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# Green synthesis of silver nanoparticles using green alga (*Chlorella vulgaris*) and its application for synthesis of quinolines derivatives

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## ABSTRACT

Nanoparticles have been used century ago but have regained their importance in recent years being simple, ecofriendly, pollutant free, nontoxic, low-cost approach, and due good atom economy. In this report, we have demonstrated the synthesis of silver nanoparticles using green algae (*Chlorella vulgaris*) which in turn was used for synthesis of biologically important quinolines. Algal extract was prepared and treated with silver nitrate solution for the synthesis of silver nanoparticles. Synthesized nanoparticles were characterized with the help of analytical tools like UV, FTIR, X-ray, and SEM and used as a catalyst for the synthesis of quinolines.

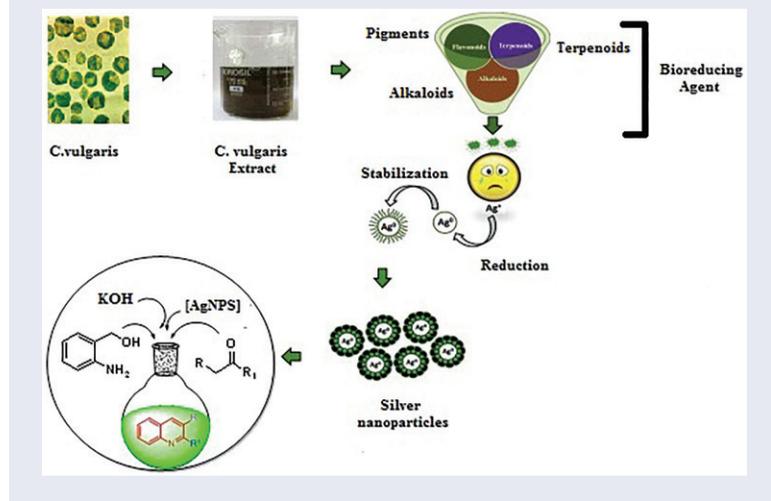
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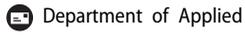
Biocatalyst; *Chlorella vulgaris*; green synthesis; quinolines; silver nanoparticles

## GRAPHICAL ABSTRACT



## Introduction

Metal nanoparticles have been synthesized extensively for a variety of applications in the areas such as chemistry, physics, life science, material science, medical science,

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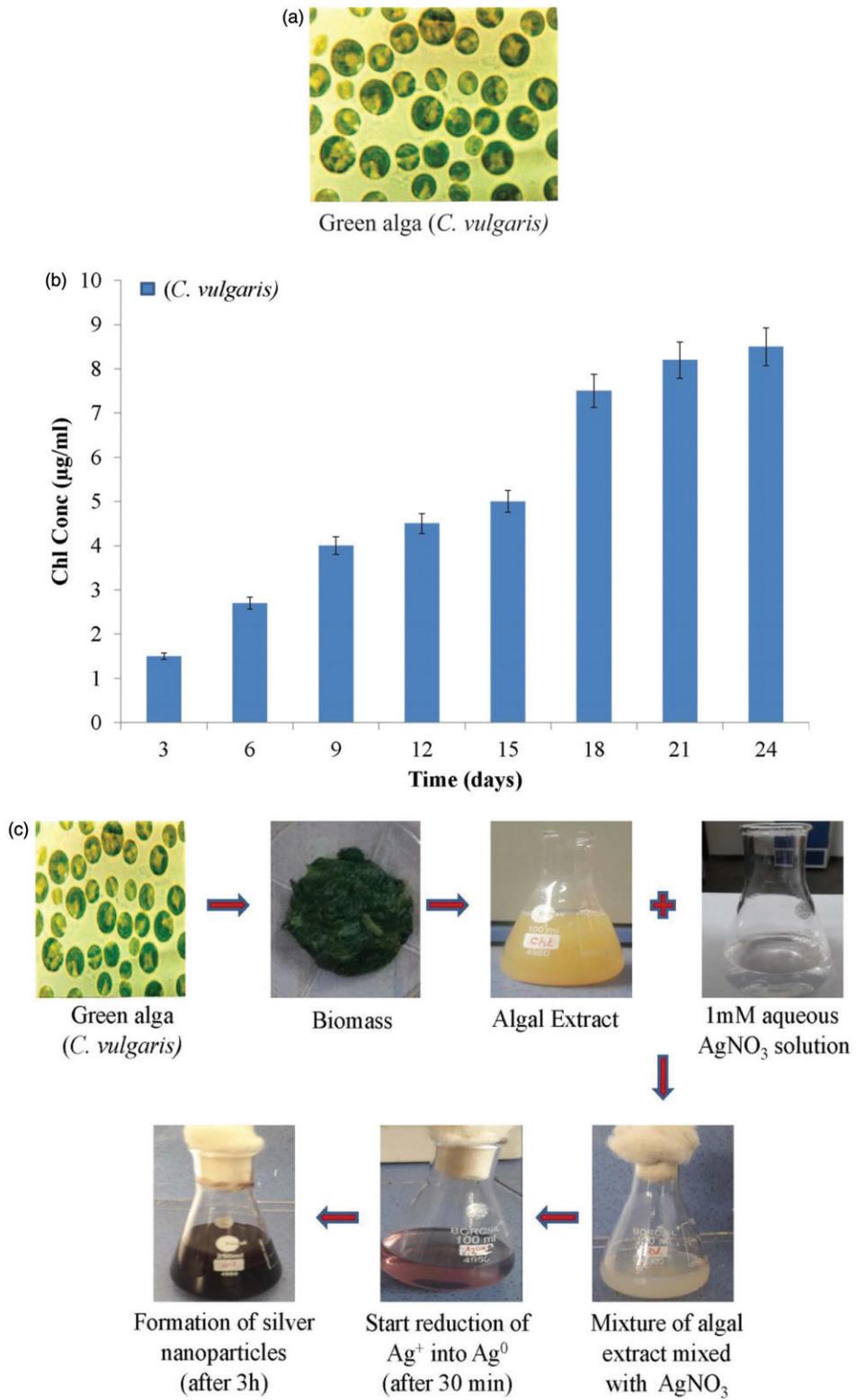
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nanomedicine, and engineering due to size and shape dependable properties. Nanoparticles possess unique optical, magnetic, electronic and catalytic properties along with their distinctive feature of size and shape.<sup>[1-2]</sup> Chemical synthesis of the metal nanoparticles requires a chemical reducing agent to convert metal ion into metal nanoparticles which involve the use of aggressive and hazardous chemicals.<sup>[3]</sup> In contrast to the chemical synthesis, green synthesis uses eco-friendly reagents as reducing agents in place of hazardous and toxic chemicals. The emphasis of green synthesis of metal nanoparticles is due to minimal impact on the ecosystem.<sup>[4]</sup> There are three criteria for green synthesis as (i) The selection of the environmental friendly solvent system. (ii) An eco-friendly reducing agent. (iii) A nanoparticle stabilizing capping agent.<sup>[5]</sup> The biological systems used for green synthesis include bacteria, fungi, plants, algae, and some other microorganisms.<sup>[6]</sup>

Green algae are considered as a source of bioactive compounds such as proteins, lipids, carbohydrates, carotenoids, vitamins, and other secondary metabolites with a range of biological activities.<sup>[7,8]</sup> Quinoline derivatives are known to have a broad scope in therapeutic, bioorganic, modern science and in the field of engineered natural science. Quinoline derivatives have been found to exhibit different therapeutic activities such as antimalarial,<sup>[9-12]</sup> antibacterial,<sup>[13,14]</sup> antifungal,<sup>[15,16]</sup> antiplatelet,<sup>[17]</sup> anticancer,<sup>[18,19]</sup> antitubercular<sup>[20]</sup> etc. Quinolines scaffold being useful for the preparation of biologically active molecules, the newer methods for their synthesis has always been explored. Although several methods are already available for the synthesis of quinolines, there is always a scope to develop simpler and cost-effective synthetic methods. In the present study, we have explored the catalytic usage of silver nanoparticles, obtained by green synthesis, for the synthesis of quinolines. This newer method of synthesis provides a one-pot route of synthesis for quinolines directly from 2-aminobenzylalcohol utilizing the catalytic efficiency of synthesized novel silver nanoparticles from green alga *C. vulgaris*. Different algal strains like red algae, brown algae, and green algae have been used to synthesize silver nanoparticles.<sup>[21]</sup> To the best of our knowledge and understanding, there are no reports on the use of silver nanoparticles (AgNPs) synthesized from *C. vulgaris* as a catalyst for this conversion.

## Results and discussion

This report discusses the synthesis of silver nanoparticles from green algae *C. vulgaris*. The synthesized silver nanoparticles were explored for the catalytic activity in the synthesis of quinolines. The complete process of the algal extract preparation and synthesis of silver nanoparticles are given in (Fig. 1c). Algal extract of green alga *C. vulgaris* was used as a potential source of reducing, capping and stabilizing agent in place of harsh chemical reagents such as sodium borohydride. The results obtained in this study are discussed in this section. Change in the color of the solution from colorless to brown, after mixing of an algal extract with 1 mM silver nitrate solution, confirmed the synthesis of silver nanoparticles. It was observed that the color intensity of the solution increases with the time of incubation. Best time for harvesting the test alga *C. vulgaris* with respect to growth was observed to be on the 15th day (Fig. 1a,b).<sup>[22,23]</sup>



**Figure 1.** (a) *Chlorella vulgaris*. (b) Growth of *Chlorella vulgaris* nutrient medium. (c) Experimental procedure for synthesis of silver nanoparticles using *Chlorella vulgaris*.

In this work, PMI (process mass intensity) was also calculated to quantify the greenness in terms of metrics. The green metrics related to nanoparticles synthesis was related to PMI and it was defined as:

$$\text{PMI} = \text{material input (kg)} \div \text{product output (kg)}$$

The PMI of synthesized nanoparticles was found to be 1.22 and yield of synthesized nanoparticles was 82%. The ideal value of PMI is 1 when PMI increases mean waste increases.<sup>[24]</sup>

The extract of green alga *C. vulgaris* was treated with the aqueous silver nitrate solution. The formation of silver nanoparticles was then characterized by visual observation means a color change from light pale yellow to reddish brown indicating the reduction of silver ions into silver nanoparticles (Fig. 1c). In this reduction process, silver nanoparticles scatter and absorb light at certain wavelength due to the resonate collective excitations of charges density at the interface between a conductor and an insulator, phenomena called Surface Plasmon Resonance (SPR).<sup>[25]</sup>

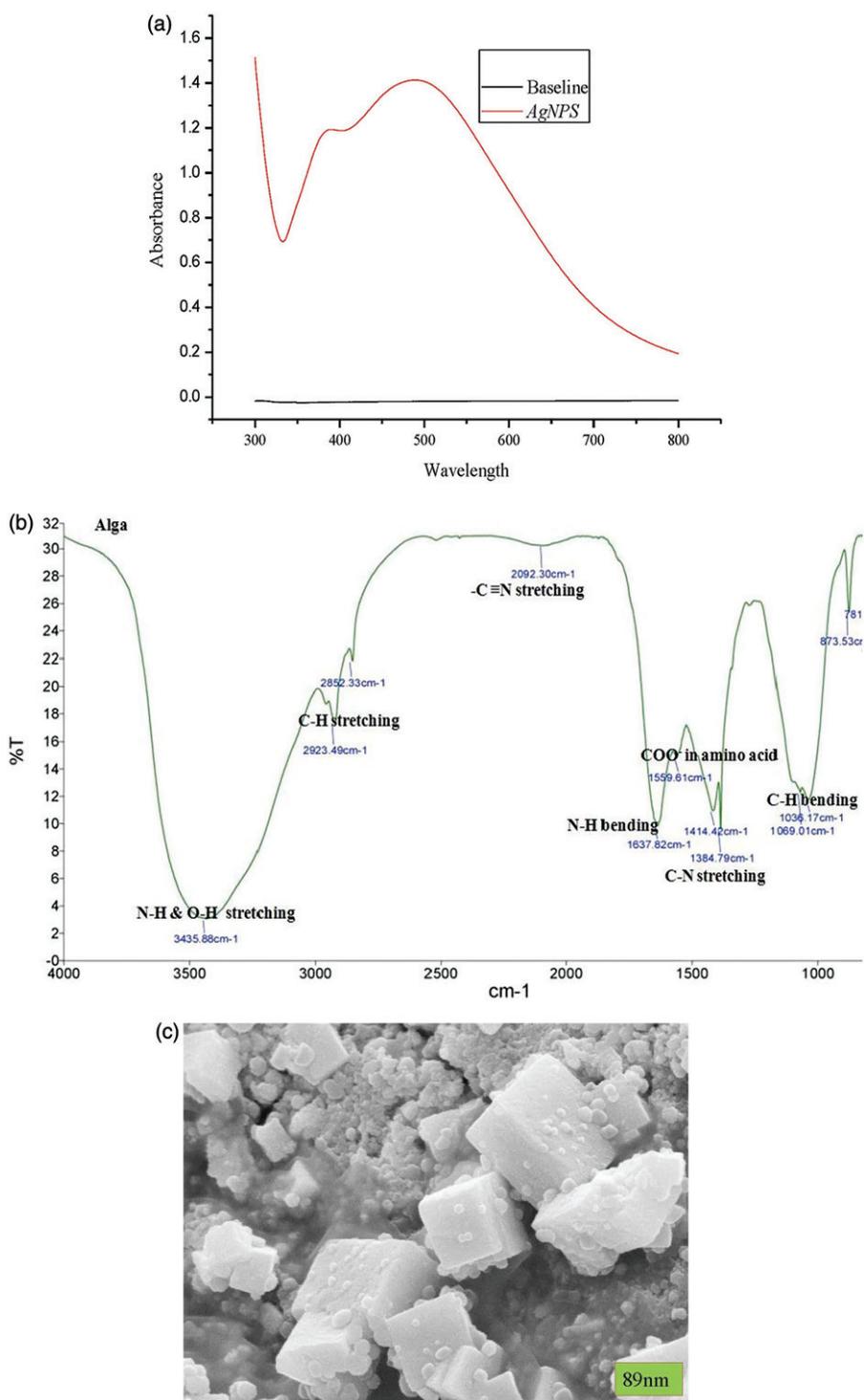
UV-visible spectrum of the silver nanoparticles synthesized by *C. vulgaris* exhibited an absorption peak around 490 nm (Fig. 2a). This peak has already been recorded for various metal nanoparticles which ranged from 2 to 100 nm in size.<sup>[26]</sup> The synthesized silver nanoparticles covered with biomolecules are well dispersed in solutions and fairly stable up to 3 months as indicated by retention of the brown color of the solution.

FTIR spectrum showed peaks at 3435.88 and 2092.30  $\text{cm}^{-1}$  due to N–H and O–H stretching vibrations.<sup>[27]</sup> 1637.82  $\text{cm}^{-1}$  peak is characteristic of N–H bending vibrations in amide of the protein as capping agent.<sup>[28]</sup> The peak at 1559.61  $\text{cm}^{-1}$  showed the presence of carboxyl group and a weak band at 1414.42 and 618.16  $\text{cm}^{-1}$  due to  $\text{COO}^-$  in the amino acid residue of the protein.<sup>[1,29]</sup> C–H bending vibrations by carbohydrates (glucose residue by C–OH bond) showed the peak at 1037.17  $\text{cm}^{-1}$ . The results of the present study have shown that hydroxyl groups have a strong ability to interact with nanoparticles (Fig. 2b). The main peaks existing in the spectrum of alga are also present in the spectrum of synthesized silver nanoparticles with lower intensities and slight shift. Therefore, it may be evidenced that proteins, polysaccharides, amides and long chain fatty acids are responsible biomolecules for bioreduction, capping, and stabilizing agents.

Scanning electron microscopy showed the synthesis of cubical, spherical, and truncated triangular shaped silver nanoparticles. Size of the synthesized silver nanoparticles was found to be in the range of 40–90 nm (Fig. 2c). These results of SEM were confirmed by reference.<sup>[30,31]</sup>

The X-ray diffraction (XRD) analysis explained the structure of silver nanoparticles. XRD measured between  $2\theta$  of 20–80 (Fig. 2d). It is indexed by JCPDS card no (04-0783). Indexing of  $2\theta$  values for 33, 36, 42, 62, 73 are 110, 111, 200, 220, 311, respectively.<sup>[27,32]</sup> Silver nanoparticles were crystalline in nature with face-centered cubic structure. Average crystalline size calculated by Debye Scherer equation was found to be 89 nm.

The synthesized silver nanoparticles were explored for the catalytic activity since there are a number of reports.<sup>[33,34]</sup> Quinoline synthesis could not be accomplished in the absence of the silver nanoparticles. This motivated us to design a direct synthesis of quinolines from 2-aminobenzylalcohol and acetyl derivatives (Scheme 1). Utilizing this



**Figure 2.** (a) UV-Visible absorbance peak at 490 nm. (b) FTIR spectrum at  $3435.88$  and  $2092.30\text{ cm}^{-1}$  due to N-H and O-H stretching vibrations,  $1637.82\text{ cm}^{-1}$  peak of N-H bending vibrations in amide of protein. (c) Scanning electron microscopy showed the presence of cubical, spherical and truncated triangular with size range 40–90 nm. (d) XRD measured between  $2\theta$  of 20 and 80.

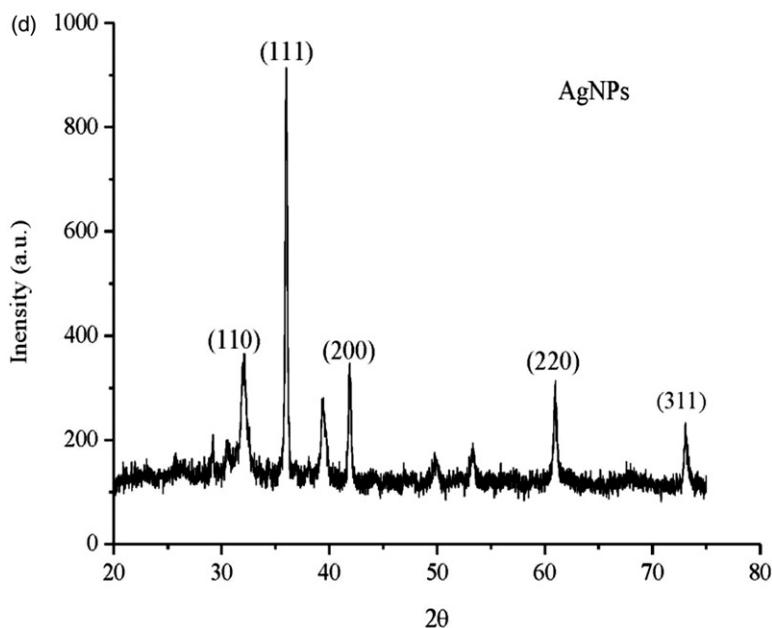


Figure 2. Continued.

synthetic scheme (Scheme 1), 19 substituted quinolines analogs were synthesized (as shown in Table 1). Progress of the reaction was checked on TLC (5% ethyl acetate/hexane as mobile phase, seen on UV light). Formation of products (4a-s) was confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and electron ionization mass spectra.

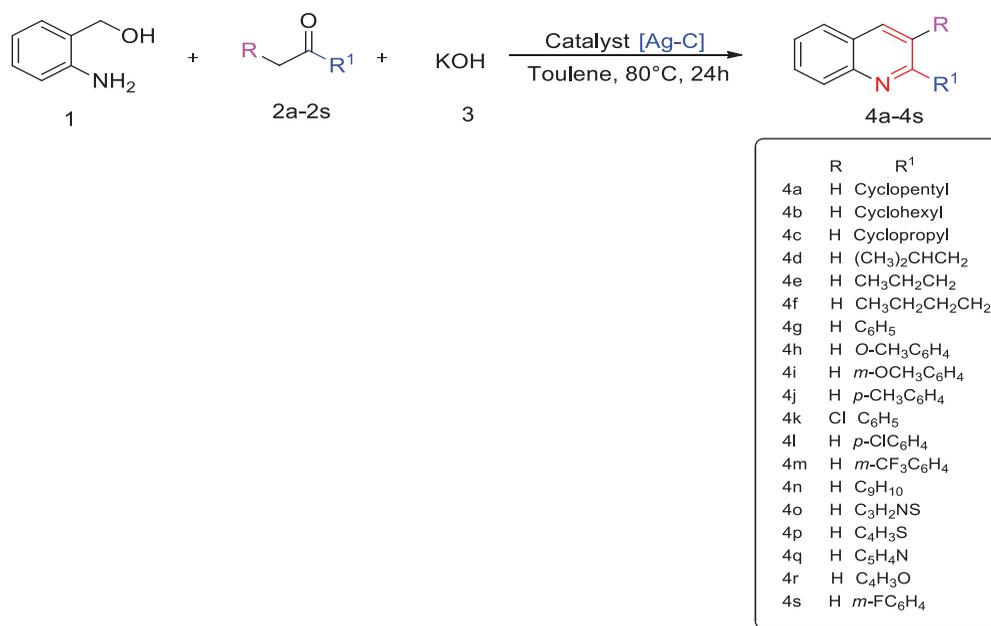
## Experimental

### Culturing and extraction of green algae

Green algae *C. vulgaris* was isolated from cement tank, central park, Delhi, India by serial dilution method, followed by plating on Chu-10 nutrient medium solidified by 1.5% agar-agar. The colonies appearing after three weeks of incubation were isolated and inoculated into liquid medium. For growth experiments, algal species were grown in an incubator at  $27 \pm 1^\circ\text{C}$ ,  $1.2 \pm 0.2$  Klux light intensity and 16:8 hr light: dark cycle in a nutrient medium.

For chlorophyll extraction the culture was centrifuged to obtain algal pellets, which were then treated with the same volume of 90% methanol, followed by heating in a water bath at  $60^\circ\text{C}$  for half an hour. Total chlorophyll was estimated by measuring the absorbance of the supernatant thus obtained at 652 and 665 nm using UV-Vis spectrophotometer

The algal biomass was shade dried. 10 g of algal biomass was taken in 500 ml Erlenmeyer flask along with 200 ml of distilled water. The mixture was boiled in an autoclave for 15 min and filtered hot through filter paper (Whatman No.1). The filtered extract was centrifuged and the supernatant was used as a reducing agent for preparing nanoparticles.



**Scheme 1.** Silver catalyzed synthesis of quinoline.

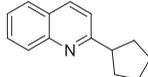
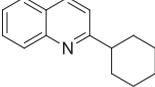
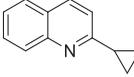
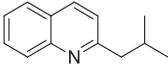
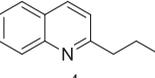
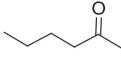
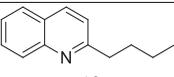
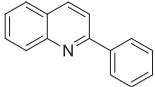
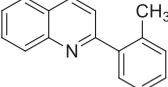
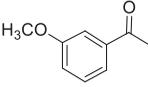
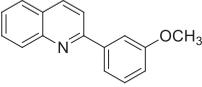
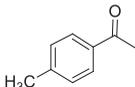
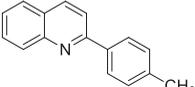
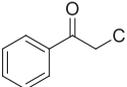
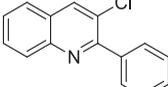
### Synthesis of silver nanoparticles

About 10 ml of algal extract was mixed with 90 ml of 1 mM AgNO<sub>3</sub> aqueous solution in 250 ml Erlenmeyer flask and stirred at room temperature for 3 h. Simultaneously, positive control of silver nitrate aqueous solution, algal extract and a negative control containing only silver nitrate aqueous solution were maintained under the same conditions. The reaction progress was regularly monitored by observing color change and recording UV-visible spectrum. In positive control the initial light pale yellow solution turned to reddish brown, indicating the formation of silver nanoparticles but in negative control, no change in color was found. After the reaction reached saturation the solution was centrifuged for 20 min and the obtained pellets were washed with deionized water to remove impurities. This process of centrifugation and washing was repeated thrice to get better isolation of nanoparticles. The obtained silver nanoparticles were then oven dried at 55 °C for 5 h.

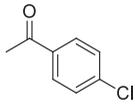
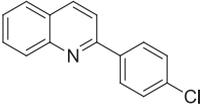
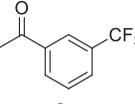
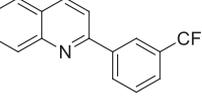
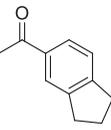
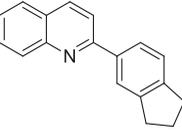
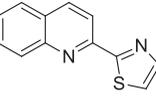
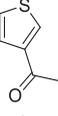
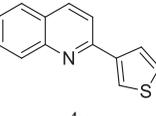
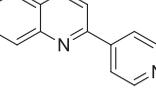
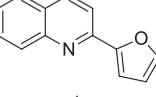
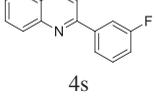
### General experimental procedure for synthesis of substituted quinolines (4a–4s)

To a solution of compound 2-aminobenzyl alcohol (250 mg, 1eq) in Toluene (5 ml) was added acetyl derivatives (1.5eq) followed by addition of potassium hydroxide (2eq) and Ag nanoparticles (0.05eq). Whole reaction mass was heated at 80 °C for 24 h. Progress of the reaction was monitored by TLC. After completion of the reaction, as confirmed by TLC, the reaction mass was brought to room temperature and quenched with water. Ethyl acetate/water work up was done. Organic layer was separated and the aqueous layer was once again extracted with ethyl acetate. All the organic layers were combined and dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to obtain crude material, which was purified by column chromatography to obtain desired material using

**Table-1.** Silver catalyzed synthesis of quinoline derivatives.

S.No	CH <sub>3</sub> COR	Product	Yield (%)	State
1	 2a	 4a	75	Liquid
2	 2b	 4b	77	Liquid
3	 2c	 4c	80	Liquid
4	 2d	 4d	78	Liquid
5	 2e	 4e	85	Liquid
6	 2f	 4f	84	Liquid
7	 2g	 4g	88	Solid
8	 2h	 4h	87	Solid
9	 2i	 4i	78	Solid
10	 2j	 4j	82	White solid
11	 2k	 4k	75	Off white solid

**Table-1.** Continued.

12	 2l	 4l	78	White solid
13	 2m	 4m	81	White solid
14	 2n	 4n	65	White solid
15	 2o	 4o	53	Solid
16	 2p	 4p	78	White solid
17	 2q	 4q	85	Brown solid
18	 2r	 4r	83	Brown solid
19	 2s	 4s	81	Brown solid

ethyl acetate/hexane as a mobile phase. Formation of the desired product was confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and electron ionization mass spectra.

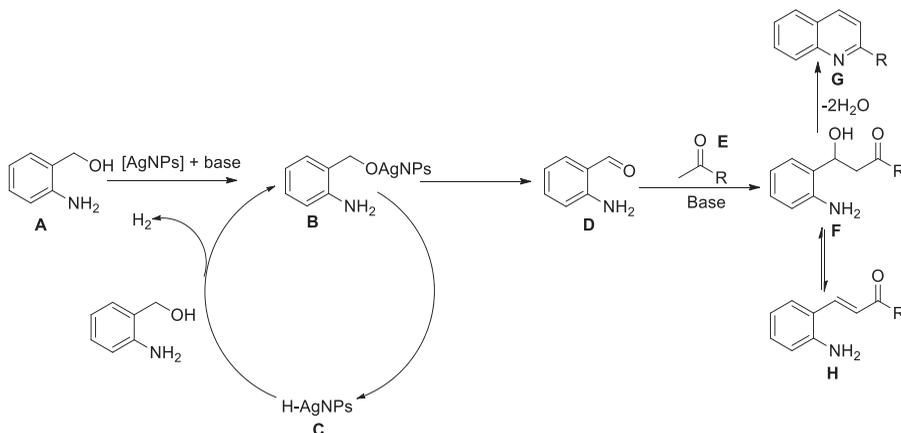
### **2-butylquinoline (4a)**

Liquid; B.P (105–120 °C, 0.1 torr);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.26 (d,  $J$  = 8.00 Hz, 1 H), 7.92 (t,  $J$  = 7.24 Hz, 2 H), 7.70 (t,  $J$  = 7.96 Hz, 1 H), 7.52 (t,  $J$  = 7.60 Hz, 1 H), 7.44 (d,  $J$  = 8.4 Hz, 1 H), 2.92 (t,  $J$  = 7.60 Hz, 2 H), 1.78–1.70 (m, 2 H), 1.40–1.31 (m, 2 H), 0.93 (t,  $J$  = 7.32 Hz, 3 H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.00, 147.38, 136.62, 129.57, 128.44, 127.49, 126.72, 125.82, 121.38, 38.80, 32.17, 22.67, 13.98; Anal. Calcd for

$C_{13}H_{15}N$ : C, 84.28, H, 8.16; N, 7.26. Found: C, 84.23; H, 8.14; N, 7.24. MS ( $m/z$ )  $[M+ ]^+$ : 186.22; HRMS  $[M+H]^+$  measured: 186.2258.

### Plausible mechanism

Initially, 2-aminobenzyl alcohol **A** reacts with AgNPs in the presence of base to form intermediate **B** which results in formation of H-AgNPs **C** and 2-aminobenzaldehyde **D** whereas as 2-aminobenzaldehyde **D** further reacts with acetyl derivative **E** in the presence of base to form intermediate **F** which finally undergo cyclization resulting in formation of desired quinoline **G** with elimination of water molecule.



### Conclusion

This study demonstrated that the aqueous extract of green alga *C. vulgaris* can reduce silver ions into silver nanoparticles and have the potential to stabilize them. The novel method for synthesis of silver nanoparticles was explored and found to be efficient for the synthesis of substituted quinolines. This provides a greener catalytic approach for the synthesis of silver nanoparticles from alga *C. vulgaris* and its application is a newer synthetic approach for biocatalytic, one-pot conversion of 2-aminobenzyl alcohol to biologically important substituted quinolines. Although this method is cheap and does not generate a toxic or harsh waste whose elimination else would have been a tedious job. Synthesis of quinoline by this method is similar to the traditional method, but when the multi-substituted 2-aminobenzyl alcohol was used, yield were either low or no product formation was observed. Industrial scale application of this method would be worth exploring taking sustainable measure into account, but it would require process optimization.

Characterization data of compounds **4a–4s** associated with this manuscript are found in the Supporting Information file.

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