IOP Conf. Series: Materials Science and Engineering **188** (2017) 012022 doi:10.1088/1757-899X/188/1/012022

The synthesis of 2-(5-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol using sodium impregnated on activated chicken eggshells catalyst

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Abstract. The novel compound of 2-(5-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol as a pyrazoline derivative has been synthesized by two-steps reaction using sodium impregnated on activated chicken eggshells (Na-ACE) catalyst. Na-ACE was primarily prepared by a simple wet impregnation of NaOH solution on activated chicken eggshells solid support. The Na-ACE catalyst produced was characterized using FTIR spectrophotometer, XRD and SEM then applied in pyrazoline synthesis. First, chalcone was prepared from the reaction of 2-hydroxyacetophenone and 3-methoxybenzaldehyde by base-catalyzed aldol condensation. This product was subsequently reacted with hydrazine hydrate to give corresponding pyrazoline. The structure elucidation of the compound using FTIR, UV-Vis, LC-ESI-MS and ^{1}H-NMR indicated the desired product has been successfully synthesized. Furthermore, the potential antioxidant activities of chalcone and pyrazoline have also been studied *in-vitro* using DPPH radical scavenging method. The results revealed that pyrazoline has a greater antioxidant activity than chalcone.

1. Introduction

Chalcone and its derivatives are known for a wide spectrum of applications in the field of pharmaceuticals and other industries [1, 2]. For example, they have been found to exhibit various biological functions as antioxidant, anti-inflammatory, analgesic, anticancer, antitubercular, antitumor, antimicrobial, and antihyperglycemic agents [3-6]. Chemically, chalcone consists of two aromatic rings separated by keto-vinyl chain, and it is a versatile intermediate for synthesizing heterocyclic molecules [7, 8]. Meanwhile, pyrazoline, a type of hydrogenated pyrazole, is a five-membered nitrogen-containing heterocyclic molecule which has broad applications, especially in medicinal chemistry. Nitrogen heterocyclic is of special interest because it constitutes an important group of natural and synthetic products, many of them show valuable biological and pharmacological performances such as antiviral, anti-inflammatory, antifungal, antibacterial, and also demonstrates unique optical properties [9, 10]. Owing to the attractive properties of both chalcone and pyrazole, novel protocols to design different molecular structures have attracted attention of many researchers [11-13].

Several synthetic protocols are available for synthesis of chalcone and its pyrazole derivatives in the absence or presence of catalysts. The catalyst of KOH/EtOH [14] and NaOH/MeOH [15] have been used in chalcones synthesis, whereas some catalysts used in pyrazole synthesis such as triethylamine [16], hydrotalcite [17], *L*-proline in ionic liquid [bmim]BF_4 [18] and in catalyst-free condition using water as green solvent [19]. Many of the previously reported protocols suffer some limitations, such as high reaction temperatures, time consuming, poisonous solvents, and/or high cost reactants [20]. Thus,

the demand for the efficient methods using single catalyst that can accommodate two-steps pyrazoline synthesis from aromatic aldehydes/ketones via chalcone has accentuated the demand to develop a novel, green routes and eco-compatible motivated the present investigation. The activated chicken eggshell (ACE) is a simple, low cost and multipurpose material which can be used as catalyst for several types of organic reactions, including transesterification [21, 22], aldol condensation [6] and Schiff base formation [23]. Because of its potential to be used in organic synthetic as heterogeneous catalyst, in this work, we will demonstrate and compare the catalytic ability of Na-modified activated chicken eggshells (Na-ACE) in two-steps reaction during pyrazoline synthesis.

2. Materials and methods

2.1. Materials

Chemicals were from analytical grade (from Sigma-Aldrich, Merck and other commercial suppliers) and used without any further purification. TLC analyses were conducted on Merck silica gel 60 F_{254} aluminium plates. Melting point measurements were performed in an Electrothermal-9100 melting point apparatus and were uncorrected. IR spectra were conducted on a Shimadzu Prestige-21 spectrophotometer in KBr pellets. XRD pattern was recorded on a D8 Bruker diffractometer (40 kV and 30 mA) with Cu-Kα radiation (0.154 nm). Catalyst morphology and its elemental content were measured on SEM-EDAX instrument Hitachi S4160 at accelerating voltage of 20 kV. UV-Vis spectra were recorded in Shimadzu 2450 spectrophotometer. LC-ESI-MS Mariner biospectrometer was used to determine the molecular weight of the products, and the exact organic structure was confirmed using ^{1}H-NMR on a 500 MHz Agilent Technology instrument.

2.2. Catalyst preparation

The Na-ACE catalyst was prepared based on our previous work and re-characterized using IR, XRD, and SEM-EDAX [6]. Briefly, the chicken eggshells were boiled in distilled water for 1 h then dried in an oven at 110ºC for 3 h. Subsequently, the chicken eggshells were activated at 900ºC for 4 h under atmospheric air in a furnace to give activated chicken eggshells (ACE). The Na-ACE catalyst was obtained by impregnating 3% NaOH solution on ACE, and followed by calcination at 700ºC for 2 h. The total basicity of the catalyst was measured by titration method [6,24].

2.3. Chalcone and pyrazoline synthesis

Chalcone synthesis: A mixture of 2-hydroxyacetophenone (1 mmol), 3-methoxybenzaldehyde (1 mmol), solvent (5 mL), and proper amount of Na-ACE were magnetically stirred. The amount of catalyst added, reaction time, temperature and organic solvent were varied to get optimum condition of chalcone synthesis. Purity of the synthesized chalcone was tested by using TLC-scanner. (*E*)-1-(2-hydroxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one, a chalcone, was obtained. Pyrazoline synthesis: chalcone (1 mmol), hydrazine hydrate (2 mmol), ethanol (5 mmol) and 30% wt. of Na-ACE were stirred for 24 h at 60ºC. The product was purified with column chromatography with gradient elution to get pure pyrazoline, 2-(5-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol for further analyses.

2.4. Antioxidant activity

Chalcone and pyrazoline were evaluated for their *in vitro* free radical scavenging activity by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [25, 26]. Stock solutions of chalcone and pyrazoline with various concentrations were mixed with DPPH ethanolic solution (0.5 mL, 0.01 mM) in 3 mL of total reaction mixture and allowed to react at room temperature. Absorbance values were measured at 517 nm every 10 min for 40 min and converted to percentage of inhibition. The percentage of inhibition activity was then calculated. The data are summarized in table 1. Percentage of inhibition = [(absorbance of control – absorbance of sample) / absorbance of control] ×100%.

3. Results and discussion

3.1 Catalyst preparation

Chicken eggshells (CE) waste can be considered as material which has more than 98% wt. calcium carbonate by mass [27]. By activating it through calcination, the activated chicken eggshells (ACE) is produced with CaO as major constituent. As the basic properties of the alkaline earth metal oxides [28, 29], ACE is a promising material used for solid support in base-catalysed organic reactions. In addition, the use of ACE has advantages such as less expensive and less toxic compared to other alkaline earth metal oxides [28]. Catalytic performance of metal oxides based heterogeneous catalysts was found to be a function of their basicity. The basic strength of ACE used as solid support in this work was found to be 33.31 mmol/g, and can be enhanced up to 35.96 mmol/g by impregnating 3% Na^+ in ACE. Impregnation of alkali metal ions could increase the basic strength of metal oxides based catalysts, and it is confirmed with earlier published articles [6, 28].

IR spectra of CE in figure 1 shows sharp peak near 1700 cm^{-1} due to the carbonyl stretching of carbonate ions in CE. The disappearance of C=O band in ACE and Na-ACE indicated that calcium carbonate in CE is fully converted to calcium oxide. A broad peak near 500 cm^{-1} is attributed to the Ca-O vibration. Meanwhile, a sharp and strong peak at 3600 cm^{-1} is attributed to the non-hydrogen bonded –OH stretching in $Ca(OH)_2$ formed during adsorpsion of water over Na-ACE. The ACE and Na-ACE are chemically similar, and our data regarding the unactivated and activated chicken eggshells agree with those previously reported work [27, 30].

X-ray diffraction analysis results for CE, ACE and Na-ACE are shown in figure 2. The typical X-ray diffraction patterns of CE are observed at the Bragg angles, 2θ = 22.93°, 29.21°, 35.68°, 39.27°, 43.04°, 47.53° and 48.43°, implying the presence of *calcite* structure in chicken eggshells waste. The XRD pattern of ACE (activated at 900°C for 4 h) has peak at 2θ = 32.09°, 37.12°, 53.64°, 64.05° and 67.11°, indicating that *lime* (CaO) is the only phase presents in the activated chicken eggshells. The major peak of the *calcite* phase (2θ = 29.21°) is not observed in the XRD pattern of ACE, signifying that the $CaCO_3$ phase was completely changed to the CaO phase. Upon Na^+ impregnation the formation of calcium hydroxide in hexagonal form was depicted due to the presence of peaks at 2θ =17.90°, 28.67°, 34.06°, 47.00°, 50.77° and 62.44°. Impregnation of Na^+ was not found to affect the structure of *lime* phase, so the two major phases in Na-ACE are CaO and $Ca(OH)_2$. XRD resulted in this work was relevant with previous investigations [27, 28, 31].

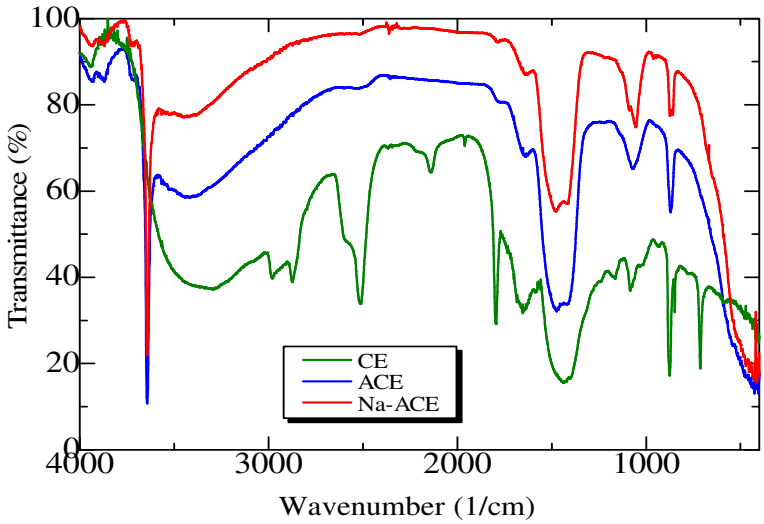


Figure 1. IR spectra of CE, ACE and Na-ACE.

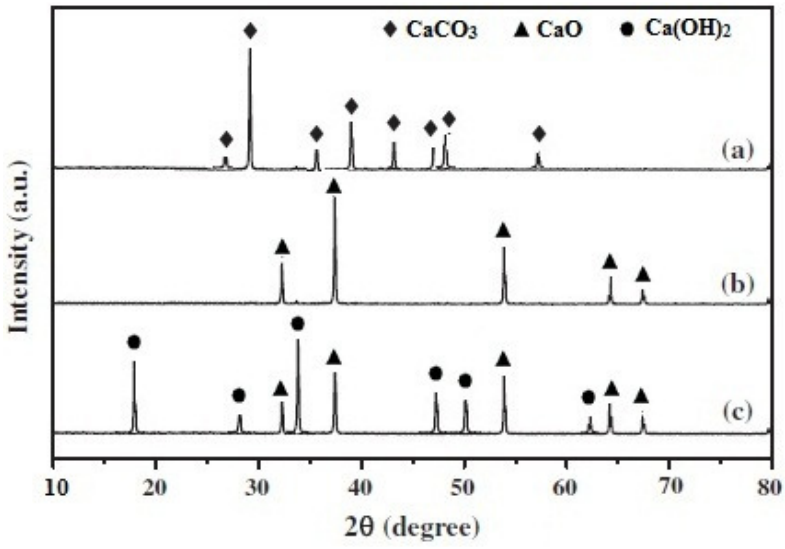
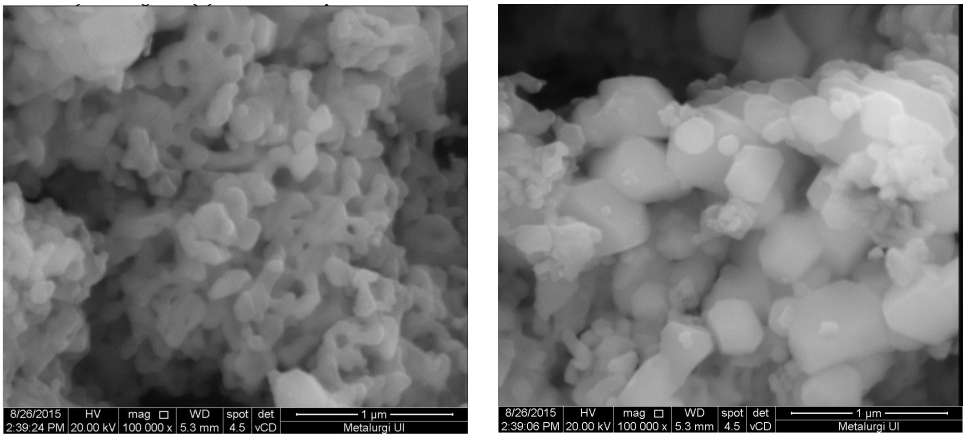


Figure 2. XRD patterns of (a) CE, (b) ACE and (c) Na-ACE.



(a) (b)

Figure 3. FESEM micrographs of (a) ACE and (b) Na-ACE.

FESEM micrograph of ACE shows that it has a porous structure and irregular shaped cluster of 1-2.5 μm sized particles as shown in figure 3(a). In Na-ACE catalyst, particles size decreased to 40-500 nm. This result is supported by calculation of the crystallite size using Debye-Scherrer equation, and the size of ACE and Na-ACE were found to be 108 and 50 nm, respectively. The data revealed that the 3% Na^+ impregnation can decrease the particles size of the activated chicken eggshells.

3.2. Chalcone and pyrazoline synthesis

Substrate 2-hydroxyacetophenone was reacted with 3-methoxybenzaldehyde using Na-ACE catalyst via base-catalysed aldol condensation, and it produced yellowish crystal of chalcone with melting point of 83-84°C. Structure elucidation of chalcone was performed from its spectroscopic data (IR, LC-ESI-MS and UV). The IR spectrum of chalcone showed an absorption peak at 1642 cm^{-1} associated to the α,β-unsaturated carbonyl group and absorption peaks at 3200 and 2960 cm^{-1} associated to the hydroxyl and C-H sp^3 group, respectively. Meanwhile, absorption at 3016 and 1491 cm^{-1} associated to the C-H sp^2 and C-C aromatic ring. In LC-ESI-MS analysis, there was only one single peak at retention time 7.57 min, and it has m/z values of 255.23 as $[M+H]^+$ and 277.24 as $[M+Na]^+$. The absorption profile of chalcone was screened from 200 to 500 nm and gave maximum wavelength at 310 and 255 nm. Analysis

using TLC-scanner indicated that the chalcone obtained was relatively pure with area chromatogram more than 99%. Chalcone obtained from step 1 was subsequently reacted with hydrazine hydrate via base-catalysed nucleophilic addition at step 2 to give corresponding pyrazoline (yellowish crystal, mp. 90-92°C). Structure elucidation of pyrazoline was performed from its spectroscopic data (IR, UV, LC-ESI-MS and ^{1}H-NMR). The IR spectrum of pyrazoline showed peaks at 3200-3380 cm^{-1} for overlap –OH and secondary –NH functional groups, at 1566 cm^{-1} for imine (C=N) group and at 3020-3050 cm^{-1} for C-H aromatic stretching. The disappearance of carbonyl peak near 1700 cm^{-1} indicated the successive transformation of chalcone to pyrazoline. Regarding the LC-ESI-MS data, there was a single peak at retention time 1.66 min with $[M]^+$ of 268.31 for $C_{16}H_{16}N_2O_2$. Analysis of pyrazoline using ^{1}H-NMR showed a singlet with integration of 3H at 2.89 ppm, assigned to the methyl protons of the methoxy group. Chemical shift between 6.90-7.78 ppm indicated there were 9 protons, including 8 proton of aromatic ring and 1 proton of N-H in pyrazole ring. Hydroxyl proton appeared in the downfield region at 10 ppm due to the hydrogen bond interaction by N atom from pyrazole ring. The UV absorption maximum of pyrazoline was at 300 and 291 nm, slightly lower than chalcone due to hypsochromic effect regarding to the number of its conjugated double bond.

To investigate the catalytic ability of Na-ACE in chalcone synthesis, several optimizations have been carried out. Figure 5a shows the chalcone yield obtained with Na-ACE catalyst, as a function of the reaction time. The reaction was carried out using 30% wt. of catalyst at 60°C in ethanol as solvent. The reaction produced chalcone with low percentage of yield (20%) at 1h, and achieved the optimum at 3h. In figure 5b, the use of 10% wt. of catalyst at 60°C for 3h contact time in ethanol, produced very low percentage of yield (~1%). The increase in the amount of catalyst used, increases the chalcone obtained, with the optimum catalyst of 30% wt. Surprisingly, increasing the catalyst amount to 35% wt. will not affect to the increase of chalcone yield. On the contrary, the chalcone yield decreased from 57% to 33% in this condition due to very strong adsorption of starting materials over Na-ACE catalyst, and will not produce the desirable product. Another reason to this phenomenon is the chalcone produced from the reaction was strongly adsorbed over Na-ACE catalyst and it was hard to handle, so the isolated yield was lower. Furthermore, when large amount of catalyst is added, the sintering and agglomeration of catalyst particles happens, and could decrease the catalytic ability of Na-ACE. Figure 5c shows that 60°C is the most suitable temperature for chalcone synthesis using Na-ACE catalyst. As depicted in figure 5d, we have performed chalcone synthesis in four different solvents. Ethyl acetate, as semi-polar solvent, gave the lowest percentage yield of chalcone by 29%. By changing the medium with more polar solvent, using t-butanol, for example the yield of chalcone increased and reached the optimum when ethanol was used in the reaction. Ethanol can dissolve all starting materials and disperse the catalyst particles well. When methanol was used, a more polar solvent than ethanol, the percentage yield was observed to be lower. The use of very polar solvents can be considered to be unsuitable condition for chalcone synthesis. The reaction scheme of chalcone and pyrazoline synthesis can be seen in figure 4.

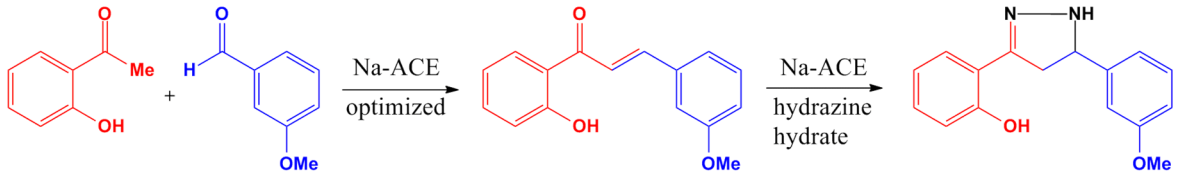


Figure 4. Reaction scheme in pyrazoline synthesis.

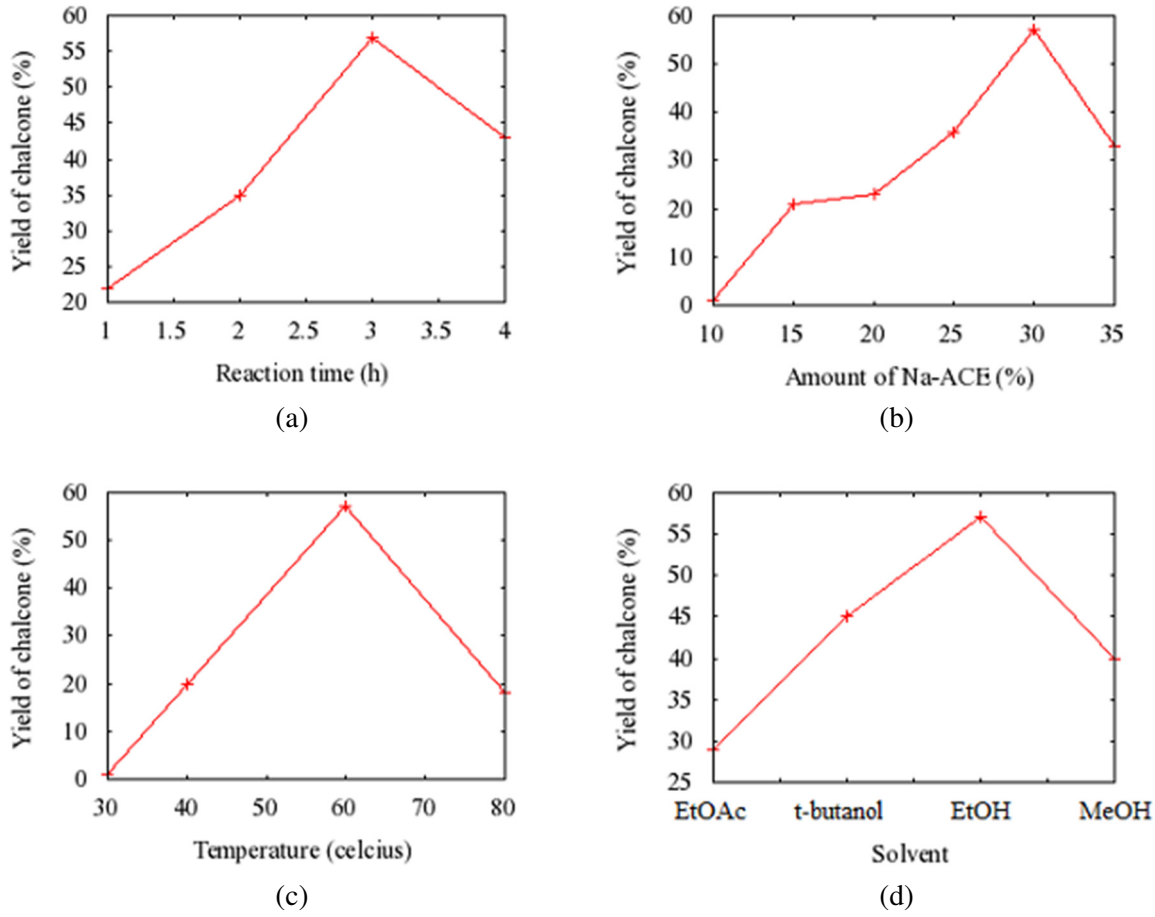


Figure 5. Optimization in chalcone synthesis (a) reaction time (b) amount of Na-ACE catalyst (c) temperature and (d) solvent.

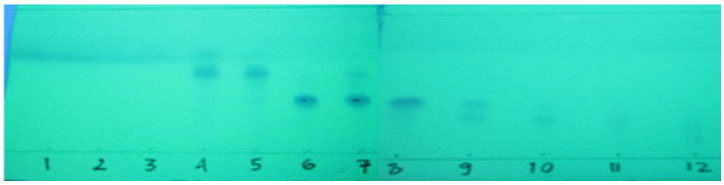
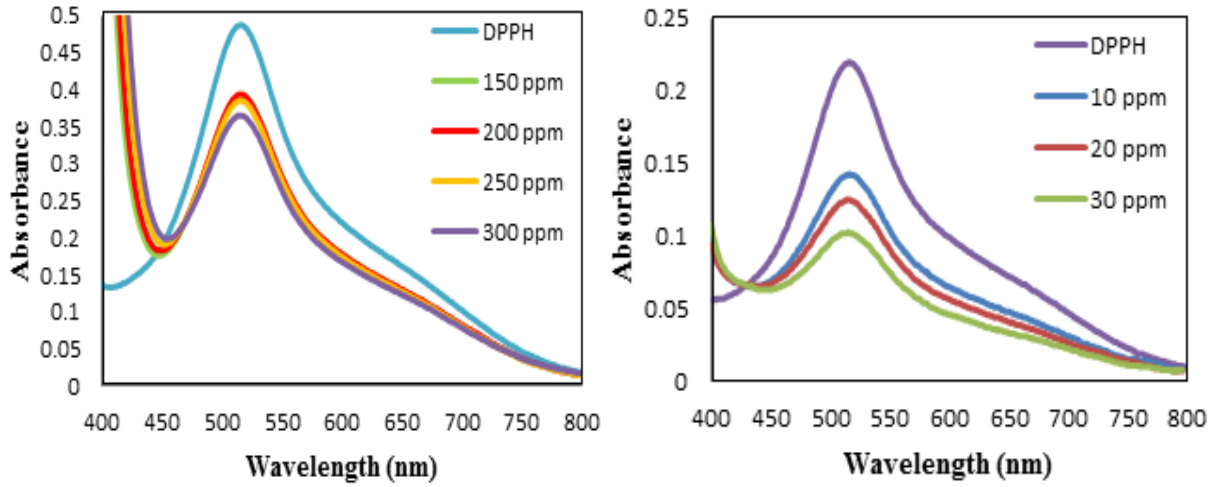


Figure 6. Undesirable by-product in pyrazoline synthesis was observed using TLC analysis. Fraction No. 6-8 is considered contains pyrazoline.

After chalcone was obtained (> 99% purity), we conducted the second step in pyrazoline synthesis. Molar ratio 1:2 of chalcone and hydrazine was used, and the reaction was performed at 60°C for 24 h by using 30% wt. catalyst amount produced very low desirable product, approximately 12% yield. Shortening the reaction time and decreasing both of the catalyst amount and temperature would produce pyrazoline lower than 10% yield. For example, when we used 30% wt. catalyst at 60°C for 10 h, 8% yield pyrazoline was isolated. Pyrazoline was not formed when the reaction was carried out at room temperature using Na-ACE catalyst. From the data, we concluded that the Na-ACE catalyst is less suitable for pyrazoline synthesis via reaction of chalcone and hydrazine hydrate than for chalcone

Table 1. Percentage inhibition by chalcone and pyrazoline.

Chalcone	DPPH inhibition (%)				Pyrazoline	DPPH Inhibition (%)			
	10 min	20 min	30 min	40 min		10 min	20 min	30 min	40 min
150 ppm	18.7	18.8	19.1	19.4	10 ppm	31.4	33.2	33.6	35.0
200 ppm	19.1	19.2	19.3	20.0	20 ppm	33.6	37.3	40.0	43.2
250 ppm	20.0	20.4	20.8	20.9	30 ppm	39.6	45.5	49.1	53.2
300 ppm	21.7	23.5	24.3	25.1	-	-	-	-	-



(a) (b)

Figure 7. Visible absorption profile of (a) chalcone and (b) pyrazoline in antioxidant test.

synthesis. Another limitation in the pyrazoline synthesis using Na-ACE was separation method of the product mixture. Undesirable product was observed in TLC analyses and need to be purified using gradient concentration column chromatography to get pyrazoline (figure 6).

3.3. Antioxidant activity

The *in vitro* antioxidant and scavenging effects of chalcone and pyrazole were evaluated by using DPPH radical scavenging method, and the results are shown in table 1. The interaction of the chalcone and pyrazoline with the radical DPPH denotes their radical scavenging performance. Both of chalcone and pyrazoline exhibit higher DPPH inhibition ability at higher concentration and longer time exposure. The full visible absorption profiles of DPPH control and sample-containing DPPH solution at different sample concentrations are depicted in figure 7. By using percentage inhibition data, the IC_{50} value for pyrazoline was calculated and found to be 26.8 ppm. On the other hand, the DPPH inhibition value for chalcone at 300 ppm and 40 min was 25.1% only. Hence, the IC_{50} value for chalcone was considered more than 300 ppm and classified as inactive antioxidative agent.

4. Conclusions

A new green approach to the synthesis of chalcone and pyrazoline using the same catalyst, sodium impregnated on activated chicken eggshells (Na-ACE), has been developed. The Na-ACE was found to be more active in catalysing aldol condensation to produce chalcone than in catalysing ring closure pyrazoline formation, with percentage yield of chalcone and pyrazoline of 57% and 12%, respectively. The future research should be focused on the catalytic modification of eggshells-based materials to improve their ability. From the bioassay, it was proven that pyrazoline, 2-(5-(3-methoxyphenyl)-4,5-

dihydro-1H-pyrazol-3-yl)phenol are potent and possessing high antioxidant and DPPH scavenging activities.

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