



Novel green synthesis of ZnO, CaO, and ZnO-CaO hybrid nanocomposites using extract of *Foeniculum vulgare* (Fennel) stalks : characterization, mechanical stability, and biological activities

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Abstract

Plant-mediated synthesis of nanoparticles is considered an eco-friendly and cost-effective approach to developing safer and more effective treatment strategies. The current study employed *Foeniculum vulgare* (FV) waste extract to mediate the green synthesis of Zinc oxide (ZnO) and Calcium oxide (CaO) nanoparticles (NPs) as well as their hybrid NPs (ZnO-CaO). The biosynthesised NPs were characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infra-red (FTIR) spectroscopy, energy-dispersive X-ray analysis (EDX), X-ray diffraction (XRD), dynamic light scattering (DLS), and scanning electron microscopy (SEM). The influence of zeta potential, size, and turbidity on the NPs stability was studied. The synthesised NPs were also evaluated for their antioxidant, anti-inflammatory, and antibacterial activities. UV-visible spectroscopy confirmed the synthesis of FV-ZnO, FV-CaO, and FV-ZnO-CaO hybrid NPs using a surface plasmon resonance peak at 271.5, 269.5, and 269 nm, respectively. FTIR analysis indicated that the plant extract contains phytochemicals that aid in the bio-reduction and stabilization of the NPs. EDX confirmed the elemental composition, and XRD showed crystallinity of the NPs. FV-ZnO revealed a hexagonal crystalline structure, FV-CaO showed a cubic lattice structure, and FV-ZnO-CaO showed a mixed ratio of crystalline and cubic phase. SEM revealed distinct characteristics for the NPs with FV-ZnO-CaO showing spherical morphology. FV-ZnO-CaO and FV-ZnO NPs exhibited low turbidity and high zeta potentials, indicating better dispersion and more stable suspensions. FV-ZnO-CaO exhibited better antibacterial activity against Methicillin-resistant *Staphylococcus aureus* than their monometallic oxide forms. Mild to moderate scavenging activities were observed for the NPs. FV-ZnO-CaO showed a 91% ability to mitigate against albumin protein denaturation. The antibacterial, free radical scavenging and anti-albumin denaturation potentials of the FV-mediated NPs can be prospectively explored for more effective treatment options.

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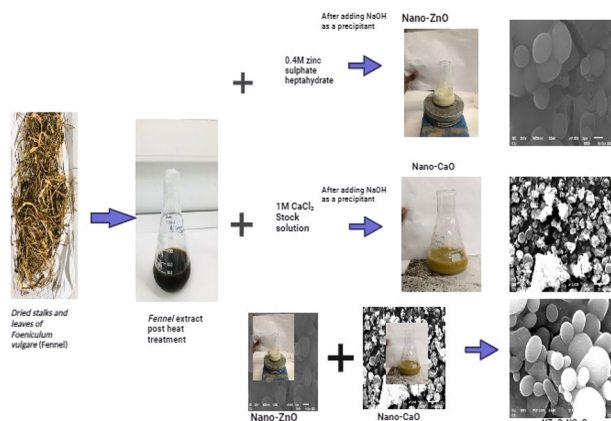
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Graphical Abstract



Keywords Green synthesis · Nanoparticles, · ZnO and CaO hybrid nanoparticles · *Foeniculum vulgare* · Anti-inflammatory

Highlights

- *Foeniculum vulgare* (Fennel) waste extract mediated the green synthesis of zinc oxide (ZnO) and calcium oxide (CaO) nanoparticles as well as their hybrid nanoparticles (ZnO-CaO).
- The nanoparticles were characterized using ultraviolet-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, energy-dispersive X-ray (EDX) analysis, X-ray diffraction (XRD), dynamic light scattering (DLS), and scanning electron microscopy (SEM).
- ZnO-CaO hybrid NPs and ZnO NPs exhibited low turbidity and high zeta potentials, indicating better dispersion and more stable suspensions.
- Plant phytochemicals assist in NP stabilization and bio-reduction.
- ZnO-CaO hybrid NPs showed enhanced antibacterial activity against Methicillin-resistant *Staphylococcus aureus* relative to their monometallic oxide forms.

1 Introduction

Nanotechnology is widely employed in various scientific fields and has become increasingly popular in biochemical, pharmacological, and medical research and practice [1]. Traditionally, different chemical and physical methods have been used to synthesise nanoparticles (NPs) including lithography, laser ablation, hydrothermal synthesis, mechanical milling, chemical precipitation, sol-gel process, and spray pyrolysis. However, significant concerns associated with conventional nanoparticle production methods include their environmental toxicity, high cost, and labour intensity [2]. Recently, green nanotechnology has been applied to produce nature-friendly and less toxic NPs using microorganisms and medicinal plants [3]. Metal oxides created by plants and microbes exhibit good stability with improved characteristics at high pressures and temperatures [4, 5]. Nanoscale materials generally have several excellent characteristics, including a sub-200 nm average size, a vast surface area, metallic qualities, zeta potential, and elastic modulus [6].

Zinc oxide nanoparticles (ZnO NPs) are nanostructured materials made from zinc oxide. ZnO NPs have distinctive

physical and chemical properties that make them highly suitable for a wide range of applications. The use of ZnO NPs in biomedicine is attributed to their properties, such as nanoscale size, excellent antimicrobial activity, biocompatibility, and biodistribution in humans [7]. They have been biosynthesised from plant extracts, including *Salvia hispanica* L., *Ziziphus jujuba* Mill., and *Albizia lebbek* (L.) Benth. as capping agents [8, 9]. Multiple studies have also demonstrated the ability to produce ZnO NPs with trace metals and other NPs with green interfaces, such as essential oils and aqueous extracts [10]. ZnO NPs have high toxicity against bacterial and fungal strains including *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas vulgaris* [7]. Like ZnO NPs, calcium oxide NPs (CaO NPs) are generally considered as biocompatible and biosafe nanomaterials with wide applications in catalysis, biomedicine, food preservation, water purification, and cosmetics [11]. The leaf extracts of *Piper betle* L. [12], *Ocimum tenuiflorum* L. [13], and *Moringa oleifera* Lam [14] have been used to produce CaO NPs.

Furthermore, hybrid NPs have been synthesised by modifying the specific characteristics of monometallic oxide NPs to enable their combination for a synergistic approach [15]. Hybrid NPs find usefulness in biomedicine, cancer research, photo-catalysis, antimicrobial research, and water treatment [16]. Modification methods, including heterojunctions and doping with various chemicals, can be used to enhance the characteristics of certain NPs, such as magnetic properties, morphology, surface charges, stability, and bio-compatibility [17, 18]. It has been reported that doping enhanced the biological activity of titanium dioxide (TiO₂) NPs and altered the surface defects, morphology, crystalline size, and photocatalytic performance of ZnO NPs [19]. In addition, black phosphorus doped with ZnO demonstrated a strong ability to disintegrate biofilms on medical implants in vitro while maintaining an excellent eradication rate in vivo [20].

Foeniculum vulgare (Mill.) is a flowering plant belonging to the Apiaceae family. It is commonly known as Fennel. The Mediterranean region is home to wild fennel, which has potent medicinal properties often said to reduce anxiety and menopausal symptoms [21, 22]. It has also been commercially cultivated in China, India, Japan, and Russia. Fennel is also used to treat stomach conditions such as abdominal pain and constipation, cough, conjunctivitis, and rheumatism. The essential oil of Fennel contains bioactive constituents, including α -thujone, α -pinene, α -terpinene, β -fenchol, β -myrcene, estragole, and limonene [23]. *F. vulgare* extract has been used to bio-synthesise gold NPs [24], silver NPs [25], selenium NPs [26], and copper NPs [27]. In the current study, *F. vulgare* was considered because of its novelty as a reducing and capping agent for synthesising ZnO NPs, CaO NPs, and ZnO-CaO NPs. Unlike several published studies that synthesise hybrid NPs by directly mixing the stock solutions of different metal salts, this study first synthesised individual ZnO and CaO NPs separately using *F. vulgare* extract. The resulting NPs were combined in various ratios to produce the hybrid ZnO-CaO NPs. The study aims to carry out green synthesis of ZnO NPs, CaO NPs, and their hybrid ZnO-CaO NPs using the aqueous extract of *F. vulgare* waste as reducing, capping, and stabilizing agents. Based on our literature search, *F. vulgare* extract has not been reported for synthesising ZnO and CaO NPs, and their hybrid ZnO-CaO NPs. Consequently, this study has achieved the synthesis of ZnO NPs, CaO NPs, and ZnO-CaO NPs using the aqueous extract of *F. vulgare* waste. The plant-mediated NPs were characterised using several analytical techniques such as UV-Vis, FTIR, EDX, BET, XRD, DLS, and SEM [28], and further evaluated for DPPH-free radical scavenging, anti-protein denaturation, and antibacterial activities. To the best of our knowledge, this is the first report on the anti-protein denaturation activity of the ZnO-CaO NPs.

2 Experimental

2.1 Reagents and chemicals

Dimethyl sulfoxide (DMSO), calcium chloride (CaCl₂), zinc sulfate hexahydrate, distilled water, Chloramphenicol (98%), and a Millipore filter (0.45 μ m) were purchased from Sigma-Aldrich, Germany.

2.2 Plant collection and preparation

Fresh fennel stalks were collected from the Yesilirmak region in Northern Cyprus. The leaf materials were dried in the laboratory at room temperature for some weeks. A pharmacologist did the botanical identification of fennel with an herbarium sample (voucher No.: CIU/PHARM/APIA/001) deposited at the Biotechnology Research Center, Cyprus International University, Haspolat, Nicosia. The dried plant material was pulverized and extracted using the method proposed by Jang et al. [29]. Briefly: A 10% *F. vulgare* stalk extract was prepared by mixing 10 g of powdered sample with 100 mL distilled water in a conical flask, stirred with a magnetic stirrer at 50 °C for 30 min. This mixture was then heated in a water bath for 10 min. Afterward, the extract was filtered through Whatman filter paper No. 5 and stored in a refrigerator.

2.3 Synthesis of ZnO NPs

ZnO NPs were synthesised following a modified version of the methods proposed by Umar et al. [8]. and Elshayb et al. [30]. Initially, 0.025 L of *F. vulgare* supernatant was heated to over 60 °C. To prepare ZnO NPs, 287.4 mg of anhydrous zinc sulfate was dissolved in 0.1 L of deionized water. The fennel extract was then combined with the zinc sulfate solution and stirred with a magnetic stirrer at 300 rpm for 12 h. Subsequently, a 40 mM solution of sodium hydroxide was added dropwise to the mixture. This resulted in a white suspension, which was centrifuged and washed with distilled water. The resulting precipitate was then dried at 350 °C in a muffle furnace.

2.4 Synthesis of CaO NPs

To prepare CaO NPs, 10 mL of a 1 M CaCl₂ stock solution was mixed with the fennel extract in a 1:1 ratio using a magnetic stirrer for approximately 30 minutes. Then, 10 mL of 1 M NaOH was added as a precipitating agent. The resulting precipitate was washed and dried in a muffle furnace at 300 °C and 500 °C, following the method suggested by Bano and Pillai [31].

2.5 Hybrid aggregation of FV-ZnO-CaO NPs

The hybrid was synthesised using the drop-by-drop mixing method, as described by Elshayb et al. [30]. and Adun et al. [32]. The individual NPs were prepared using different mixing methods and adjusting the temperature to facilitate bond breakage and formation. FV-CaO and FV-ZnO were mixed in 1:1, 1:2, and 2:1 ratios at 50 °C with constant stirring at 3000 rpm for several hours. The mixtures were repeatedly washed with distilled water to remove the base component. Once thoroughly mixed, the beads were centrifuged, filtered, and stored at −4 °C.

2.6 Characterization of NPs

The biosynthesised ZnO, CaO, and ZnO-CaO nanomaterials were characterized using various analytical techniques such as Ultraviolet-visible (UV-vis) spectroscopy, Fourier-transform infra-red (FTIR) spectroscopy, energy-dispersive X-ray analysis (EDX), Brunauer-Emmett-Teller (BET), X-ray diffraction (XRD), Dynamic light scattering (DLS), and Scanning electron microscopy (SEM). XRD was utilized to characterize the crystallinity, lattice parameters, and atomic organization of the NPs and their hybrid. The X-rays were applied at 40 kV and 30 mA at a 2θ° angle using an X-ray diffractometer (Malvern Panalytical, Spain). Scherrer equation was used to determine the size of crystallites as given below [33]:

$$D = \frac{(k\lambda)}{\beta \cos \theta} \quad (1)$$

Where λ is the wavelength of the X-rays, β is full width at half maximum (FWHM) in radians (obtained from the origin plot of XRD), θ is the Bragg angle in degree, k is the Scherrer constant (0.94–1 for spheres), and D is the crystallite size in nm (accurate for sizes ≤ 200 nm) [33]. SEM was used to evaluate the nanostructure, size distribution, and agglomeration of the biosynthesised NPs. The samples were examined with an EVO electron microscope (Zeiss, USA) at an acceleration voltage of 10 kV, and the images obtained were analyzed with ImageJ software. FTIR analysis was employed to determine the composition of the functional groups and structures within the NPs using an IR Prestige infra-red spectrophotometer (Shimadzu, Japan) across the spectrum range of 4000–400 cm^{-1} . UV-Vis spectroscopy analysis was used to monitor the synthesis of the NPs. UV-vis spectra of the synthesised NPs were able to determine the surface plasmon resonance (SPR) using XYZ UV-Vis spectrophotometer (Shimadzu 2354, Japan). The NPs were diluted with Milli-Q water and filtered, and a spectrum search was conducted at wavelengths between 250 nm and 700 nm. DLS is generally used to identify the sizes of particles within a solution and emulsion. It is based

on Brownian motion, wherein the smaller particles in the samples exposed to light inside a zeta-sizer machine tend to travel faster within the liquid, providing data on diffusion speed. The Malvern Panalytical ZS XPLOER (United Kingdom) was used in this study. A BET surface area analyzer was used to calculate the surface area, pore size, and pore volume using the NPs with N_2 adsorption-desorption isotherm. The elemental composition of the biosynthesised NPs was analyzed using EDX.

2.7 Turbidity and stability assessment

The degradation of metal oxide nanoparticles (MON) was investigated using a turbidimeter. A 15 mL sample of a solution containing MON of known weight was suspended in a 10% phosphate buffer saline (PBS) solution and incubated at 37 °C for 14 days while being stirred at 100 rpm. Measurements were recorded daily using the turbidimeter (Eutech TN-100, Singapore).

2.8 DPPH free radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) stock solution was prepared by dissolving 0.04 g DPPH with 100 mL of methanol in a volumetric flask (1.01 mM). Various concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.6 mg/mL, and 0.3 mg/mL) of the nanoparticle were also prepared. About 1 mL of each nanoparticle solution was mixed with 3 mL of the DPPH solution and incubated for over 40 minutes in the dark. The resulting solution was measured at 517 nm using a UV-visible spectrophotometer. A solution containing 1 mL methanol and 3 mL DPPH stock solution served as control. Ascorbic acid was used as the standard drug. The formula below was used to compute the percentage of DPPH inhibition [34].

$$\% \text{ of DPPH inhibition} = 100 \times [\text{Vt}/\text{Vc} - 1] \quad (2)$$

where Vt is the absorbance of the test samples, and Vc is the absorbance of control.

2.9 Protein denaturation assay

A protein denaturation assay was used to assess the anti-inflammatory potential of the NPs as previously described with slight modification [1] with modifications. Initially, 2.6 mL of a phosphate buffer solution (pH range 6–7) was combined with 2 mL of NPs/standard drug of varying concentrations (5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, and 0.6 mg/mL). Then, 0.4 mL of egg albumin was cautiously added to the mixture. After thoroughly mixing the colloidal solution, it was incubated at 35 °C for 15 min and heated on a magnetic stirrer at 70–80 °C. The mixture was allowed to cool to room temperature. The samples were transferred to a

microtiter plate and measured at 630 nm. Diclofenac potassium was used as a reference drug. The percentage of protein inhibition was calculated as follows.

$$\% \text{ protein inhibition} = 100 \times [V_t/V_c - 1] \quad (3)$$

where V_t is the absorbance of the test samples, and V_c is the absorbance of standard drug (control).

2.10 Antimicrobial activity

The antimicrobial activity was assessed using the well diffusion (Kirby Bauer) assay [35]. Methicillin-resistant *Staphylococcus aureus* (MRSA B4420) and *Escherichia coli* (0157) were obtained from the Biotechnology Research Center, Cyprus International University, Haspolat, Nicosia. Each sterile Petri dish, measuring 9 cm in diameter, was prepared with 20 mL of nutrient agar. After the agar was solidified, a standardized volume (200 μ L) of a 24 h bacterial suspension was evenly spread on the plates. Wells of about 6 mm in diameter were bored in the agar using a sterile well borer. Different concentrations of the samples were added to each well. To facilitate the diffusion of extracts into the agar, the plates were initially incubated at room temperature and then transferred to a 37 °C incubator for 24 h. Zones of inhibition (mm) were measured after the incubation period. Chloramphenicol (30 μ g/disk) was used as the positive control. Experiments were conducted in triplicate.

2.11 Statistical analysis

The results were presented as mean $\pm$ standard error of the mean (SEM) ($n = 3$). Statistical analysis was performed on the data using GraphPad Prism 8.02 (263). The level of statistical significance was set at $P < 0.05$.

3 Results and discussion

3.1 Optical studies

3.1.1 UV-visible analysis of ZnO, CaO, and ZnO-CaO NPs

Aqueous extract of *F. vulgare* waste was used to biosynthesise zinc oxide nanoparticles (ZnO NPs), calcium oxide nanoparticles (CaO NPs), and their hybrid nanoparticles (ZnO-CaO NPs). The plant *F. vulgare* has been reported to contain phytochemicals, which include trans-anethole, estragole, fenchone, α -thujone, limonene, quercetin, epicatechin, rutin, caffeic acid, kaempferol, oleanolic acid, and naringin [23]. When the *F. vulgare* extract is added to the colourless aqueous solutions of zinc sulfate and calcium chloride, the reaction mixtures turned cloudy (brownish-milky) or milky white with

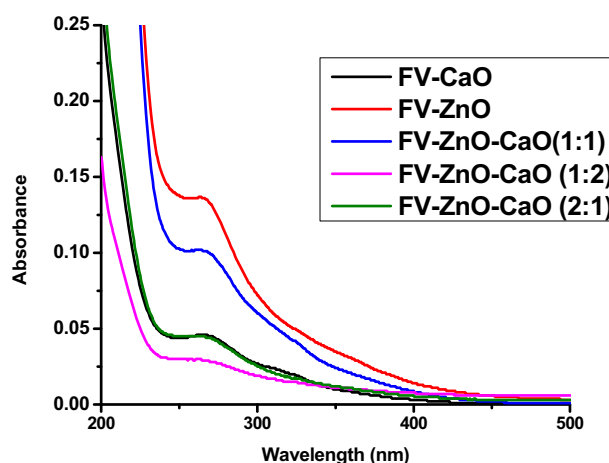


Fig. 1 UV spectra of FV-ZnO, FV-CaO, and FV-ZnO-CaO using *F. vulgare*

a precipitant (NaOH), indicating the formation of ZnO and CaO NPs, respectively. These phyto-compounds mediate the synthesis of ZnO, CaO, and ZnO-CaO NPs. The UV-visible spectroscopic technique established the formation of NPs by measuring absorbance within the range of 200 and 800 nm. The metal reduction and metal oxide NPs production were assessed by the surface plasmon resonance (SPR). FV-CaO, FV-ZnO, and the FV-ZnO-CaO (ratio 1:1) NPs showed characteristic SPR absorptions at 269.5 nm, 271.5 nm, and 269 nm, respectively, in the UV spectra (Fig. 1) [36]. The SPR absorbance wavelength reflects such NPs properties as the shape of the NPs, particle size, dielectric properties, and degree of aggregation of the particles [37]. The energy band gap values for FV-CaO, FV-ZnO, and the FV-ZnO-CaO NPs were 4.6 eV, 4.57, and 4.61 eV, respectively. Based on literature data, all synthesised nanoparticles are classified as semiconductors [38]. The band gap energy for the hybrid and individual nanoparticles is determined using the equation:

$$E_g = hc/\lambda_g \quad (4)$$

Where E_g is band gap energy, h is Planck's constant, c is light velocity, and λ_g is wavelength [38].

3.1.2 FTIR analysis of ZnO, CaO, and ZnO-CaO NPs

FTIR spectroscopy was used to examine the biosynthesised NPs using *F. vulgare*, as shown in Fig. 2. The FTIR spectra of FV-ZnO, FV-CaO, and FV-ZnO-CaO show similar patterns but distinct functional groups. FV-ZnO showed peaks at 3476, 1611, 1111, 961, and 783.1 cm^{-1} in the FTIR spectrum. FV-CaO exhibited peaks at 3644, 3383.1, 1408, 2008, 1404.1, and 874 cm^{-1} . FV-ZnO-CaO showed peaks with wavenumbers at 3410, 2154, 1992.4, 1423, 1119, and 877.8 cm^{-1} . The peaks at 3644, 3476, and 3410 cm^{-1} correspond to the stretching vibration of O–H, absorbing for alcohols or phenols. Peaks at 2363 and 1992.4 cm^{-1} are

associated with C–H stretch [39]. Peaks at 1423 and 1408 cm^{-1} could indicate C–H bending. Peaks at 1119 and 1111 cm^{-1} correlate to C–O stretching vibration, indicating alcohols, ethers, or phenols [40]. The peak at 1611 cm^{-1} represents C = C stretching. Peaks at 961.8 cm^{-1} , 858.3 cm^{-1} , 877.8 cm^{-1} , and 874 cm^{-1} denote strong C = C bending, indicating an alkene. These peaks are consistent with previous studies on hybrid NPs, demonstrating similarities across wavenumbers above 2000 cm^{-1} [41]. Secondary metabolites such as triterpenes, flavonoids, phenols, alcohols are present in plants and have been shown to play an important role in NPs biosynthesis [37]. The main phytochemicals in *F. vulgare* extract may reduce the metallic Zn^{2+} and Ca^{2+} ions to ZnO NPs and CaO NPs, respectively.

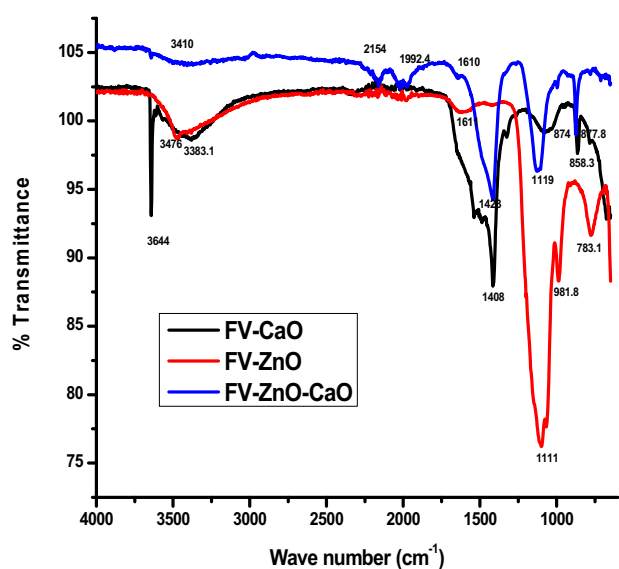


Fig. 2 FTIR spectroscopy of FV-ZnO, FV-CaO, and FV-ZnO-CaO

3.2 Surface morphology and elemental composition

3.2.1 Dynamic light scattering (DLS)

DLS was used to measure the size distribution of the bio-synthesised (NPs) size distribution. Tiny particles such as NPs are best characterized by size, shape, and morphological properties. According to the British Standards Institution, nanoscale materials, including nanoparticles, fall within the size range of 1–1000 nm [42]. In this study, FV-ZnO-CaO, FV-CaO, and FV-ZnO NPs were produced by employing *F. vulgare* as a capping agent that reduces aggregation, controls size, and improves the stability of NPs. In the DLS spectrum, the particle sizes ranged from 18 to 68 nm, with a mean zeta size of 47.48 nm for FV-ZnO-CaO NPs (Fig. 3A). For FV-CaO NPs, the particle sizes ranged from 100 to 150 nm, with maximum size distribution at 128.6 nm. In the DLS spectrum of FV-ZnO NPs, the particle sizes ranged from 150 to 175 nm with maximum size distribution at 162.3 nm. This means that, according to the DLS spectrum, most of the particles in FV-ZnO NPs have an effective hydrodynamic diameter of around 162.3 nm. The observed relatively good symmetry in the DLS spectra of the NPs indicates fairly uniform or less variable particle sizes of the FV-mediated NPs [43].

3.2.2 Scanning electron microscope (SEM) and energy dispersive spectrum (EDX)

The surface morphology of the bio-synthesised FV-CaO, FV-ZnO, and FV-ZnO-CaO hybrid NPs was analyzed using SEM. The SEM images reveal distinct characteristics of the nanoparticles. In Fig. 4A, FV-CaO NPs appear rough on the sides, unevenly shaped, and clustered. FV-ZnO NPs in Fig. 4B display circular, smooth features clustered together and

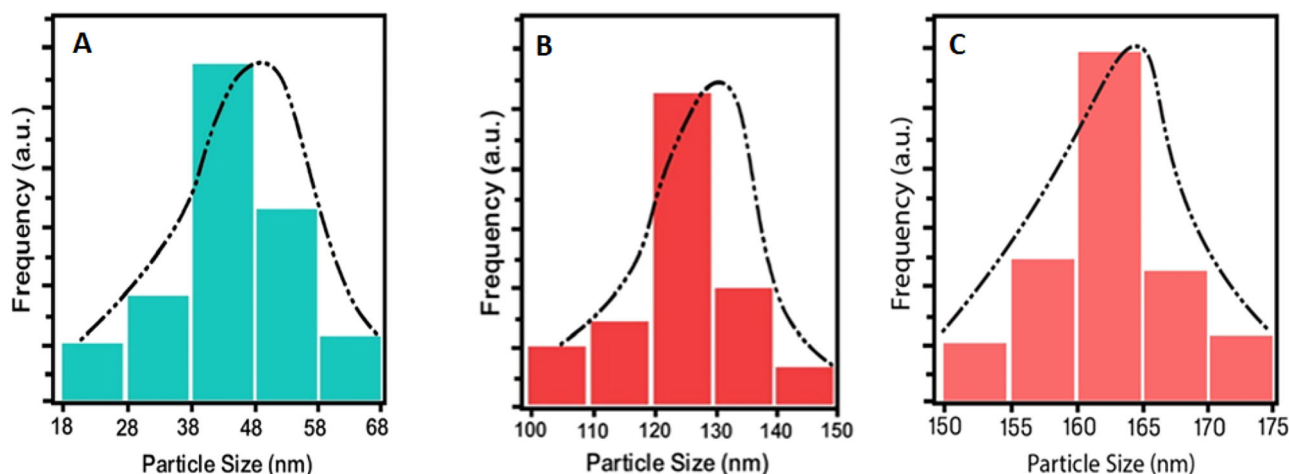


Fig. 3 DLS particle size distribution of FV-ZnO-CaO (A), FV-CaO (B), and FV-ZnO (C)

Fig. 4 SEM images of (A) FV-CaO, (B) FV-ZnO, and (C) FV-ZnO-CaO NPs

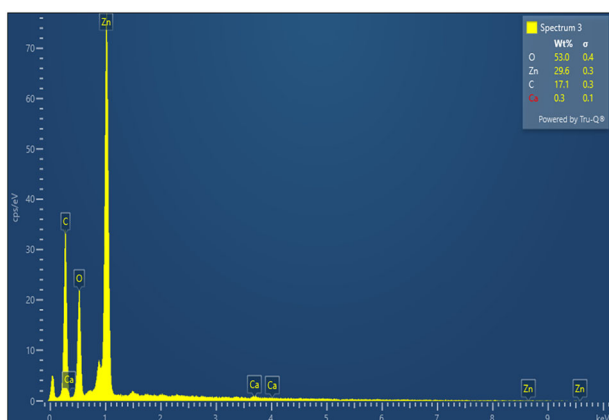
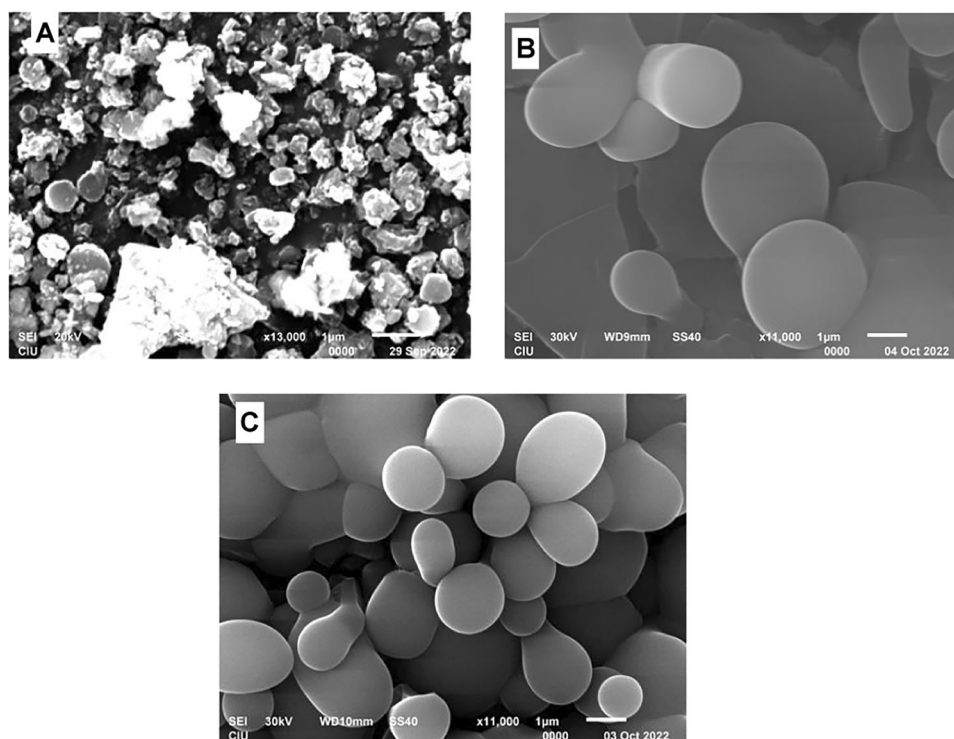


Fig. 5 EDX analysis for FV-ZnO-CaO NPs

appear dark. FV-ZnO-CaO NPs in Fig. 4C appear spherical, smooth, and more clustered than to FV-CaO. The hybrid (FV-ZnO-CaO NPs) exhibits a raised appearance with upward-pointing clustered spheres. The EDX spectrum of the hybrid NPs is shown in Fig. 5. Since the elements present in ZnO-CaO NPs have well-defined peaks at certain energy levels, their presence in the EDX spectrum can therefore be used to confirm the synthesis of ZnO-CaO NPs. The EDX spectrum shows peaks that correspond to zinc, calcium, and oxygen. The production of ZnO NPs was established in the EDX spectrum by the peaks of Zn at 1.0, 8.6, and 9.6 keV and O at 0.5 keV. The formation of CaO NPs was confirmed spectrum by the peaks of Ca at 0.3, 3.7, and 4.0 keV.

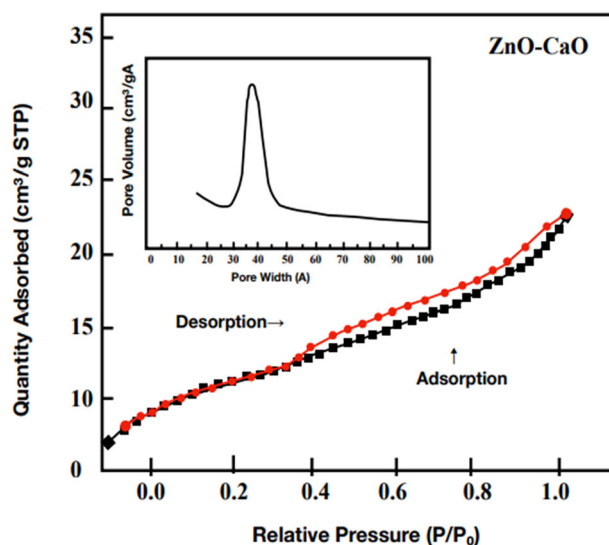


Fig. 6 BET surface area with N₂ adsorption-desorption isotherm for FV-ZnO-CaO

3.2.3 Brunauer-Emmett-Teller (BET)

The pore size, pore synthesised hybrid's pore size, volume, and surface area distribution of the synthesised hybrid were studied using BET surface area analysis as represented in Fig. 6. The FV-ZnO-CaO hybrid NPs have pore diameters between $2 \leq 50$ nm. The NPs are, therefore, described as mesoporous [44]. The mesoporous structures make them suitable for various applications such as controlled drug

delivery, catalysis, biosensing, and environmental remediation. In Fig. 6, the adsorption-desorption curve for FV-ZnO-CaO follows a type IV isotherm, the hysteresis loop is type H4, and the pore size distribution is 37 nm.

3.2.4 X-Ray diffraction (XRD)

The crystallinity of the NPs and hybrid was analyzed using XRD at 2θ . As shown in Fig. 7, the prominent peaks for FV-ZnO NPs were at 32° , 34.4° , and 36.3° . The prominent peaks for FV-CaO NPs were at 31.7° , 34° , and 45.4° . For FV-ZnO-CaO NPs, the peaks were at 32° , 34.4° , and 36.24° . Comparing the XRD peaks of the three NPs with the Miller indices, the diffraction peaks at 2θ values of 35° , 48° , 65° , and 79° are consistent with the Miller indices of (111), (200), (220), and (311), respectively. This also agrees with previously reported studies [45]. FV-ZnO-CaO NPs appear to comprise both the hexagonal phase of ZnO and the cubic phase of CaO [46]. Although the metallic oxide NPs and their hybrid NPs essentially exhibited similar peaks, they varied in intensity. This supports the fact that different combinations will affect the extent to which a particular component is expressed. This is because the organic components of NPs tend to influence the range of peaks in green synthesised NPs and hybrids [47]. As the peak ranges were consistent with the various individual components, the particles may be said to constitute a hexagonal polycrystalline and a face-centred cubic lattice [8]. The data from this study are in tandem with the database of the International Center for Diffraction Data (ICDD). Based

on ICDD No. 98-002-9272, the XRD spectra confirm that the ZnO NPs have a polycrystalline hexagonal structure (JCPDS card No. 52-0278) [46]. The data obtained for the CaO was consistent with the pure cubic phase of CaO NPs indexed on the ICDD Card No. 004-0777&00-017-0912 [48]. The crystallite size was determined to be 17.8 ± 1.0 , 13.7 ± 1.0 , and 45.3 ± 1.0 nm for FV-ZnO-CaO, FV-ZnO, and FV-CaO, respectively.

3.3 Zeta potential and stability profile

3.3.1 Turbidity and stability

The stability of the NPs was ascertained based on their zeta potential. In addition, their degradation was assessed by observing the Turbidity of the various primary hybrids. The FV-ZnO-CaO, FV-CaO, and FV-ZnO particle complexes were monitored for 14 days, after which they were examined using a turbidimeter in NTU. Generally, the NPs and hybrid varied based on the extent of dissociation/breakdown evident in the 37°C solution after 24 h.

As shown in Fig. 8, FV-ZnO-CaO had the lowest Turbidity after 14 days, whereas FV-CaO had the highest turbidity. In general, the rate of Turbidity declined over the 14-day period, reaching a relatively stationary phase. This may be attributed to the hydrogen bond responsible for the underlying aggregation. The application, biocompatibility, and biodegradability of NPs are influenced by various characteristics, hence the need for colloidal NP stability. NPs tend to aggregate under certain conditions, such as temperature or the influence of a buffer. The results from this study reveal that NPs with lower Turbidity (FV-ZnO-CaO and FV-ZnO) tend to exhibit less aggregated particles than those with higher Turbidity (FV-CaO). Consequently, FV-ZnO-CaO and FV-ZnO NPs are likely to be more stable. Low Turbidity reduces nanoparticle aggregation, suggesting that particles in a light pathway contribute to

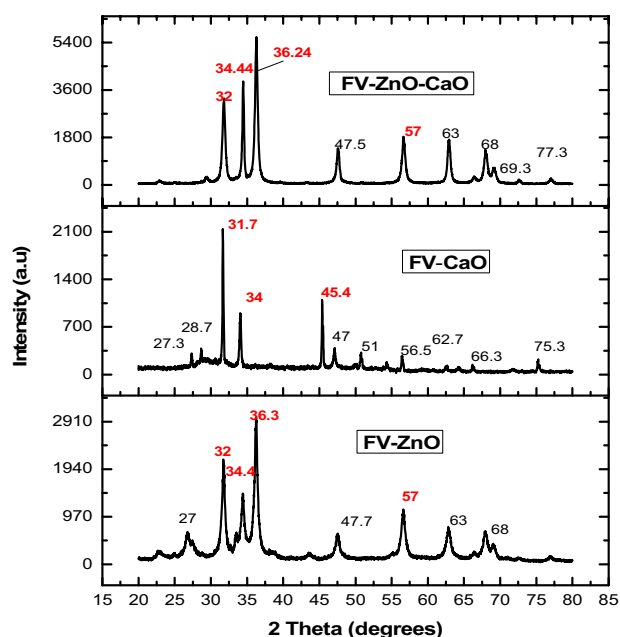


Fig. 7 XRD patterns of biosynthesised FV-ZnO, FV-CaO, and FV-ZnO-CaO NPs

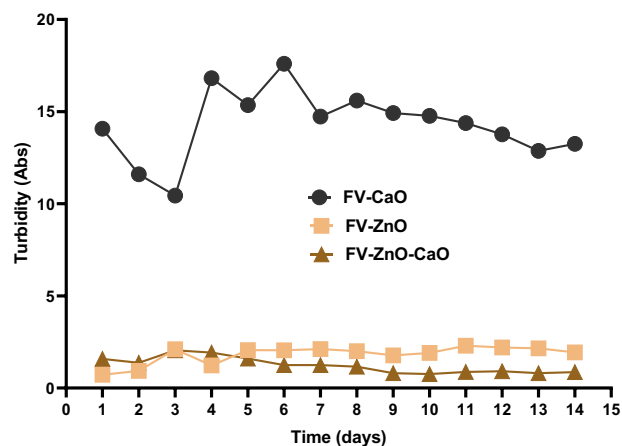


Fig. 8 Turbidity study of FV-ZnO, FV-NCAO, and FV-ZnO-CaO NPs

Table 1 Hybrid formulations and their zeta potentials

Number code	Hybrid formulation and ratio	pH	Zeta potential (mV)	Stirring speed (rpm)	Stirring time (Hours)
FV-ZnO	N/A	N/A	−19	300	3
FV-CaO	N/A	N/A	−6	300	3
NC1	FV + 50% ZnO + 50% CaO	8.5	−25.71	300	3
NC2	FV + 75% ZnO + 25% CaO	8.14	−35.5	300	3
NC3	FV + 25% ZnO + 75% CaO	9.23	−12.65	300	3

scattering, which increases Turbidity [49]. Although FV-ZnO-CaO NPs appeared to be more stable than the mono-metallic oxide NPs, as the 14-day period progressed, the Turbidity of all the NPs decreased and became almost static by the fourteenth day (Fig. 8). It has been reported that Turbidity tends to decrease with a decrease in wavelength and sampling/reading time intervals [50].

3.3.2 Zeta potential and stability

Table 1 illustrates how various formulations affect the zeta potential. The average refractive index of each hybrid was obtained and used to infer the zeta potential. The stability of colloidal FV-ZnO, FV-CaO, and FV-ZnO-CaO is strongly influenced by their zeta surface potential, regardless of whether it is positive or negative. The zeta surface potentials of FV-ZnO, FV-CaO, and FV-ZnO-CaO were −19 mV, −6 mV, and −25.71 mV, respectively.

The stability of nanoparticles (NPs) in suspension depends on the zeta potential, which also influences the initial adsorption of NPs onto cell membranes by affecting their electrostatic interactions with the membrane surface. For example, the endocytosis absorption rate is highly affected by the particle size [51]. The zeta potentials of FV-ZnO, FV-CaO, and their hybrid NPs were compared, taking into account the mixing ratios, stirring time, and stirring speed, which were kept constant for all hybrid samples. As shown in Table 1, the code NC3 ratio mix of FV-ZnO-CaO had the least stable zeta potential of −12.65 mV, whereas the code NC2 ratio mix of FV-ZnO-CaO had the most stable zeta potential of −35.5 mV. The other NP's primary hybrids were all anionic. Notably, the mixtures with higher volumes of ZnO exhibited a significant increase in their negative ionic strength, to the same degree as those that had the with individual particles in at 25% and 50%, respectively. With the increase in pH value of the solution, the zeta potential of the nanofluid is far away from the isoelectric isoelectric point, and a higher zeta potential maintains high repulsion, which can keep the nanoparticles dispersion dispersed more stable stably and uniform uniformly [52].

The zeta potential varied as the concentration changed. The pH values also changed with the concentration. A previous study confirmed that, at certain concentrations, whether low or high, the zeta potential tends to change, with an accompanying accompanied by an increase in particle size [53]. Similar studies have also affirmed confirmed that certain properties are strongly dependent on the concentration of the different metal oxide nanoparticles (NPs) scattered dispersed in water; as the particle concentration increased, the zeta potential of the given nanoparticle at a given pH (pH = 6) increased from 6.7 mV to 8.2 mV [54]. Another study on clay-rich and clay-poor sandstones found different zeta potentials at the same pH [55]. The stability of NPs and synthesised hybrid is ascertained by measuring their zeta potentials. As the surface charge increases, the electrostatic repulsion between particles intensifies, leading to reduced agglomeration, potentially lower Turbidity, and enhanced stability of the particle suspension system—provided other conditions, such as ionic strength, are controlled [56]. The agglomeration and aggregation rates of particles must be considered while designing drug-delivery systems. However, the medium of nanoparticle dissolution in which nanoparticles dissolve can affect the extent of their stability. In some cases, the extent of cationic and anionic activity may also influence cellular uptake, as extremely cationic particles are harmful to in vivo cells [57].

3.4 Biological activities

3.4.1 Evaluation of DPPH free radical scavenging potential

DPPH free radical scavenging activity was used to assess the antioxidant potential of FV-ZnO, FV-CaO, and FV-ZnO-CaO NPs biosynthesised from *F. vulgare* extract. DPPH scavenging assay is a popular method for evaluating the antioxidant activity of NPs [58]. From the results, it was noted that the scavenging activity was concentration-dependent. The scavenging activities of the biosynthesised NPs were lower than that of the standard drug, ascorbic acid. As shown in Fig. 9, the scavenging activity of *F. vulgare* extract ranged between 15% and 85.1%,

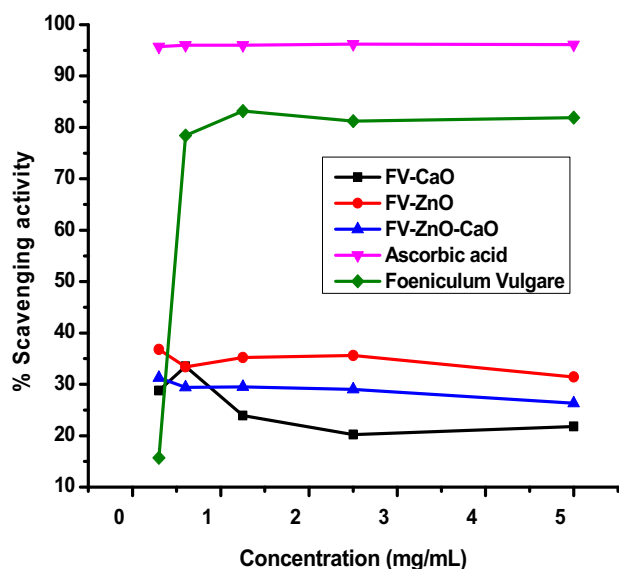


Fig. 9 % DPPH scavenging activity of NPs synthesised using *F. vulgare* extract

concentration-dependently. Ascorbic acid showed exhibited the highest scavenging activity, reaching 96.2% at a concentration of 10 mg/mL. FV-ZnO exhibited 29–40% scavenging activity against free radicals across the tested concentrations. The activity was followed by FV-ZnO-CaO, with which exhibited a % DPPH scavenging activity that ranged ranging from 25% to 35%. Several studies have demonstrated the ability of green-synthesised NPs to resist oxidative stress conditions [4]. The antioxidant activity of ZnO NPs was consistent with previous studies. ZnO NPs produced using *Coccinia abyssinica* (Lam.) Cong. tuber extract showed IC_{50} values of 127.74 μ g/mL against DPPH radicals [58]. The metal ions present within the NPs may be responsible for the moderately high scavenging activity levels of FV-ZnO, FV-CaO, and FV-ZnO-CaO.

3.4.2 Evaluation of protein denaturation inhibitory activity

Denaturation of protein can lead to inflammatory responses. The biosynthesised NPs were evaluated for their anti-protein denaturation activities over a concentration range as shown in Fig. 10. At 5 mg/mL, the highest albumin denaturation inhibitory activity was found to be 90%, 79%, and 71.6% for FV-ZnO-CaO, FV-ZnO, and FV-CaO NPs, respectively, compared to diclofenac which had an activity of 284%. Biosynthesised CuO and ZnO NPs from plant have demonstrated good inhibition potentials against protein denaturation, with activity comparable with the standards. A study has reported that an NP formulation showed significantly higher anti-inflammatory activity than the bulk standard drug formulation at the same dose [59]. Another study reported that plant-mediated silver NPs showed anti-

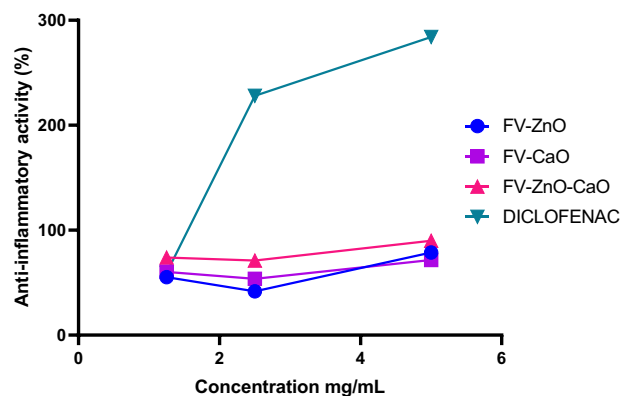


Fig. 10 Protein denaturation inhibitory activity of NPs synthesised using *F. vulgare* extract

Table 2 Antibacterial activities of the NPs against *E. coli* and Methicillin-resistant *S. aureus* (MRSA)

Samples	Methicillin-resistant <i>S. aureus</i> Zones of inhibition (mm)	<i>E. coli</i>
FV-ZnO	9	13
FV-CaO	–	6
FV-ZnO-CaO	13	9
Amoxicillin	9	Nt
Ciprofloxacin	Nt	17
Chloramphenicol	19	20

nt Not tested, – No growth.

inflammatory activity of greater than 80% [60]. In addition, green-synthesised silver NPs were reported to have high anti-inflammatory activity in rats with cfa-induced arthritis [61].

3.4.3 Evaluation of antibacterial activity

The Agar diffusion method was used to test the effects of biosynthesised NPs against Gram-positive and Gram-negative Methicillin-resistant *S. aureus* (MRSA) and *E. coli*, respectively. Zones of inhibition (mm) were measured for the NPs and compared to standard drugs. The activities of the *F. vulgare*-mediated ZnO, CaO, and ZnO-CaO NPs were reported in Table 2. According to the results, FV-ZnO-CaO NPs exhibited the highest activity with a zone of inhibition of 13 mm against MRSA. FV-ZnO NPs had zone of inhibition of 9 mm. However, FV-CaO NPs did not exhibit any visible inhibition of MRSA growth. Chloramphenicol (19 mm) and amoxicillin (9 mm) were used as reference drugs. This indicates that FV-ZnO-CaO NPs showed more activity against MRSA than amoxicillin. In addition, FV-ZnO had the highest activity against *E. coli* (zone of inhibition, 13 mm). FV-ZnO-CaO showed a zone of inhibition value of 9 mm. Unlike the MRSA, *E. coli*

showed some sensitivity to FV-CaO with a zone of inhibition value of 6 mm. Some studies have demonstrated that the mechanisms of antibacterial activity of metallic NPs include the ability to penetrate the bacterial cell wall, the generation of free radicals, and the inactivation of cellular proteins by metal ions (such as Zn^{2+} and Ca^{2+} ions) [35]. According to SEM and TEM images, ZnO NPs exhibited their antibacterial activity by first compromising the bacterial cell wall, thereby gaining entrance. Next, the NPs accumulate in the bacterial cell membrane, causing damage to the microorganism's vital metabolic functions [7, 62–64].

4 Conclusion

In this study, FV-ZnO, FV-CaO, and FV-ZnO-CaO NPs were synthesised using the aqueous extract of *F. vulgare*. The NPs were characterized using FTIR, DLS, EDX, BET, XRD, and SEM. UV-visible spectroscopy confirmed the synthesis of the NPs. FTIR revealed functional groups that might have been involved in the bio-reduction process. XRD confirmed the hexagonal crystalline phase of FV-ZnO, the cubic lattice of FV-CaO, and the mixed phase of the FV-ZnO-CaO hybrid. DLS spectra of the NPs showed relatively good symmetry, indicating fairly uniform particle sizes. SEM images showed surface morphological characteristics of the biosynthesised NPs, while EDX analysis confirmed their elemental compositions. The turbidity test conducted on the NPs revealed that the hybrid system (FV-ZnO-CaO NPs) was more stable than the monometallic oxide forms. In addition, the NPs showed good scavenging activity in concentration-dependent manner. The antibacterial studies demonstrated that the NPs inhibited the growth of Methicillin-resistant *S. aureus* and *E. coli*. The hybrid NPs were observed to demonstrate 91% albumin denaturation inhibitory potential, an activity relatively higher than that of the corresponding monometallic oxide NPs. Overall, the green synthesis of ZnO and CaO NPs and their hybrid NPs has been demonstrated in this study using the aqueous extract of *F. vulgare* waste. The NPs exhibited high stability and good biological activities. ZnO, CaO, and their hybrid NPs may provide effective and eco-friendly therapeutic alternatives in the future.

Data availability

Data used in this study cannot be shared publicly at this time. However, it is available from the corresponding author C.E.O upon reasonable request.

Author contributions CEO conceptualized experimental design, performed experimental research, analysis, methodology development, and the original draft preparation; DK conceptualized the experimental

design, supervised the methodology development and research performance, and edited the final manuscript. DBA: Writing—review & editing, Writing—original draft, Project administration. YAA: Writing—review & editing, Formal analysis, Data curation. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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