IJPSR (2019), Volume 10, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 May 2019; received in revised form, 15 September 2019; accepted, 24 September 2019; published 01 October 2019

SYNTHESIS OF PURE AND BIO MODIFIED CALCIUM OXIDE (CaO) NANOPARTICLES USING WASTE CHICKEN EGG SHELLS AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

Srimathi Pasupathy * and Manivannan Rajamanickam

Department of Chemistry, Government Arts College (Autonomous), Kumbakonam - 612001, Tamil Nadu, India.

Keywords:

CaO,
XRD, Antimicrobial,
Bio-modified CaO, Nanoparticle

Correspondence to Author:
Srimathi Pasupathy

Research Scholar,
Department of Chemistry,
Government Arts College
(Autonomous), Kumbakonam -
612001, Tamil Nadu, India.

E-mail: sanmathi87@gmail.com

ABSTRACT: Calcium oxide nanoparticles (CaO NPs) gain great value in the areas of energy storage and drug delivery systems. Due to good porosity, it finds its part in storage systems and its biocompatibility earns it a good value in drug delivery and gene transfection. Synthesis of nanoparticles by waste materials and plants of metal oxide is gaining considerable interest due to environmentally friendly reactants and room temperature synthesis. This is most using method of preparation of nanoparticles as it makes use of pollution-free chemicals and encourages the use of non-toxic solvents such as water and plant extracts. In this present study, the calcium oxide nanoparticles synthesized by the eco-friendly green synthesis using environmentally begin waste chicken egg shells. CaO nanoparticles show better antibacterial activity. CaO was bio modified by *Cissus quadrangularis*, *Acalypha indica*, *Solanum nigrum*, *Phyllanthus niruri* to enhance the antibacterial activity, then the antibacterial activity of pure CaO and bio modified CaO was investigated against *B. substilis*, *S. aureus* and *E. coli* species. The synthesized nanoparticles have been characterized by XRD. Antimicrobial activity of synthesized nanoparticles were also analyzed in this study.

INTRODUCTION: Nanotechnology, a newly evolved discipline aims the creation, manipulation and application of structures in the nanometer size range. Over a past few decades, nanoparticles have been extensively applied in various fields of public interest. Nanotechnology has the potential to revolutionize the pharmaceutical industry with new tools for the molecular treatment of diseases, and rapid disease detection.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.10(10).4731-37

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(10).4731-37

It advances materials with a nano-dimension provides several means for innovative design of nano-size drug delivery systems (Nano systems) to overcome biological barriers in order to direct the drug [1].

Nanoparticles have also been successfully used to enhance the immobilization and activity of catalysts, in medical and pharmaceutical nano-engineering for delivery of therapeutic agents, in chronic disease diagnostics, and in sensors. The increasing adaptability of clinical microbial strains to antimicrobial drug resistance demands highly effective compounds for the treatment of critical microbial infection. Nanoparticles have demonstrated antimicrobial activities; the development of novel applications in this field makes them an attractive alternative to conventional dispensesions. Metal oxide nanoparticles (NPs) are known to possess strong antimicrobial properties. Inorganic metal oxides are being increasingly used for antimicrobial applications and these nanoparticles are well known for inherent antimicrobial activity [2]. In this study, a new antimicrobial nanoparticle (CaONPs) was synthesized and assessed for antimicrobial activities using a panel of bacterial and fungal pathogen.

CaO is of particular interest as it is regarded as a safe material to human beings and animals and it has excellent antimicrobial potential and adeptness to indolent microbial endotoxin [3, 4]. Due to idiosyncratic structural and optical properties of CaO it acts as a potential drug delivery agent [5]. There are many reports on the preparation of Calcium oxide nanoparticles from chemical methods. However, only few biogenic syntheses are being reported in literature [6]. CaO nanoparticles can be used as bactericides, adsorbents, and in particular as destructive adsorbents for toxic chemical agents. Drugs have grown beyond therapeutic agents to growth factors and have turned more quantized. Calcium oxide nanoparticles with its nanostructures are very feasible to the applications in drug delivery systems [7, 8]. A variety of nanoparticle-based therapeutics have improved the efficacy and reduced the toxicity of drugs, subsequently, making them potential candidates to overcome the separation and purification of cells, biological barriers and targeted drug delivery agents [9], and chemotherapeutic agents [10].

Calcium oxide and calcium acetate are accepted as food additives. Calcium oxide FCC food grade helps as a preservative, acid regulator, dough conditioner and prevents food elements from aggregation and increases its bioavailability. In addition, metal oxides nanoparticles demonstrate antimicrobial in previous studies, which may be produced from its alkalinity and presence of active oxygen particles. Metal oxides generally considered safe for human and animals comparing to other organic materials [11]. In recent years, inorganic agents have been used increasingly for control of microorganisms in various applications. The key advantages of inorganic agents are improved safety and stability compared with organic antimicrobial agents. Basic metal oxides such as MgO and ZnO, have been shown to exhibit antibacterial activity, where the particle size of the oxides appears to have an impact [12].

Recent studies have shown that green biologically based methods using microorganisms and plants to synthesize nanoparticles are safe, inexpensive, and an environment-friendly alternative. Both micro-organisms and plants have long demonstrated the ability to absorb and accumulate inorganic metallic ions from their surrounding environment. These attractive properties make many biological entities efficient biological factories capable of significantly reducing environmental pollution and reclaiming metals from industrial waste [13]. Recently, the biological synthesis of nanoparticles using plants and plant extracts appears to be to an attractive alternative to conventional chemical synthesis and the more complex culturing and isolation techniques needed for many micro-organisms. Moreover, combinations of molecules found in plant extracts perform as both reducing and stabilizing (capping) agents during nanoparticle synthesis [14]. These biological molecules are chemically complex but have the advantage of being environment-friendly. All results obtained form the basis for further application in food-relevant fields. Until now, no relevant references on this topic have been reported. Thus the study is to provide an overview of recent trends in synthesizing nanoparticles *via* biological entities and their potential applications.

METHODOLOGY:

Collection of Egg Shell: Empty chicken eggshells were collected from household waste and washed with warm tap-water. The adhering membranes were separated manually. The egg shells samples were washed and cleaned, then sun-dried for two days followed by drying oven at 110 °C for 2 h. Dried and cleaned egg shells were then crushed and grounded with blender into egg shells powder form. The resulting material was denoted by nano-CaO [15].

Synthesis of Calcium Oxide Nanoparticle: CaO was synthesized from chicken eggshell by physical method. Collected eggshells were washed with distilled water and dried it in the open air for 48 h. After that the eggshell was crushed using pestle & mortar by physical method, for 30 min.

The crushed powder was formed and this powder was treated at above 700 ºC for 7 h. The gaseous state CO_2 was evaporated and form pure CaO Nanoparticles were presented which used for further studies [15].

Preparation of Plant Extract: 100 g of Fresh leaves of *Cissus quadrangularis, Acalypha indica, Solanum nigrum, Phyllanthus niruri* were cleaned with de-ionized water. 10 g of fresh leaves of *Cissus quadrangularis* was crushed and then boiled with 100 ml of distilled water at 60 °C for 30 min.

Then it was filtered with Whatman filter paper. The extract was obtained and it was stored at 4 °C for future use. The similar manner other plants were cooled to extract. The collected plant extract was used for the synthesis of metal oxide nanoparticles [16].

Preparation of Bio Modified CaO Samples: 0.2 g of CaO powder was mixed with 20 ml of DI water and stirred vigorously for half an hour. Then to this mixture 4 ml of leaves extract was added drop by drop and stirred for 90 min. The final product was centrifuged and then rinsed with water and ethanol to remove the impurities and then kept in an oven at 100 °C, thus the bio modified CaO nanoparticles were obtained. The same procedure was repeated for other samples.

Antimicrobial Activity Assay: The preliminary antimicrobial susceptibility was tested by using the agar-well diffusion method as described by Saravanan *et al*., 2015. [17] Test organisms *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis*, *Escherichia coli* (ATCC 8739), were obtained from Centre for Laboratory Animal Technology and Research, Sathyabama University, Chennai. Each test strain was inoculated in Mueller Hinton liquid medium (5 ml broth) and incubated in a temperature-controlled shaker (120 rpm) at 37 °C for 18 h.

Well Diffusion Assay: Bacterial growth with 0.5 McFarland standard was inoculated into nutrient agar plates using sterile cotton swab. About 5 mm size well was made and different concentrations 50, 75, 100 µl of CaO were added into it. Sterile 1mM CaO solution was used as blank exhibited no activity against any of the used organisms. Gentamycin (10 µg) was used as a standard drug (CLSI, 2005). The plates were incubated at 37 °C for 24 h, and the zone of inhibition (ZOI; mm) appearing around the wells was recorded All the plates were observed for zone of inhibition after incubation at 37 °C for 24 h [18].

Minimum Inhibitory Concentration (MIC): The antimicrobial activities were evaluated through the determination of the minimum inhibitory concentration (MIC) by the two-fold serial microdilution method in 96-well microtiter plate. Hundered µl of dilution 5×10^5 CFU/g of the microorganisms and 100 µl of the spices or CaO NPs were pippeted in the wells (beginning with the concentration 250 mg/ml for spices and 600 µg/ml for CaO NPs). Positive and negative controls were also prepared. Then the plates incubated at 37 °C for 24 h. MIC is the concentration at which the antimicrobial agent visually inhibits the growth of the microorganism [19].

Characterization of Synthesized Nanoparticles: XRD analysis of pure and bio doped CaO sample was carried out using powder X-ray diffraction (PXRD) measurements were performed on PAN analytical Xpert Pro X-ray diffractometer using Cu-Kα radiation (λ = 0.154 nm) at 40 kV, at a scanning rate of 2 °C $min^{-1.}$

RESULTS AND DISCUSSION:

Synthesis of Cao Nanoparticles from Egg Shell: The CaO nanoparticles from egg shell were collected after oven treatment. After treatment, the egg shells were crushed and powdered. The resulting material was denoted by nano-CaO. The gaseous state CO_2 was evaporated and form pure CaO Nanoparticles were presented which used for further studies.

Antibacterial Activity of Plant Extract Doped with Synthesized CaO Nanoparticles: The plant extract from four different species were evaporated and used for antimicrobial activity by doping with synthesized CaO nanoparticles.

Antibiogram was done by disc diffusion method [20] using plant extracts. Petri plates were prepared by pouring 30 ml of Nutrient Agar medium for bacteria. The test organism was inoculated on a solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/

fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Escherichia coli* and *Bacillus subtilis, S. aureus*, were spread on Nutrient agar plates for bacteria. Using sterile forceps, the standard antibiotic disc containing gentamycin were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 h for the bacteria and at room temperature (30 ± 1) for 24-48 h. Each sample was tested in triplicate.

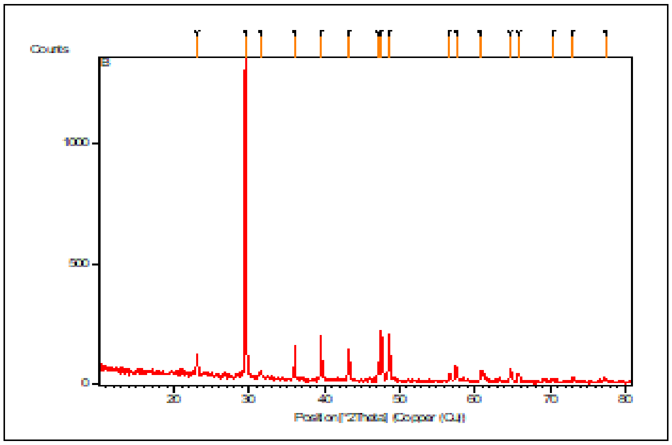


FIG. 1: XRD PATTERNS OF SAMPLE A

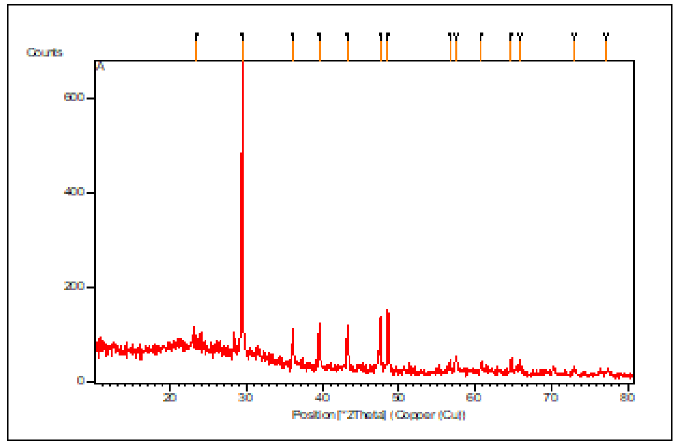


FIG. 2: XRD PATTERNS OF SAMPLE B

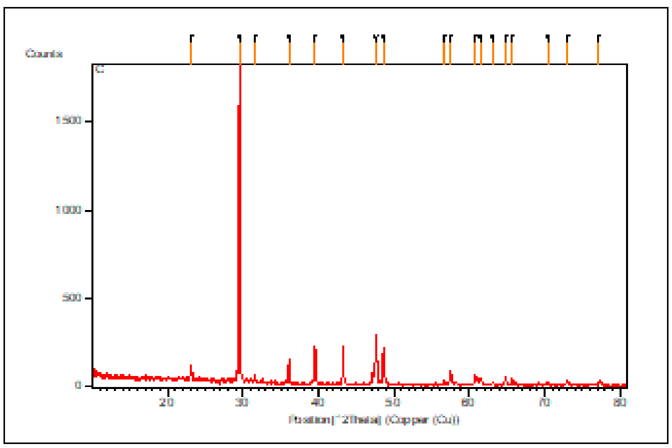


FIG. 3: XRD PATTERNS OF SAMPLE C

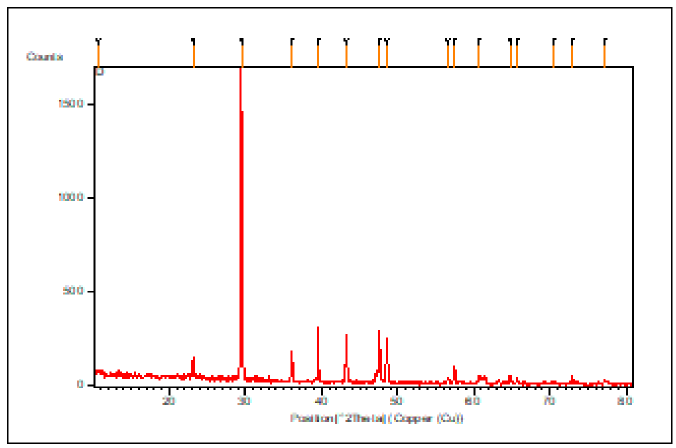


FIG. 4: XRD PATTERNS OF SAMPLE D

In the present study, the antibacterial activity of synthesized pure CaO nanoparticles derived from chicken egg shells and bio modified CaO nanoparticles were studied using agar well diffusion method against gram-positive and gram-negative bacterial strains. The pure CaO nanoparticles show good antimicrobial activity particularly in 100 µg/ml. The pure CaO shows 14mm zone of inhibition against *B. subtilis*, 13mm against *S. aureus* and 16 mm against *E. coli*. The antibacterial activity was carried out with four different concentrations (25, 50, 75, 100 µg/ml). Sample C and sample E shows good antimicrobial activity, Sample C produces 19 mm zone of inhibition against *B. subtilis*, 18 mm against *S. aureus* and 20 mm against *E. coli*. Sample E shows 19 mm zone of inhibition against *B. subtilis*, *S. aureus* and *E. coli*. Sample B and Sample D inhibit *B. subtilis* and *S. aureus*. The results are represented in **Table 1** and **2** and **Fig. 5**. These nanoparticles were compared with standard antibiotic.

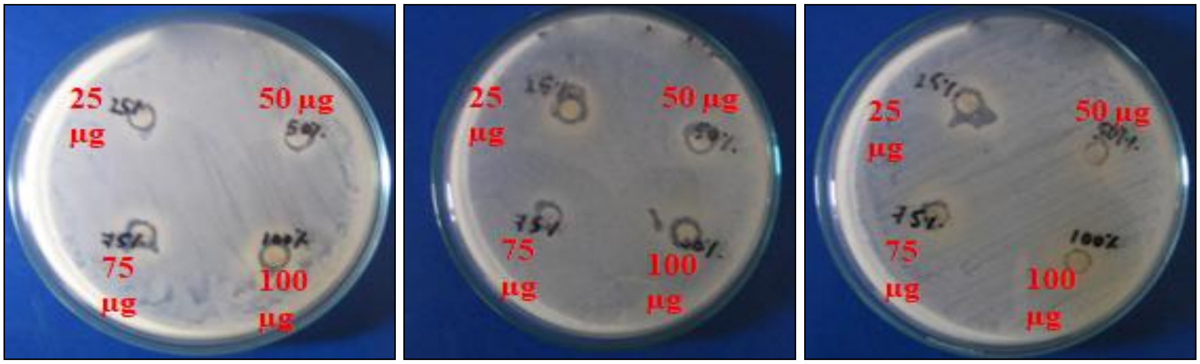
TABLE 1: ANTIMICROBIAL ACTIVITY OF PURE CaO

S. no.	Test Bacterial strains	Zone of inhibition in mm in diameter (µg)				
		25	50	75	100	standard
1	*Bacillus subtilis*	R	8	11	14	19
2	*S. aureus*	R	9	10	13	21
3	*E. coli*	R	6	12	16	19

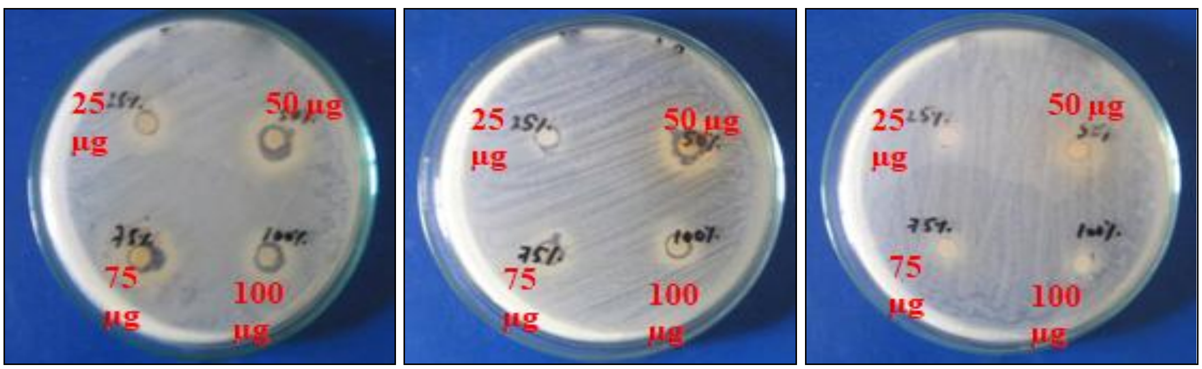
TABLE 2: ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT DOPED WITH SYNTHESIZED CaO NANOPARTICLES

Test Bacterial strains	Zone of inhibition in mm in diameter																
	Cissus quadrangulari (µg) Sample B				*Acalypha indica* (µg) Sample C				*Solanum nigrum* (µg) Sample D				*Phyllanthus niruri* (µg) Sample E				Std (µg)
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	Gm
B. subtilis	R	7	10	17	R	5	11	19	R	6	10	17	R	6	12	19	19
S. aureus	R	8	11	17	R	6	13	18	R	8	14	19	R	8	12	19	21
E. coli	R	9	-	-	R	-	-	20	R	-	-	6	R	8	12	19	19

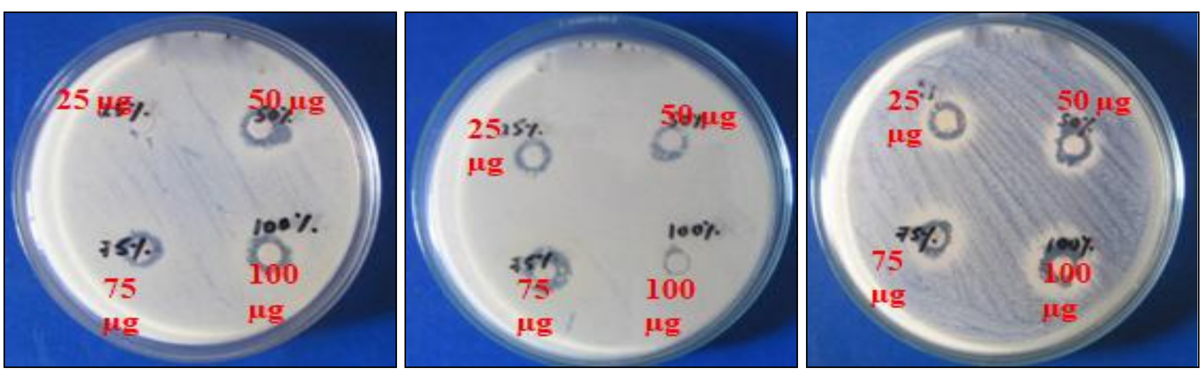
(µg) – microgram; R- Resistant; (-)- No inhibition



Escherichia coli



Bacillus subtilis



Streptococcus pneumoniae

FIG. 5: ANTIBACTERIAL ACTIVITY OF PURE AND BIO MODIFIED CaO

Yuki Ohshima *et al*., 2015 [21] reported that heated egg shell powder showed excellent antimicrobial activity. Antibacterial activity of green synthesized CaO nanoparticles with dopant *Mentha pipertia* was studied by Ijaz *et al*., 2017 [22]. From the literature, it was proved that the antibacterial activity increases due to the addition of the plant extract that is bio dopants.

In this present study also the maximum zone of inhibition was observed for sample E modified against the bacterial strains of *Staphylococcus aureus, Bacillus substills* and *E. coli.* Similarly, Plant mediated CaO nanoparticles with *Phyllanthus niruri* showed *maximum* antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli.* Another sample of plant extract showed activity against was observed in Gram-positive bacteria only. This shows the sample E *Phyllanthus niruri* has potential antimicrobial activity when doped with CaO nanoparticles than other plant extracts. The results were represented in **Table 1** and **Table 2**.

XRD analysis of synthesized nanoparticles showed that bio modified CaO nanoparticles were in crystalline in nature. Allbio-doped samples displayed a potent antibacterial activity when compared to standard antibiotic Gentamicin (MIC 100 µg/ml) indicating that these plants could be a good source for the antibacterial to combat MDR bacterial infections. Among them the species *Acalypha indica* shows excellent antibacterial activity against *E. coli* bacteria at 100 µg. Other species show optimum antibacterial at 100 µg concentration.

XRD Analysis: XRD analysis of pure and bio doped CaO sample was carried out using an X-Ray diffractometer equipped with Cu Kα radiation (λ=0.15406nm) source. The 2θ values of Bio doped CaO ranging from 20°-80°. A comparison of XRD spectrum with the bio doped CaO nanoparticles formed in our experiments was in the form of nanocrystals. The peaks at 2θ values of 29.39º, 39.56 º, 43.23 º, 47.65 º, and 48.80º corresponding to 111, 200, 105, 211 and 310. The crystallite size of the prepared nanoparticles are calculated by the Debye Scherrer equation,

$$D = 0.9\lambda/\beta \cos\theta$$

Where D is the crystallite size, β = is the full width at half-maximum ($FWHM_{hkl}$) of an hkl peak at 2θ value and θ value is the half of the scattering angle.

CONCLUSION: The present study was obtained by simple and economic friendly method of synthesized calcium oxide nanoparticles. The synthesized nanoparticles showed good anti-microbial activity against test microorganisms. The study also showed higher photocatalytic activity. Hence, the calcium oxide nanoparticles synthesized using waste chicken egg shells acts as an antibiotic.

ACKNOWLEDGEMENT: The authors express their gratitude to Principal, and The Head of the Department and staff members of Department of Chemistry, Government Arts College (Autonomous), Kumbakonam for all the encouragement and facilities rendered in my work.

CONFLICT OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Saravanan M, Ramachandran B, Hamed B and Giardiallo M: Barriers for the development, translation and implementation of nanomedicine: an African perspective. Journal of Interdisciplinary Nano Medicine 2018; 3: 3.
2. Kadiyala U, Nicholas A, Kotov and Epps JSV: Antibacterial metal oxides nano particles challenges in interpreting the literature 2018; 24(8): 896-03.
3. Wang L, Hu C and Shao L: The antimicrobial activity of nanoparticles: present situation and prospects for future, International Journal of Nanomedicine 2017; 1227-49. http.//.dx.doi.org/10.2147/UN-S121956.
4. El-aziz DMA and Yousef NMH: Antimicrobial effects of Calciumoxide (CaO) Nanoparticles and some spices in Minced Meat 2017; 3: 38-45.
5. Butt AR, Ejaz S, Baron JC, Ikram M and Ali S: CaO nanoparticles as a potential drug delivery agent for biomedical applications, Digest Journal of Nanomaterials and Biostructures 2015; 10: 799-09.
6. Gedda G, Pandey S, Lina YC and Wu HF: Antibacterial effect of CaO nano-plates fabricated from shrimp shells, The Royal Society of Chemistry Green Chem 2015, Doi.10.1039/c5Gc00615e
7. Antharaman A, Ramalakshmi S and Mary G: Green synthesis of calcium oxide nanoparticles and its applications. Int Journal of Engineering Research and Application 2016; 6: 27-31.
8. Balaganesh AS, Sengodan R, Ranjithkumar B and Chandarshekar: Synthesis and characterization of porous calcium oxide nanoparticles (CaO NPS). International Journal of Innovative Technology and Exploring Engineering 2018; 8: 2278-75.
9. Patra JK, Das G, Fraceto LF, campos EVR, Rodriguez-Torres MDP, Acosta–Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S and Shin HS: Nano based drug delivery systems: Recent developments and Future Prospects 2018, 16: 71
10. Suja A and Sarathy VP: Biomedical applications of calcium oxide nanoparticles - a spectroscopic study. Int J Pharm Sci Rev Res 2018; 49: 121-25.
11. Butt AR, Ejaz S, Baron JC, Ikram M and Ali S: CaO Nanoparticles as a potential drug delivery agent for biomedical applications. Digest Journal of Nanomaterials and Biostructures 2015; 10: 799-09.
12. Doaa M, Abd EA and Naeima MHY: Antimicrobial Effects of calcium oxide nanoparticles and some spices in minced meat. ARC Journal of Animal and Veterinary Science 2017; 3: 38-45.
13. Ghanemi A and Boubertakh B: Biological tools to deal with pollution: selected advances and novel perspectives. International Jou of Public Health Science 2014; 3: 57-62.
14. Monaliben S, Derek F, Shashi S, Suraj KT and Gérrard EJP: Green synthesis of metallic nanoparticles *via* biological entities. Materials 2015; 8: 7278-08.
15. Ashok CS, Kumar MK, Chakra CS, Rao V and Dayakar T: Calcium oxide nano particles synthesized from chicken Egg shells by physical method. International Conference on Emerging Technology in Mechanical Sciences 2015.
16. Mosaddegh E and Hassankhani A: Preparation and characterization of nano-CaO based on eggshell waste: Novel and green catalytic approach to highly efficient synthesis of pyrano[4,3-b]pyrans. Chinese Journal of Catalysis 2014; 35: 351-56.

17. Ashwini A, Ramalakshmi S and Mary G: Green synthesis of calcium oxide nanoparticles and its applications. Int Journal of Engineering Research and Application 2016; 6: 27-31.
18. Saravanan D, Radhakrishan M and Balagurunathan R: Bioprospecting of bacteria from less explored ecosystem; Journal of Chemical and Pharmaceutical Research 2015; 7: 852-57.
19. Saravanan D and Radhakrishnan M: Antimicrobial activity of pigments produced by fungi from Western Ghats. Journal of Chemical and Pharmaceutical Research 2016; 8: 634-38.
20. Krishnan M, Dey DK, Sharma C and Khang SC: Antibacterial activity of Weissella confuse by disc diffusion method 2019; 14: 3. https://doi.org/10.3229/bip.v14i3.41545.
21. Ohshima Y, Takada D, Namai S, Sawai J, Mikio K and Hotta M: Antimicrobial characteristics of heated Egg shell powder. Biocontrolscience 2015; 20(4): 239-46.
22. Ijaz U, Bhatti IA, Mirza S and Ashar A: Characterization and evaluation of antibacterial activity of plant meadiated Calcium oxide (CaO) nanoparticles by employing *Mentha pipertia* extract. Published on 3, 2017. IOP Publishing Ltd. Material Research Express 2017; 4: 10.

How to cite this article:
Pasupathy S and Rajamanickam M: Synthesis of pure and bio modified calcium oxide (CaO) nanoparticles using waste chicken egg shells and evaluation of its antibacterial activity. Int J Pharm Sci & Res 2019; 10(10): 4731-37. doi: 10.13040/IJPSR.0975-8232.10(10).4731-37.