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Anticancer activities of *Sesbania grandiflora* leaf mediated CaO nanoparticles

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Abstract
The CaO nanoparticles, synthesized via *Sesbania grandiflora*, were characterized using TG-DTA, XRD, FTIR, EDS and HR-TEM techniques. The structural analysis revealed cubic structure with average crystallite size being 55 nm. The recorded FTIR spectrum exhibited the occurrence of Ca-O band and the EDS analysis confirmed the presence of Ca and O, thereby, ascertaining the formation of CaO nanoparticles. The recorded HR-TEM image demonstrated the shape of the synthesized CaO nanoparticles to be spherical in nature. *Sesbania grandiflora* mediated CaO nanoparticles were explored for their Anti-ancer activity and found to be effective against the cell lines such as HeLa and MCF-7.

Keywords: CaO NPs, *Sesbania grandiflora*, EDS, HR-TEM, anticancer cell lines

Introduction
Nanoscience deals with the preparation of nanoparticles of various forms, shapes, chemical composition and controlled dispersity, while nanotechnology is associated with their potential applications in diversified fields [1]. Due to their unique physicochemical features, the utility of the nanoparticles has been widely explored [2]. The applicability of nanocrystalline calcium oxide, in various fields such as biology, therapy, detection and diagnostics and microelectronics has been noteworthy [3-6].
Sesbania grandiflora, being a medicinal plant, is used to treat many diseases and disorders. Its leaves can be used to treat thrombosis, diarrhea, inflammatory illnesses and bacterial infections [7, 8]. Leaf extract can be utilized to cure bronchitis, cough, vomiting, wound ulcers, diarrhea and dysentery. Its flowers have been reported to exhibit a good antibacterial and antifungal activity. Moreover, the roots of this plant, in its powder form, can be applied externally to mitigate the rheumatic swelling [9-12].
In the current work, Calcium oxide nanoparticles have been biosynthesized using the *Sesbania grandiflora* leaves and characterized using different techniques to ascertain the formation of nanoparticles. Besides, the efficacy of these bioinspired CaO nanoparticles as Anticancer agent has also been evaluated.

Materials and Methods
Preparation of Leaf Extract
The *Sesbania grandiflora* (Agathi) leaves, were cleansed with water to remove the adherent dust particles. 10 g of leaves was soaked in 100 ml of distilled water and heated at 60°C for 45 minutes. Finally, it was filtered and the filtrate was stored for further process.

Preparation of CaO Nanoparticles
For the preparation of CaO nanoparticles, 5 g of the precursor Calcium nitrate tetrahydrate [Ca $(NO_3)_2$ $.4H_2O$] was added to 50 ml of the leaf extract. Then, it was heated at 60 ºC and stirred well until it turned into white color. The cooled solution was taken in a silicon crucible and was annealed at 900 ºC for 2 hours to remove the unreacted precursor present in the prepared sample. The final CaO nanopowder was collected and stored for further analysis.

Instruments
The thermal behaviour of the as-prepared CaO nanoparticles was examined using NETZSCH-STA 449 F3 JUPITER. The Structural analysis was done by employing SHIMADZU XRD 6000 X-ray diffractometer with Cu Kα radiation (1.5406 Å). The X-ray tube was operated at 40 KV with a current rating of 30 mA and the scanning speed of 0.05 second per step. In the present study, FTIR analysis of the nanopowder was performed using PERKIN ELMER system in 4000–400 cm^{-1} region. The chemical composition was ascertained by employing EDAX (Bruker). HRTEM (JEOL - JEM - 2100F) technique was adopted to investigate the morphology of the synthesized material and the SAED pattern was recorded.

Anticancer activity
The Anticancer activity of the biosynthesized CaO nanoparticles was evaluated by adopting the following standard procedure:
$1x10^5$ cells per well were seeded in 24-well plates and incubated at 37 ^{0} C with 5% CO_2. The sample was inserted after the cell reached confluence and incubated for 24 hours. The sample was removed from the well after incubation and washed with phosphate-buffered saline (pH 7.4). 100 µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2 -thiazolyl) 2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. Following the incubation period, 1ml of DMSO was injected into each well. Using a UV Spectrophotometer and DMSO as a blank, the absorbance at 570 nm was measured. Measurements were taken and the concentration required for 50% inhibition (IC_{50}) was calculated graphically. The percent cell viability was measured using the following formula:

% cell viability = A_{570} of treated cells / A_{570} of control cells × 100

Results and Discussion

Thermal analysis

The thermal characteristic of the as prepared sample of CaO nanoparticles was analyzed by TG-DTA technique (Fig. 1). From Fig.1 it is obvious that the first stage of weight loss (≈ 21.08%) occurred, with an endothermic peak, at 366.94 °C which may be due to the removal of locally bound water molecule. The second weight loss of approximately 34.82%, induced by decomposition of $CaCO_3$, occurred around 511.71 °C.

The third and final stage of weight loss of about 10.22% was recorded between 651.27 °C and 800 °C. It may be attributed to the decomposition of chemically bound chromophoric groups and the residual organic constituents of the precursor.

These thermal variations may be due to the presence of organic phase phytochemicals adsorbed on the surface of the synthesized CaO powder. Similar observations have been reported by early researchers [13].

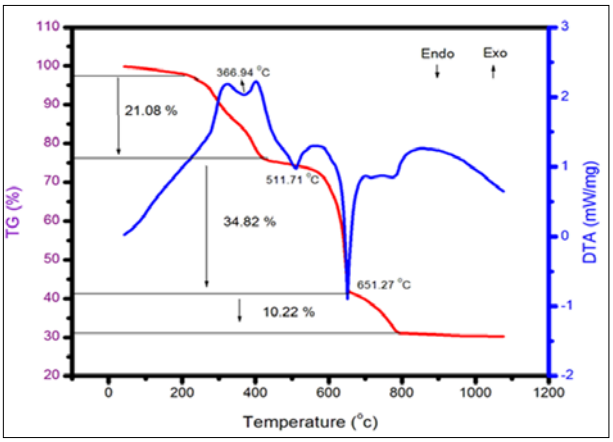

Fig 1: TG-DTA curve for as-prepared CaO nanopowder using *Sesbania grandiflora* leaf extract

As the CaO nanopowder synthesized by the present green method exhibited thermal stability for temperature above 800 ºC, the calcination temperature was fixed at 900 ºC.

Structural analysis

Fig.2 represents XRD spectrum recorded for the biosynthesized CaO nanopowder, using *Sesbania grandiflora* leaves as the reducing agent. The spectrum depicts well intensity peaks corresponding to different planar orientations and the peaks are matching very well with the standard JCPDS card no. 00-004-0777, confirming the cubic structure for the synthesized CaO nanopowder [14,15]. The Crystallite size (D) and the corresponding Strain (ϵ) values were calculated using the following equations 1 & 2 and the calculated values are tabulated in Table -1:

$$D = K\lambda / \beta \cos\theta \quad (1)$$

$$\epsilon = \beta \cos\theta / 4 \quad (2)$$

Where β is the Full Width at Half Maximum (FWHM), λ is the wavelength of the X-ray source used (1.54060 Å) and θ is the Bragg angle. The Crystallite size and Strain values were also determined by Williamson-Hall (W-H) method (Equation 3).

$$\frac{\beta cos\theta}{\lambda} = \frac{1}{D} + \frac{\varepsilon \sin\theta}{\lambda} \quad (3)$$

Further, Fig. 3 portrays the W-H plot recorded for CaO nanopowder, synthesized via *Sesbania grandiflora* leaves. The measured Crystallite size (D) and the Strain values (ϵ), using Scherrer method and W-H plot method, were compared and found to be in good agreement (Table-2).

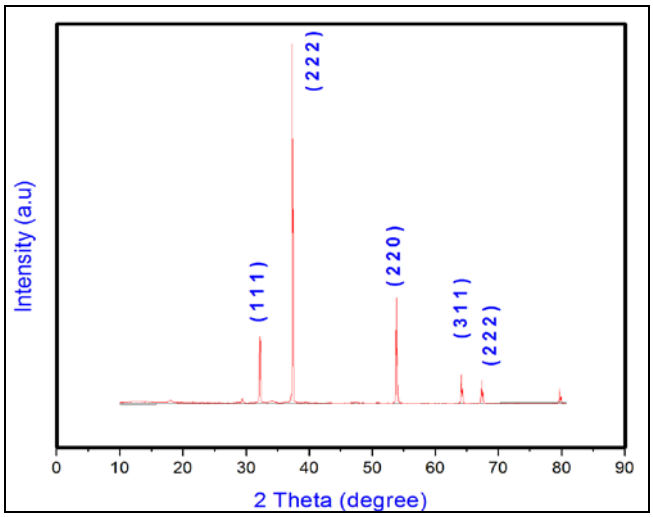

Fig 2: XRD pattern of bio-prepared CaO nanopowder using *Sesbania grandiflora leaf* extract

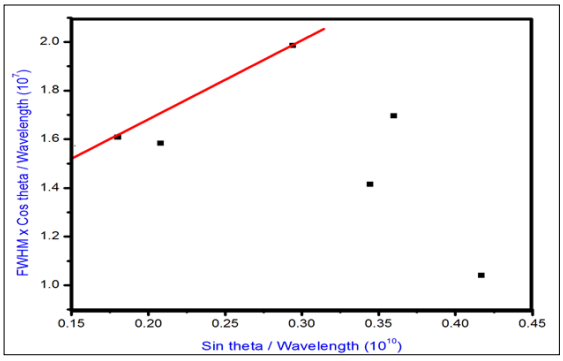

Fig 3: Williamson–Hall plot of biosynthesized CaO nanoparticles using *Sesbania grandiflora* leaf extract

Table 1: XRD analysis of Biosynthesized CaO nanopowder using *Sesbania grandiflora* leaf extract

Position (2θ)	h k l	FWHM	Height (cts)	Ref. Intensity (%)	d-spacing (Å)	Crystallite Size (D) (10^{-9} m)	Dislocation Density (10^{14})	Micro strain (10^{-3})	Lattice Constant (a)
32.2280	(1 1 1)	0.1476	3176	34.53	2.7776	56.066	3.1811	5.3789	4.8005
37.3904	(2 0 0)	0.1476	10763	100.00	2.4051	56.864	3.0925	4.5896	4.8103
53.8828	(2 2 0)	0.1968	4106	38.15	1.7015	45.316	4.8695	2.2931	4.8127
64.1347	(3 1 1)	0.1476	1518	14.11	1.4520	63.563	2.4750	2.4803	4.8160
67.3587	(2 2 2)	0.1800	1391	12.93	1.3890	53.077	3.5495	1.8112	4.8084

The average crystallite size (D = 55±5.923 nm ε = 3.310 ±1.406

Table 2: Comparison of Debye–Scherrer and W-H methods

Calcination temperature	Method	Crystallite Size (D) (nm)	Microstrain(ε) ($x10^{-3}$)
900 °C	Debye–Scherrer	55	3.310
	W-H Method	65	3.329

FTIR Analysis

The FTIR spectrum of the biosynthesized CaO nanopowder was recorded in the region of 4000−400 cm^{-1} and represented as Fig. 4. The sharp peak at 3643 cm^{-1} may be attributed to the physical absorption of water [17], while the broad band at 3435 cm^{-1} may be due to hydroxyl (–OH) group and Ca $(OH)_2$ present on catalyst surface [18].

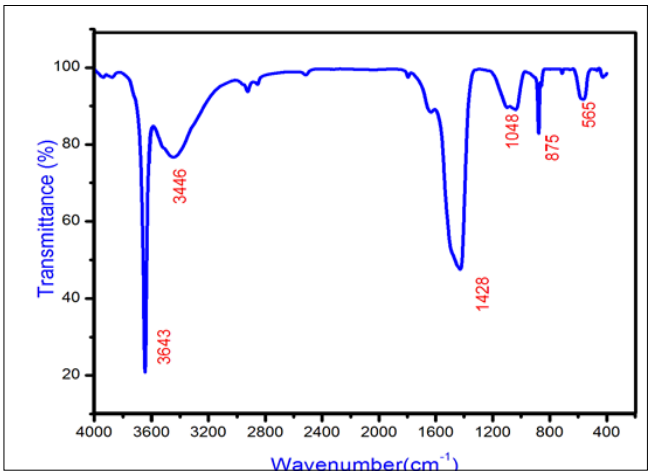

Fig 4: FTIR spectrum of biogenic CaO NPs

The prominent absorbtion peak at 1428 cm^{-1} may occur due to C-O group. The sharp peak at 875 cm^{-1} may be attributed by the formation of Ca-O-Ca bonding while peak at 565 cm^{-1} may be characteristic of the Ca-O bonding[11]. Besides these peaks, no other bands occurred in the FTIR spectrum indicating the purity of the synthesized sample.

EDS analysis

The EDS spectrum was recorded for the bioinspired CaO nanopowder (Fig.5). From the figure, it is crystal clear that Calcium and Oxygen were present predominately along with traces of Silicon and Copper.

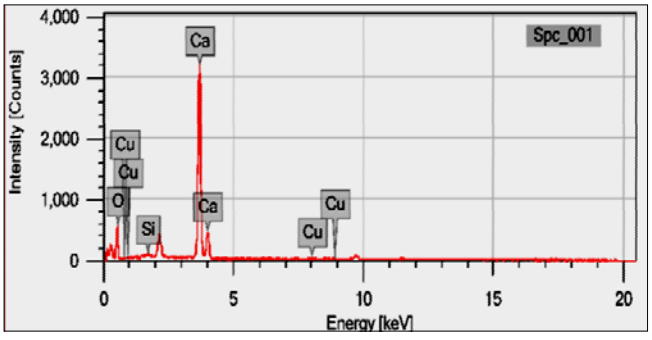

Fig 5: EDS spectrum for the synthesized CaO nanopowder

Table - 3 represents the EDS data. On the basic of the atomic percentage, the presence of Cu and Si is negligible compared to that of Ca and O. The presence of Silicon may be due to the leaf extract [16].

Table 3: EDS data for CaO nanopowder synthesized by Green (leaf extract) method

Element	Weight %	Atomic %
O	40.14	36.64
Ca	58.68	62.80
Si	0.18	0.16
Cu	1.00	0.39
	100	100

The ratio between Oxygen and Calcium (O/Ca) was calculated and found to be 0.5834. This calculated ratio confirmed the presence of oxygen vacancies in the synthesized CaO nanopowder, which may favour a wide range of applications for *Sesbania grandiflora* mediated CaO nanopowder, especially, as a potential antimicrobial agent.

HR-TEM image

As seen in Fig. 6(a), HR-TEM image demonstrated the spherical shape for the synthesized CaO nanoparticles with particle size of about 58 nm. Fig. 6(b) shows the lattice fringes with d spacing of 0.241 nm, corresponding to (2 0 0) reflecting plane. The recorded SAED pattern, corresponding to the (2 0 0) plane confirmed the crystalline nature of the synthesized calcium oxide nanoparticles [Fig. 6(c)].

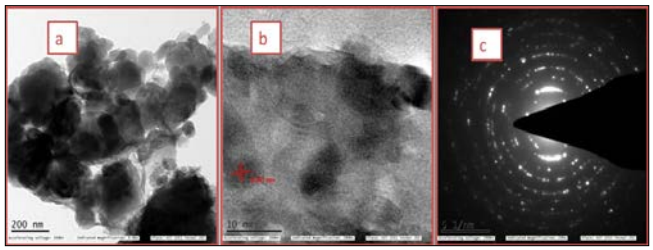

Fig 6: (a & b) HR-TEM images and 6(c) SAED pattern for the biosynthesized CaO nanoparticles

Anticancer activity

In the present study, standard HeLa (Cervical) and MCF-7 (Breast) cell lines have been used to carry out in vitro cytotoxic study on the effect of the biosynthesized CaO nanopowder on human cancer cell lines.

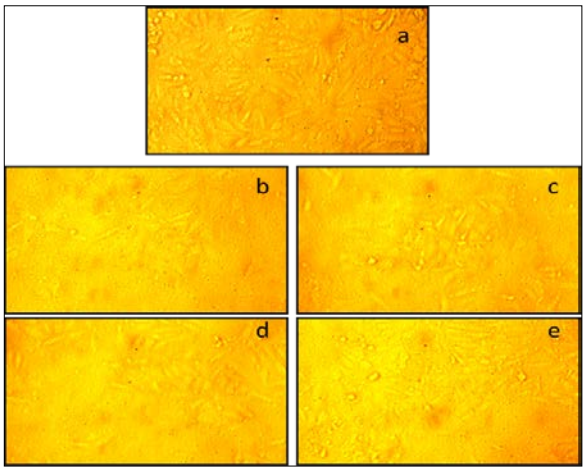

Fig 7: Cytotoxic effect of biosynthesized CaO NPs on *HeLa* cell lines for different concentrations a) Control, b) 1000 µg/ml, c) 250 µg/ml, d) 62.5 µg/ml and e) 31.2 µg/ml

Fig.7 and Fig.8 represent the cytotoxic effects of different concentrations of the bioinspired CaO nanopowder on HeLa cell lines and MCF-7 cell lines respectively.

Normal cell lines were also used to equate the changes in therapy with other concentrations of cell lines. After treatment with 7.8 µg/ml of drug, cell viability in the case of HeLa cell lines decreased from 100 percent to 78.46 percent, indicating the efficacy of the prepared CaO nanopowder.

In a similar fashion, treatment was also performed for MCF-7 cell lines at a concentration of 7.8 µg/ml and the cell viability was found to decrease from 100 percent to 75.71 percent. As the concentration of the synthesized CaO nanoparticles increased, it is found that the cell viability decreased and the corresponding values have been tabulated [Table - 4 & 5].

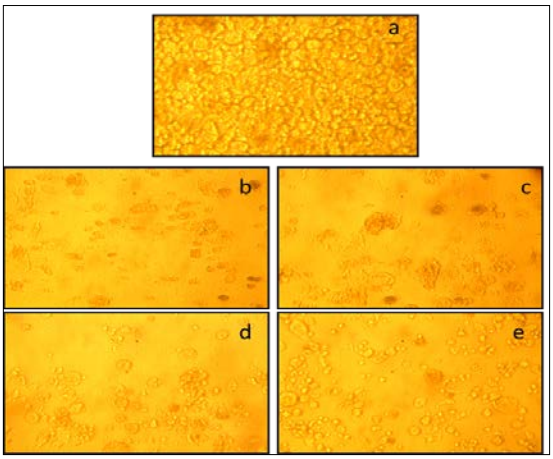

Fig 8: Cytotoxic effect of biosynthesized CaO NPs on *MCF-7* cells for different concentrations a) Control, b) 1000 µg /ml, c) 250 µg /ml, d) 62.5 µg /ml and e) 31.2 µg /ml

For a concentration of 1000 µg/ml of the bioinspired CaO nanoparticles, the cell viability of about 12.30 percent for HeLa and 12.85 percent for MCF-7 cell lines was noticed. IC_{50} values were calculated and found to be 58.91µg /ml and 59.12µg /ml for HeLa and MCF-7 cell lines respectively (Fig.9 and 10).

Therefore, it is understood that the *Sesbania grandiflora* mediated CaO nanoparticles were selective against the choosen human cells. Moreover, IC_{50} value for HeLa cell line has been detected to be the lowest, confirming the fact that the synthesized nanopowder has the potential to destroy the cancer cells at the lowest concentration. Similar findings indicating the Anticancer activity of CaO NPs have been reported by earlier workers [19-22].

Table 4: Anticancer activity of bioinspired CaO nanopowder on *HeLa* cell lines

S.No	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.08	12.30
2	500	1:1	0.15	23.07
3	250	1:2	0.22	33.84
4	125	1:4	0.26	40.00
5	62.5	1:8	0.32	49.23
6	31.2	1:16	0.37	56.92
7	15.6	1:32	0.44	67.69
8	7.8	1:64	0.51	78.46
9	Cell control	-	0.65	100

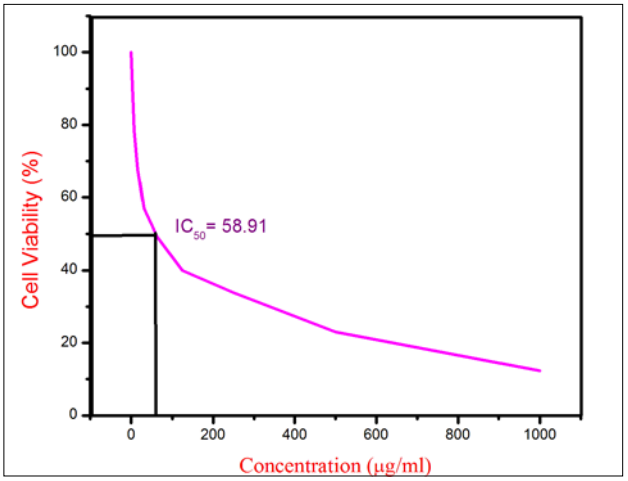

Fig 9: Effect of Green synthesized CaO NPs on HeLa cell lines

Table 5: Anticancer activity of bioinspired CaO nanopowder on *MCF-7* cell lines

S.No	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.09	12.85
2	500	1:1	0.15	21.42
3	250	1:2	0.21	30.00
4	125	1:4	0.27	38.57
5	62.5	1:8	0.34	48.57
6	31.2	1:16	0.41	58.57
7	15.6	1:32	0.47	67.14
8	7.8	1:64	0.53	75.71
9	Cell control	-	0.70	100

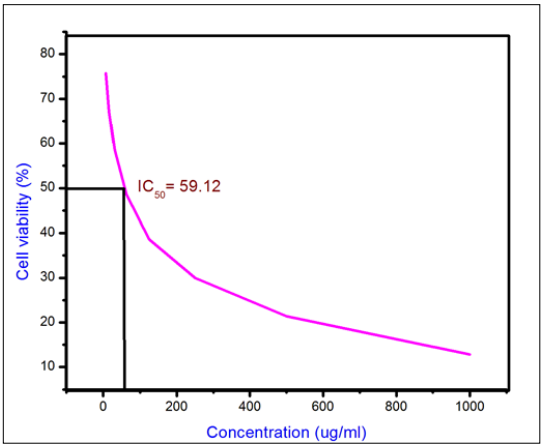

Fig 10: Effect of Green synthesized CaO NPs on MCF-7 cell lines

4. Conclusion

Sesbania grandiflora, a medicinal plant, has been used as the reducing agent to synthesis CaO nanoparticles. The structural analysis indicated cubic structure with average crystallite size being 55 nm.

The recorded FTIR spectrum and the EDS analysis confirmed the presence of Ca and O and thereby the formation of CaO nanoparticles. HR-TEM with SAED pattern exhibited the single crystalline nature of the synthesized nanoparticles. The bioinspired CaO NPs, tested against the human cell lines such as HeLa and MCF-7, were found to be effective.

Therefore, it is concluded that the *Sesbania grandiflora* mediated CaO nanoparticles may be used as a preferential candidate for Anticancer activity.

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Conflict of Interest
The authors declare that there is no potential conflict of interest among them.

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