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Synthesis and radical scavenging evaluation of 4-(3-(2-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol

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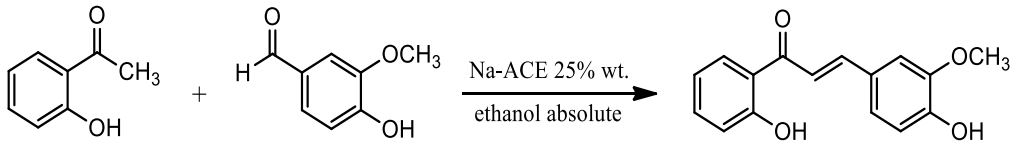
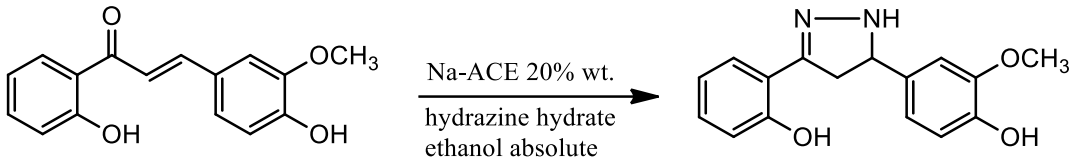
Abstract
Sodium impregnated on activated chicken eggshells (Na-ACE) has been utilized as an eco-friendly and green catalyst for the synthesis of a pyrazoline derivative, 4-(3-(2-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol via chalcone intermediate. The highest yield of chalcone (65%) was obtained from the reaction with 25% wt. catalyst at 60 ºC for 8h. Meanwhile, the best yield of pyrazoline (54%) was isolated from the reaction with 20% wt. catalyst at 60 ºC for 4h reaction time. The structure of organic compounds was confirmed using FT-IR, UV-Vis and LC-MS. In free radical scavenging evaluation, pyrazoline derivative exhibit antiradical activity with IC_{50} value of 20.45 μg/ml.

Keywords: Free radical, chalcone, pyrazoline, solid catalyst, Na-ACE

1. Introduction
Pyrazoline is a class of heterocyclic compounds which is rarely found in natural products. Pyrazoline and its derivatives have been known to have various biological activities such as anti-inflammatory [1], antitumor and antimalarial [2], analgesic [3] and antibacterial [4]. With their biological advantages and potential as drug candidates, it is necessary to develop green synthesis methods of pyrazoline derivatives.
Synthesis of pyrazolines can be carried out by reacting α, β-unsaturated carbonyl compounds with hydrazine or its derivatives in acidic or alkaline conditions [5]. Chalcone, a type of α, β-unsaturated carbonyls, is a multifunctional organic compounds in the synthesis of various heterocycles [6]. Chalcone is an intermediate of flavonoids with C6-C3-C6 carbon skeleton. It can be synthesized through Aldol condensation reactions using several types of catalysts like NaOH [7], KOH [8], acidic ionic liquids [9], activated carbon [10] and kaolin [11]. These catalysts can promote the formation of chalcones in good yields, but some of them have limitations such as corrosive, require complex reactor/ equipment setting and use of toxic solvents [12, 13]. Thus, environment-friendly materials or protocols are needed for the synthesis of chalcone and pyrazoline compounds.
In this study, pyrazoline compound, 4-(3-(2-hydroxyphenyl)-4, 5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenol will be synthesized from the reaction between chalcone compound 3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one and hydrazine hydrate. Meanwhile, chalcone compound will be prepared from the reaction between 2-hydroxyacetophenone and vanillin via Aldol condensation (Scheme 1 and 2). Sodium impregnated on activated chicken eggshells (Na-ACE) will be used as heterogeneous catalyst to speed-up these reactions. The material has been prepared under same conditions in our previous work. Furthermore, the ability of chalcone and pyrazoline compounds to inhibit free radical will be evaluated in vitro using DPPH method.



Scheme 1: Synthesis of chalcone



Scheme 2: Synthesis of pyrazoline from chalcone

2. Materials and Methods

2.1 General

All chemicals used in this research were analytical grade and purchased from commercial suppliers, such as 2-hydroxyacetophenone (Aldrich), vanillin (Merck), ethanol (Merck), ethyl acetate (Merck), hexane (Merck), deionized water (PT. Brataco) and hydrazine hydrate (Aldrich). Sodium impregnated on activated chicken eggshells (Na-ACE) catalyst has been prepared in our previous work [13]. FTIR spectra were recorded on Shimadzu Prestige 21 spectrophotometer, whereas UV-Vis spectra on Shimadzu 2450 UV-Vis spectrophotometer. Confirmation of the structure of chalcone and pyrazoline was done by LC-MS instrument.

2.2 Synthesis and optimization of chalcone compound

In an ordinary 50 ml Erlenmeyer flask, the mixture of 2-hydroxyacetophenone (1 mmol), vanillin (1 mmol), ethanol (5 ml) and certain amount of catalyst (0-30% wt.) was gently stirred for various reaction time (4,6,8 and 10 h). To get optimized condition for chalcone synthesis, temperature of reaction was also varied (rt, 45, 60 and 75 ^{o}C). Hot ethanol was added to the reaction mixture, then it was filtered. Solvent was evaporated to get crude product. Finally, crude product was purified by recrystallization [14].

2.3 Synthesis and optimization of pyrazoline compound

In a 50 ml Erlenmeyer flask, the mixture containing chalcone (0.25 mmol), hydrazine hydrate (0.6 mmol), ethanol (5 ml) and varied amount of Na-ACE catalyst (0-30% wt.) was gently stirred for various reaction time (2,3,4 and 5 h) and at some temperature variation (rt, 45, 60, and 75 ^{o}C. The reaction mixture was filtered, and filtrate was evaporated. Pure pyrazoline was obtained after recrystallization from hot ethanol [14].

2.4 Evaluation of free radical scavenging

Free radical scavenging activity of chalcone and pyrazoline compound was tested in vitro by DPPH method based on literature [15]. Initially, 1 mg/ml DPPH solution in methanol was prepared as stock. After that, 100 µl of stock DPPH solution was added to the different concentrations of samples. Then the solution was made up to 3 ml with methanol. After incubation for 15 min, absorbance of samples was measured at 517 nm. The ability of chalcone and pyrazoline to scavenge free radical was calculated using following equation.

Scavenging (%) = [(Ac-As)/Ac]*100

where Ac is the absorbance of control and As is the absorbance of sample.

3. Results & Discussion

3.1 Synthesis of chalcone

The preparation of chalcone was carried out via Aldol condensation reaction using equimolar amounts of aromatic ketone and aldehyde in the presence of sodium impregnated on activated chicken eggshells (Na-ACE) catalyst. Before attempting a detailed catalytic reaction, a noncatalytic synthesis of chalcone between 2-hydroxyacetophenone and vanillin was examined, and it was found that under the experimental condition (60 ^{o}C, 8 h), only trace formation of chalcone was observed. It indicates that the Aldol condensation reaction is not occur in the absence of Na-ACE catalyst (Table 1 Entry 1). To optimize the synthesis, amount of catalyst was varied from 0-30% wt. It was found that the highest yield of chalcone was isolated in the use of 25% wt. of catalyst at 60 ^{o}C in 8 h reaction time (Table 1 Entry 5). Prolonging reaction time or increasing temperature did not increase the yield of chalcone.

The FT-IR spectrum of prepared chalcone showed strong absorption bands in the range 1640-1650 cm^{-1} due to the C=O stretching conjugated with the double bond and 1580-1585 cm^{-1} due to olefinic C=C stretching (Fig. 1a). Also, the spectrum showed bands in the range 1461-1560 cm^{-1} due to the aromatic double bond, while the phenolic -OH group showed a broadband in the range 3300-3600 cm^{-1}. Chalcone had maximum wavelength at 344 nm, it was greater than its starting materials due to the conjugated double bonds (Fig. 1b). Also, mass spectrum analysis that depicted in Fig. 1c, the m/z at 271.27 showed $[M+1]^{+}$ value for the prepared chalcone, whereas the m/z at 293.27 showed $[M+Na]^{+}$ fragment. Further, pure chalcone compound (Fig. 1d) will be used as starting material in pyrazoline synthesis.

Table 1: Optimization parameter in chalcone synthesis

Entry	Catalyst (%wt.)	T (^{o}C)	Time (h)	Yield (%)
1	Free	60	8	Trace
2	10	60	8	45
3	15	60	8	55
4	20	60	8	58
5	25	60	8	65
6	30	60	8	59
7	25	rt	8	40
8	25	45	8	44
9	25	75	8	60
10	25	60	4	52
11	25	60	6	56
12	25	60	10	62

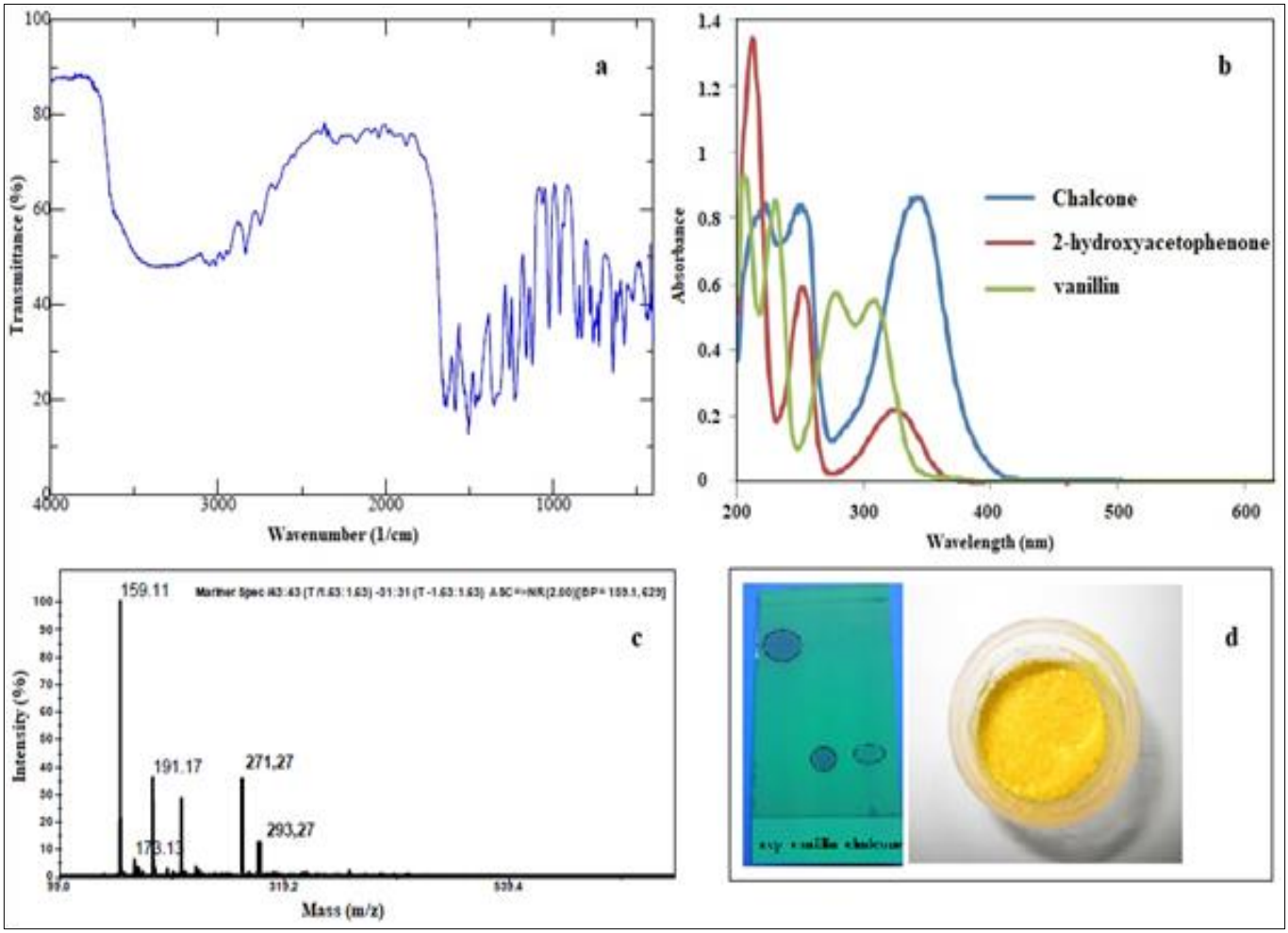


Fig 1: (a) FTIR, (b) UV-Vis, (c) Mass spectra and (d) TLC and solid product of chalcone compound, 3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one

3.2 Synthesis of pyrazoline

Synthesis of pyrazoline compound 4-(3-(2-hydroxyphenyl)-4, 5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenol was first carried out between chalcone compound 3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one and hydrazine hydrate in ethanol absolute in the absence of Na-ACE catalyst at 60 ºC for 4 h. We found this condition to yield a trace of pyrazoline. It was suggested that the catalyst plays an important role in formation of product. For optimization of experimental conditions, the influence of different reaction parameters such as amount of catalyst, temperature and time was investigated (Table 2). In order to investigate the effect of amount of Na-ACE catalyst, the pyrazoline synthesis was carried out using different weight of catalyst ranging from 10-25%. It was found that 20% wt. catalyst gave highest yield of pyrazoline (Table 2 Entry 4). To see the effect of temperature, the reaction was carried out in four different temperatures. At room temperature (using 20% wt. catalyst for 4 h), only 33% yield of pyrazoline was obtained. Increase in the reaction temperature affects the pyrazoline yield positively up to 60 ºC and further increase to 75 ºC could not affect the higher yield. Also, reaction time was varied from 2 to 5 h and it was found that 4 h was optimum time to produce pyrazoline.

The pyrazoline compound showed in the FTIR spectrum the stretching bands of NH group at 3318 cm^{-1} and C=N moiety at 1609 cm^{-1}. Also, absorption bands at 3417 cm^{-1} assigned to the phenolic -OH functionality (Fig. 2a). Pyrazoline had maximum wavelength at 354 nm (Fig. 2b). In the mass spectrum of pyrazoline compound the peak observed at m/z = 285.34 is due to the $[M+1]^+$ cation (Fig. 2c). Obtained pyrazoline was stored as yellow solid for further study.

Table 2: Optimization parameter in pyrazoline synthesis

Entry	Catalyst (%wt.)	T (ºC)	Time (h)	Yield (%)
1	Free	60	4	Trace
2	10	60	4	40
3	15	60	4	48
4	20	60	4	54
5	25	60	4	51
6	20	rt	4	33
7	20	45	4	39
8	20	75	4	42
9	20	60	2	28
10	20	60	3	36
11	20	60	5	52

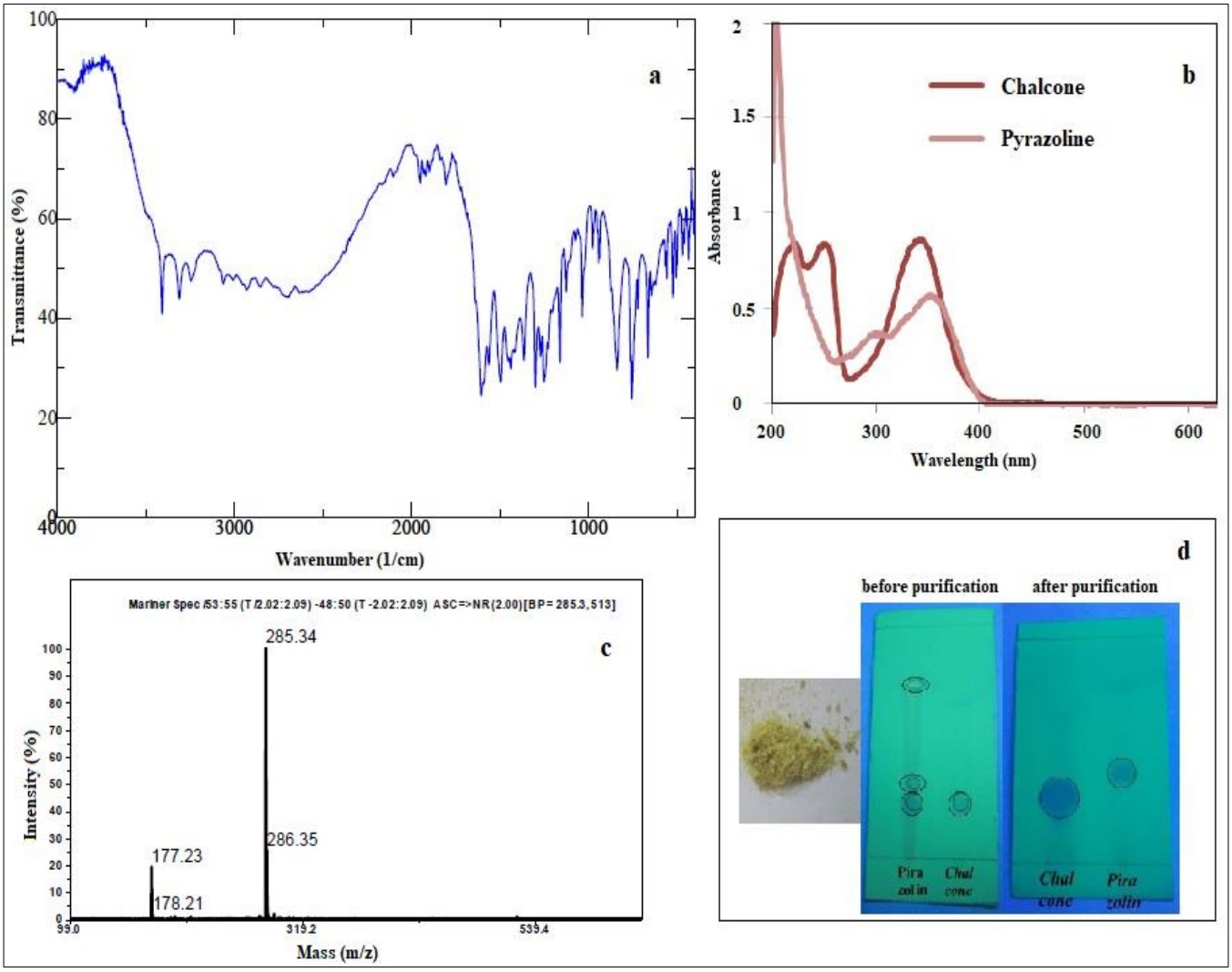


Fig 2: (a) FTIR, (b) UV-Vis, (c) Mass spectra and (d) Solid product and TLC chromatogram of pyrazoline compound, 4-(3-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenol

3.3 Evaluation of free radical scavenging activity

Free radical scavenging activity of prepared chalcone and pyrazoline was evaluated in vitro using DPPH method. DPPH is a stable free radical which has maximum wavelength at 517 nm. It could react with hydrogen donating compounds to form DPPH-H. Consequently, absorbance of DPPH decreases during transformation of DPPH to its reduced form, DPPH-H [15]. The degree of discoloration indicates the scavenging activity of the tested samples. Fig. 3 showed absorption profile of DPPH containing samples. Increase in sample concentration can decrease the absorption of DPPH at 517 nm. It indicates higher scavenging ability of the samples. The IC_{50} values of the tested chalcone and pyrazoline were 233.16 and 20.45 µg/ml, respectively.

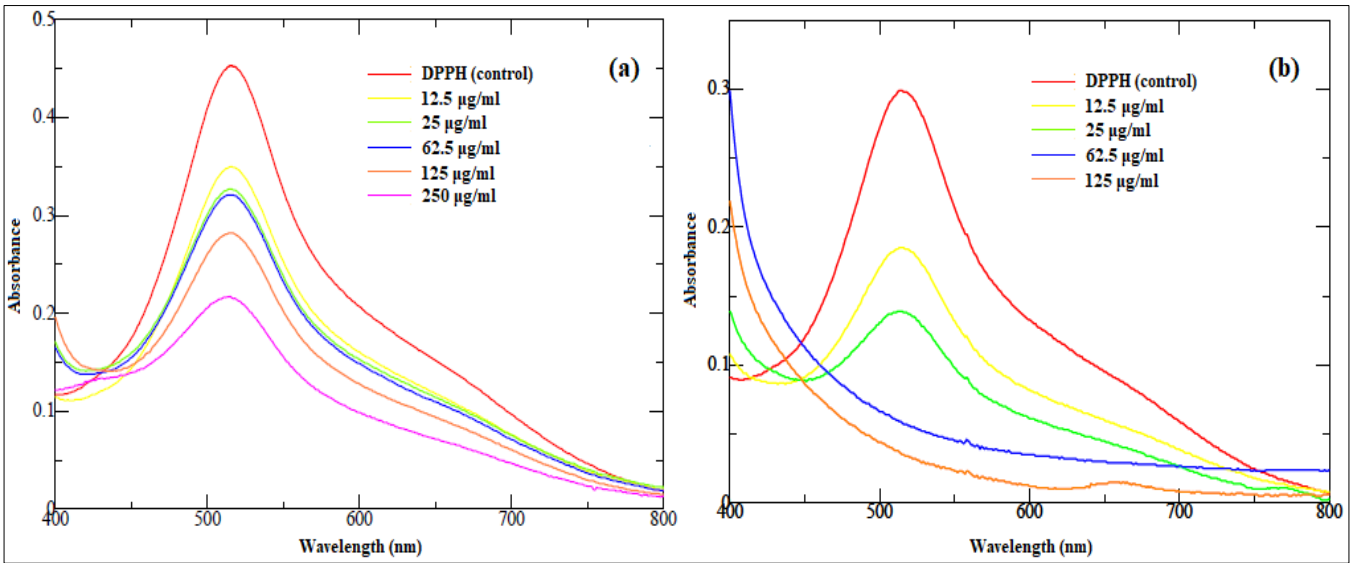


Fig 3: Visible absorption profile of DPPH solution in addition of various sample concentration: (a) chalcone and (b) pyrazoline

4. Conclusions

Chalcone compound, 3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one and pyrazoline compound, 4-(3-(2-hydroxyphenyl)-4, 5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenol were successfully synthesized in medium yield using sodium impregnated on activated chicken eggshells (Na-ACE) catalyst. The pyrazoline showed potent free radical scavenging activity with IC_{50} value of 20.45 μg/ml.

5. Acknowledgments

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