Citrate-capped superparamagnetic iron oxide (Fe₃O₄-CA) nanocatalyst for synthesis of pyrimidine derivative compound as antioxidative agent

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Abstract. The development of a recyclable catalyst based on magnetic nanoparticles has attracted an increasing interest as the emerging application in the heterogeneous catalyst field. Superparamagnetic iron oxide nanoparticle with citric acid as capping agent was successfully obtained from iron (III) chloride solution via two steps synthesis. The first step involving the formation of magnetite nanoparticle by bioreduction using Sargassum Sp, then its surface was modified by adding citric acid solution in the second step. The structural, surface morphology and magnetic properties of the nanocatalyst were investigated by various instrumentations such as scanning electron microscope with energy dispersive (SEM-EDS), and particle size analyser (PSA). Fe₃O₄-CA was then applied as reusable catalyst for Knoevenagel condensation of barbituric acid and cinnamaldehyde to produce (E)-5-(3-phenylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione. The optimum condition of this reaction was achieved by using 7.5% mole of catalyst at 50°C for 6 h to give 83% yield. Some spectroscopy techniques such as UV-Vis, FTIR, LC-MS and ¹H-NMR were used to confirm the product’s structure. Furthermore, the synthesized compound has an attractive antioxidant activity based on the in-vitro analysis using DPPH method.

1. Introduction

In recent years, the development of Fe₃O₄ as magnetic nanoparticle became a trending topic due to their multiple functions, including information storage, electronic devices, rapid biologic separation, and drug delivery [1]. Fe₃O₄ was successfully synthesized by the reduction of ferric chloride solution using Sargassum Sp. brown seaweed extract, applying a green chemistry concept. Initially, the ferric chloride hydrolyzes to form ferric hydroxide and release H⁺. The presence of aldehyde group in Sargassum Sp. extract reduced the ferric chloride to form Fe₃O₄ nanoparticle, and the other side of the aldehyde was oxidized to form the corresponding acid [2, 3]. However, the nanoparticle with no stabilizer will cause agglomeration. To solve this problem, the surface of Fe₃O₄ must be stabilized with citric acid through the coordination bond [4]. Fe₃O₄ capped by citric acid can be used as catalyst to form pyrimidine derivative compound. The properties of nano-sized Fe₃O₄-CA such as high surface area shows an important role in catalytic performance. The advantages of using this nanocatalyst are its low toxicity and cost [5], can be easily separated, and recycled from the product by an external magnet [6]. Bioactivity of pyrimidine derivative compound as an antioxidative agent was measured by UV-Vis spectrophotometer using DPPH method and interpreted as IC₅₀ [7].
2. Materials and methods

2.1. General
Chemicals used were from pro-analytical grade. The Sargassum Sp. was obtained from Banten Province (Binuangeun Sea). The chemical used in this research were analytical grade from commercial suppliers.

2.2. Synthesis of Fe₃O₄-CA nanocatalyst
Fe₃O₄ nanoparticles were prepared based on earlier work with modification [3]. In short, FeCl₃·6H₂O (4 mmol), FeCl₂·4H₂O (1 mmol), Sargassum Sp. extract (5 mL) and deionized water (50 mL) were stirred in a 250 beaker glass at room temperature, and the mixture was adjusted to pH 10 by adding NaOH. After 2 h, the product will be capped by adding 1% of citric acid solution, the reaction temperature was raised up to 60°C and completed for 1 h with continuous stirring. The product was dried in the oven at 50°C and characterized using SEM-EDS and PSA [8].

2.3. Synthesis of pyrimidine derivative compound and its bioactivity
In a 250 Erlenmeyer flask, 1 mmol cinnamaldehyde, 2 mmol barbituric acid, 5% mol Fe₃O₄-CA were added to methanol as solvent and stirred for 6 h at 50°C. When the reaction was completed, the catalyst can be removed easily from the product using an external magnet. The product was recrystallized by adding hot ethanol and characterized with UV-Vis, FTIR, LC-MS, and NMR. The bioactivity was measured by UV-Vis spectrophotometer using DPPH method according to previous research [7, 9].

3. Results and discussion
The citrate-modified Fe₃O₄ was prepared based on our earlier work and re-characterized by SEM, EDS, and PSA [10]. Figure 1 is the morphology of Fe₃O₄ and Fe₃O₄-CA by SEM. Fe₃O₄ has granular grain. The other side of Fe₃O₄-CA has granular and fiber grain. The different surface of these compound show that the Fe₃O₄ was successfully capped by citrate from citric acid. Elemental analysis using EDS for Fe₃O₄ indicated that the sample contains Fe (65.33%), O (24.47%), C (8.31%) and the trace of Na element (1.89%). After capping process of Fe₃O₄ with citrate, there was an increase of carbon content in the sample. The result of EDS analysis of Fe₃O₄-CA is as follows: Fe (51.36%), O (38.49%), and C (10.14%). The re-synthesized catalyst has similar characteristic, and it is comparable with our previous work [10].

Figure 2 shows the particle size of Fe₃O₄ and Fe₃O₄-CA. From this measurement, the average particle size of Fe₃O₄ is 62.5 nm and 89.0 nm for Fe₃O₄-CA. The increasing of Fe₃O₄-CA particle size caused the capping of Fe₃O₄ surface by citrate and it increased the radius of particle. The Fe₃O₄-CA was then applied in synthesis of pyrimidine derivative compound. From FTIR analysis, the peak at 1567 cm⁻¹ might be assigned to mono-substituted benzene. The peak at 1694 cm⁻¹ signified the presence of secondary amide. The absorption at 1748 cm⁻¹ is attributed to the C=O vibration. The peaks at around 2851 and 3188-3071 cm⁻¹ are attributed to the C-H sp² and N-H functional group, respectively.

![Figure 1. SEM micrographs of (a) Fe₃O₄ and (b) Fe₃O₄ modified with citrate.](image-url)
The maximum wavelength of this pyrimidin derivative compound is 371 nm as shown in figure 3. Additionally, the LC-MS gave the M+1 base peak at 243 (Figure 3), means the molecular weight of this compound is 242 g mol$^{-1}$. Further, $^1$H-NMR (500 MHz, DMSO-d6) was used to confirm the structure. The peaks were appeared at 7.48 ppm ($m$, 5H, phenyl); 7.69 ppm ($d$, 1H, CH, $J$ = 15.6 Hz); 8.01 ppm ($d$, 1H, CH, $J$ = 11.9 Hz); 8.44 ppm ($t$, 1H, CH); 11.25 ppm ($s$, 2H, 2NH). From the LC-MS and $^1$H-NMR results, it was showed that the compound is (E)-5-(3-phenylallylidene)pyrimidine-2,4,6(1H,3H,5H)-trione ($C_{13}H_{10}N_2O_3$). The bioactivity of this pyrimidin derivative compound was measured using DPPH method. The result showed an antioxidative activity through the changes of violet colour from DPPH to yellow colour by adding 1 mL sample. The IC$_{50}$ of this compound is 8.57 ppm.
4. Conclusions
Magnetic nanoparticle Fe₃O₄ was successfully synthesized using Sargassum Sp. as stabilizer and it was successfully capped by citrate to form Fe₃O₄-CA. The carboxyl group on its surface acted as catalyst for synthesis of pyrimidine derivative compound. This compound’s structure was confirmed by some instruments such as FT-IR, LC-MS, and NMR. From DPPH test, we can conclude that the compound is very active as antioxidative agent.

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