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SYNTHESIS AND BIOLOGICAL INVESTIGATION OF 3,3,6,6-TETRAMETHYL-9-STYRYL-1,8-DIOXOOCTAHYDRO-XANTHENE PROMOTED BY Fe_3O_4-SUPPORTED CITRIC ACID AS A MAGNETICALLY RECOVERABLE CATALYST

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ABSTRACT

A green and attractive protocol was developed to produce 3,3,6,6-tetramethyl-9-styryl-1,8-dioxooctahydroxanthene using magnetically separable Fe_3O_4 modified by citric acid (Fe_3O_4-CA) as catalyst. After catalyst characterization using XRD, Particle Sizer, SEM and EDAX, the Fe_3O_4-CA was then applied in dioxooctahydroxanthene synthesis with several optimizations, such as % wt. of catalyst, reaction time, temperature and solvent. The best protocol was found when the reaction is conducted using 5% wt. of catalyst at 50°C for 6 h reaction time in methanol, with the highest yield of 80%. The product obtained was confirmed by FTIR, UV-Vis, LC-MS, ^{1}H- and ^{13}C-NMR analysis. In biological investigation, dioxooctahydroxanthene showed higher radical scavenging activity with IC_{50} of 24.17 ppm, whereas it has comparable antibacterial activity than cinnamaldehyde as its precursor.

Keywords: magnetite, dioxooctahydroxanthene, citric acid, antioxidative, antibacterial

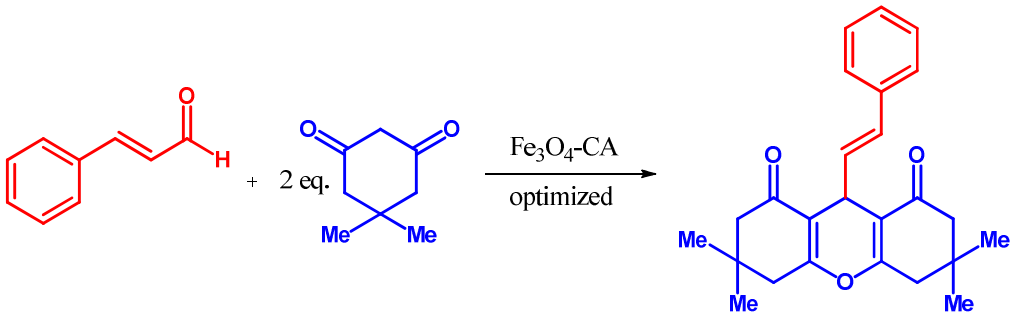
INTRODUCTION

Xanthenes are very appealing class of O-containing heterocycles with extensive number of natural origins and synthetic derivatives.[1-3] A structural diversity of xanthenes and their derivatives reveal potential use in the field of pharmaceutical and medicinal chemistry as anti-bacterial[4,5], anti-inflammatory[6] and antioxidative agents[7], and it is also used in laser technologies.[8] Latterly, tremendous attempts have been made in developing the scope of the synthesis of 1,8-dioxooctahydroxanthene via condensation of suitable aldehydes and 1,3-diketones or their relatives. Several protocols have been reported such as using ionic liquid[9], solvent-free system with various catalysts[10–12], homogeneous[13,14] and heterogeneous catalysts[15-17], as well as ultrasound[18] and microwave –assisted.[19] Some of the above-mentioned methods have one or more negative points such as high-cost reactants, long reaction time, toxic media, harsh reaction condition and leaching of active species of the catalysts.[1,13,20] Therefore, to evade these limitations, the development of a novel readily available catalyst with superior catalytic ability, simple work-up and rapid reaction time is highly necessitated.

In the past decade, the applicability of solid catalysts in organic transformation is become interesting field because they are removable from the reaction media by simple separation and reusable.[20,21] Withal, composite nanoparticles are the reasonable strategies because a huge surface to volume ratio, thus increasing catalytic performance to acquire higher yield of organic products.[20,22] Thereunto, magnetic nanocatalysts, have enamored great attention in both academic and industrial stakeholders because they have unique characteristics compared to conventional materials, readily available, very simple separation from reaction mixture by external magnetic field and high degree of chemical stability in all kinds of organic and inorganic media.[23,24]

One kind of the magnetic nanoparticles that can be used as catalyst or catalyst support is magnetite (Fe_3O_4). In the previous investigation, Ali Maleki and co-workers utilized Fe_3O_4/chitosan composite to speed-up one-pot four-component synthesis of 1,4-dihydropyridines.[20] Meanwhile, Saumya Nigam et al. and Elham Cheraghipour et al. have prepared citrate-stabilized Fe_3O_4 as colloidal nanoparticles in

biomedical applications, especially in the treatment of hyperthermia.[25,26] According to the work performed by Camillo A. Navarro research group, aqueous citric acid is a promising green catalyst for synthesis of 1,8-dioxooctahydroxanthene.[1] Therefore, herein, we will present a preparation of Fe_3O_4-supported citric acid nanoparticles and explore it as reusable and magnetically separable nanocatalyst for the efficient synthesis of 3,3,6,6-tetramethyl-9-styryl-1,8-dioxooctahydroxanthene from cinnamaldehyde and dimedone.



Scheme-1: Reaction scheme for the green synthesis of 1,8-dihydrooctahydroxanthene

EXPERIMENITAL

General and Instrumentation

Chemicals, including cinnamaldehyde and dimedone were analytical standard (obtained from Sigma-Aldrich, Merck and others commercial shop) and used without any further purification. Elemental and morphological analysis of the catalyst has been carried out by Scanning Electron Microscope tandem Energy Dispersive on JEOL JED-2300. Functional group analysis was carried out using Shimadzu Prestige-21 in KBr pellet. Powder X-Ray Diffraction (XRD) patterns were recorded in the range of 10^0–80^0 with scan speed 2^0/min in a D8 Bruker diffractometer (40 kV and 30 mA) with Cu-Kα radiation (0.154 nm). ^{1}H-NMR analysis was performed using JEOL JSM 500 MHz spectrometer, whereas ^{13}C-NMR was conducted on JEOL JSM 125 MHz spectrometer. Ultraviolet-visible absorption was screened on Shimadzu 2450 spectrophotometer and LC-MS analysis was done by Mariner Biospectrometer.

Catalyst Preparation

The magnetite-supported citric acid (Fe_3O_4-CA) catalyst was prepared by two-stage synthesis. In the first step, as a catalyst support, magnetite nanoparticles were generated by mixing 1 mmol iron(III) chloride, 0.5 mmol iron(II) chloride and 50 mL aquadest. The mixture was gently stirred and then sodium hydroxide was added drop-wise until the reaction mixture reached the pH 10. The resulting solid was heated overnight using an ordinary oven and collected as magnetite nanoparticles. In the second step, citric acid solution (1% b/v) was added to magnetite (2 g). The mixture was heated at 60ºC and stirred for 60 min to yield Fe_3O_4-CA nanocatalyst.

Synthesis of 1,8-dioxooctahydroxanthene and Optimization Procedures

In a 25 mL round-bottom flask, cinnamaldehyde (1 mmol), dimedone (2 mmol), various amount of Fe_3O_4-CA and solvent (5 mL) were stirred at different temperatures. The amount of catalyst, reaction time and solvent were varied to get the best protocol for this synthesis. The Fe_3O_4-CA was separated by external magnet, and organic product was obtained by cooling the rest of reaction mixture.

Biological Evaluation

Antioxidant activity of the 1,8-dioxooctahydroxanthene was evaluated using DPPH method according to the previous procedure.[27] Briefly, stock solutions of xanthene with various concentrations (5, 10, 15 and 25 ppm) were mixed separately with DPPH methanolic solution. The mixtures were incubated at 37ºC for 30 min. After that, absorption maxima of solution were monitored at 517 nm and converted to % inhibition of free radicals. The minimum inhibitory concentration (MIC) of 1,8-dioxooctahydroxanthene was determined by using two fold serial dilution of Mueller-Hinton Broth (MHB) methods according to the previous investigations.[28,29] In this method, various concentrations of newly organic product were

prepared from 10,000 to 4.88 ppm in sterile tubes No. 1-12. The bacterial strains used in this test were E. coli, S. aureus and S. typhi. Chloramphenicol was used as positive control

RESULTS AND DISCUSSION

Catalyst Characterization

Figure-1 shows XRD pattern of magnetite-supported citric acid catalyst. The XRD patterns both in Fe_3O_4 and Fe_3O_4-CA are very similar, indicating the observed peaks can be attributable due to the presence of Fe_3O_4 (magnetite) phase. There are five dominant peaks recorded in XRD diffractogram with 2θ values of 30.32°, 35.66°, 43.32°, 57.13° and 62.58°, which are in good agreement with the reported data JCPDS Card No. 88-0315 and with previous research.[25,30]

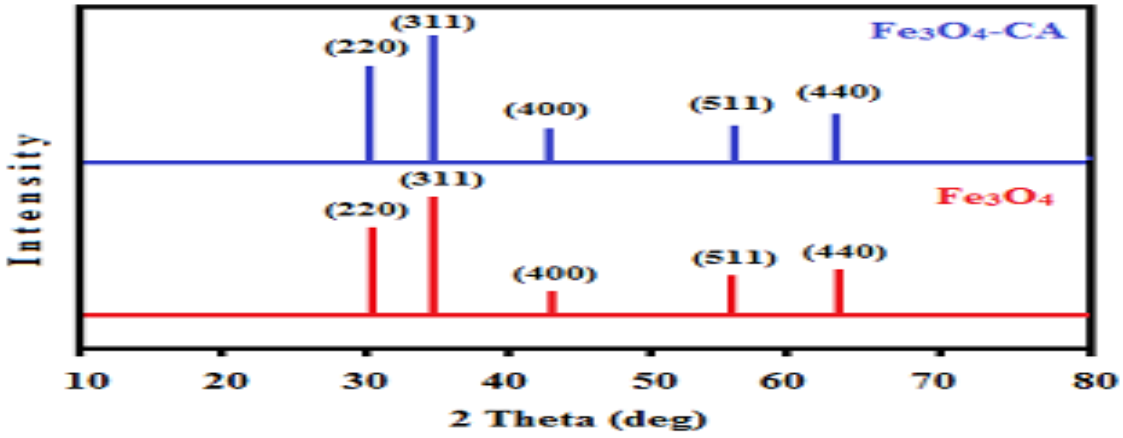


Fig.-1: XRD representation of Fe_3O_4 and Fe_3O_4-CA

Size distribution of Fe_3O_4 was measured using Particle Sizer and expressed in radius (nm). Measurement was repeated seven replications with the radius as follows: 17.7, 22.6, 22.9, 23.8, 25.1, 44.5 and 56.3 nm, gave the average radius of 30.4 nm, thus the diameter of Fe_3O_4 was 60.8 nm. After treatment of Fe_3O_4 with 1% of citric acid solution, radius of the synthesized Fe_3O_4-CA was measured also by seven replications as follows: 27.5, 39.1, 41.0, 44.6, 51.1, 52.0 and 60.3 nm with the average radius of 45.1 nm. Therefore, the average diameter of the Fe_3O_4-CA catalyst was found to be 90.2 nm. The increasing size of the particles of Fe_3O_4 to Fe_3O_4-CA was attributed to the impregnation of citric acid over the surface of Fe_3O_4. The size distribution was depicted in Figure-2.

The major elements present in synthesized Fe_3O_4 are Fe (68.50%) and O (22.67%), sodium present in trace due to the use of sodium hydroxide during nanoparticles synthesis. After modification with citric acid, different percentage of elements composition was found as Fe (48.72%), O (36.11%) and C (15.17%). The presence of carbon in elemental analysis indicated the successive modification of Fe_3O_4 to form Fe_3O_4-CA. analysis using SEM indicated that Fe_3O_4-CA has larger particle size than Fe_3O_4 (Fig.-3). This result has supported the analysis using particle sizer.

Spectral analysis of 1,8-dioxooctahydroxanthene

After catalyst separation using magnet, the rest of mixture was cooled to get solid product (mp. 174-175°C). The chemical functionalities of the yellow crystal were characterized by FTIR. An intense peak appeared at 2958 cm^{-1}, attributed to the stretching mode of C-H sp^3 bond. The band at around 1660 cm^{-1} appears due to C=O stretching vibration. The band at 1453 cm^{-1} represents mono-substituted benzene ring. Another band at 3050 cm^{-1} represents C-H sp^2 from aromatic ring and olefin in the structure of dioxooctahydroxanthene. In UV-Vis analysis, this product has absorption maximum at 371 nm, in agreement with yellow color observed.

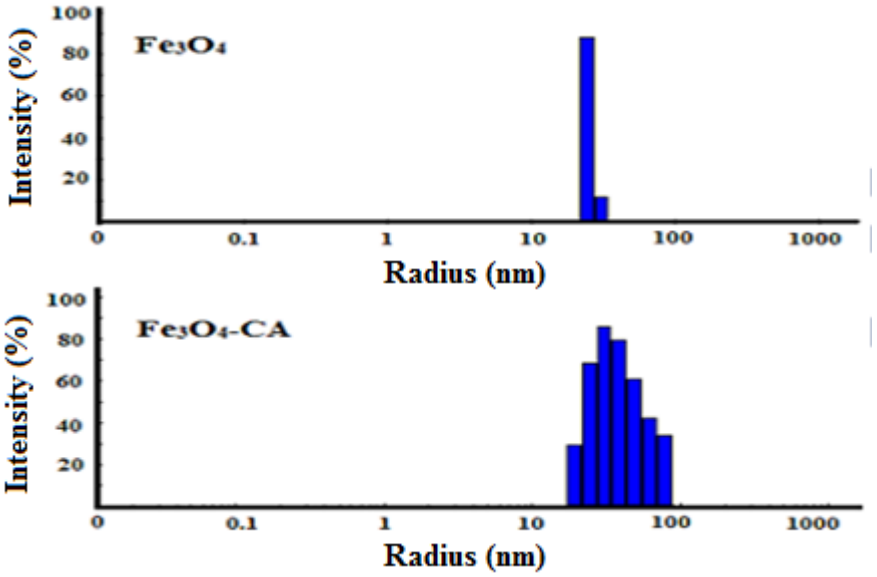
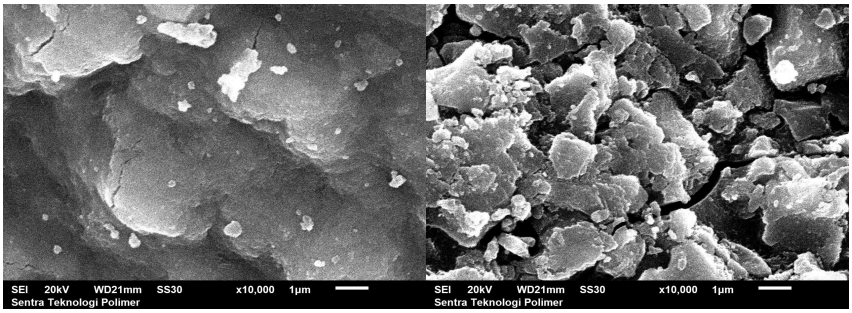


Fig.-2: Size distribution of catalyst



(a) (b)

Fig.-3: SEM images of (a) Fe_3O_4 and (b) Fe_3O_4-CA

(a) (b)

Fig.-4: (a) Magnetically separable catalyst and (b) Crystal of product

Mass spectrum has confirmed the formation of 3,3,6,6-tetramethyl-9-styryl-1,8-dioxooctahydro- xanthene from cinnamaldehyde and dimedone promoted by Fe_3O_4-CA. this compound has molecular formula of $C_{25}H_{28}O_3$ (376.0 g/mol).The highest peak with m/z 377.50 is associated with $[M+1]^+$ of the synthesized xanthene. The ^{13}C-NMR analysis of 1,8-dioxooctahydroxanthene indicating the presence of carbonyl group of ketone at 199.58 ppm, aromatic carbon at 128-138 ppm, C=C originally from cinnamaldehyde skeletal at 129.50 ppm (C-10) and 127.10 ppm (C-11). C=C in fused ring have chemical shift at 165.88 and 115.31 ppm (C-5 and C-6). Moreover, from ^{1}H-NMR analysis, indicating the successive 1,8-dioxooctahydroxanthene formation due to the existence of peaks at 1.12 ppm (12 H) for four methyl

group, 6.25-6.26 ppm (2H) for olefinic protons with trans configuration, 7.13-7.24 ppm (5 H) for aromatic protons and 4.27 ppm (1 H) for methine proton.

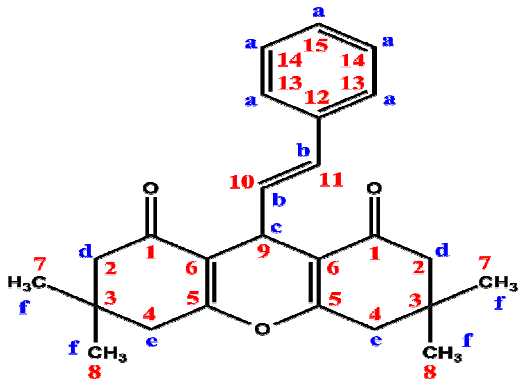


Fig.-5: Skeletal formula with different type of C and H in NMR analysis

The best protocol for 1,8-dioxooctahydroxanthene synthesis

Table-1 represents different conditions for 1,8-dioxooctahydroxanthene synthesis. 5% wt. of the catalyst is the best composition for this synthesis with the output of 80% chemical yield. Increasing the catalyst amount in the reaction mixture will not increase the yield of product (Entry 1 – 4). The use of 5% wt. of catalyst at 50°C in methanol for 2 h reaction time only produce low chemical yield at about 65% (Entry 5), indicated the reaction is still go to completion. Prolonging reaction time to 6 h will increase the yield of xanthene to 80% (Entry 2). The additional reaction time from 6 to 8 h has not effect in increasing the yield (Entry 7). Furthermore, the best temperature for the 1,8-dioxooctahydroxanthene synthesis is 50°C, whereas methanol acts as the best solvent to accommodate polarity of all reactants that combine each other.

Table-1: Synthesis of 1,8-dioxooctahydroxanthene in different condition

Entry	% wt. catalyst	Time (h)	T (°C)	Solvent	Yield (%)
1	2.5	6	50	Methanol	73
2	5	6	50	Methanol	80
3	7.5	6	50	Methanol	77
4	10	6	50	Methanol	75
5	5	2	50	Methanol	65
6	5	4	50	Methanol	70
7	5	8	50	Methanol	78
8	5	6	25	Methanol	29
9	5	6	75	Methanol	76
10	5	6	100	Methanol	75
11	5	6	50	Water	37
12	5	6	50	Citric acid	45
13	5	6	50	Ethanol	77
14	5	6	50	Solvent-free	51

The recoverability of Fe_3O_4-CA was determined by repeating the xanthene synthesis using the same catalyst from previous running. The yield of xanthene only decreased from 80% to 68% after 5th running (Fig.-6). A little loss of catalytic ability is indicated the high stability of the catalyst and it represents a bright future in developing this catalyst for industrial purpose.

Antioxidant and antibacterial evaluation

From antioxidant evaluation, 1,8-dioxooctahydroxanthene has approximately twice higher activity than its starting material, cinnamaldehyde in inhibiting DPPH free radical in methanolic solution (Table-2). 1,8-

dioxooctahydroxanthene has IC_{50} of 24.17 ppm, whereas cinnamaldehyde has IC_{50} of 43.05 ppm. Meanwhile, this product has comparable antibacterial performance against E. coli, S. aureus and S. typhi at different concentration (Table-3).

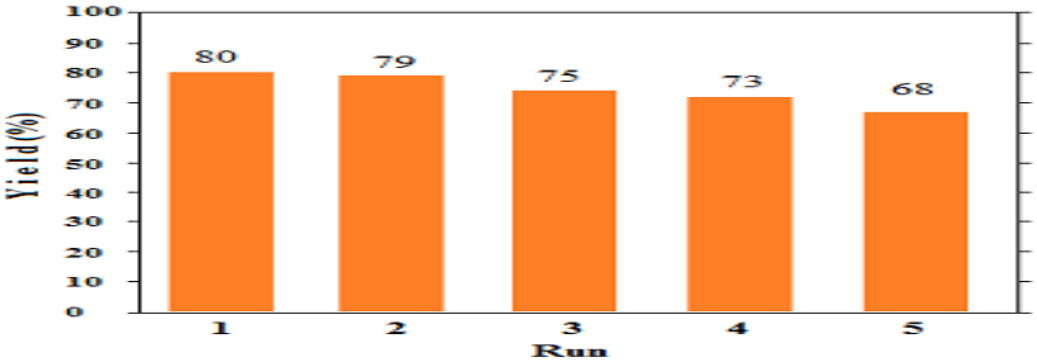


Fig.-6: Reusability of Fe_3O_4-CA

Table-2: Radical scavenging activity of cinnamaldehyde and product

Sample	Concentration (ppm)	Inhibition (%)
Cinnamaldehyde	10	15.11
	20	44.68
	100	90.88
dioxooctahydroxanthene	5	13.28
	10	20.80
	15	34.13
	25	51.11

Table-3: Antibacterial activity of cinnamaldehyde and product

Sample	Replication (n)	MIC (ppm) in different bacteria		
		E. coli	S. aureus	S. typhi
Cinnamaldehyde	1	2500	5000	78.125
	2	1250	5000	78.125
	3	1250	5000	78.125
Dioxooctahydroxanthene	1	5000	5000	2500
	2	5000	5000	2500
	3	5000	5000	2500

CONCLUSION

The synthesis of newly 1,8-dioxooctahydroxanthene from cinnamaldehyde and dimedone has been done by catalytic protocol of Fe_3O_4-CA. The best conditions for synthesis are using 5% wt. of catalyst, 6 h reaction time at 50°C in methanol as solvent gave the percentage of yield of 80%. Furthermore, The Fe_3O_4-CA has been used 5 times in the same reaction procedure, and can be highly recovered and reused with little loss of catalytic ability. Therefore, the Fe_3O_4-CA is a reasonable catalyst to be develop further to accommodate the synthesis of xanthene derivatives.

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