(3d)** enhanced the activity. Incorporation of -OH group into the benzene ring enhance radical scavenging activity exhibiting dominant activity among analogues. The increasing orders of DPPH activity of the synthesized compounds are as follows: **3d>3b>3a>3c**.

CONCLUSION

The compounds under study were synthesized via Claisen-Smidth condensation using the MAOS method. The synthesis of compounds **(3a-c)** gave good yields (88-99%). We are still looking for a better synthesis method for compound **(3d)** to increase its yield. The radical scavenging activities of the synthesized compounds were evaluated using DPPH radical assay. Although most of the compounds showed considerable inhibition percentage, the compound **(3d)** were the most active. From the present study, conclusion could be drawn that the nature of the substituent on the aromatic ring played a critical role in exerting the antioxidant activity.

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