



# Phytochemically Synthesized Silver Nanoparticles using *Ocimum Basilicum* Leaf Extract Potentiate Cytotoxicity

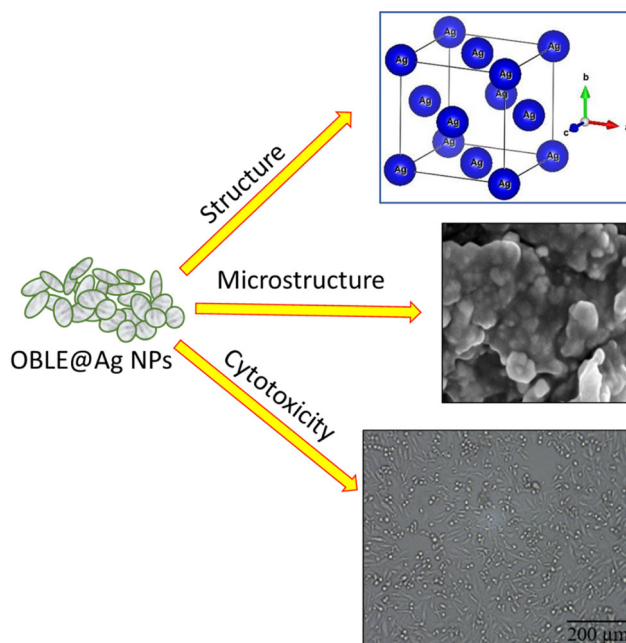
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## Abstract

A study was conducted to develop a cost-effective and eco-friendly method for synthesizing silver nanoparticles (AgNPs) using *Ocimum Basilicum* leaf extract (OBLE). The physical and chemical properties of the synthesized AgNPs were analyzed. The X-ray diffractogram of OBLE-mediated AgNPs (OBLE@AgNPs) exhibited face-centered cubic crystallinity with space group  $Fm\bar{3}m$ , which was confirmed by Rietveld refinement studies. Fourier Transform Infrared (FTIR) spectroscopy identified the functional groups of the phytochemicals present in OBLE at  $3416\text{ cm}^{-1}$  and  $1652\text{ cm}^{-1}$ , indicating the presence of phenolic ( $-\text{OH}$ ) content belonging to OBLE. The formation of AgNPs was confirmed by a characteristic hump at 403 nm in the UV-Vis spectrum. The granular microstructure of OBLE@AgNPs showed well-interlinked grains with agglomeration and an average grain size of 22 nm. The anticancer activity of OBLE@AgNPs was evaluated against MDA-MD 231 breast cancer cell lines, and the results indicated dose-dependent cell viability with an  $\text{IC}_{50}$  value of  $67.83\text{ }\mu\text{g/mL}$ . The present study demonstrated a simple and environment-friendly method for synthesizing AgNPs using OBLE, which could potentially be used in various biomedical applications.

## Graphical abstract



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**Keywords** AgNPs · *Ocimum Basilicum* · Cytotoxicity · Breast cancer · MDA-MB 231 cell line

## Highlights

- OBLE@AgNPs exhibit face-centered cubic crystal structure with  $Fm\bar{3}m$  space group.
- OBLE@AgNPs showed well-interlinked grains with an average grain size of 22 nm.
- FTIR spectrum of OBLE@AgNPs confirmed the presence of phytochemicals of OBLE.
- Low  $IC_{50}$  ( $= 67.83 \mu\text{g/mL}$ ) was obtained for the cytotoxic activity of OBLE@AgNPs against MDA-MD 231 cancer cell line.

## 1 Introduction

Nanotechnology culminates various fields including materials science, energy harvesting, medicines, optical fiber, sensors, cosmetics, antimicrobials, photocatalysis, and agriculture [1]. Materials at the nanoscale behave differently than their bulk counterparts because nanomaterials possess a high surface area-to-volume ratio and a characteristic feature of quantum confinement [2]. Metal nanoparticles (MNPs) display an important class of nanomaterials as these find applications in all areas of science and technology. MNPs can be synthesized using various methods such as physical method, chemical method, and biological method. The biosynthesis of metal nanomaterials is advantageous over other methods because the biogenic synthesis technique is cost-effective, environmentally benign, energy-efficient, and easy to transport [3]. Besides, other methods include costly instrumentation, toxic chemicals, and difficult to transport. There exist several reports depicting the synthesis of MNPs using plant derivatives including leaf, fruit, stem, roots, peel, and seed extract. Amongst various metal nanoparticles such as copper (Cu), gold (Au), and silver (Ag), Ag nanoparticles (NPs) are broadly studied owing to their potential applications [4]. AgNPs are easy to fabricate and non-toxic in nature. Various studies reported anticancer, antifungal, antibacterial, and antiviral actions of AgNPs are available [5–8]. Many chemical and physical routes are employed to synthesize AgNPs including co-precipitation, thermal evaporation, microwave synthesis, etc. Although these techniques are costly and provide good yields with pure crystallinity, still there are some disadvantages associated with them as the use of toxic precursors, the release of harmful byproducts, and complications in purification. Contrary to these, the green route or biosynthesis of AgNPs is an economical and environment-friendly approach that utilizes non-toxic solutions derived from plant extracts, algae, bacteria, etc. Making use of various plant parts such as leaves, stem, or root to get plant extracts is striking and beneficial because of freedom from harmful and toxic substances and it also comprises complex microorganism culturing. The sacred

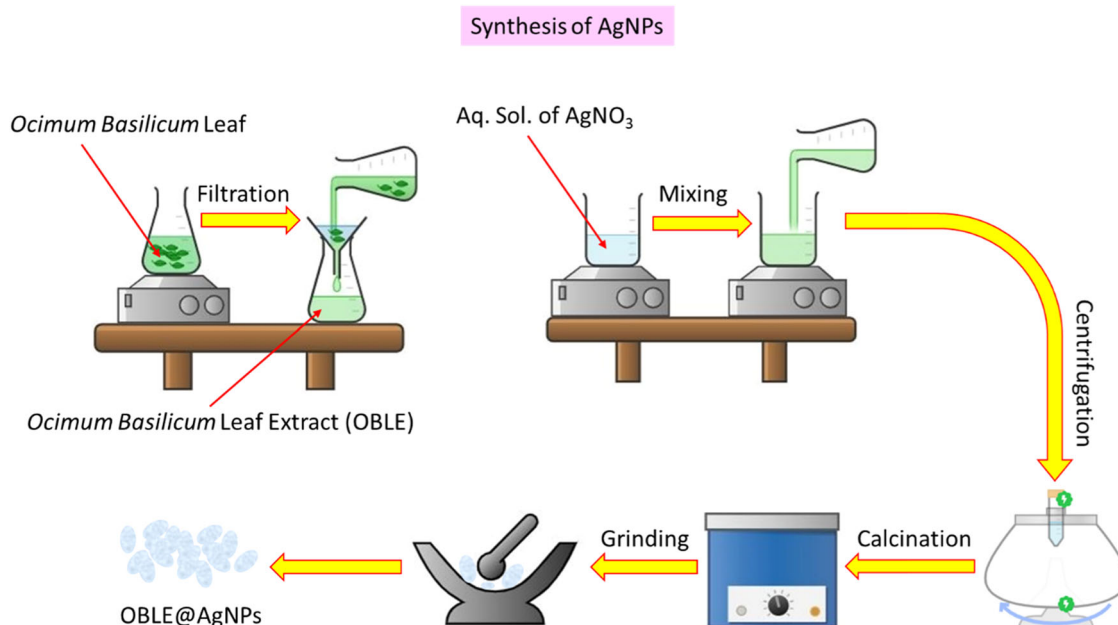
herb *Ocimum Basilicum*, popularly known as Tulsi or holy basil, was used in the current study for green route synthesis of Ag NPs. It contains phytochemicals named as linalool, ursolic acids, alkaloids, glycosides, tannins, carvacrol, rosmarinic acid, aromatic molecules, and many more. *Ocimum Basilicum* has been utilized in Indian tradition and Ayurveda for many years for immunity-building, stress relief, digestion, and the cure of the common cold. Additionally, helpful for [9, 10]. The biosynthesis of AgNPs aroused the interest of researchers. Bharadwaj et al. synthesized AgNPs using fruit extract of *Diospyros malabarica* and confirmed its efficacy as an antibacterial and anti-cancer agent [11]. *Ocimum sanctum* was used by Krishna et al. to create silver nanoparticles, which have powerful antibacterial properties against harmful bacteria. As a result, AgNPs are essential to nanomedicine [12]. The effectivity of green route synthesized AgNPs using different plant extracts against different cancer cells has been reported by various researchers in their reports [13–15]. Rudrappa et al. synthesized AgNPs using *Plumeria alba* leaf extract and examined their cytotoxic activity against the Glioblastoma U118 MG cancer cell line [16]. Rashidipour et al. prepared AgNPs via the green route using olive leaf extract and observed improved anticancerous effect against MCF-7 cancer cell line [17].

In the present report, AgNPs were synthesized using *Ocimum Basilicum* leaf extract, and their structural, optical, and microstructural properties were investigated. Additionally, the cytotoxic potential of *Ocimum Basilicum* leaf extract-mediated AgNPs [OBLE@AgNPs] was investigated against a breast cancer cell line (MDA-MB-231).

## 2 Experiment

### 2.1 Materials

*Ocimum Basilicum* leaves were collected from the local garden at Chandigarh University, Mohali, Punjab India. Analytical grade silver nitrate ( $\text{AgNO}_3$ ) was purchased from MERCK, India.



**Fig. 1** Schematic representation of steps involved in green synthesis of AgNPs

## 2.2 Preparation of leaf extract

The fresh leaves of *Ocimum Basilicum* were plucked from the local botanical garden and then washed with methanol and deionized water to remove the dust entities. The leaves were chopped and contained into the beaker with 100 mL of deionized water and then boiled at 100 °C until the volume of solution was reduced to 3:4 by volume. The prepared leave extract was then filtered out using Whatman No. 1 filter paper and stored at 4 °C for further use.

## 2.3 Synthesis of AgNPs

The stoichiometric quantity of AgNO<sub>3</sub> was weighed using a high-precision weighing balance and then dissolved into 40 mL of deionized water. The solution was magnetically stirred until it became homogenous and transparent. Now 60 mL of prepared extract was poured into the beaker containing Ag salt to maintain the ratio 2:3 (v:v) and kept on stirring for 2 h with the addition of OBLE the color of the solution transformed from pale green to dark brown, which was an indication of reduction of Ag<sup>+</sup> to Ag<sup>0</sup>. Further, the solution was filtered out using Whatman No. 1 filter paper and the collected precipitates of AgNPs were dried in an oven at 100 °C for 2 h as shown in Fig. 1.

## 2.4 Characterization of AgNPs

To determine the crystal structure of Biosynthesized AgNPs X-ray Diffractometer (Rigaku, Japan) fitted with Ni

monochromata and Cu source of wavelength 1.5406 Å was used. The phytochemicals attached to AgNPs were observed by implementing FTIR (PerkinElmer). The optical characteristics of OBLE@AgNPs were carried out using a UV-Vis spectrophotometer (PerkinElmer). The granular microstructure and surface morphology of synthesized AgNPs were illustrated using a field emission scanning electron microscope (FESEM), (Hitachi).

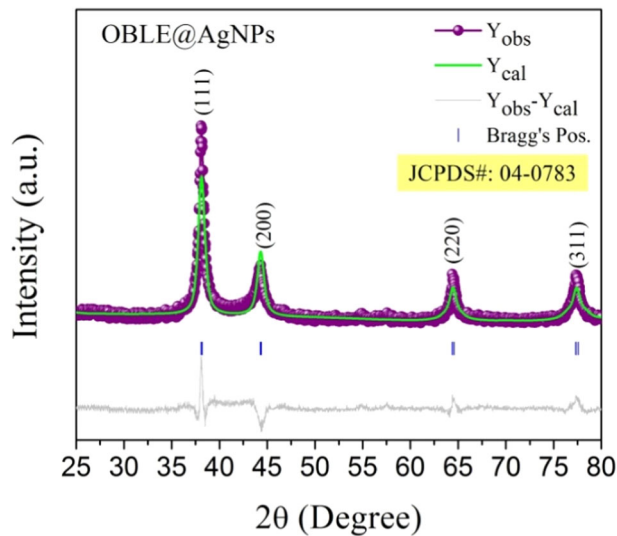
## 2.5 Cytotoxic activity

### 2.5.1 Anticancer activity

MDA-MB 231 cell lines used in this study were obtained from the National Centre for Cell Science (NCCS) in India. To maintain these cells, they were cultured in Dulbecco's Modified Eagle Medium supplemented with 4.5 g/L of glucose and L-glutamine (Cat. 12-604 F, Lonza Bioscience), 10% fetal bovine serum (Cat. 10270106, Gibco), and penicillin-streptomycin (100 units/mL) (Cat. 1524002, Gibco). The cultured cells were incubated in a CO<sub>2</sub> incubator held at a constant temperature of 37 °C, with a controlled atmosphere of 5% CO<sub>2</sub> and 95% humidity. The culture medium was refreshed every 2 days to ensure optimal cell growth and maintenance.

### 2.5.2 MTT Assay

To assess the potential cytotoxicity of OBLE@AgNPs on the MDA-MB 231 cell line, we conducted an MTT assay.



**Fig. 2** XRD pattern of *Ocimum Basilicum* leaf extract mediated AgNPs [OBLE@AgNPs]

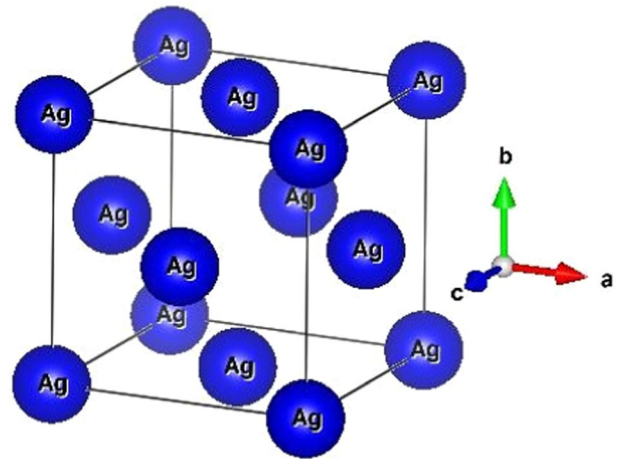
Breast cancer cells were initially seeded at a density of  $1 \times 10^4$  cells per well in 96-well culture plates and then incubated at 37 °C in an environment containing 5% CO<sub>2</sub> and 95% humidity for a period of 1 day. Subsequently, the cultured cells were exposed to varying concentrations of OBLE@AgNPs, including 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL, and further incubated for 48 hours. Following the incubation period, the cells were rinsed with a phosphate-buffered saline solution. Next, 100 µL of a 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well and allowed to incubate for 4 hours. The resulting insoluble formazan crystals were then dissolved in 100 µL of dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm with reference to 630 nm using a microplate spectrophotometer (Epoch, USA). To determine the percentage of cell viability, the obtained data was compared to untreated cells, which served as the control group. To ensure accuracy and reliability, this experiment was replicated three times, and the standard deviation was calculated to assess the consistency of the results.

### 2.5.3 Statistical analysis

The experimental results were presented as the mean value along with the standard deviation. To assess the statistical significance of these results, a one-way analysis of variance (ANOVA) was initially conducted, followed by a post-hoc Tukey test for further analysis and comparison. The values  $****p < 0.0001$ ,  $***p < 0.001$  were considered as significant levels between the experimental and control sets.

**Table 1** Illustrate refined structural parameters of OBLE@AgNPs

Composition	Atomic Positions			Lattice constant (Å)	Cell Volume (Å <sup>3</sup> )	Statistical parameter
	x	y	z			
OBLE@AgNPs	0	0	0	4.088	68.34	$\chi^2 = 1.82$ $R_{\text{Bragg}} = 7.73$ $R_F = 4.04$



**Fig. 3** Face-centred cubic unit cell of AgNPs

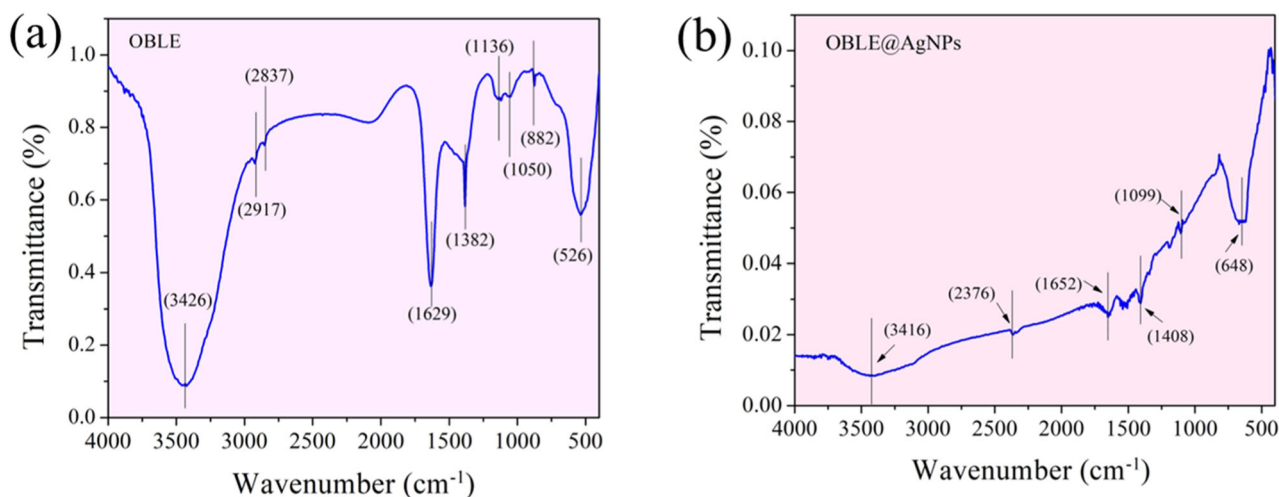
Cell viability (%) was calculated using the following formula (1) [18]:

$$\text{Cell viability}(\%) = \frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of control cells}} \times 100 \quad (1)$$

## 3 Results and discussion

### 3.1 Structural analysis

Structural information of OBLE@AgNPs was determined using the X-Ray diffraction technique. Rietveld refinement method was employed to find the lattice constant, cell volume, and crystallite size of OBLE@AgNPs. To fit the XRD pattern, linear interpolation background profile, pseudo-voigt function, and space group  $Fm\bar{3}m$  were selected [19]. Figure 2 demonstrates the Rietveld refined XRD profile of OBLE@AgNPs. High-intense peaks were assigned with Miller planes (111), (200), (220), and (311), respectively. No trace of secondary phase and impurities were observed confirming the phase pure synthesis of AgNPs. The observed XRD pattern exactly matches with JCPDS#: (04-0785) [20]. The lattice constant, unit cell volume, and satisfied parameter are given in Table 1. The



**Fig. 4** FTIR spectrum of (a) OBLE and (b) OBLE@Ag NPs

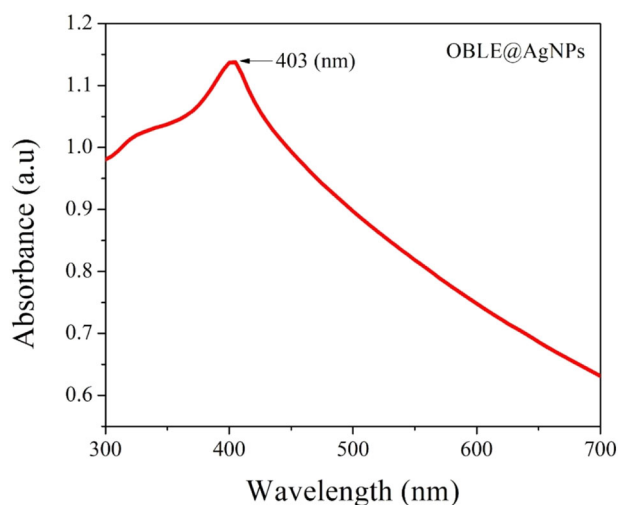
face-centered cubic crystal structure of AgNPs is shown in Fig. 3. The average crystallite size was determined using Debye Scherrer's formula (2) [21]:

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (2)$$

The mean crystallite size was found to be 21 nm.

### 3.2 FTIR spectra analysis

The functional groups of OBLE and OBLE@AgNPs were detected by recording the FTIR spectra within a wavenumber ranging from  $400\text{ cm}^{-1}$ – $4000\text{ cm}^{-1}$ . Figure 4(a) shows the FTIR spectrum of OBLE. A broad bend appeared at  $3426\text{ cm}^{-1}$  showing the existence of a hydroxyl (-OH) group belonging to H-bonded alcohols and phenols present in OBLE. The peak at  $2917\text{ cm}^{-1}$  arises from C-H stretching and symmetric stretching of methoxy groups [22]. The band at  $1629\text{ cm}^{-1}$  corresponds to the N-H bending of primary amines. The bands at  $1382\text{ cm}^{-1}$  are associated with the C-N stretching of aromatic amine groups. In the region around  $1050\text{ cm}^{-1}$ , the absorption peaks correspond to the C-C stretching of alcohols, carboxylic acids, ethers, and esters [23]. These absorption peaks are indicative of the molecular configuration of different functional groups within the extract. Figure 4b depicts the FTIR spectrum of as-synthesized OBLE@AgNPs. It can be clearly visualized from the figure that a broad characteristic hump located at  $3416\text{ cm}^{-1}$  belonging to phenol groups present in OBLE and also there exists a kink at  $1652\text{ cm}^{-1}$  which attributes to -OH hydroxyl group [22]. The presence of kink at  $1099\text{ cm}^{-1}$  governs the attachment of phytochemicals such as carboxylic acids and ethers belonging to OBLE. This confirms



**Fig. 5** UV-Vis spectra of OBLE-mediated Ag NPs

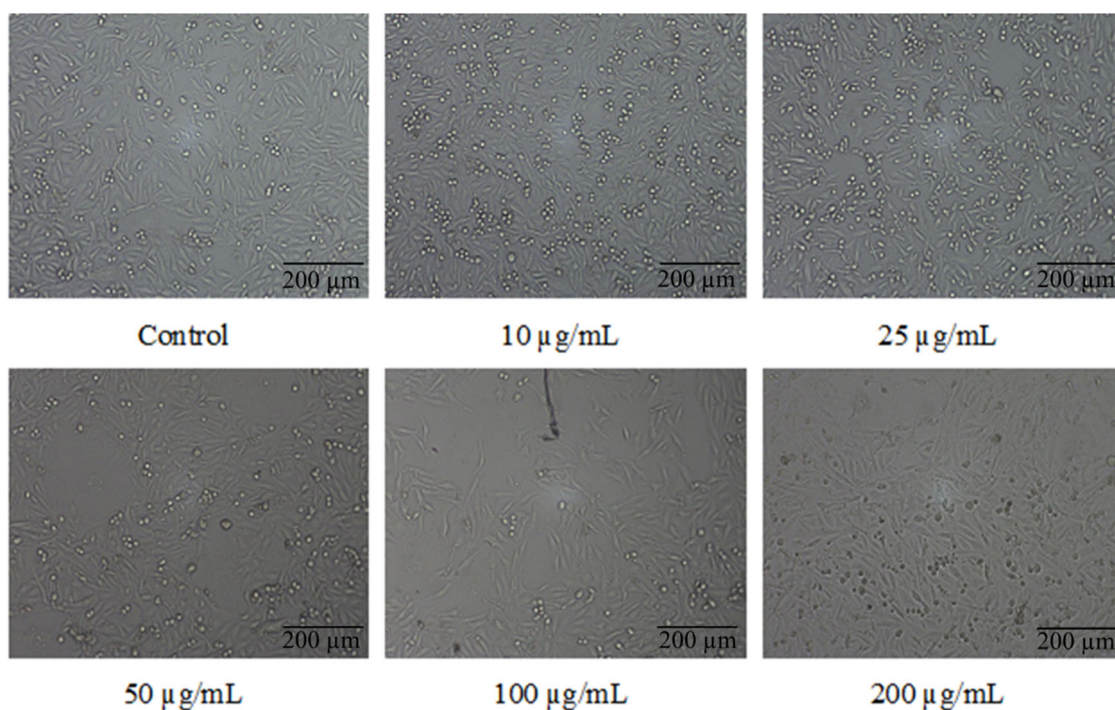
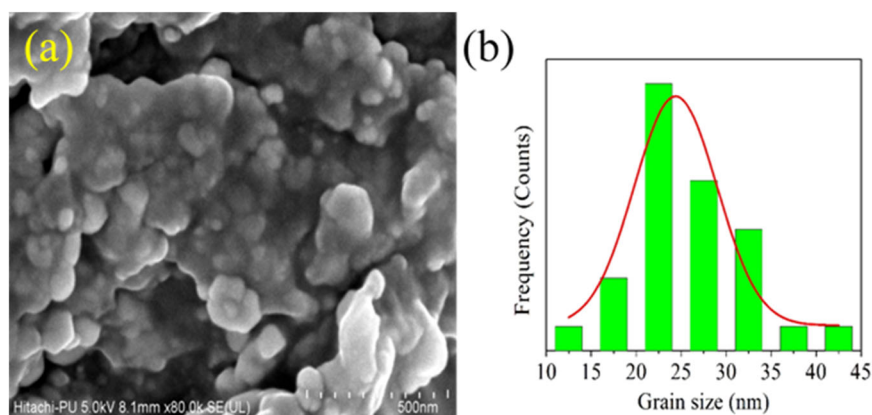
the presence of phytochemicals of OBLE which act as stabilizing, reducing, and capping agents. The high frequency bends positioned at  $648\text{ cm}^{-1}$  belong to Ag stretching vibrations which is a manifestation of pure phase synthesis of AgNPs using phenolic content of OBLE. Fozia et al. and Yilmaz et al. also observed similar results for AgNPs synthesized using plant derivatives [24, 25].

### 3.3 UV-visible spectra studies

UV-Vis spectra were recorded within the wavelength range of 300–700 nm at room temperature as shown in Fig. 5. A characteristic surface plasmon peak appeared at  $\sim 403\text{ nm}$  confirming the formation of AgNPs using green route synthesis [26]. A small hump between 300–350 nm belongs to the phytochemicals present in OBLE. Analogous UV-Vis



**Fig. 6** FESEM image of (a) OBLE@Ag NPs and (b) Bar graph shows the distribution of grains



**Fig. 7** Anticancer activity of OBLE@AgNPs at varying concentration against MDA-MB-231 cancer cell line

spectral result of AgNPs synthesized using *Rheum emodi* leaf extract reported by Sharma et al. which also supports the present findings [27].

### 3.4 Microstructural analysis

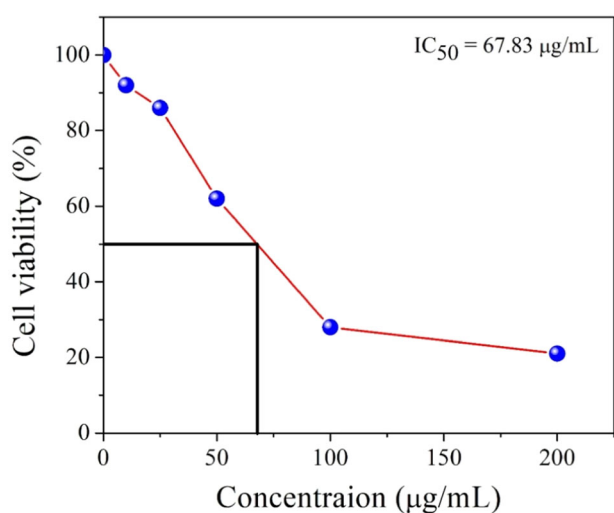
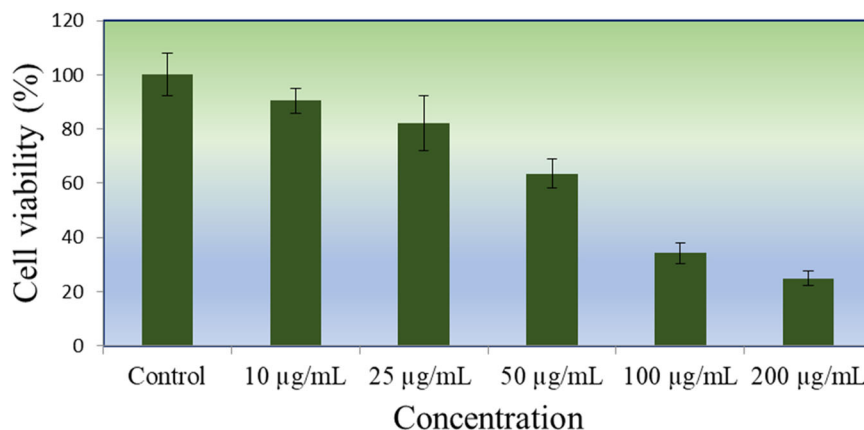
The granular microstructure and surface morphology of OBLE@AgNPs were depicted using the FESEM. Figure 6 shows the FESEM micrograph of OBLE@AgNPs. The granular microstructure of OBLE@AgNPs exhibits well-interlinked and homogeneously distributed grains with small traces of agglomeration [28]. The histogram shown in the inset of Fig. 6 shows the distribution of grains. The bar graph was fitted with the Gaussian distribution function to determine

the exact mean grain size of as-synthesized OBLE@AgNPs. The average grain size of OBLE@AgNPs was found to be 22 nm. Gopinath et al. and Jayanthi et al. also observed similar results for biosynthesized AgNPs [29, 30].

### 3.5 Anticancer studies

The cytotoxic effect of OBLE@AgNPs was explored on a human breast cancer cell line (MDA-MB-231) through an MTT assay. Different concentrations of OBLE@AgNPs were studied for cytotoxic effects against breast cancer cell lines MDA-MB-231 as shown in Fig. 7. The dose-dependent decrease in cell viability was observed in the response of OBLE@AgNPs for cytotoxicity [31]. At lower

**Fig. 8** Cell viability of OBLE@Ag NPs after 48 h of incubation time



**Fig. 9** IC<sub>50</sub> value of OBLE@Ag NPs

concentrations, the cell viability of OBLE@AgNPs was found to be 91.2 % and with the increase in concentration to 200 µg/mL, a decrease in cancer cell viability was found to be 21 % as depicted in Fig. 8. After an incubation period of 48 h, OBLE@AgNPs is found to have an IC<sub>50</sub> value of 67.83 µg/mL on MDA-MB-231 cancer cells (Fig. 9). Many reports are available for anticancer effects of AgNPs on different cell lines which are synthesized by green route using various plant extracts [32–34]. Rudrappa et al. synthesized AgNPs using *Penicillium brasilianum* NP5 and examined cytotoxic potential against the MDA-MB 231 cell line with an IC<sub>50</sub> value of 41.93 µg/mL [35]. Chakraborty et al. investigated the anti-cancerous properties of *Galphimia glauca* leaf extract-mediated AgNPs on SK-HEP1 liver cancer cell line and demonstrated low IC<sub>50</sub> = 19.12 µg/mL [36]. Bhat et al. also reported cytotoxic activity of *Pyrrostegia venusta* leaf extract-mediated AgNPs against kidney cells (COS-7 cell line) [37, 38]. Various phytochemicals found in plant extracts like saponins, tannins, and glycosides are reported as anti-cancerous agents inhibiting cancer

cell growth [31]. The cytotoxic effect of OBLE@AgNPs may also be attributed to the triggering of reactive oxygen species (ROS) causing cancerous cell loss through damaged cellular components. The morphological changes in the cell shape such as the shrinking of cells at various regions indicate cytotoxicity [36].

## 4 Conclusion

In summary, polycrystalline AgNPs were prepared using *Ocimum Basilicum* leaf extract (OBLE). Structural characterization revealed the phase pure synthesis of AgNPs with face-centered cubic center crystallinity as derived by Rietveld refinement. The existence of phenolic phytochemicals (such as eugenol and linalool) of OBLE as appeared at 3416 cm<sup>-1</sup> and 1652 cm<sup>-1</sup> which act as stabilizing, reducing, and capping agents were detected in the FTIR spectrum of OBLE@AgNPs. The optical properties were investigated using the UV-Vis spectrum which depicted a characteristic broad hump centered at 403 nm, which supports the formation of AgNPs. Microstructural characterization revealed that OBLE@AgNPs possessed interlinked homogeneous microstructure with an average grain size of 22 nm. The anti-cancerous activity of OBLE@AgNPs was obtained through MTT assay against MDA-MB-231 which showed cell viability as a function of concentration of OBLE@AgNPs with IC<sub>50</sub> = 67.83 µg/mL. Overall, the present study showcased the potential candidature of OBLE@AgNPs for combating breast cancer cells.

## Data availability

Availability of data and materials Data will be available on request.

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**Author contributions** NA: Conceptualization. Bishali: Data Curating. NK: Supervision. DA: Reviewing. PP: Sample Preparation. AV: Editing. RSP: Data analysis. VR: Cytotoxic activity. GK: Draft writing. SH: Data analysis

## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** In the present study, no animal and human are involved and the manuscript doesn't require ethical approval.

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Consent to publish** The study carried out in the present manuscript is a novel work and didn't involve any data from any individual and doesn't require consent from any individual.

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