Nanoscale Reports

DOI:10.26524.nr.3.1

3(1) (2020) 1-9

RESEARCH ARTICLE

OPEN ACCESS

Facile Synthesis of Calcium Oxide Nanoparticles from the Carica Papaya Leaf Extract with the Significantly Enhanced Antibacterial Activity

B. Dhivya[1], K. Sujatha[2], A.P. Sudha[2]

Abstract

The conventional inethods for the synthesis of metal oxides intake large mnount of hazardous còemicals, the best proinising alternative is the use of plant extracts. In this wodc calcium Dxide nanoparticles of 16 nm size with the cubic shape were synthesized usingthepapayaleafextract bythesimple greenerroiiteusinpcalcium chloride as a soince material by co-piecipitation method. By using the XRD (X-Ray Diffiaction), FTIR (Fourier Transforin Infrared Spectroscopy), SEM (Scanning Election Microscope) and EDAX (Energy Dispersive X-ray Analysis). the structural, surface inorphology, fiinctional groiip and the antibacterial activity of the synthesized calcium oxide nanoparticles were annlyzed. The XRD patlem of the CaO nanoparticles was well matched with the slandard value and the crystalline size obtained using the Scherer formula was ló nm. The elemental composition of the prepmd sample was conflrmed by the EDAX resiilt. The piesence of the functional groups of the synthesized CaO nanoparticles was confumed by the FTIR anaÇsis (4000-400 cm°'). The cubic iuorphology wu identified from the SEM image and the grain size ranges from 125-218 nin. The CaO nanoparticles were fuither evaluated for their antibacterinl activity against Staphylococcus aiireus and klebsielia pneumonia and from the result it was found lhat CaO nanoparticles was active against both gram pDsitive sud grani negativebacteiia.Therefore, it may be an emergiug platform for new medicines

Keywords: CaOnanoparticles. Green synthesis, XRD. Antibacteriail properties.

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How to cite this article: B. Dhivya, K. Sujatha, A.P. Sudha, Facile Synthesis of Calcium Oxide Nanoparticles from the Carica Papaya Leaf Extract with the Significantly Enhanced Antibacterial Activity. *Nanoscale Reports* 3(1), 1-9. Retrieved from https://nanoscalereports.com/index.php/nr/article/view/39

Source of support: Nil

Conflict of interest: None.

Received: 1 March 2020 **Revised:** 2 April 2020 **Accepted:** 4 April 2020

1. Introduction

In order to solve the various environmental crises, nanotechnology offers the numher of opportunities to tackle it. Nanotechnology is a fascinating as well as the novel area of research in modern science and technology.[1, 2] It gains great attenfion in almost all the fields.[1, 3] At the nanosize, the properties of the materials are enhanced and are more reactive than the conventional materials. Nanosoucture metal oxides have many unique physical, chemical, electrical and magnetic properties. Therefore that creates an extensive research scope for the technical applications. A new set of items are discovered by making the slight change in the manufachiring techniques.

The metal oxides nanoparticles like chromium oxide nanoparticles are dangerous and a cancer causing sulistance when it is present in abundance.[4] Amedea *et al.*, reported that the synthesis of bismuth trioxide nanoparticles involves complex procedures and require extreme conditions.[5] Cytotoxicity in human liepatocellular carcinoma cells induced by the CtiO nanoparticles was analyzed by the Siddiqui *et al.*[6] Whereas, calcium oxide nanoparticles has following advantages that including the antibacterial property.

Calcium oxide is cheap and toxic free substance[7] and had a wide range of applications in almost every field. CaO is used to treat the waste water and in the pollution control devices to neutralize the level of sulphur and ni6-ogen oxide in the smoke, as a food additive, as an insecticide, fungicides and as poultry feed. When heated near its melting point it pi'odtices a bright light lii the nineteenth century, CaO was used as a light source. Researchers attract more to synthesize CaO due to these unique properties.

Bharathiraja *et al*., had prepared calcium oxide nanoparticles, which was used as an effective filtration aid for purification of vehicle gas exhaust.[8] Ashwin *et al*., had analyzed the photo catalytic activity of calcium oxide nanoparticles.[9] The calcium oxide was synthesized from the soya bean oil, and reported by Masota *et al*., about its application in the biodiesel production.[10] Hasna *et al*., had prepared calcium oxide used as cement for the dental application.[11] Luiit *et al*., reported the antibacterial property of the CaO synthesized using the waste egg shells.[12]

There are many methods of synthesizing nano metal oxides such as microwave assisted method, chemical co-precipitation, thermal deposition and so on. However, those methods suffer from the limitations such as high temperature[13] and pressure,[14] expensive[15] and complicated steps to be involved.[16, 17] There is an availability of the diverse range of the plant species and many researchers were reported using green synthesis.[1, 2] M.Sundrarajan *et al*., had synthesized the TiO_2 nanoparticles using nyctanthes arbor-tristis leaves extract [18]. Shikuo et at., reports the capsicum leaf extract assisted synthesis of Ag nanoparticles.[19] The use of the biological materials like plant extracts, fungus and algae for the synthesis of nanoparticles will help to uplift their environmental friendliness and compatibility of the bio-medical applications.[2, 3] Besides, green synthesis avoids the production of the unwanted and the harmful by-products during the synthesis of nanoparticle.[3]

Scientific name	Carica papaya
Common name	Papaya
Class	Mangnoliopsida
Genus	Carica
Division	Magnoliophyta
Family	Caricaceae
Parts used	Leaves, Friuts, Barks

Papaya is naturally rich in calcium and vitamin A, C, E, K and B and therefore it have many medical applications. It is used to treat dengue fever, reduces cancer risks and lowers sugar level of blood and so on. So Papaya leaf extract can be used as a reducing agent in the synthesis of nanoparticles. Rajeswari *et al*., reported the photo catalytic and photovoltaic applications of synthesized ZnO nanoparticles.[20] Biosynthesis of TiOz nanoparticles using papaya leaves for the photo catalytic application was analyzed by Harpeet *et al*.[21] In this paper, we reported that the carica papaya was successfully used as a bio relucmnt for the rapid synthesis of CaO nanoparticles by co-precipitafion method and their anfibacterial properties were reviewed.

2. Experimental Procedure

2.1 P1ant Colletion and Materials

Papaya leaves were collected from Thindal, Erode district and the chemical s used were calcium chloride pellets and sodium hydroxide solution.

2.2 Preparation of Papaya Leaf Extract

The extract was prepared from fifty grams of fresh leaves of papaya. The leaves were washed and cut into small pieces which were then boiled with 100 ml distilled water for 30 minutes. The colorless aqueous solution was changed into dark green colour. Extract was subjected to room temperature and then filtered through the Whatmann no. 1 filter paper to remove the bio materials. The extract was stored for further experiments.

2.3 Preparation of Calcium Chloride Solution

13 g of calcium chloride pellets were dissolved in the 50 ml of distilled water. The mixture was stirred for 15 minutes. The homogeneous and colorless calcium chloride solution was obtained.

The reaction medium contains 30 ml of calcium chloride solution and 30 ml of papaya leaf extract The precipitating agent sodium hydroxide solution (NaOH) of 15 ml was added drop wise and the pH value was maintained at 10. The prepared solution was then stirred for 20 minutes at room temperature and then solution was incubated for 30 minutes. The precipitate was harvested by centrifuging the reaction mixture and the filtrate was washed with the distilled water and ethanol to remove the secondary metabolites and other organic impurities. The precipitate was dried in the oven at 100°C for 6 hrs and then calcinated at 400°C for an hour using muffle furnace. The product was ground by mortar and pastel to obtain the fine

4. Synthesis of Calcium Oxide

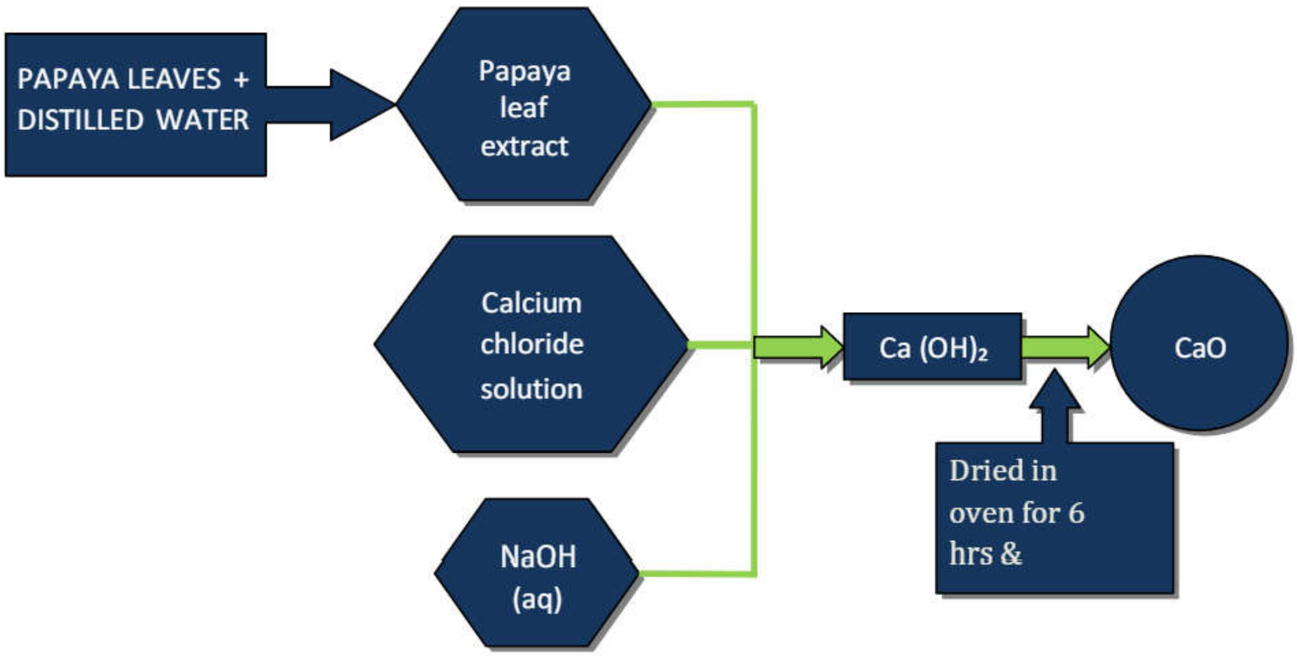

Fig. 1: Flowchart of biosynthesized CaO Nanoparticles

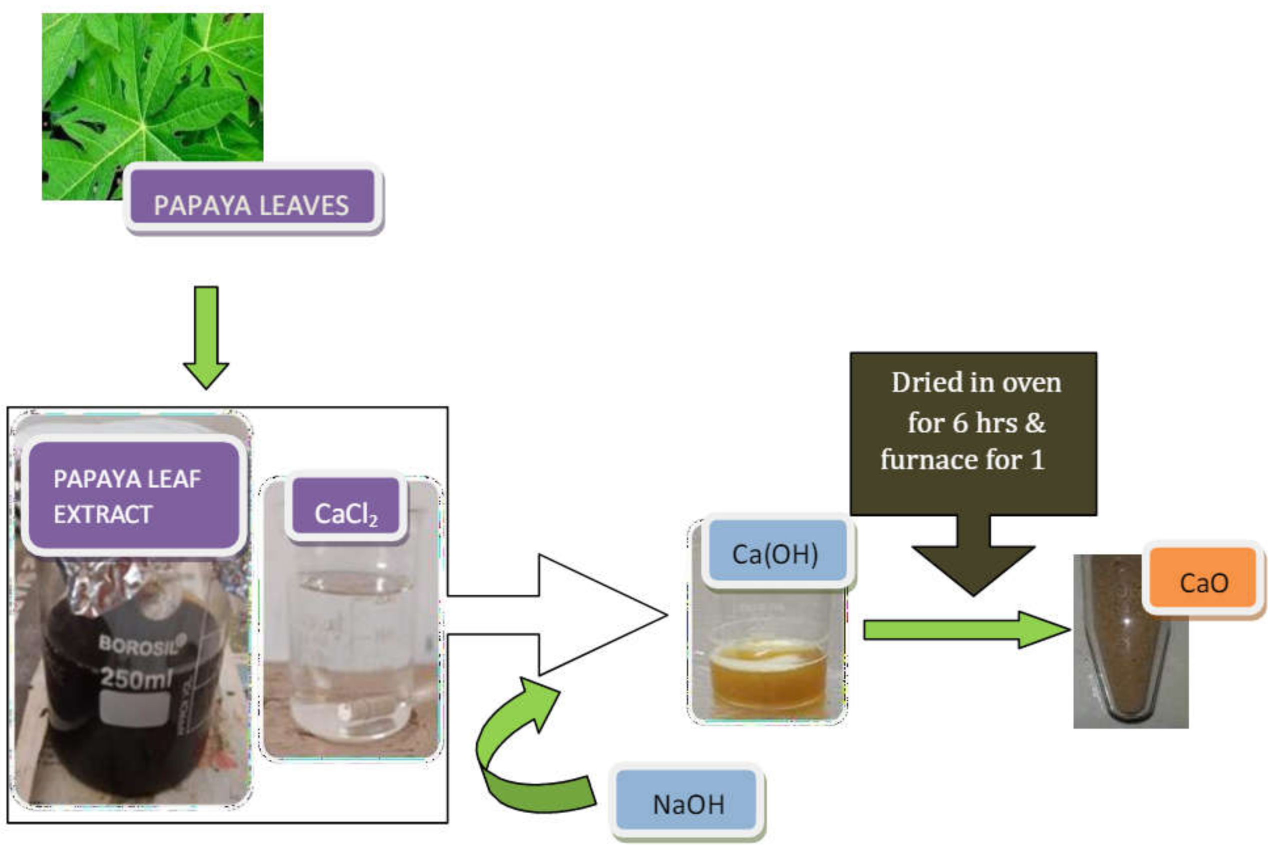

Fig. 2: Schematic representation of Biosynthesized CaO Nanoparticles

yellow powder. The obtained yellowish calcium oxide nanopowder was used for further analysis.

2.5 Characterization of the Synthesized CaO Nanoparticles

Biosynthesized calcium oxide nanoparticles were successfully characterized by the following techniques. The crystallite size and the structural properties of the synthesized sample was investigated by the X-ray diffraction (LABX XRD 6000) using the Bruker Diffractometer, operating with the voltage = 40.0 (kV) and current 30.0 (mA), where CuKo radiation (1.5406 A°) was used as an X-ray source. The sample was scanned in the range 20=0° to 90°. The grain size, the morphology and the elemental composition of the synthesized nanoparticles was observed by the MODEL PHILIPS XL-30 Scanning Electron Microscope (SEM) with an attached energy dispersive X-ray (EDAX) analyzer.

2.6 Antibacterial Activity

The antibacterial activity of the green synthesized CaO nanoparticles were carried out against the four selected bacterial strains include Gram positive microorganisms such as Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 3160), Gram negative microorganisms such as E.coli (MTCC 1698) and Klebsiella pneumoniae (MTCC10309) obtained from IMTECH, Chandigarh by agar well diffusion method. Agar plates and the petridish were sterilized at 121°C for about 30 minutes at 15 lbs pressure. The bacteria were swabbed on the surface of the agar plates. Well was prepared by using cork borer and loaded with 100 pi of each sample to the distinct well with sterile distilled water as negative control and Vancomycin (30 mcg/disc) as positive control. The experiment was carried out under the sterile condition and the sample loaded plates were incubated at 37 r C for 24 hours to observe the zone of inhibition. Zone of inhibition of the sample against the microorganisms was shown in Fig.3

3. Results and Discussion

3.1 X-ray Diffraction Analysis

The X-Ray diffraction tool was used to analyze the purity and crystallite size of the synthesized sample. The XRD data was recorded in 20 value ranges from 0° to 90°.The XRD plot of biosynthesized CaO nanoparticles (Fig. 4) shows the poly crystallite nature and the characteristic peaks appeared at 20 = 38.2134°, 28.2404° and 47.2151° corresponds to the lattice planes (200), (111) and (220) that revealed the formation of cubic phase of CaO nanoparticles. The diffraction pattern was well indexed and in good agreement with the standard values (JCPDS NO. 77-2376).[9] No extra peaks were observed for synthesized CaO nanoparticles. The average crystallite size of the calcium oxide was calculated by using the Scherer formula.[22, 23]

$$D = k\lambda / \beta.\cos\theta \text{ (Å)} \quad (1)$$

Where, D is the mean crystallite size, A is the wavelength of the incident beam, p is the hill width at half maximum and 0 is the Bragg angle.

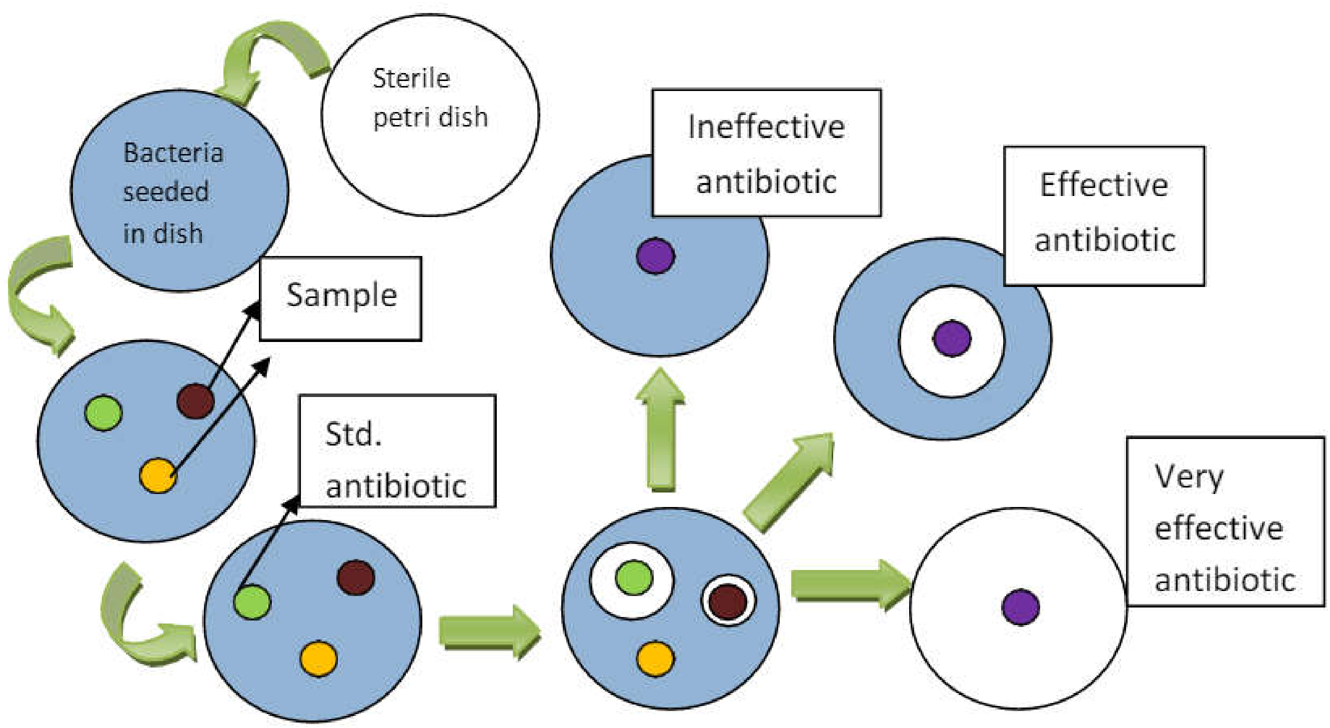

Fig.3: Antimicrobial activity of Biosynthesized CaO nanoparticles

The interplanar spacing is calculated from the Bragg's equation,

$$2\, d_{hkl} \sin\theta = m\lambda \quad (2)$$

Where, dhki is the interplanar spacing (m=l), (hkl) lattice plane index, 2 is the lattice constant and h, k and 1 are the integers. Table 1 gives the crystallite size and the spacing between the consecutive planes along with the dislocation density and micro strain.

The lattice constants were calculated from the equation of cubic system using the method of least squares

$$1/d^2 = (h^2 + k^2 + l^2)/a^2$$

Where,

$$a^2 = (/i^2 + k^2 + l^2)\, d^2 \quad (3)$$

Substituting (2) in (3), we get

$$a = \frac{n\lambda\ h^2 + k2 + l2}{2 \sin\theta}$$

Dislocation density is

$$\delta = 1/ D\ 2\ \text{Lines/m}^2 \quad (4)$$

Micro strain arises due to the lattice misfit and then calculated by,

$$£ = \beta.\cos\theta / 4 \quad (5)$$

Thus, the crystallite size of calcium oxide was 16.2 nm.

Fourier Transform Infrared Spectroscopy [FTIR] analysis

The result of FTIR Analysis for synthesized CaO nanoparticles was depicted in Fig. 5. The band at 877.61 cm 1was due to the Ca-O-Ca stretching vibration.[9] The peak at 1074.35 cm 1corresponds to the C-N stretching of the amines and the broad peak observed at 377.67 cm^{-1} corresponds to 0-H stretch caused by alcohols, phenols.[12] The broad band at 1627.92 cm 1was attributed to the -H bonding[24] and 2357.01 cm 1 represents the N-H bonds due to presents of amide groups.[12] The various band range for the stretching and the bending vibrations were shown in Table 2.

3.3. Scanning Electron Microscope [SEM] analysis

Fig.6 revealed the surface morphology of calcium oxide nanoparticles using SEM analysis. The morphology and the grain size of the calcium oxide synthesized using papaya leaf extract were calculated by SEM results. The SEM image exhibits cubic structure nanoparticles which were uniformly distributed and average grain size of the calcium oxide nanoparticle was found to be 125-218 nm.[25]

Table 1: Structural Parameters of the biosynthesized Calcium Oxide nanoparticles

2θ deg	d spacing (Å)	FWHM (deg)	Ciystallite size (D)	Dislocation density(δ) x 1015 lines/ metre^2	Micro strain (S) x 10-3m	Lattice planes hkl	Lattice parameter (Å)	Unit cell Volume x 10*23m^3 (nm)
38.2134	2.3532	0.5269	16.6665	3.60	2.1723	200	4.7064	1.0425
28.2404	3.1575	0.5192	16.5367	3.65	2.3360			
47.2151	1.9235	0.5880	15.3999	4.22	2.3509			

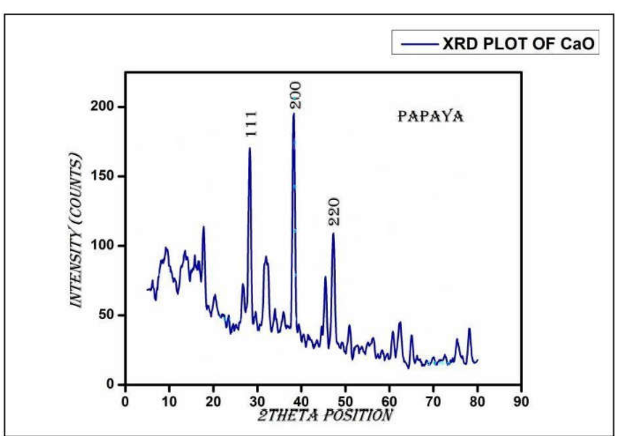

Fig.4: XRD pattern of Biosynthesized Calcium Oxide nanoparticles

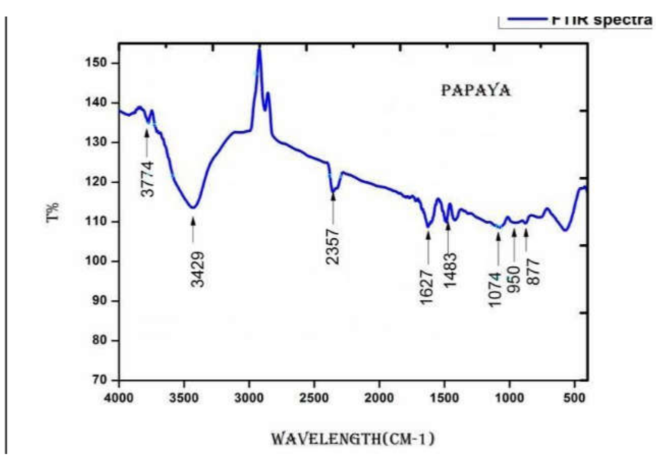

Fig. 5: FTIR analysis of Biosynthesized Calcium Oxide nanoparticles

Table 2: Stretching and the bending vibrations of Biosynthesized Calcium Oxide nanoparticles

S.No	Band Range	Type of Vibration	Appearance
1.	877.61	Ca-0 Stretching	Strong
2.	950.91	C= C Bending	Strong
3.	1074.35	C-0 Stretching	Strong
4.	1483.26	O-H Bending	Medium
5.	1627.92	C= C Stretching	Strong
	2357.01	N-H Stretching	Strong
6.	3429.43		Medium
7.	3774.69	O-H Stretching	Medium / Strong

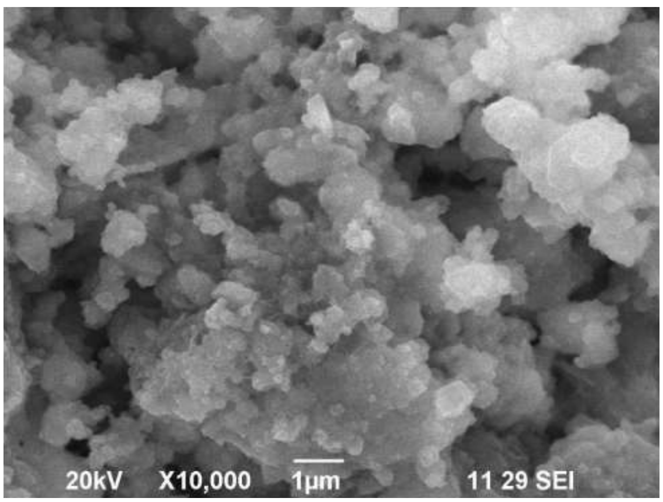

Fig.6: SEM image of Biosynthesized Calcium Oxide nanoparticles

Energy Dispersive X-ray Analysis [EDAX] analysis

Fig.7 shows the EDAX spectra of the CaO synthesized via papaya leaf extract. The elemental composition of the synthesized sample was analyzed through the EDAX analysis. From the result, the sample was mainly composed of Ca and 0 elements. Two strong signals from the Ca atom (58.86%) together with the 0 atom (41.14%) were observed from the EDAX analysis [26]. Table 3 shows both the weight and the atomic percentage of the calcium and oxygen. There was no evidence for the presence of other elements or impurities.

Antibacterial activity

Bio synthesized Calcium Oxide nanoparticles were tested against the gram positive such as Bacillus cereus (MTCC 430), and gram negative bacteria such as E. coli (MTCC 1698) and Klebsiella pneumonia (MTCC10309) according to the agar

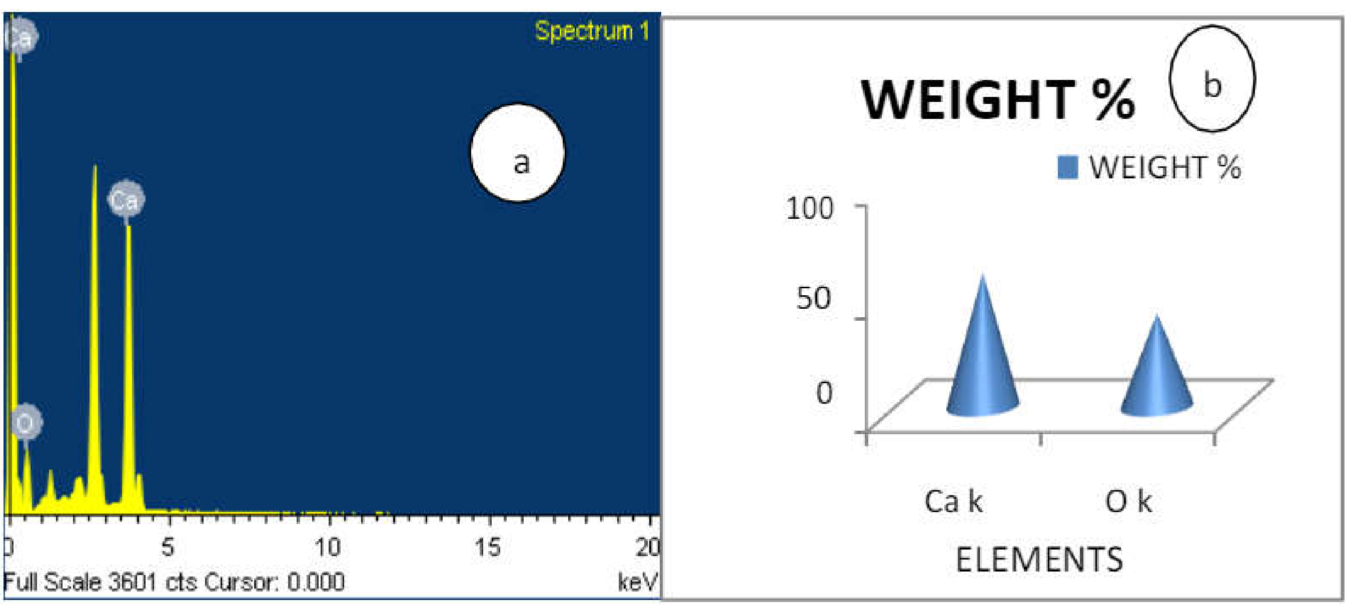

Fig. 7A and B: (a) EDAX spectra of Biosynthesized calcium oxide and (b) Weight percentage of the elements calcium and oxygen

Table 3: EDAX Analysis of the Bio synthesized Ca 0 nanoparticles

Element	Weight %	Atomic %
Ca k	58.86	78.19
Ok	41.14	21.81

well diffusion method. Vancomycin was used as a standard antibiotic during the test. 50 pi and 100 pi of the sample were taken for the antibacterial study to determine the zone of inhibition. From the result, bio synthesized Calcium Oxide nanoparticles shows good antibacterial activity against

Table 4: Zone of inhibition of Biosynthesized calcium oxide nanoparticles

		Zone of Inhibition in Diameter (mm)			Std. Antibiotic (Vancomycin) 30 meg
		Control	PI		
S.No	Microorganisms	100 µl	50 µl	100 µl	
1 Bacillus cereus	Nil	12	14	30	
2	Escherichia coli	Nil	14	16	25
3	Klebsiella pneumonia	Nil	15	20	31

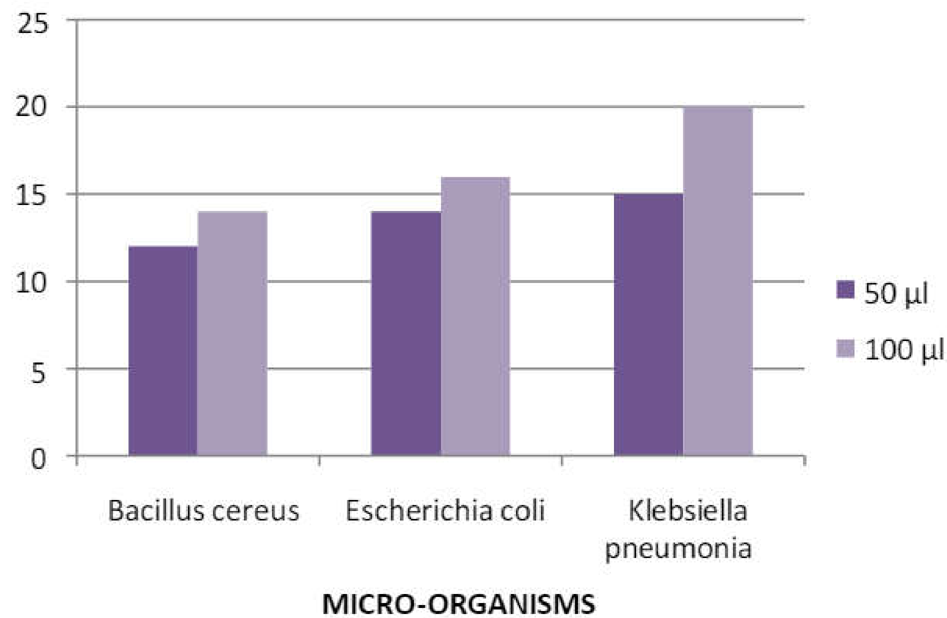

Fig. 8: Graphical representation of the Biosynthesized Calcium Oxide nanoparticles

Fig.9: Antibacterial activity of Biosynthesized Calcium Oxide nanoparticles against Bacillus cereus, E.coli and Klebsiella pneumonia

Klebsiella pneumonia and the inhition zone was 0.2 cm for 100 pi and 0.15 cm for 50 pi. This reveals that, activity was noted on various bacteria's and antibacterial effect was greater in Klebsiella pneumonia than the other bacterial species (Staphylococcus aureus, Escherichia coli) [Fig.8 and Fig.9].[27].Zone of reticence for the various concentration of the sample against the microorganisms was shown in Table 4

4. Conclusion

CaO nanoparticles had been successfully synthesized based on the green approach using carica papaya leaf extract. One of the significances of this study is the use of the carica papaya in plant mediated route which makes the process economically and more ecologically friendly compared to the chemical synthesis. This paper demonstrated the use of the papaya leaf extract as a reducing agent. The analytical tools like XRD, FTIR, SEM and EDAX confirmed the formation of the CaO nanoparticles. By using the XRD , we had obtained the crystallite size of nm and the XRD pattern shows the polycrystalline nature of the calcium oxide nanoparticles. The cubic structure of the CaO nanoparticles was observed by the SEM analysis. FTIR shows the peak at 877.61 for the CaO nanoparticles. EDAX Analysis shows the elemental composition of Ca (58.86%) and 0 (41.14%). Calcium oxide nanoparticles exhibit the good antibacterial activity against Klebsiella pneumonia. This is the effective route of synthesis of nanoparticles involving decreased amount of chemicals..

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