Synthesis of Marine Vertebrate Mediated Metal Nanoparticles and Evaluation of its Catalytic Efficacy in Organic Transformation

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Abstract: **The current scenario highlights a growing importance towards synthesizing new nanomaterials using numerous routes persistently with groundbreaking contributions. Most considerably, the biological synthesis, well known as green synthesis is considered as an imperious additive to synthesis nanoparticles. This technology implies the production of nanoparticles with greater scientific interest without harming the environment. In this quest, the biosynthesis is the use of living organism towards the synthesis of nanomaterials. A suitable green methodology to synthesize a series of novel *dihydropyrimidinones* and *dihydropyrimidinthiones* derivatives from heterocycles by one pot three component tandem reactions using copper nanoparticles as a catalyst has been described. The catalytic activity of stabilized copper nanoparticles (CuNPs) from the fish scales of *Sardinella longiceps* in the one pot three component condensation reactions were investigated under microwave irradiation.**

Keywords*: *Copper, Sardinella longiceps, Fish scales, Dihydropyrimidinones, Microwave.

I. INTRODUCTION

Nanotechnology implies the design of particles with nanodimensions with respect to shape, size, controlled diversity, chemical composition and possible uses for human benefits.[1] Nanoparticles are produced by chemical and physical methods which are costly, non-dependable and potentially dangerous to the environment. Instead nanoparticles produced by biological methods seem to gain interest as they are made-up to be low-cost and environmental.[2] The manufacture of nanoparticles can be largely defined in two approaches. The first method is the top down which involves a material go through significant size reduction via physical or chemical processes.[3-4] The second, bottom up method forms nanoparticles through the assemblage of atoms, molecules, and smaller particles or monomers.[5] Regrettably, many of the chemical and physical processes used in both approaches suffer from several downsides such as generally expensive, have high energy requirements, low material conversion rates and technically complex. Additionally, several of these processes employ unsafe chemicals such as organic solvents, reducing agents and non-ecofriendly stabilizing agents.[6] For the reason of the shortcomings related with conversional manufacturing developments, there has been an increasing interest in emerging new ecofriendly production technologies established on the principles of green chemistry.[7]

Copper nanoparticles which has gained significant consideration in the past two decades due to its unusual properties, leading to probable applications in many varied fields. Copper nanoparticles are very attractive due to their heat transfer properties such as high thermal conductivity.[8-10] Its high surface area and small size have enhanced their use in material science and have increased their demand. Their properties are size reliant and also depend upon surrounding medium of nanoparticles.[11]Multicomponent reactions (MCRs) succeed a significant role due to the ability to prepare target compounds with more efficiency by the reaction of three or more compounds together in a single step.[12-16] 'Microwave-accelerated' chemical synthesis in solvents along with under solvent-free conditions have observed an explosive growth. The prominent features of microwave irradiation often leads to easier workup, shorter reaction times, increased yields, matches with green chemistry protocols and enhance the regio and stereo selectivity of reactions. In fact, the great utility of microwave-assisted synthesis fortified the researchers to increase the efficiency of several organic syntheses. Microwave methods offer an efficient and harmless technology conforming to green chemistry necessities. [17]

II. MATERIALS AND METHODS

A. *Chemicals Used*

All the reagents were purchased commercially and the solvents were purified by standard methods.

B. *Collection of Fish Scales*

Fish scales of *Sardinella longiceps* were collected from the local fish market (Ukkadam, Coimbatore, Tamilnadu). The scales were washed with tap water followed by distilled water to remove the unwanted debris.

C. *Preparation of Fish Scales Extract*

The collected fish scales were shade dried for about two weeks and then finely powdered. Fine powdered fish scales (12g) was placed in a 500ml Erlenmeyer flask containing 450ml distilled water and heated at 80°C for 30 minutes. This was centrifuged at 4000 rpm for 20 minutes and the supernatant was then filtered. The extract was stored in a refrigerator at 4°C for further analysis.

D. *Biosynthesis of Nanoparticles*

$CuSO_4.5H_2O$ (2g) was dissolved in 4ml of double-distilled water and 5ml of 10% of fish scales extract was added to it. The mixture was then refluxed at 100°C with continuous stirring and heated for 1 hour until the color changed to dark brown, which indicates the formation of copper nanoparticles. These were then filtered, centrifuged and the obtained precipitate was washed with distilled water.

E. *Synthesis of 2-hydroxy-4-formyl quinoline/ 2-formyl-4-methoxy quinolone*

2-hydroxy-4-formyl quinoline/ 2-formyl-4-methoxy quinoline was synthesized from comparing with reported literatures.[18]

D. *General Procedure for the Synthesis of 3, 4-dihydropyrimidin-2(1H)-ones/ thiones*

A mixture of equimolar of aromatic aldehyde/ 2-hydroxy-4-formyl quinoline/ 2-formyl-4-methoxy quinoline, ethyl acetoacetate/ methyl acetoacetate and urea/thiourea was homogenised using a glass rod in 5ml of ethanol to which a suspension of copper nanoparticles was added and subjected to microwave irradiation. The completion of the reaction was monitored by TLC. After completion of the reaction, it resulted in precipitation of the desired 3,4-dihydropyrimidin-2(1H)-ones/ thiones. The precipitated solid was filtered, dried and washed with petroleum ether to remove any residual starting material and then recrystallized from ethanol to give the pure product. The catalyst was recovered after evaporation of the aqueous layer and used for subsequent reaction without losing any substantial activity. The products were identified by physical data by comparing with those of reported literatures.[19]

III. RESULTS AND DISCUSSION

A. *Characterization of Gelatin*

The gelatin present in the fish scales extract of *Sardinella longiceps* were characterized using UV-Visible spectroscopy.

1) *UV-Vis Analysis:*

The gelatin present in the fish scales extract of *Sardinella longiceps* were characterized by means of UV-Visible spectrophotometer. The maximum absorption peak was obtained at **231nm**. The absorption spectra of gelatin is showed in the **fig. 1**

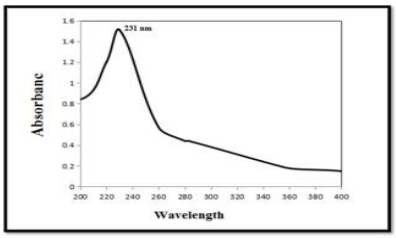


Fig. 1 UV-Visible absorption spectra of gelatin in fish scales of *Sardinellalongiceps*

B. *Characterization of Synthesized Copper Nanoparticles*

The biosynthesized copper nanoparticles were characterized using various analytical techniques.

1) *UV-Vis Analysis:*

The synthesized copper nanoparticles from aqueous extract of *Sardinellalongiceps* were characterized by means of UV-Visible spectrophotometer. The maximum absorption peak was obtained at the range of **585nm**. The absorption spectra of synthesized copper nanoparticles is showed in the **fig. 2**

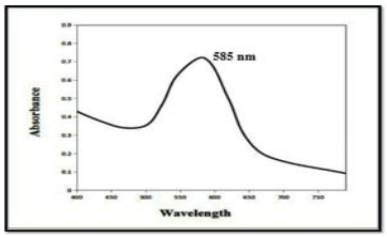


Fig. 2 UV-Visible absorption spectra of copper nanoparticles synthesized using fish scales of *Sardinella longiceps*

2) X-Ray Diffraction Study:

The XRD pattern of the synthesized copper nanoparticles (**fig. 3**) confirms the size of nanoparticles (**Table 1**) and the percentage of crystallinity (**Table 2**). The experimentally obtained X-ray diffraction angle were compared with the standard diffraction angle of Cu specimen.Three peaks at 2θ values of 43.502, 50.623 and 74.277 deg corresponding to (111), (200) and (220) planes of copper have been observed.

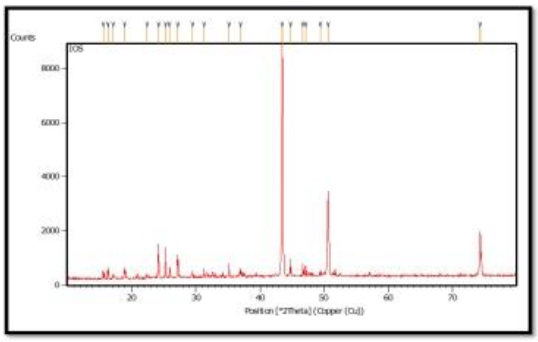


Fig. 3 X-ray diffraction pattern of synthesized copper nanoparticles

TABLE 1. SIZE OF THE PARTICLE FOR THE SYNTHESIZED COPPER NANOPARTICLES

S.No.	2 θ (deg)	Hkl	FWHM (deg)	Size of the particle (D) nm
1.	43.5023	(111)	0.0036	41.52
2.	50.6236	(200)	0.0033	45.5
3.	74.2772	(220)	0.0024	47.88

The sizes of the synthesized copper nanoparticles were calculated from powder XRD pattern using **Debye - Scherrer's**formula,

$$D=k\lambda/\beta \cos\theta$$

where,

D is the particle size can be calculated using the equation
k is Scherrer's constant≈ 0.94
λ is the wavelength having value 0.1547
β is the Full Width for Half Maximum for the diffracted peak (FWHM)
θ is the Bragg's angle for the peak

Hence, the particle size obtained to be less than 50 nm. The percentage crystallinity was calculated from XRD analysis was observed to be 75.87%

TABLE 2. PERCENTAGE CRYSTALLINITY OF SYNTHESIZED COPPER NANOPARTICLES.

Synthesized nanoparticles	**Total intensity of stronger peaks (counts)**	**Total intensity of broader peaks (counts)**	**% Crystallinity** $\%X_c = [I_C/(I_C+I_A)]\times100$
Copper	18,708.51	5,947.65	75.87%

3) Scanning Electron Microscope (SEM) Study:

SEM analysis was performed to observe the surface morphology of the copper nanoparticles synthesized from fish extract of *Sardinella longiceps*. The shapes of the copper nanoparticles were observed to be cubical in shape. The SEM image of synthesized copper nanoparticles is showed in the **fig. 4**

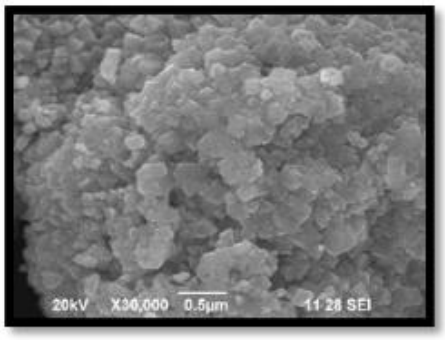


Fig. 4 Scanning electron microscopic image of copper nanoparticles synthesized using fish scales of *Sardinella longiceps*

C. Synthesis of dihydropyrimidinones **(9a)**

A mixture of equimolar of 2-hydroxy-4-formyl quinoline **(5)**, urea **(6b)** and ethyl acetoacetate **(7a)** was homogenized using a glass rod in 5ml of ethanol to which a suspension of copper nanoparticles was added and subjected to microwave irradiation. The completion of the reaction was monitored by TLC. After completion of the reaction, it resulted in precipitation of the desired product **(9a)**.The precipitated solid was filtered, dried and washed with petroleum ether to remove any residual starting material and then recrystallized from ethanol to give the pure product.

*D. Characterization of Resultant Dihydropyrimidinones Product **9(a)***

The obtained dihydropyrimidinones **9(a)** product from Biginelli reaction was characterized by various techniques.

1) FT-IR Analysis:

The IR spectrum of the purified product **9(a)** gave sharp bands at 3384 cm^{-1} and 3264 cm^{-1} corresponding to N-H stretching , 1680 $cm^{-1}$and 1662 cm^{-1} to C=O stretching. The FT-IR spectrum for the synthesized dihydropyrimidinones (9a) is showed in the fig. 5

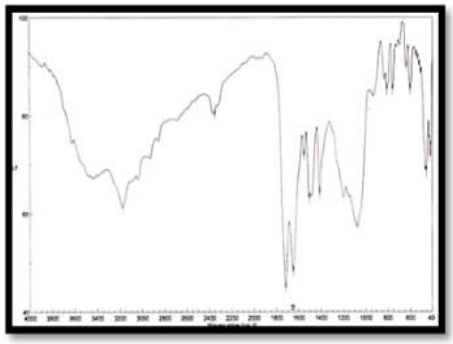


Fig. 5 FT-IR spectrum of the compound 9(a)

2) Proton NMR Spectroscopy:

^{1}H NMR studies of compound **9(a)** reveals the appearance of sharp singlet at δ 5.10 ppm which corresponds to that of the CH of the pyrimidone ring indicating the fusion of the quinoline aldehyde to the dihydropyrimidone ring. The two singlets at δ 5.98 and 7.25 ppm were accounted for NH groups. A broad peak corresponding to OH group was observed at δ 12.36 ppm. The

aromatic protons appeared between δ 7.46 to 8.27 ppm for five protons. A multiplet around δ 3.86 ppm corresponds to OCH_2-. A singlet around δ 1.89 ppm corresponds to the three methyl protons of CH_3 and a triplet at 1.47 ppm corresponds to the three methyl protons of OCH_2CH_3.The proton NMR spectrum of the compound 9(a) is shown in the fig. 6

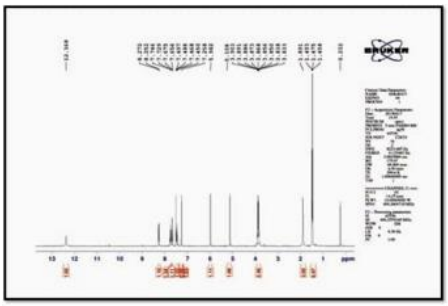


Fig. 6 ^{1}HNMR spectrum of the compound 9(a)

3) *^{13}C NMR Spectroscopy:*

The^{13}C NMR of the compound **9(a)** under investigation confirms the presence of 17 carbons. The ^{13}C NMR spectrum of the resultant dihydropyrimidinones **9(a)** shown in the fig. 7

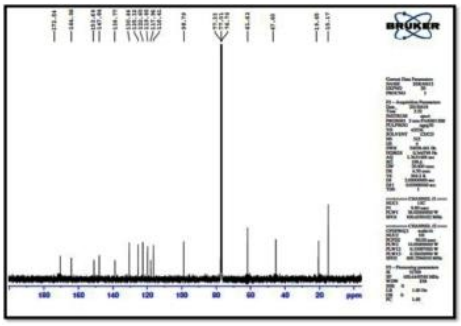


Fig. 7 ^{13}C NMR spectrum of the compound 9(a)

4) Electron Spray Ionization Mass Spectrum:

The ESI Mass spectrum m/z 327.33 is in agreement with the molecular formula of the compound, thus identifying the compound **9(a)** to be ***5-ethoxycarbonyl-4(2'-hydroxy quinolin)-6-methyl-3,4 dihydropyrimidin-2(1H)-one*** by all the spectral studies. We extended the reaction to all the other derivatives. The ESI Mass spectrum of obtained dihydropyrimidinones **9(a)** showed in the **fig. 8**

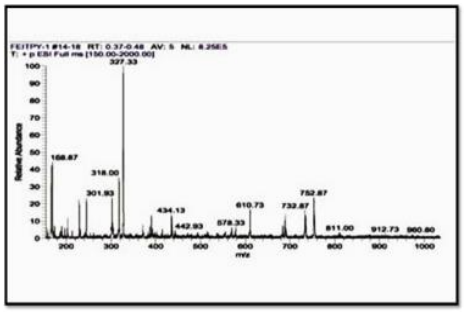


Fig. 8 ESI mass spectrum of the compound 9(a)

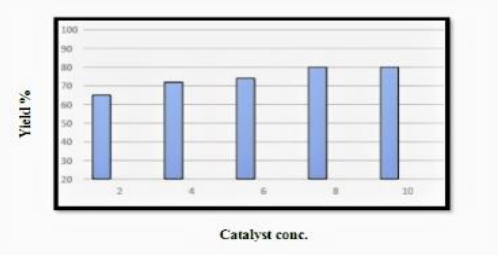
D. Effect of Catalyst Concentration on the Substrate Selectivity

Because of their extremely small size and relatively large surface area, the efficiency of the organic transformation can be increased by the use of nanosized catalyst. The primary attention was to enhance the effect of catalyst on the reaction condition. The catalyst concentration was diversed over a range of 2-10 mol% copper nanoparticles.

Table 3 shows the effect of catalyst concentration on the reaction of 2-hydroxy-4-formyl quinolines**5,** ethyl acetoacetate **7(a)** and urea **6(b)**. The resultant dihydropyrimidinones **9(a)** showed increase in yield with the increase in catalyst concentration from 2 to 8 mol %. Beyond concentration of 8 mol% more catalyst exists than that required by the reactant molecules. A supplementary addition of the catalyst had no noticeable effect on the yield. Hence the rate of the reaction does not increase on addition of catalyst. Thus 8 mol% of the catalyst was used in all further reactions.

The differences of the amount of catalyst are summarized in **Table 3** under solvent free microwave irradiation, best results were obtained.

TABLE 3. EFFECT OF CATALYST CONCENTRATION ON THE YIELD FOR THE SYNTHESIS **9(a)**



Catalyst conc. mol%	2	4	6	8	10
Yield %	65	72	74	80	80

Fig. 9 Effect of catalyst concentration on the yield, for the synthesis of 9(a)

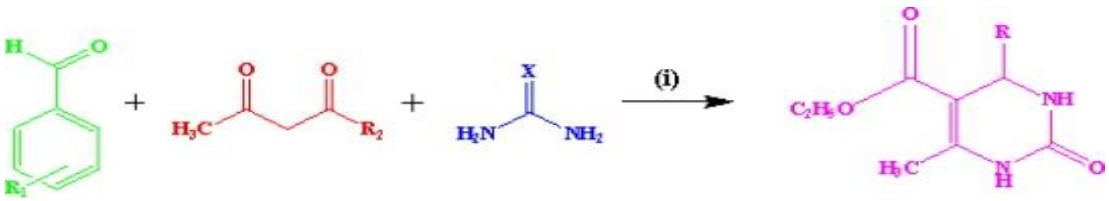
E. Effect of Variation of Microwave Power

The typical reaction under microwave also carried out using different powers. The model reaction was studied varying microwave power (120, 160, 240, 360, 400, 500 W) during the optimization of reactions. We noted that up to 360 W, there is an increase in the yield with a corresponding reduction in reaction time as the power rises. Thus the different reaction conditions were optimized at 360 W. After the course of the reaction, the copper nanoparticles were recorded by centrifuging the aqueous layer. By simple filtration, the catalyst was easily recovered by simple filtration after dilution of the reaction mixture with ethyl acetate and was reused after being vacuum dried.

TABLE 4. EFFECT OF MICROWAVE IRRADIATION (IN WATTS) ON REACTION TIME (IN MINS) AND PRODUCT YIELD (IN %)

Power, W	Reaction time, min	Yield,%
120	20	32
160	14	46
240	11	59
360	5.5	80

F. Synthesis of Functionalised Dihydropyrimidone (DHPMs) / Dihydropyrimithione (DHPMTs)



1 **2** **3** **4a-4p**

1a: $R_1 = H$
1b: $R_1 = CH_3$
1c: $R_1 = O\ CH_3$
1d: $R_1 = Cl$

2a: $R_2 = CH_3$
2b: $R_2 = C_6H_5$

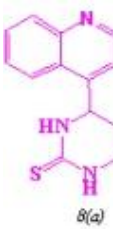
3a: X=O
3b: X=S

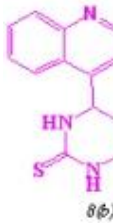
(i) = copper nanoparticles

Scheme 1. Synthesis of dihydropyrimidinones/thiones catalyzed by copper nanoparticles

TABLE 5. SYNTHESIS OF DIHYDROPYRIMIDONE (DHPMS)/ DIHYDROPYRIMITHIONE (DHPMTS) VIA BIGINELLI REACTION CATALYZED BY COPPER NANOPARTICLES

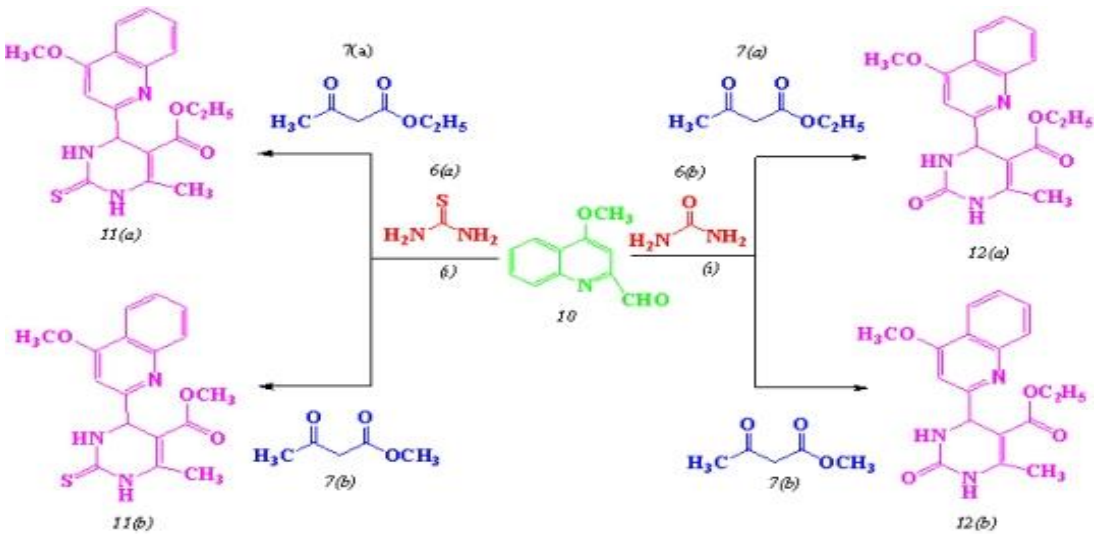
SI.no.	Aromatic Aldehyde	1,3-Dicarbonyl compound	Urea / Thiourea	Product	Observed mp°(C)	Reported[19] mp°(C)	Yield%
1	1a	2a	3a	**4a**	209	209-211	97
2	1b	2a	3a	**4b**	214	215-216	96
3	1c	2a	3a	**4c**	140	139-141	97
4	1d	2a	3a	**4d**	208	207-208	93
5	1a	2b	3a	**4e**	198	199-201	97
6	1b	2b	3a	**4f**	217	215-216	92
7	1c	2b	3a	**4g**	204	202-203	93
8	1d	2b	3a	**4h**	211	210-213	96
9	1a	2a	3b	**4i**	205	205-207	95
10	1b	2a	3b	**4j**	196	195-198	94
11	1c	2a	3b	**4k**	155	152-154	93
12	1d	2a	3b	**4l**	191	190-192	96
13	1a	2b	3b	**4m**	203	203-205	96
14	1b	2b	3b	**4n**	218	216-218	92
15	1c	2b	3b	**4o**	141	139-141	94
16	1d	2b	3b	**4p**	194	192-193	95





(i) Copper nanoparticles/ 10ml of ethanol/Microwave irradiation

Scheme 2. Schematic pathway for the synthesis of densely functionalized dihydropyrimidone (DHPMS)/ dihydropyrimithione (DHPMTS) involving 2-hydroxy-4-formyl quinoline



(i) Copper nanoparticles/ 10ml of ethanol/Microwave irradiation

Scheme 3. Schematic pathway for the synthesis of densely functionalized dihydropyrimidone (DHPMS)/ dihydropyrimithione (DHPMTS) involving 2-formyl-4-methoxy quinoline.

TABLE 6. SYNTHESIS OF DIHYDROPYRIMIDONE (DHPMS)/ DIHYDROPYRIMITHIONE (DHPMTS) VIA BIGINELLI REACTION CATALYZED BY COPPER NANOPARTICLES

SI. No	Quinoline Aldehyde	Urea / thiourea	1,3 Dicarbonyl compound	Product	Observed Mp°C	Reported[18] Mp°C	Yield (%)
1	5	6a	7a	**8(a)**	216	216-217	96
2	5	6a	7b	**8(b)**	222	223-224	97
3	5	6b	7a	**9(a)**	205	204-205	96
4	5	6b	7b	**9(b)**	212	211-214	98
5	10	6a	7a	**11(a)**	206	207-210	95
6	10	6a	7b	**11(b)**	231	230-233	97
7	10	6b	7a	**12(a)**	158	156-159	94
8	10	6b	7b	**12(b)**	179	178-181	93

To analyze the influence of catalyst and ratio of the components, equimolar mixtures of 2-hydroxy-4-formyl quinolines **5**, ethyl acetoacetate **7(a)**, urea **6(b)** and copper nanoparticles (8 mol%) were taken in 5ml of ethanol, combined and irradiated in synthetic microwave oven (360 W) by interval of 10s. The mixture was cooled, transferred into crushed ice and extracted with ethyl acetate after the completion of the reaction. The organic layer was dried over sodium sulphate and to give the crude product. The crude products were purified by crystallization in ethanol.

Further the optimized conditions were similarly applied for the synthesis of other derivatives and the structures of the newly synthesized compounds were established from their spectral data.

Fig. 10 Recrystallisation of dihydropyrimidine product 9(a) from ethanol

G. Catalytic Reusability

The stability and activity of the catalyst was verified in the recycle use experiments. The reusability of the catalyst was observed in the one pot three component reactions. For large scale operations and industrial point of view, the reusability of the catalyst is important. The nano catalyst was separated from the reaction mixture by simple centrifugation after the completion of the reaction which was washed with ethanol and acetone to remove the organic compounds followed by filtration and dried in an oven for further use. The recovered catalyst was dried and weighed. The copper nanoparticles were recycled for three times and observed that the catalytic activity of the catalyst was restored within the limits and there was no actual loss in the yield of the product which supports the stability and activity of the catalyst.

IV. CONCLUSION

The present study involves the biosynthesis of copper nanoparticles from *Sardinella longiceps* fish scale extract and evaluation of catalytic activity via Biginelli reaction. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, Scanning electron microscope and X-ray diffraction studies. Efforts has been made to synthesize nanoparticles in a greener route. The Biginelli reaction occurs with diversely substituted aromatic aldehydes and heterocycles. We have developed a safe, economic, quick and one pot synthetic protocol for the synthesis of DHPMs. The synthesis of 3,4-dihydropyrimidinones via Biginelli reaction leads to high yield in the presence of copper nanoparticles. The obtained purified products were characterized by FT-IR analysis, Proton NMR, ^{13}C NMR and ESI spectroscopy. The method also provides environmental advantages such as clean reaction, low loading of catalyst, short reaction times and use of various substrates and pure products in good to excellent yield.

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