Catalytic Activity of Natural Silica for Green Synthesis of 3,4-Dihydropyrimidinone Derivatives and Antibacterial Activity

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ABSTRACT

This research describes the synthesis of 3,4-dihydropyrimidinone derivatives using natural silica as a novel source catalyst *via* the developed one-pot three component Biginelli reaction protocol. Compared to the classical method, this environmentally friendly synthetic method is efficient, simple, and short reaction times with higher yields (80-98%). The derivatives showed good potential on antibacterial activity against both gram-positive and gram-negative bacteria with the MIC values of 3.125-25 mg mL^{-1}.

Keywords: 3,4-dihydropyrimidinone, Antibacterial activity, Catalyst, One-pot synthesis

INTRODUCTION

In recent years, pharmacological properties of 3,4-dihydropyrimidinone (DHPM) and their derivatives have been reported, which include antihypertensive, calcium channel blockers, antiviral, antitumor, antioxidant, anticancer, anti-inflammatory, antibacterial, antifungal, anti-epileptic, antimalarial and anti-HIV activities (Kappe, 2000, Patil et al., 1995, Russowsky et al., 2006, Yu et al., 2007). The Classical synthesis of DHPMs was first reported by Biginelli reaction (Biginelli, 1983) that involves the three-component one-pot condensation of an aldehyde, *β*-ketoester and a urea or thiourea under strong acidic conditions. However, this method

requires harsh reaction conditions, long reaction times and affords low yields. According to their pharmacological properties of DHPMs, interest in this reaction has increased rapidly and several protocols for the synthesis of DHPMs have been developed using different types of catalysts and conditions to improve this reaction that aimed to overcome these drawbacks of the original reaction including ultrasound irradiation (Li et al., 2003) and microwave irradiation (Banik et al., 2007) methodologies. Different types of catalysts have been previous studied such as Brønsted acids (Yu et al., 2007), Brønsted bases (Shen et al., 2010), Lewis acids (Kumar et al., 2001), SiO_2-$CuCl_2$ (Kour et al., 2014), nanosilica-supported tin(II) chloride (Ghomi et al., 2013), silica sulfuric acid (Salehi et al., 2003), silica chloride (Karade et al., 2007) and so on. However, many of previous reported methods have some drawbacks such as the use of toxic, volatile and inflammable solvents, expensive reagents, long reaction times, harsh reaction conditions, difficult work-ups and low yields of products.

Therefore, the search for new catalyst, environmentally friendly reaction conditions, clean, milder and more efficient method is considerable current interest. In this study, we report preliminary results employing natural silica which synthesize from corn cob as catalyst for the synthesis of DHPMs by Biginelli reaction under solvent free conditions and their antibacterial activity.

METHODOLOGY

### Preparation of natural silica by precipitation method

The fresh corn cob (35 kg) were collected from Phayao province, Thailand and then washed with water, chopped into small pieces and dried in oven at 110°C for 10 hours. The corn ash was prepared under to firing at 600°C for 6 hours. Silica was extracted from corn cob ash with a modified previous method (Kalapathy et al., 2002). 250 ml portions of 3N NaOH were added to 15 g corn cob ash samples and constant stirring for 2 hours to dissolve the silica and produce a sodium silicate solution. The solution was filtered through whatman No. 41 ashless filter paper, and the residue was washed with 20 mL boiled distilled water. The filtrate was allowed to cool to room temperature and the pH of the solution was reduced with 5N H_2SO_4 to 2.0 with constant stirring. During the precipitation, the pH of the solution was adjusted to 10 with NH_3 in order to obtain the precipitant. The precipitant was filtered and dried in oven at 120°C for 12 hours.

Characterization of catalyst

The catalyst was thoroughly characterized by various instrumental techniques. X-ray diffraction (XRD) was used for determination of silica morphology and chemical components. The major chemical groups present in the silica were identified by FTIR.

General procedure for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones catalyzed by natural SiO_2

In the present improved protocol as exhibited in Scheme 1, a mixture of 1 mmol aromatic aldehydes (**1**), 1 mmol ethyl acetoacetate (**2**), 1.2 mmol urea (**3**), and SiO_2 (10 %w/w) were mixed together in a reaction vessel and the reaction mixture was then heated at 80°C. The reaction was monitored by TLC until the completion. After the completion of the reaction, the mixture was cooled at room temperature and filtered through Whatman No. 1 filter paper to separate the silica. The crude products were recrystallized with ethyl alcohol. All synthesized compounds were characterized using NMR, IR spectroscopy and melting point. Selected data of the structure analysis (Entry 1, Table 1): 5-(ethoxycabonyl)-4-(phenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (4a) is as follow; white needle crystal, mp 205.7-206.8°C; ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 1.05 (t, 3H, *J* = 7.2 Hz, CH_3), 2.20 (s, 3H, CH_3), 3.95 (q, 2H, *J* = 7.2 Hz, OCH_2), 5.10 (d, *J*=2.8 Hz, 1H, CH), 7.20 -7.30 (m, 5H, Ar-H), 7.70 (s, 1H, NH), 9.15 (s, 1H, NH); IR (v_{max}.; Neat, cm^{-1}): 3245 (N-H stretching (secondary amines)), 3116 (C-H stretching (aromatic)), 2979 (C-H stretching), 1724 (C=O stretching (ester)), 1700 (C=O stretching (amide)), 1648 and 1465 (C=C stretching), 1221 (C-O stretching) with respect to previously reported spectral data (Kour et al., 2014).
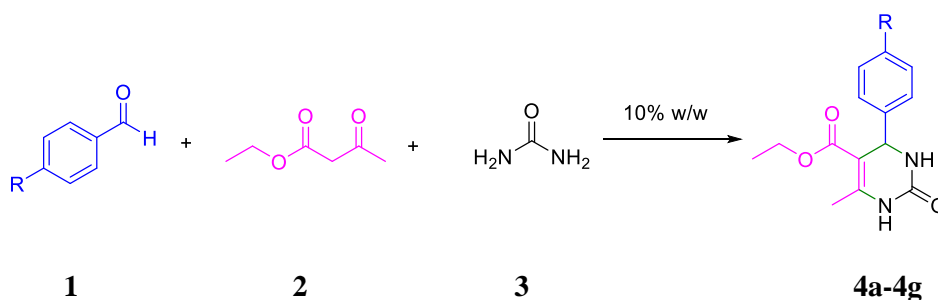
Scheme 1 Natural SiO_2 catalyzed synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones

Antibacterial assay

The antibacterial activity of the DHPM products was determined using the agar diffusion modified method (Khamsan et al., 2011). Bacterial strains were obtained from School of Medical Science, University of Phayao. The microorganisms used were: *Bacillus cereus*, *Staphylococcus aureus* (Gram positive bacteria) *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative bacteria). In brief, 50 mg of the DHPM products were dissolved in definite volumes of DMSO to give solution of 50 mg/mL concentration. The bacterial strains inoculum was diluted in sterile 0.85% Saline to obtain turbidity visually comparable to a McFarland standard No. 0.5 (10^{7-8} CFU/mL). Every inoculum was spread in agar plates. In each of these plates, 4 wells (9 mm) were cut out using sterile cork borer and 100 μL of the DHPM products was applied into the wells. A positive control was also assayed to check the sensitivity of the tested organisms using the presently available antibiotics: 100 μL (50 μg/mL) of ampicilin. A negative control was also assayed using 100 μL of DMSO. The plates were incubated at 37 ºC for 24-48 hours. The antibacterial activities were measured by the zone of inhibition expressed in mm.

The *in vitro* Minimum Inhibitory Concentration (MIC) assay

The highest dilution of the DHPM products that still retained an inhibitory effect against the growth of a microorganism was known as minimum inhibitory concentration (MIC). The MIC values of the DHPM products were determined by the disc diffusion modified method (Bauer et al., 1966).

In brief, twenty µL of each sample serial dilution solutions (1.5625, 3.125, 6.25, 12.5, 25.0 and 50.0 mg/mL) was carefully added into the sterile filter paper 6.0 mm disc, then the disc was placed on the surface medium plate. The control disc was impregnated with 10% DMSO and placed in the Petri dish center. The Petri dishes were incubated at 37 ºC for 24 h and the diameters of the zone of inhibition were measured in mm.

RESULTS AND DISCUSSION

Characterization of catalyst

The natural silica was prepared from corn cob by precipitation method affording the silica product in high yield (0.20%). The broad X-ray diffraction pattern showed that the silica is predominantly amorphous which is corresponding to amorphous solids (Kalapathy et al., 2002). The XRD pattern showed the major crystalline structure of silica in the type of SiO_2 Cristobalite (Figure 1).
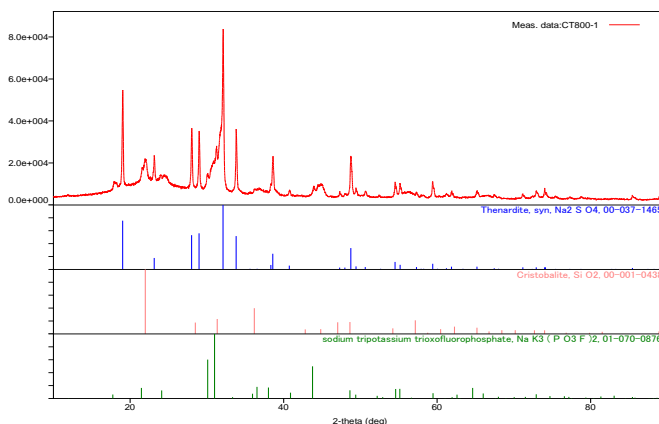

Figure 1 XRD pattern of silica from corn cob

The major chemical groups present in silica were identified by FTIR spectral that showed broad band at 3210 to 3485 cm^{-1} which is due to the stretching vibration of the O-H bond from the silanol groups (Si-OH) and is due to the adsorbed water molecules on the silica surface (Javed et al., 2011). The band at 1055 to 1099 cm^{-1} is due to the Si-O-Si asymmetric stretching vibration of silica, while the band at 795 cm^{-1} is belong to Si-O-Si symmetric stretching vibration. The band at 467 cm^{-1} assigned to O-Si-O bending vibration modes (Fig 2).
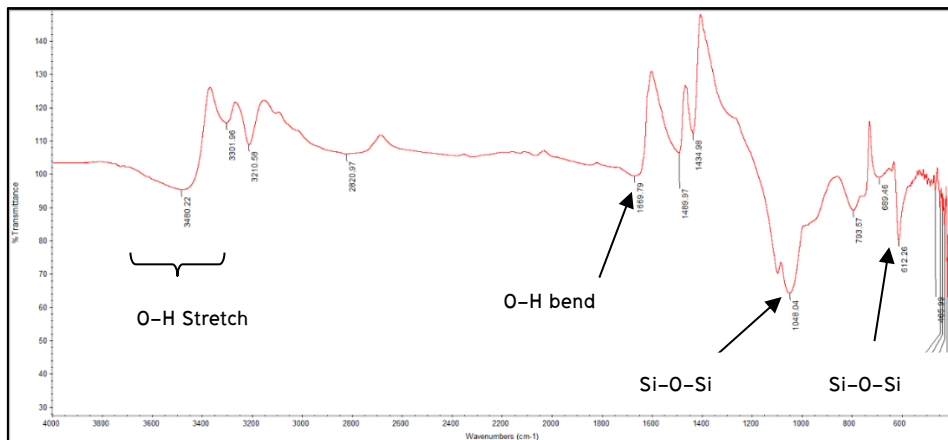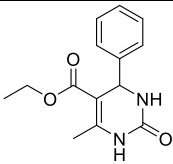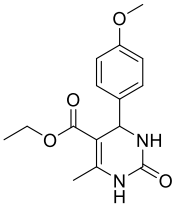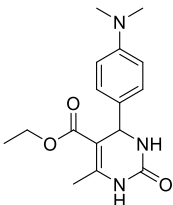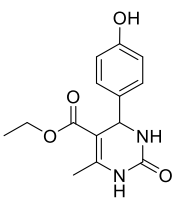
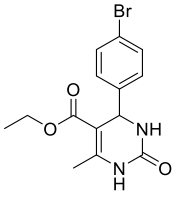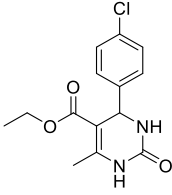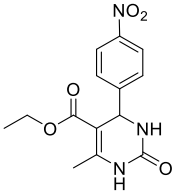**Figure 2** FTIR spectrum of silica from corn cob

Natural SiO₂ catalyzed synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones

This study aims to evaluate the effect of the natural silica as catalyst for the synthesis of DHPMs and their antibacterial activity. To establish the optimal reaction conditions, we carried out a set of model reactions by investigating various parameters such as the reaction time, amounts of the catalysts and molar ratios of reactants. The best conditions were obtained when 10 %w/w of silica, 1 equivalent of both benzaldehyde and ethyl acetoacetate and 1.2 equivalent of urea were mixed and heat at 80ºC under solvent free condition, corresponding product in excellent yield (4a, 98%) in short experimental time (2 h). This method was also carried out without the catalyst. In the absence of silica, compound 4a was obtained after 3 h in low yield (48%). To study the model reactions of this process, several aromatic aldehydes were studied and are summarized in Table 1. The results showed that all aromatic aldehydes have reacted with ethyl acetoacetate and urea under these conditions smoothly afford the corresponding DHPMs in good to excellent yields (80-95%). All products were characterized by comparison of their IR and ¹H NMR spectral analysis previously reported. Comparison with the classical Biginelli method (Biginelli, 1983), this modified protocol has the advantages include simple catalyst preparation, milder reaction conditions, short reaction times, easy recovery, remarkably improved the yields of DHPMs and reusability of the catalyst with consistent activity as well as environmentally friendly method. Furthermore, to explore the advantages of this natural silica catalyzed synthesis of 3,4-dihydropyrimidin-2(1*H*)-one by comparison with other catalysts such as montmorillonite KSF (Bigi et al., 1999), silica sulfuric acid (Salehi et al., 2003), silica-chloride (Karade et al., 2007) zeolite-supported HPA (Moosavifar et al., 2012), and cellulose sulfuric acid (Rajack et al., 2013), the results showed that this natural silica is an efficient catalyst corresponding higher yield and solvent free conditions. Furthermore, several aromatic aldehydes carrying either electron releasing or electron withdrawing substituents were also studied. The results showed that the developed methodology is suitable for other functional groups, such as hydroxyl, nitro, methoxy, amino and halides under reaction conditions affording high yields. A proposed reaction mechanism for the formation DHPM is by activating

the aromatic aldehyde on active silica and followed by nucleophilic addition of urea to forming the iminium intermediate and interacts with enol form of ethyl acetoacetate followed by cyclization and dehydration to achieve DHPM product as shown in Scheme 2.

Table 1 Natural SiO_2 catalyzed synthesis of 3,4-dihydropyrimidin-2(1H)-ones

DHPMs[a]	reaction time[b] (h)	Isolated Yield[c] (%)	mp (°C)		References
			Experiment	Reported	
4a	2	98	205.7 - 206.8	203-205	(Saher et al., 2016)
4b	3	95	202.3 - 203.9	201-202	(Karade et al., 2007)
4c	3	86	256.8 - 257.3	255-257	(Karade et al., 2007)
4d	2	90	223.2 - 225.7	225-228	(Saher et al., 2016)

Structure	Time (h)	Yield (%)	mp (found)	mp (lit.)	Reference
4e	2	82	213.2 - 214.6	212-213	(Gopinath et al., 2015)
4f	2	80	212.5 - 213.7	212-213	(Azizian et al., 2006)
4g	2	92	208.7- 209.2	207-209	(Azizian et al., 2006)

[a]All DHPMs were performed using aromatic aldehydes (1 mmol), ethyl acetoacetate (1 mmol), urea (1.2 mmol), and SiO_2 (10 % w/w)

[b] The reaction was monitored by TLC until the completion, [c] Isolated yield
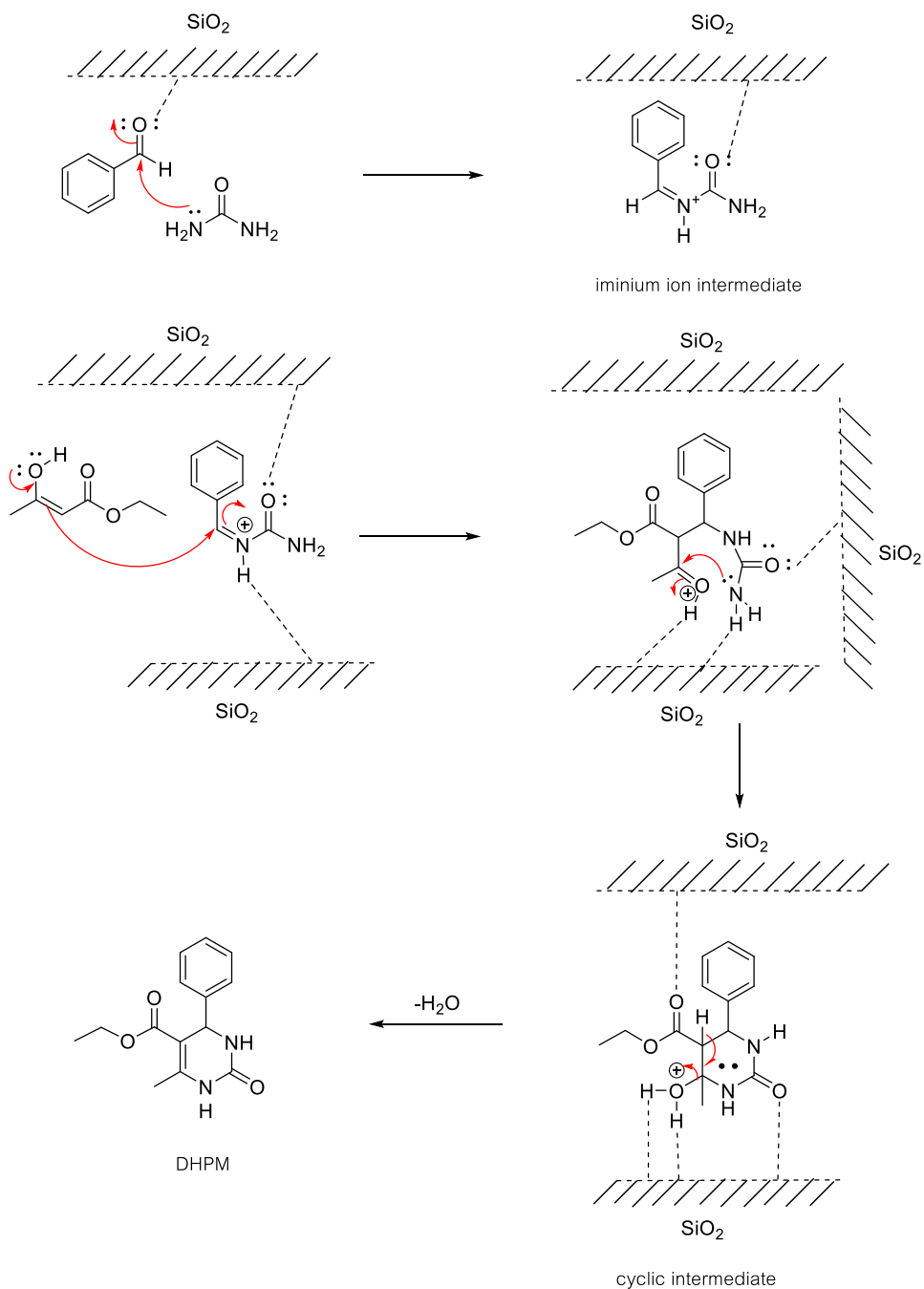
Scheme 2 A plausible mechanism of natural SiO$_2$ catalyzed synthesis of 3,4-dihydropyrimidin-2(1H)-one

Antibacterial assay

The agar well diffusion method was used to evaluate the *in vitro* antibacterial activity by measuring the inhibition zone against the test microorganisms. The preliminary antibacterial screening test indicated that all DHPM derivatives showed antibacterial activities against *B. cereus*. Compounds 4b, 4f and 4g have shown broad spectrum activity against both gram-positive and gram-negative bacteria tested and compound 4c possessed selective antibacterial activity against *B. cereus* while, compounds 4b, 4e, 4f and 4g showed moderate activity against *S. aureus* and *E. coli* with their respective diameter of inhibition zones shown in Table 2.

Table 2 Antimicrobial activity of DHPM derivatives

Samples	Zone of inhibition (mm)[a]			
	Gram positive bacteria		Gram negative bacteria	
	B. cereus	*S. aureus*	*E. coli*	*P. aeruginosa*
4a	18	-[b]	-	14
4b	18	14	14	14
4c	12	-	-	-
4d	10	-	-	14
4e	12	14	14	-
4f	14	12	14	14
4g	12	14	18	18
DMSO[c]	-	-	-	-
Ampicilin[d]	17	39	31	12

[a]Diameter of inhibition zones (mm), [b]No activity
[c]Negative control, [d]Antibiotics used as positive control

The minimum inhibitory concentration (MIC) value was defined as the lowest sample concentration that provided their high efficacy against microorganisms by the disc diffusion method (sample concentration range of 1.5- 50.0 mg mL^{-1}). The results of the MIC assay of DHPMs are shown in Table 3. Compound 4c and 4g exhibited the highest antibacterial efficacy against *B. cereus*, compound 4e and 4f showed the highest antibacterial activity against *S. aureus*, compound 4e exhibited the highest activity against *E. coli*, while compound 4a and 4f possessed the highest activity against *P. aeruginosa* with the MIC values of 3.125 mg mL^{-1}, respectively. Compound 4c exhibited the highest selective antibacterial efficacy against *B. cereus* at 3.125 mg

mL^{-1} concentration. Therefore, according to these results, the synthesized DHPMs might be another potential source for the discovery of new antibacterial drugs.

Table 3 MIC evaluation of DHPM derivatives

Microorganisms	Concentration (mg mL^{-1})						
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**
B. cereus	12.5	12.5	3.125	12.5	6.25	25	3.125
S. aureus	-[a]	12.5	-	-	3.125	3.125	25
E. coli	-	12.5	-	-	3.125	12.5	6.25
P. aeruginosa	3.125	6.25	-	6.25	-	3.125	12.5

[a]Not tested

The reusability of catalyst was also examined in the model reaction that performed under optimal conditions for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-one (4a). After the completion of the reaction, the catalyst was filtered to separate from the reaction mixture, washed with hot ethanol, dried, weighed and reused in next reaction. The crude product was recrystallized from hot ethanol. The possibility of recycling the catalyst was repeated four times. The results demonstrated that the catalyst has high efficiency even after 5 run reuses in 85–98% yield as shown in Figure 3.
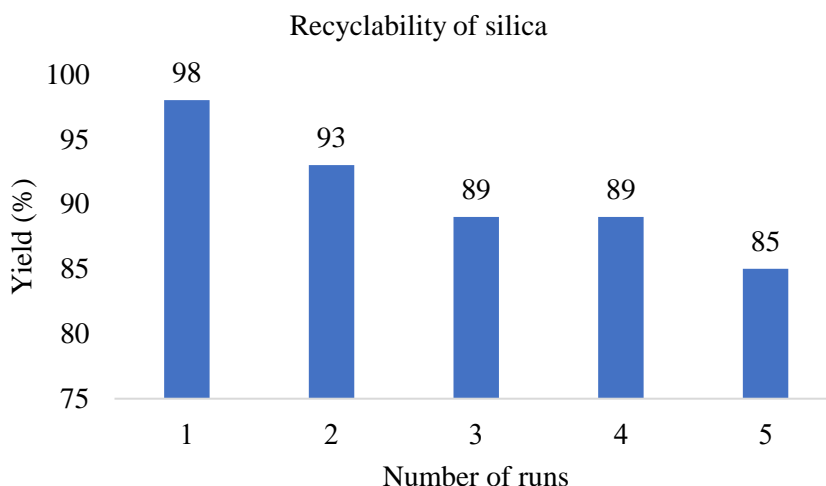

Figure 3 Recycling of natural SiO$_2$ catalyst for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-one

CONCLUSIONS

In conclusion, the efficient protocol for the synthesis of DHPMs has been reported using natural silica from corn cob which is simple, short reaction times and environmentally friendly method with good to excellent yields (80-98%) as well as reusability of catalyst. Derivatives showed a good potential on antibacterial activity against both gram-positive and gram-negative bacteria with the MIC values of 3.125-25 mg mL^{-1}.

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