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Short Communication

Biosynthesis of calcium oxide nanoparticles using Ocimum sanctum (Tulsi) leaf extracts and screening its antimicrobial activity

Vijay L. Gurav[a,*], Rajesh A. Samant[a], Satish B. Manjare[b], Urmila K. Patil[b], Sana R. Solkar[b], Shivani S. Moghe[b]

[a] *Department of Chemistry K.C. College,Charchgate, Mumbai Maharashtra (India)-400020*

[b] *Department of Chemistry, Ratnagiri Sub-centre, University of Mumbai P-61 MIDC Mirjole Ratnagiri (M. S.) India 415639*

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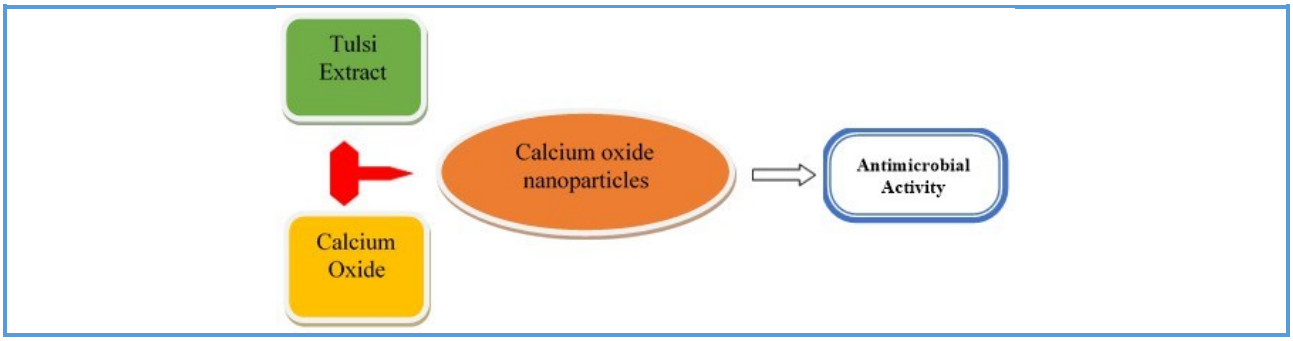
ABSTRACT

In this study, waste egg scale and inexpensive biowaste were employed to remove calcium ions. The potential of egg scales for removal of calcium ions from aqueous solutions was investigated. Preparation of calcinated egg shell powder, tulsi leaf extract, nanoparticle, and growth of bacteria were done to study biosynthesis of calcium oxide nanoparticle onto waste egg scales. Development of green nanotechnology has attracted a great deal of attention from researchers towards eco-friendly biosynthesis of nanoparticle. In this study, biosynthesis of stable calcium nanoparticles was conducted using tulsi (*Ocimum sanctum*) leaf extract. These biosynthesized nanoparticles were characterized using X-ray diffraction (XRD) analysis. The results revealed that, *O.sanctum* leaf extract can reduce Ca-ions into calcium oxide nanoparticles within 40 min of reaction time. It was found that, this method can be used for rapid and eco-friendly biosynthesis of stable calcium oxide nanoparticles with the size ranging from 40 to 70 nm.

Graphical Abstract



Corresponding author, email: vlgurav83@gmail.com (V. L. Gurav).
Tel.: 9860027889.

Introduction

In recent years, nanotechnology has attracted many researchers from various fields such as biotechnology, physics, chemistry, material sciences, engineering, and medicine [1]. Nanoparticles are synthesized using physical and chemical methods; however, they are suffering from some drawbacks including, expensive reagent, hazardous reaction condition, longer time, and tedious process to isolate nanoparticles [2]. So, developing new methods for the synthesis of nanoparticles requires inexpensive reagent, less drastic reaction condition, and eco-friendly. Recently, calcium oxide nanoparticles have attracted much attention of researchers due to its application in wound dressings and bioracidal properties, potential industrial use such as gas sensors, catalytic process, high temperature superconductors and solar cells. Ca oxide nanoparticles can be synthesized using a) vapour deposition, b) electrochemical reduction, c) radiolysis reduction, d) thermal decomposition, e) chemical reduction of calcium metal salt and f) room temperature synthesis using hydrazine hydrate and starch [3]. In recent, green synthesis of Ca-oxide nanoparticles was achieved using plant extract. Ocimum sanctum (local name Tulsi) is a traditional medicinal plant of India has a source of bio-reduction and stabilizers. Constituent of Tulsiare alkaloids, glycosides, tannins, saponins and aromatic compounds. It is used for the treatment of headaches, coughs, diarrhoea, constipation, and kidney malfunctions. Recent interest on Ocimum has resulted from its inhibitory activity against HIV-1 reverse transcriptase and platelet aggregation induced by collagen and ADP22 (adenosine 5-diphosphate). Ocimum sanctum leaf extracts have been used in the synthesis of calcium oxide nanoparticles and gold nanoparticles [3]. To the best of our knowledge, the use of Ocimum sanctum leaf extract at room temperature for greener synthesis of Ca oxide nanoparticles from waste egg shell as a source of Calcium has not been reported. Hence, the present study was carried out to synthesize and characterize the Ca- oxide nanoparticles (from waste egg shell) using Ocimum sanctum leaf extract. An eggshell is the hard. The outer covering of an egg consists mostly of calcium carbonate, a common form of calcium. The rest is made up of protein and other minerals. Calcium is an essential mineral that is abundant in many foods, including dairy products. Lower amounts are also found in many leafy and root vegetables. In the past decades, eggshell powder processed from hen eggs has been used as a natural calcium supplement [4]. Eggshells are roughly 40% calcium, with each gram providing 381–401 mg. Half an eggshell may provide enough calcium to meet the daily requirements for adults, which is 1,000 mg per day. Eggshell typically consists of ceramic materials constituted by a three-layered structure, namely the cuticle on the outer surface, a spongy (calcareous) layer and an inner lamellar (or mammillary) layer. The spongy and mammillary layers form a matrix composed of protein fibres bonded to calcite (calcium carbonate) crystal. The two layers are also constructed in such a manner that there are numerous circular openings (pores). This structure permits gaseous exchange throughout the shell. The outer surface of the eggshell is covered with a mucin protein that acts as a soluble plug for the pores in the shell. The cuticle is also permeable to gas transmission. The chemical composition (by weight) of by-product eggshell has been reported as follows: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%). The Egg Shell Membrane possesses an intricate lattice network of stable and water-insoluble fibres and has high surface

area resulting in various applications such as adsorbent [4].

Experimental

Preparation of calcined egg shell powder

Eggshells were collected from local egg market, washed with de-ionized water and dried in sunlight and oven. The crushed eggshells were kept it in muffle furnace for 7-8 h at 700 °C, and then cooled in airtight container (Figure 1) [5].

Preparation of tulsi extract

20 g fresh leaves of *O. sanctum* were collected and washed three times with distilled water to remove dust particles. Leaves were finely chopped and added to 100 mL of D/W. Heat at 100 °C for 1 h. After boiling, the mixture was cooled and filtered with Whatman paper No. 1 Filtrate was collected and was stored at 4 °C for further analysis (Figure 2) [3].

Preparation of egg shell solution

5 g of egg shell powder and 20 mL of concentrated HCl was taken in a beaker. Extract the above solution with distilled water and finally diluted to 100 mL with distilled water.

Preparation of nanoparticles

5 mL of leaf extract was added to 45 mL of calcined egg shell solution and 10 mL of conc. HCl solution. The solution were heated on sand bath for 30 min. and then cooled. After heating and cooling mixture was filtered with Whatman paper No. 1. Filter was stirred for 40 min at 900 rpm using Centrifuge Machine for bio reduction. [3].

Growth of bacteria

4.2 gm nutrient agar and 2 gm agar agar were mixed well. Prepared 0.9% saline suspension by adding 0.9 gm NaCl 10 mL distilled water. Both the agar medium and suspension was autoclaved at 121 °C for 15 min. Medium were pour in petri plate and allow it to cool solidify. Prepared culture suspension by mixing loop full of culture in suspension. 0.1 mL of culture suspension was spread on the medium plate until dry. Then, they were incubated at 37 °C for 24 h. The zone of inhibition was measured [6], and it was found that the prepared nanoparticle was antimicrobial.

Figure 1. Calcined egg shell powder

Figure 2. Leaves of Tulsi

Instrument specification

Weighing Balance Contech-223 with 0.0001 mg accuracy was used. Double-distilled water was produced from Borosil quartz double distillation plant. XRD Bruker-D_2 phaser is used for XRD Spectra.

Results and Discussion

X-Ray diffraction

XRD spectra of natural and calcined eggshell samples were obtained with CuKα radiation (λ = 0.15406 nm) at 30 kV, 16 mA, scan speed of 8.0 θ/min and scan range 5–90 °θ. Figure 3 shows a X-ray diffraction spectrum of calcinated eggshell. Main peak appeared at 2θ = 29.35. In addition, this spectrum shows several peaks at 2θ = 32.3, 53.9, 64.2, 67.5, and 79.7. Comparing the XRD peak information of Figure 4 with JCPDS file, the peaks are well matched with that of limestone (CaO). Figure 4 reveals the XRD spectrum of the egg shell nanoparticles of tulsi extract and calcined eggshell. The main peak appears at 2θ = 29.37. In addition, several peaks appeared at 2θ = 35.94, 39.36, 43.14, 47.47, 48.48. On comparing this XRD peak information of Figure 4 with the JCPDS file shows good agreement with the standard JCPDS NO. 77-2376, the peaks were well matched with that of egg shell nanoparticles. The size of Calcium oxide nanoparticle was calculated using Scherrer formula: $D = {}^{k\lambda}/_{\beta \cos \theta}$

where D is the crystallinity size(A^0), λ is X-ray wavelength in (A^0),β is the full width at half maximum(red), θ is Bragg diffraction angle, k is constant(0.94). The size of nanoparticle was found to be in an range of 40-70 nm.

Figure 3. XRD spectra of egg shell (After Calcination)

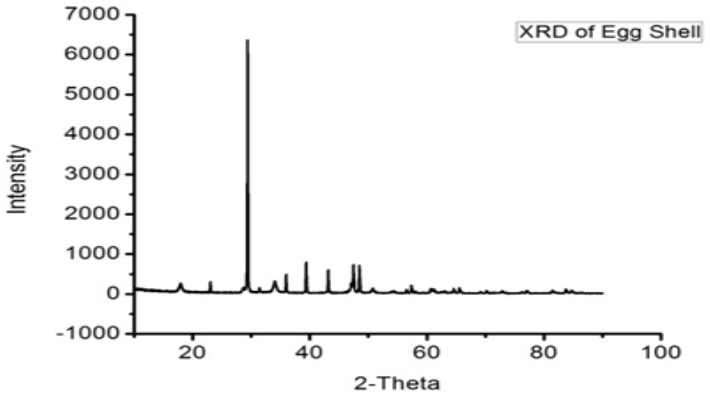
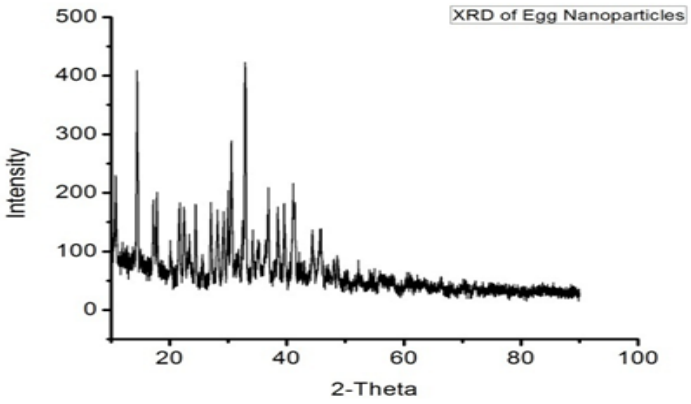


Figure 4. XRD spectra of egg nanoparticles



IR Spectra of Calcinated egg shell

IR Spectra of Calcinated egg shell clearly indicate egg shell completely converts to calcium oxide.

Surface analysis of calcinated samples

The surface morphology of the calcinated egg shell was observed using SEM analysis. The size of the nanoparticle is between 35 nm to 70 nm.

Table 1. IR data for Calcinated egg shell

Entry	Band position	Functional groups
1	3851 cm^{-1}	Stretching vibration of O-H
2	3704 cm^{-1}	Stretching vibration of O-H
3	3638 cm^{-1}	Stretching vibration of O-H
4	1523 cm^{-1}	Due to CaO
5	1404 cm^{-1}	Due to $CaCO_3$
6	869 cm^{-1}	Out-plane deformation of C=O
7	706 cm^{-1}	In-Plane deformation of C=O

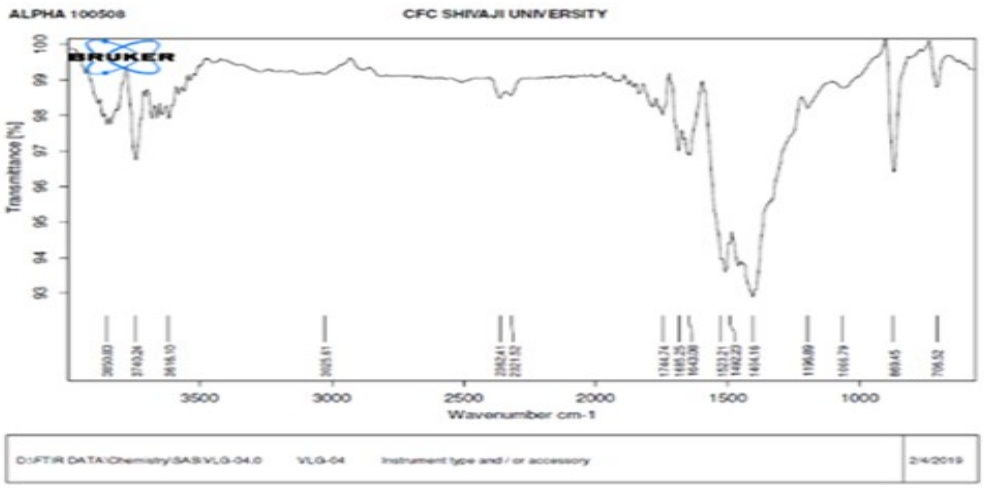


Figure 5. IR spectra of calcinated egg shell

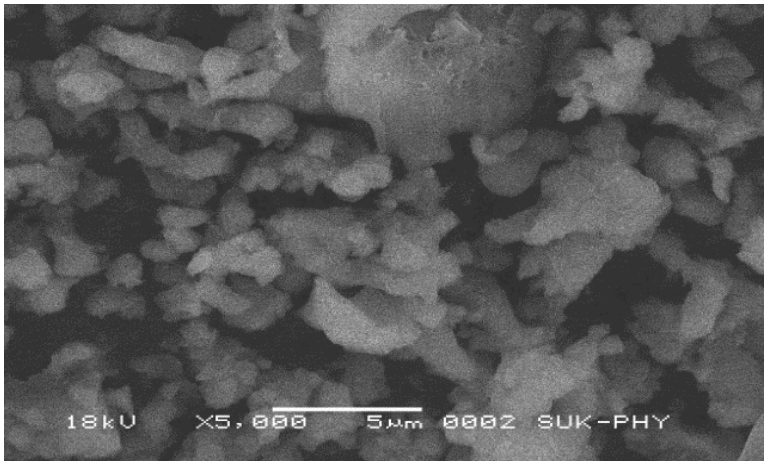


Figure 6. SEM image of Calcinated egg shell

Antimicrobial activity

Prepared nanomaterial was tested for its antimicrobial activity with gram +ve /-ve bacteria.Prepared sample was found to be antimicrobials (Figure 7 and 8).

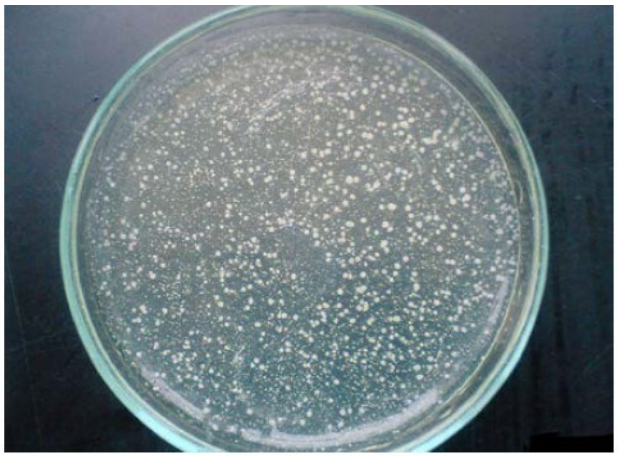


Figure 7. Shows growth of bacteria (without Adding sample)

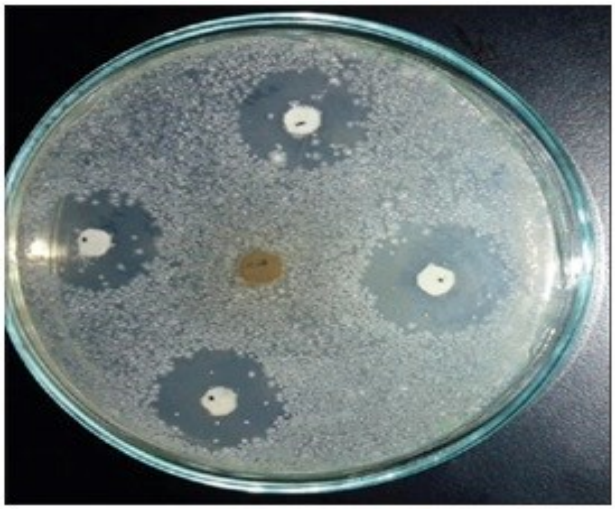


Figure 8. shows antimicrobial activity (with adding sample)

Conclusions

This study showed that, leaf extract of O. sanctum can be used efficiently for the biosynthesis of calcium oxide nanoparticle using tulsi leaf extract. The synthesized calcium oxide nanoparticles were seen to be stable. These nanoparticles were found to be crystalline in nature, possessing antimicrobial properties. Results showed that, this method can be applied for rapid, cost effective, and eco-friendly way for the synthesis of calcium oxide nanoparticles which can be used further in various industrial and medical applications.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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