



# Biogenic Synthesis and Characterization of Calcium Oxide Nanoparticles Using Bark Extract of *Syzygium cumini* and an Evaluation of Their Cytotoxicity and Wound Healing Properties

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## Abstract

The current study focuses on synthesis of biogenic calcium oxide nanoparticles (CaO NPs) using the bark extract of *Syzygium cumini*, through green chemistry approach. It deals with the development of CaO NPs using *Syzygium cumini* bark, which is ethnomedicinally relevant and has a multitude of applications. The green synthesis technique ensures sustainability by the use of natural reducing agents and less release of hazardous wastes. Synthesized CaO NPs were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and thermogravimetric analysis (TGA). The XRD analysis confirmed a cubic crystalline structure with an average particles size ranging from 48 to 52 nm. SEM and EDX analysis showed a spherical morphology with some aggregation, while elemental analysis confirmed the presence of Ca, O, Na, and C. FTIR spectroscopy identified functional groups that acted as capping and stabilizing agents. The thermal stability of CaO NPs was determined by TGA with loss of weight due to moisture evaporation and organic decomposition. Cytotoxicity assays showed dose–response manner, 88.37% cell viability at 20 µg and 61.97% at 100 µg, which indicates probable biomedical applications. Scratch assay demonstrated the nanoparticle ability to promote wound healing and tissue regeneration. *S. cumini*-mediated CaO NPs can be translated to potential biomedicine applications such as wound healing, antimicrobial agents, and drug delivery.

**Keywords** *Syzygium cumini* · Bark extract · Biogenic synthesis · Nanoparticles · Calcium oxide

## Introduction

Nanotechnology is an evolving field of science with production and fabrication of matters at the nanoscale ranging from 1 to 100 nm. Using this technology, wide range of nanoscale materials are produced, incorporated, and used in various fields [1]. Nanotechnology is changing many fields, and in medicine, it has some of the most exciting developments

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[2]. Green synthesis is an effective method which is used to synthesize desired nanoparticles using eco-friendly compounds such plants, microorganisms, and fungi [3]. The green chemistry method is designed to reduce wastes, minimize the use of hazardous chemicals, and enhance sustainability. It has relatively lower conditions of the reaction, diminished energy requirement, and mild interaction conditions [4]. *Syzygium cumini* belongs to the family Myrtaceae. Its common names include black plum, jamun, and Indian blackberry. It is found in the Asian subcontinent, South America, Madagascar, and Eastern Africa [5]. The ripe fruits are used for the production of health drinks, squashes, jellies, and wine [6]. Its seeds are used to treat disease like diabetes mellitus. Leaves and bark of *S. cumini* shows the antioxidant, antiproliferative activity [7], anti-inflammatory, neuropsychopharmacological, antidiabetic [8], antimicrobial, anti-HIV, antifertility, anorexigenic [9], antileishmanial, antifungal, anti-diarrheal, gastroprotective, anti-ulcerogenic [10], and radioprotective activities. Roy et al. [11] synthesized CaO NPs via chemical synthesis approach and determined its anti-bacterial activity against *Staphylococcus epidermidis* (MTCC 435), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida tropicalis* (NCIM 3110), whereas Anantharaman et al. [12] produced green tea leaves mediated CaO NPs by green chemistry method and evaluated its photocatalytic and antimicrobial activity.

The current study aims to synthesize and characterize the CaO NPs using bark of *S. cumini* and assesses its cytotoxicity and wound healing activity using L929 cell lines.

## Materials and Methods

### Chemicals and Reagents

All the chemicals and reagents with 99% of purity were obtained from Sigma Aldrich, Mumbai.

### Preparation of *S. cumini* Bark Extract

*S. cumini* bark was collected from Chittoor, Edappadi (Taluk), Salem District, Tamil Nadu. The bark was washed with tap water followed by distilled water to ensure the removal of dust. Five grams of *S. cumini* bark was taken and ground into paste with 100 mL distilled water. Extract was collected and kept at 80 °C water bath for 30 min. Then, it was filtered using Whatman filter paper No. 1. Collected extract was stored in a sterile container at 4 °C for further analysis.

### Synthesis of CaO NPs

0.1 M calcium nitrate was prepared for 100 mL and mixed with 100 mL of *S. cumini* bark extract. It was kept at 80 °C in magnetic stirrer with 500 rpm. pH 8 was maintained using 1 N sodium hydroxide solution. This mixture was stirred well until it turns into white color. The solution was calcinated at 60 °C for 1 h using hot air oven. Powdered *S. cumini* bark extract mediated CaO NPs were collected and stored for future studies.

## Characterization of Synthesized CaO NPs

The *S. cumini* bark mediated CaO NPs were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), energy-dispersive X-ray spectroscopy (EDX), and thermogravimetric analysis (TGA). XRD analysis was performed to identify the nature and phases of CaO NPs [13]. FTIR analysis was used to assess the functional groups present in both *S. cumini* bark extract and *S. cumini* bark mediated CaO NPs [14]. SEM analysis was carried out to find the surface morphology and structure of CaO NPs [15]. TG analysis was conducted to analyze the thermal stability and thermal decomposition of the synthesized CaO NPs [16].

## Cell Cytotoxicity of *S. cumini* Bark Extract Mediated CaO NPs Using MTT Assay

The study was conducted to determine the cytotoxicity of *S. cumini* bark extract mediated CaO NPs. Fibroblast cell lines (L929) were grown in the Dulbecco's modified Eagle's medium (DMEM) and maintained at 37 °C in a CO<sub>2</sub> incubator with 5% humidity using standard protocol [17]. Twenty to 100 µg of *S. cumini* bark extract mediated CaO NPs were prepared and treated with the grown L929 cell lines. It was kept for incubation for 24 h at optimum conditions along with an untreated cell as control. Later, 50 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye was added to the centrifuged L929 fibroblast cell lines and kept at 37 °C for 3 h. After incubation, 200 µL of phosphate buffer saline (PBS) and 200 µL of acid propanol were added, and incubate at dark condition for overnight. The count of living cells was determined using the presence formazan derivative. The derivatives were quantified by UV-visible spectroscopy. Absorbance was measured at 650 nm using micro titer plate reader (Bio RAD, USA). Percentage of cell viability was calculated.

$$\text{Percent viability} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

## Wound Healing Potential of *S. cumini* Bark Extract Mediated CaO NPs Using Scratch Assay.

The wound healing potential of *S. cumini* bark extract mediated CaO NPs was determined using scratch assay. L929 fibroblast cells were grown in the in vivo condition and seeded on the 6-well plates with 100,000 cells/well and those cells were maintained at 37 °C for 5% humidity at CO<sub>2</sub> incubator. Using sterile micropipette tip, a scratch of ~0.4–0.5 mm in width was made. The cell surface was cleaned using serum-free culture medium to remove the dislocated cells. Then, the *S. cumini* bark extract mediated CaO NPs were added to the scratch and wound closure was closely monitored using inverted phase contrast microscope from 0 to 24 h for both control and sample treated cells. The test was carried out using the standard protocol of Hulkower and Herber [18]. Area of cell migration was calculated using ImageJ software.

## Results and Discussion

### XRD Analysis

The XRD analysis was used to determine the crystalline nature of the green synthesized CaO NPs. Figure 1 displays the XRD pattern of *S. cumini*-mediated CaO NPs and it discloses that the nanoparticles were cubic structured and crystalline nature with the average size of 52 nm, which was calculated by Debye–Scherrer’s equation (JCPDS No. 77–2376). The sharp peaks were found at 28°, 34°, 52°, 53°, and 55° which corresponds to the (111), (200), (220), (311), and (222). The XRD analysis was done by Kumar et al. [19] and discovered the cubic structure and crystalline nature. Anantharaman et al. [12] green synthesized the lattice crystal structure of CaO NPs using papaya leaves and confirmed by XRD analysis.

### FTIR Analysis

Fourier transform infrared spectroscopy was crucial to characterize the functional groups and the chemical moiety of *S. cumini*-mediated CaO NPs. Figure 2a and b demonstrates the functional groups of *S. cumini* (bark) and *S. cumini*-mediated CaO NPs. The peaks of *S. cumini* bark extract such as 3317, 3217, ~2900, ~2113, 1635, 1234, 1049, 594, and 462  $\text{cm}^{-1}$  show the presence of O–H stretching, C–H stretching of aromatic rings and alkyl halides, C≡C or C≡N stretching vibrations, C=O or amide bond stretching, and metal

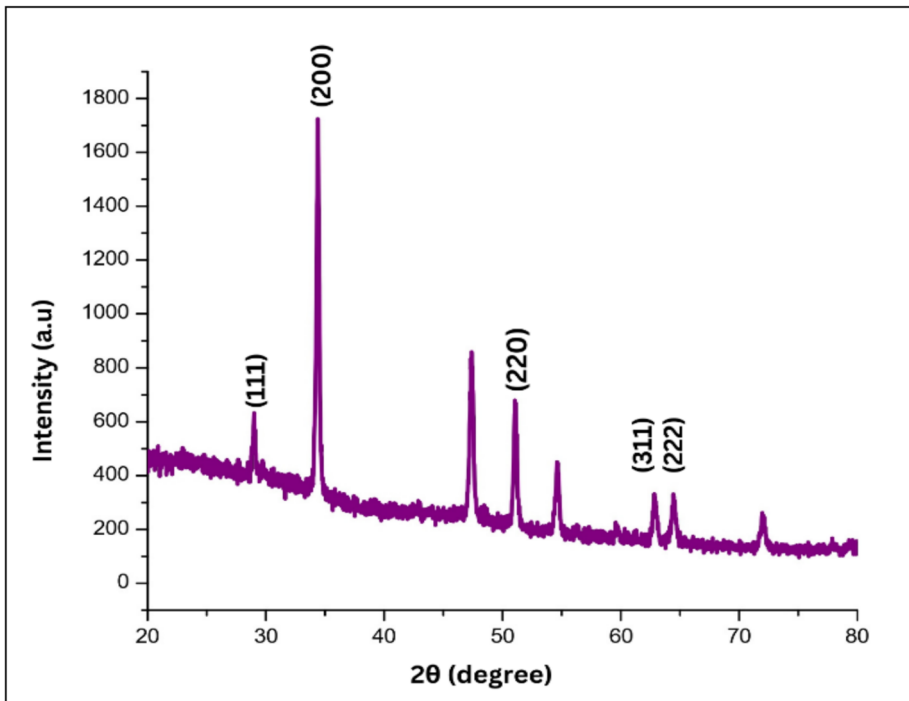
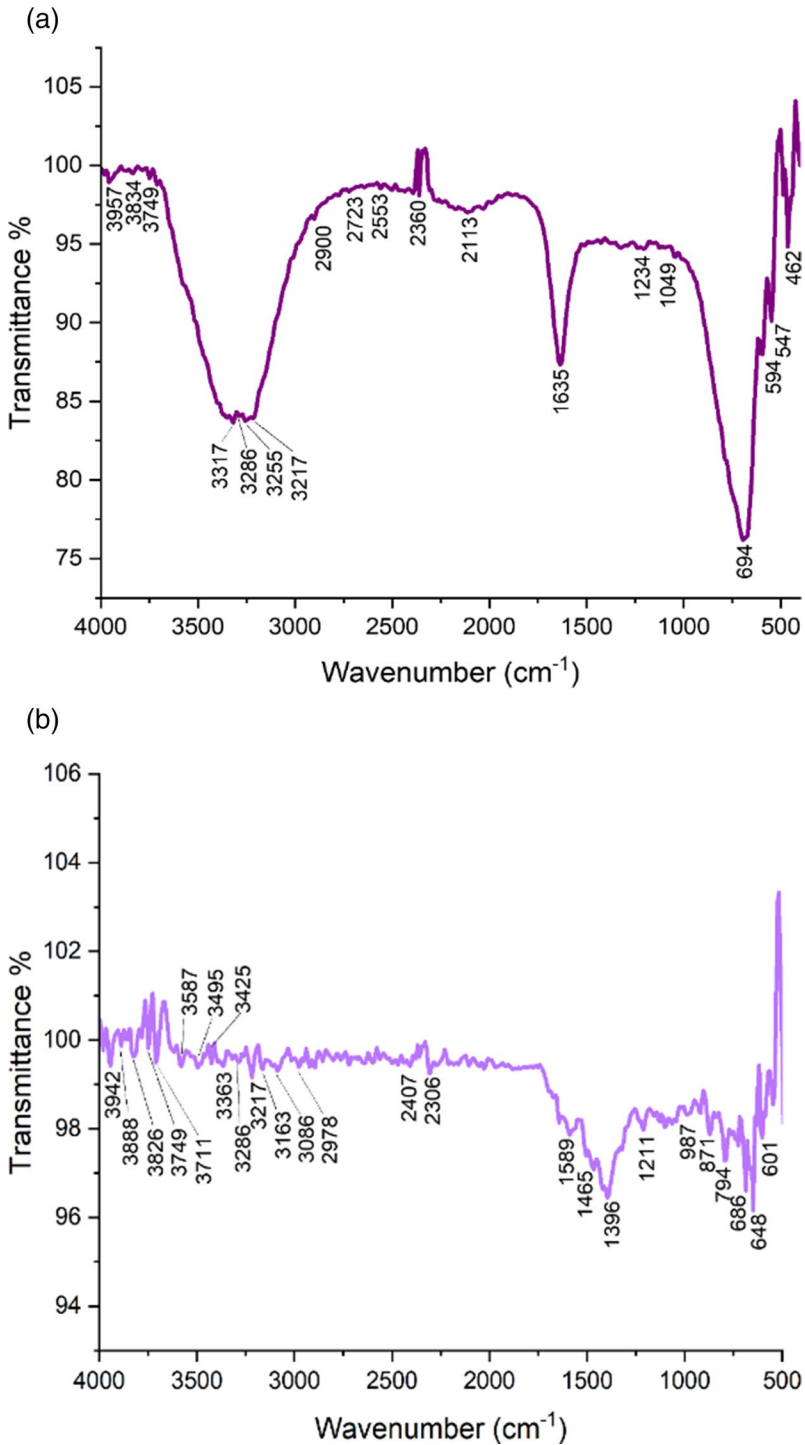


Fig. 1 XRD analysis of *S. cumini* bark extract mediated CaO NPs



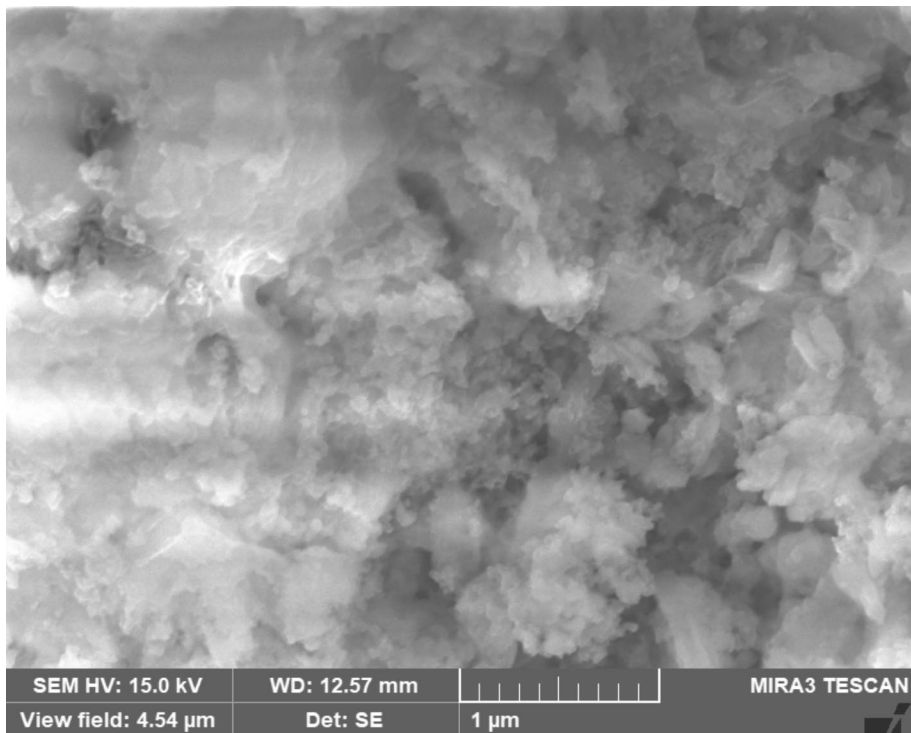
**Fig. 2** **a** FTIR analysis of *S. cumini* bark extract. **b** FTIR analysis of *S. cumini* bark extract mediated CaO NPs

oxide linkages, whereas the peaks of CaO NPs such as 3942, 3280, 2978, 3068, 1589, 1465, ~1211, 648, and 601  $\text{cm}^{-1}$  display the presence of O–H stretching, C–H stretching vibrations, C=O stretching vibrations, C–O or C–N stretching, and M–O (strong peaks indicate metal oxide). It is clearly proving that the biomolecules shifted from extract to the surface of nanoparticles. Comparably, the FTIR spectroscopy of CaO NPs was done by Prabhavathi et al. [20] using sol–gel method showing the strong absorption peaks in the regions around 437  $\text{cm}^{-1}$  that denotes C–H stretching. And the occurrence of peak at 3423  $\text{cm}^{-1}$  represents the O–H stretching of water molecules.

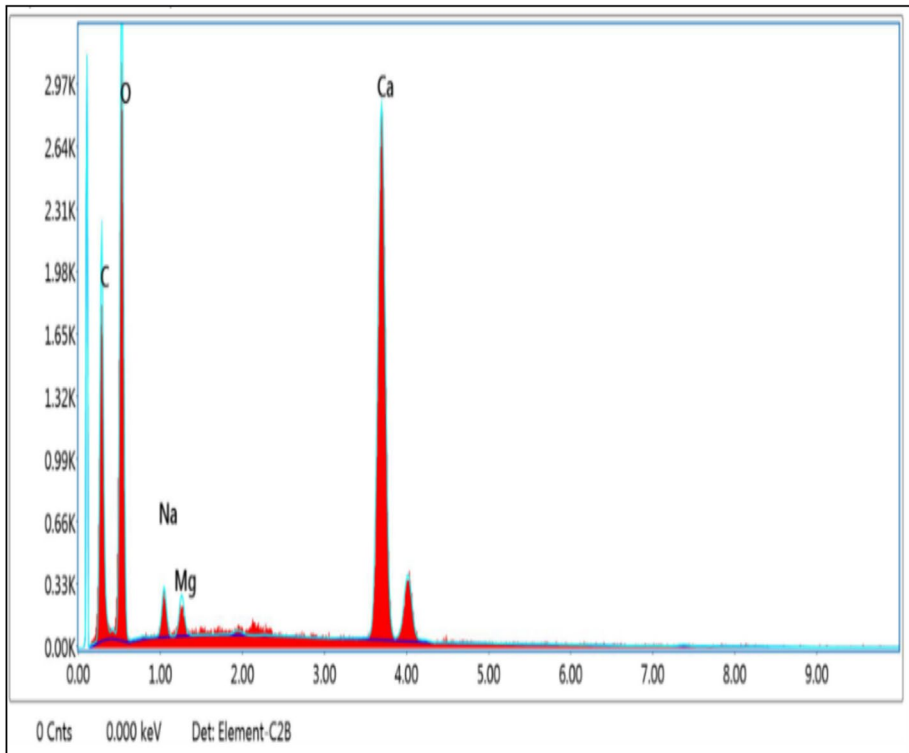
### SEM and EDX Analysis

Figure 3 shows that the SEM image of *S. cumini* bark mediated CaO NPs exhibits a spherical with aggregates and displays the average particle size of 52 nm. Similarly, Ramola et al. [21] synthesized CaO NPs using green tea extract and characterized. The SEM analysis of green tea extract mediated CaO NPs shows the agglomeration and porous.

Figure 4 illustrates that the EDX spectrum of *S. cumini* bark mediated CaO NPs confirms the presence of Ca, O, Na, and C, which verify the elemental composition of the nanoparticles. Thus, the detection of peaks of Ca and O in the spectrum ensures the successful formation of CaO NPs, while the presence of peaks for Na and C could be ascribed to the plant residue or impurities. Raizada et al. [22] performed EDX analysis for CaO NPs and concluded the presence of Ca, O, and C.



**Fig. 3** SEM analysis of *S. cumini* bark extract mediated CaO NPs



**Fig. 4** EDX spectrum analysis of *S. cumini* bark extract mediated CaO NPs

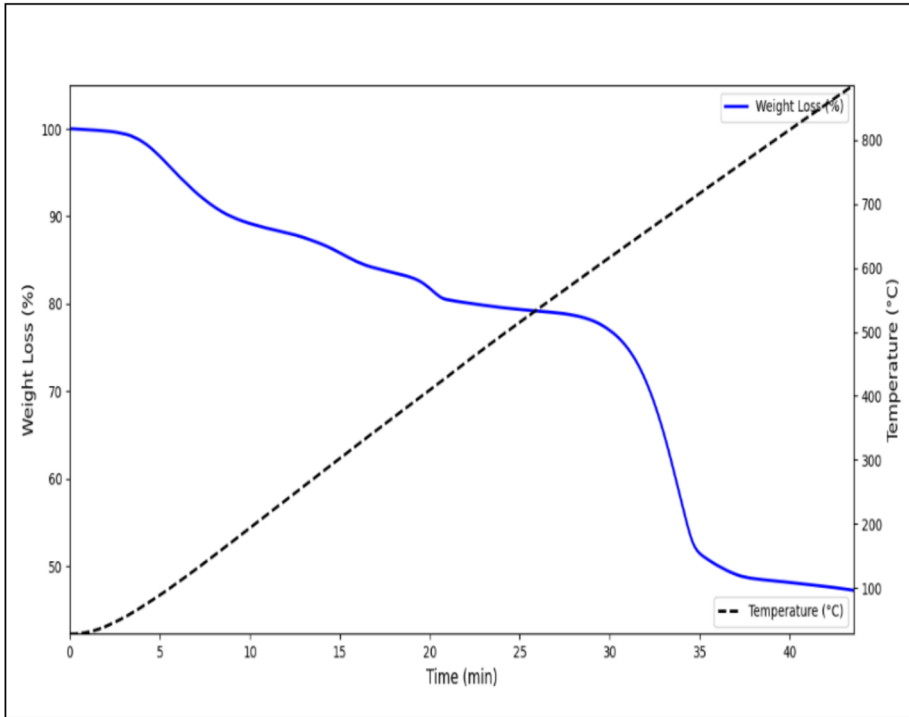
### Thermogravimetric Analysis (TGA)

TG analysis of the *S. cumini*-mediated CaO NPs was carried out in the temperature between 27 °C and 900 °C at a heating rate of 10 °C/min under an air atmosphere. TG analysis of *S. cumini* bark mediated CaO NPs was shown in Fig. 5. The weight loss up to 300 °C was for the moisture evaporation and followed by a further loss of mass due to structural dehydration between 300 and 500 °C. Between 500 and 750 °C, the weight loss was significant and most likely accounted by the decomposition of carbonates or hydroxides leading to the stable formation of metal oxides. At temperatures higher than 750 °C, the weight remains stable, thus suggesting thermal stability. Similarly, TG analysis was conducted on the CaO NPs by Mirghiasi et al. [13] using direct thermal decomposition method and revealed the weight loss from 43 to 375 °C, 375 to 480 °C, and 480 to 650 °C due to the vaporization.

### Biological Applications

#### MTT Assay

Table 1 shows the cytotoxicity of *S. cumini*-mediated CaO NPs in fibroblast (L929) cell lines. On the lowest concentration of 20 µg of *S. cumini*-mediated CaO NPs was exhibited



**Fig. 5** TG analysis of *S. cumini* bark extract mediated CaO NPs

**Table 1** Cytotoxicity of *S. cumini*-mediated CaO NPs

Samples	Concentrations (µg)	% of viability	% of cytotoxicity
Control cells (without treatment)		100%	No cytotoxicity
<i>S. cumini</i> -mediated CaO NPs	20	88.37	11.63
	40	81.71	18.29
	60	75.64	24.36
	80	68.86	31.14
	100	61.97	38.03

88.37% cell viability. On the other hand, at the maximum of 100 µg of *S. cumini*-mediated CaO NPs was recorded 61.97% cell viability. It concluded that the *S. cumini*-mediated CaO NPs hold less toxicity. CCK-8 assay was performed to assess the in vitro cytotoxicity of CaO<sub>2</sub> and PAA-CaO<sub>2</sub> nanoparticles by Yu et al. [23] using fibroblast (L929) cell lines.

### Scratch Assay

Table 2 represents the area of cell migration in *S. cumini*-mediated CaO NPs-treated fibroblast (L929) cell lines. In the control cell line, cell movement was minimal, with the mean

**Table 2** Wound healing potential of *S. cumini*-mediated CaO NPs

Components	Hour	Area	Mean	Minimum	Maximum
Control	0	746,496	114.351	2	255
	24	746,496	116.105	10	255
<i>Syzygium cumini</i> -mediated CaO NPs	0	746,496	131.950	5	255
	24	746,496	119.925	18	242

migration area slightly increasing from 114.351 to 116.105 due low cell motility. However, CaO NPs-treated cell lines shows higher (131.950) migration area at 0 h and lower (119.925) migration area at 24 h. The reduction may be due to the affection of cell viability or interference of the CaO NPs in the cellular process like cell migration, proliferation, and apoptosis. Likewise, Handral et al. [24] conducted the wound healing scratch assay using SiO<sub>2</sub> NPs, TiO<sub>2</sub> NPs, and ZnO NPs and concluded that TiO<sub>2</sub> NPs and ZnO NPs exhibited 50% wound healing activity whereas SiO<sub>2</sub> NPs did not show any wound healing/cell proliferation activity.

Table 3 displays the comparison for physicochemical and biological properties of *S. cumini*-mediated CaO NPs and *Ficus carica*-mediated CaO NPs.

## Conclusion

In this research, green chemistry method was employed to successfully synthesize CaO NPs using *S. cumini* bark extract. The synthesized nanoparticles were assessed and characterized by various standard assays. XRD confirmed a cubic crystalline structure of the nanoparticle with an average particle size from 48 to 52 nm. SEM and EDX analysis reveal that CaO NPs appear to be predominantly spherical, some aggregating, whereas the elemental analysis confirms the presence of Ca, O, Na, and C in it. Biological assays like scratch assay and MTT assay demonstrated that CaO NPs hold less toxicity and significant wound healing activity. Further, *S. cumini*-mediated CaO NPs can be used for various other applications like biomedicine (wound healing, antimicrobial agents, drug delivery, and medical dressings), environmental remediation (water purification and heavy metal removal), and agriculture (soil enhancement and pathogen control).

**Table 3** The comparison for physicochemical and biological properties of *S. cumini*-mediated CaO NPs and *Ficus carica*-mediated CaO NPs

Properties	<i>S. cumini</i> -mediated CaO NPs	<i>Ficus carica</i> -mediated CaO NPs [25]
Nature	Crystalline	Crystalline
Shape	Spherical with aggregates	Irregularly shaped and non-uniformed particles
Size	52 nm	68 nm
Elemental composition	Ca, O, C, Na	Ca, C, O, Na, Mg, P, S
Biological properties	Wound healing properties	Antibacterial activity and antibiofilm activity

**Author Contribution** Dr. Rajiv Periakaruppan: conceptualization, supervision, funding acquisition, and project administration. Mr. Logeshwaran Anandhan: investigation and writing—original draft preparation. Dr. Vanathi Palanimuthu: revision and editing. Mr. Nithish Kathiravan: revision and editing. Dr. Karungan Selvaraj Vijai Selvaraj: data curation. Dr. Noura Al-Dayan: data curation.

**Availability of Data and Materials** Not applicable.

## Declarations

**Ethics Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Competing Interests** The authors declare no competing interests.

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