Fe$_3$O$_4$/eggshells composite as green catalyst for Hantzsch condensation in acridine synthesis

A H Cahyana, E Saepudin, M Surya and B Ardiansah

Department of Chemistry, FMIPA, Universitas Indonesia, Kampus Depok, 16424, Jawa Barat, Indonesia

Corresponding author: endang.saefudin@sci.ui.ac.id

Abstract. Acridine derivatives are a class of nitrogen heterocycles which have broad spectrum of important biological properties and pharmaceutical applications. Low-cost eggshells waste material was used as source of CaCO$_3$ to modified magnetic Fe$_3$O$_4$ particles prepared from co-precipitation to serve as reusable heterogeneous catalyst. The synthesized eggshells based catalyst is characterized by X-ray diffraction (XRD) and TEM studies. The activity of the catalyst has been investigated in Hantzsch condensation reaction with good yields and simple work-up procedure. The synthesis of acridine derivative was achieved through a one-pot, three-component condensation of dimedone, aryl amine and aryl aldehydes utilizing Fe$_3$O$_4$/eggshells composite as catalyst under mild conditions. Maximum yield obtained is 91.3% with reflux condition, amount of catalyst 0.025 g, and 1.5 h reaction time. Catalyst used can be separated magnetically and can be reused up to three times without significant decrease of yield. The synthesized compound was characterized using IR, UV-Vis and LC-MS.

Keywords: acridine, Hantzsch condensation, Fe$_3$O$_4$/eggshell

1. Introduction

Indonesia is one of the world largest cinnamon producer. Cinnamon production in Indonesia reached 91,400 ton in 2014, surpassing China (71,146 ton) and Sri Lanka (16,766 ton) [1]. However, Indonesia sold cinnamon at low price because it is often marketed or exported as unprocessed cinnamon bark stick [2]. Cinnamon powder or cinnamon bark oil usually used in culinary as an flavoring or food additive and medicine. Exploration of cinnamon potential is important to rise the price value of Indonesia’s cinnamon.

Main constituent of cinnamon bark oil is (E)-cinnamaldehyde. Cinnamon bark oil can be obtained using Soxhlet extraction and steam distillation, and its content ranging from 62 to 90% depend on extraction technique [3]. Utilization of (E)-cinnamaldehyde as precursor in organic synthesis can be a way to increase its value.

One of the simple reaction that can be done using (E)-cinnamaldehyde as precursor is Hantzsch condensation reaction. The reaction involves aldol reaction of aldehyde and 1,3-dicarbonyl, followed by imine formation and cyclization forming pyridine skeleton [4]. Acridine, a tricyclic compound with similar structure with anthracene with pyridine ring in center, can be obtained from Hantzsch condensation reaction using dimedone as 1,3-diketone. Acridine possess many biological activities, from antitumor [5], anticancer [6], antifungal [7], cytotoxic [8], and antibacterial [9]. Research on synthesis of acridine has been widely done by researchers around the world, mainly focused on improving catalyst, such as using ionic liquid [10], amberlyst [11], functionalized silica [12], and refluxing water [13]. Improvement of catalyst mainly focused around green chemistry concept. This article reports our research in synthesis of 1,8-dioxoaclidine using Fe$_3$O$_4$ coated on...
Figure 1. SEM Image of Fe\textsubscript{3}O\textsubscript{4}/eggshell using (a) 1000x magnification and (b) 5000x magnification.

eggshell particles. FeO\textsubscript{3} is reported to have catalytic activity on acridine synthesis [14], meanwhile eggshell particle as FeO\textsubscript{3} support has been proven to increase yield of xanthene, where the proposed synthetic mechanism is similar with Hantzsch synthesis [15].

2. Materials and methods
All chemicals were on analytical grade, purchased from Fluka Chemical Company, Merck and Sigma-Aldrich. Silica gel 60F-254 with a mobile phase consist of ethyl acetate and n-hexane (1:1) solvents was used in thin layer chromatography (TLC) analysis. Fourier Transform Infrared Spectroscopy (FTIR) characterization was performed by using Shimadzu 8400 spectrometer with KBr background. UV-Vis absorption recorded with Shimadzu UV mini type 1240. Mass spectroscopy analysis was conducted with Xevo G2-S Qtof with J12CHA 602G serial.

2.1. General procedure for the Fe\textsubscript{3}O\textsubscript{4}/eggshell synthesis
Fe\textsubscript{3}O\textsubscript{4}/eggshell was prepared using modified method of previous studies [14, 16]. Eggshell was collected from domestic waste. Collected eggshell was rinsed, separated from the membrane, and boiled in NaOH 1 mol.L\textsuperscript{-1}. After boiling, eggshell dried and crushed, to further screened through a sieve to get < 100 μm size. FeO/eggshell as a catalyst was prepared using coprecipitation method. FeCl\textsubscript{3} (0.02 mol) and FeCl\textsubscript{2} (0.01 mol) was dissolved in 1.5 M HCl solution. Both solution was added dropwise to NaOH solution (1.5 mol.L\textsuperscript{-1}) that was heated to 65 °C and stirred vigorously at 60 °C. 20 g of previously prepared eggshell powder was added and then stirring continues for 0.5 hour. Black precipitate then collected by filtering and washed by distilled water to neutralize excess acid.

2.2. General procedure for the synthesis of 1,8-dioxoacridine synthesis
A mixture of aniline (0.5 mmol), dimedone (1.0 mmol), and cinnamaldehyde (0.5 mmol) was dissolved in ethanol in the presence of FeO/eggshell as catalyst. Reaction mixture stirred and heated to 80 °C (reflux). After completion of reaction as monitored by TLC, reaction mixture was separated from catalyst, and cooled to room temperature. Distilled water was added to mixture in 1:1 portion, and left overnight. Precipitated compound then separated, heated to evaporate excess ethanol and water, and then recrystallized and washed by hot water. Obtained product were further characterized by FT-IR spectroscopy and LC-MS with mobile phase acetonitrile and water using gradient concentration technique.

3. Results and discussion
3.1. Characterization of FeO/eggshell catalyst
FeO/eggshell particle formed as black precipitate. The particle showed magnetic properties and can be separated when it is suspended in liquid. SEM-EDX analysis on catalyst powder shown irregular pattern and nonuniform size of the eggshell particle (figure 1). Eggshell particle is successully attached by FeO\textsubscript{3} particle, as confirmed by attracting suspended catalyst with magnet. Elemental composition of one random point in catalyst by EDX is shown in the table 1.
Table 1. Chemical composition of Fe₃O₄/eggshell catalyst as analyzed by EDX

| Elements | Fe | O  | C  | Ca | Al |
|----------|----|----|----|----|----|
| % wt.    | 40.6 | 37.3 | 12.3 | 6.7 | 1.5 |

Figure 2. FTIR spectra of Fe₃O₄/eggshell catalyst

Figure 3. X-ray diffractogram of Fe₃O₄/eggshell catalyst

FTIR analysis of catalyst (figure 2) showed a sharp peak at 1400–1500 cm⁻¹ indicating presence of carbonyl functional group contained in eggshell as CaCO₃. Meanwhile, Fe-O absorption recorded at 500–600 cm⁻¹ [17]. Broad peak at 3300–3600 cm⁻¹ appeared due to water vapor contamination during analysis.

XRD analysis of Fe₃O₄/eggshell showed peak at 2θ = 23.18; 29.56; 36.14; 39.58; 47.70; 48.68; and 57.66 indicated presence of CaCO₃, and 2θ = 30.60; 35.66; 43.36; 56.88; 61.78 indicated presence of magnetite (figure 3) [18,19].

3.2. Synthesis and Characterization of 1,8-dioxoacridine

Catalytic activity of Fe₃O₄/eggshell catalyst was studied using one pot three component synthesis of 1,8-dioxoacridine (figure 4). Condensation reaction occurs between three components; cinnamaldehyde, aniline, and dimedone; forming 1,8-dioxoacridine. Resulting product is yellow solid, soluble in ethanol but insoluble in water and n-hexane. Reaction conditions were optimized by varying reaction time, temperature, and catalyst amount. In addition, reusability of Fe₃O₄/eggshell catalyst also examined using optimized reaction conditions. Reaction mechanism is likely to follow Hantzsch condensation reaction mechanism, starting with aldol product formation. Few seconds after mixing all reactant with catalyst, yellow insoluble product obtained. Further examination by comparing reaction with dimedone and cinnamaldehyde alone in base condition, followed by TLC analysis, it is concluded that both reaction formed same product.
Table 2. Optimization of reaction condition results

| No | Time (min) | Catalyst (mg) | Temp. (°C) | Yield (%) |
|----|------------|---------------|------------|-----------|
| 1  | 60         | 16.6          | 80         | 76.2      |
| 2  | 90         | 16.6          | 80         | 88.2      |
| 3  | 120        | 16.6          | 80         | 87.8      |
| 4  | 150        | 16.6          | 80         | 78.8      |
| 5  | 90         | 16.6          | 30         | 77.8      |
| 6  | 90         | 16.6          | 50         | 78.1      |
| 7  | 90         | 0             | 80         | 69.0      |
| 8  | 90         | 8.8           | 80         | 79.9      |
| 9  | 90         | 25.4          | 80         | 91.3      |

Figure 4. Reaction scheme of 1,8-dioxoacridine synthesis and obtained product

Figure 5. FTIR spectra of synthesized 1,8-dioxoacridine

Figure 6. Mass spectrum of synthesized 1,8-dioxoacridine

FTIR analysis of products shows broad absorption at 3300-3500 cm⁻¹, indicating presence of hydroxyl group in the compound (figure 5). Other absorption occurs at ~1450 cm⁻¹, possibly indicating presence of carbonyl group in acridine. Absorption occurred at lower wavenumber than normal carbonyl shift (1670-1820 cm⁻¹) due to effect of conjugation. Medium absorption at 1000-1200 cm⁻¹ indicating C-N stretch in acridine skeleton.

LCMS analysis of product resulted a single dominant peak (as base peak) in mass spectrum at m/z = 470, fit with the [M+H]⁺ value with the reaction product. Mass spectrum analysis resulted single dominant peak (figure 6) and only tiny other peak because energy of electron used in LCMS analysis is the lowest one in the instrument, in order to get the M⁺ value as a base peak and minimizing fragmentation.

Optimization result shown on table 2. Optimum reaction time obtained is 90 min. Longer reaction time resulted in lower yield obtained, possibly due to side reaction of product decomposition. Optimization of reaction temperature was done with optimum time obtained. Results shown that yield
obtained is proportional to reaction temperature. Condensation reaction needs heat to occur, and higher temperature favor condensation reaction. Meanwhile, variation of catalyst amount also directly proportional to yield obtained. However, reaction still occurred when no catalyst added. It is important to note that normally Hantzsch condensation reaction catalyzed by base, and presence of aniline acted as catalyst for the reaction itself, so with no catalyst added, reaction still occurred. Fe₃O₄/eggshell catalyst reusability was tested by running 4 reaction using same catalyst, washed by ethanol between usage. Obtained yield indicated the catalyst can be reused, first three run showed no significant decrease in yield, and fourth run the yield decreased by 10.6% (figure 7).

3.3. Antioxidant assay result
Series of 1,8-dioxoacridine solution in ethanol with concentration of 5; 10; 50; 100; and 300 ppm used for this experiment. Inhibition percent after reaction were calculated from blank absorbance (A₀) and test solution absorbance (A) as follow [20]:

\[
\% \text{ inhibition} = \frac{A_0 - A}{A_0} \times 100\%
\]

From the calculation, IC₅₀ value was obtained using regression analysis of the curve (figure 8). IC₅₀ value obtained for the compound is 36.03 ppm. This result is far lower than cinnamaldehyde and dimedone that posses IC₅₀ value above 500 ppm [21].

4. Conclusions
A low-cost, reusable and harmless catalyst, Fe₃O₄/eggshell had been synthesized using simple precipitation method. The catalyst showed excellent catalytic activity in 1,8-dioxoacridine synthesis with yield up to 91.3%. In addition, using multi-component reaction techniques maximizes atom economy, utilization of renewable and waste material (eggshell waste), and reusable property of catalyst is an important properties that is in accordance with green chemistry principle. Catalyst used can be reused up to three times without significant decrease in yield.
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