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ORIGINAL ARTICLE

Boswellia serrata-mediated zinc oxide nanoparticles-coated cotton fabrics for the wound healing and antibacterial applications against nosocomial pathogens

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Abstract
The incorporation of textile technology and medical science has emerged into a new field known as medical textiles. Here, we formulated zinc oxide nanoparticles from *B. serrata* resins (BS-ZnONPs) and coated it on cotton fabrics for antibacterial and wound healing applications against the nosocomial pathogens. The synthesized BS-ZnONPs were characterized by UV-spectrophotometer, transmission electron microscope (TEM), and Fourier transform infra-red (FT-IR) analyses. The BS-ZnONPs were coated on the cotton fabrics and the same was confirmed by the scanning electron microscope (SEM) and energy-dispersive X-ray (EDX) studies. The antibacterial property of BS-ZnONPs-coated fabrics was examined against nosocomial pathogens, i.e., *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Psuedomonas aeruginosa*, *Escherichia coli*, *Klebsiella oxytoca*, and *Staphylococcus aureus*. The findings of characterization studies such as UV, TEM, FT-IR, SEM, and EDX revealed the development of BS-ZnONPs. The fabricated BS-ZnONPs treatment at various doses, i.e., 5–30 µG/mL did not show any cytotoxicity to the L929 cells. The BS-ZnONPs demonstrated the considerable wound healing activity. The synthesized BS-ZnONPs and its coated cotton fabrics remarkably inhibited the growth of nosocomial pathogens such as *K.pneumoniae*, *A.baumanii*, *P.aeruginosa*, *E.coli*, *K.oxytoca*, and *S.aureus*. Our findings from the current study proved that the *B. serrata* is a good source of ZnONPs. The synthesized BS-ZnONPs and its treated cotton fabrics could be a promising antibacterial agent for the prevention of nosocomial infection.

Keywords Nosocomial infection · Zinc oxide nanoparticles · *Boswellia serrata* · Wound healing

Abbreviations

BS-ZnONPs	Zinc oxide nanoparticles from *Boswellia serrata*
NaOH	Sodium hydroxide
FT-IR	Fourier transforms infra-red spectroscopy
SEM	Scanning electron microscopy
EDX	Energy-dispersive X-ray spectroscopy
TEM	Transmission electron microscope
MHA	Mueller Hinton agar
MTT	3-(4, 5-Dimethylthiazoyl-2-yl)-2, 5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide

Introduction

Nosocomial infection is a major global public health issue, which threaten the patient's life, prolonged hospitalization, and high treatment costs with increased mortality

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(Apisarnthanarak et al. 2017). This kind of infection was attained after 48 h of hospitalization, a wound treatment lasts up to 30 days in a hospital (Zhang et al. 2021). Nearly, 90% of nosocomial infections were due to the gram-negative and -positive bacterial species, also mycobacteria, viruses, fungi, and protozoan. The *Streptococcus*, *Staphylococcus*, *Enterococcus*, *Escherichia coli*, and *Klebsiella pneumoniae* are the major kinds of nosocomial pathogens (Haque et al. 2018). The most prevalent source of nosocomial infections are in-person transmissions, medical devices, healthcare personnel, and contaminated materials. The medical fabrics are regarded as one of the major vehicles of pathogens transmission (Soussan R 2019). The textiles are the good medium for the growth of microbial strains and textiles are regarded as infection transmission vehicles (Owen and Laird 2020). The secretions of sweating and exfoliation give a good environment for the proliferation of microbial strains. A previous study proved that the hospital fabrics contain the pathogens (Kramer and Assadian 2014). Certainly, pathogens can live on the fabrics for a week and even after industrial wash (Kampf 2020).

Currently, the awareness on the antimicrobial materials like dressings, paintings, packaging, and surfaces was augmented because of the higher events of nosocomial infections and increased microbial resistance towards existing antimicrobials (Maghimaa and Alharbi 2020). The many developments on the integration of polymer matrices and antimicrobial agents were continuously studied to minimize and prevent the microbial infections (Mahira et al. 2019). Explicitly, the research on the new antimicrobial nanoagents has amplified over last few years, since antimicrobial nanocomposites are the most talented substitutes in the exploration of novel antimicrobials (Jimenez et al. 2016). The research interest is turned into the metallic and/or metal oxide nanoparticles because of their extensive antimicrobial potentials at low concentrations (Dumbrava et al. 2019).

Developments in the nanotechnology provide several synthesis techniques, which permit gaining nanoparticles with controlled size and magnitude that could be effective for disease management. Metal oxide nanoparticles are distinguished by huge surface area and crystalline nature, and possess both physical and chemical characteristics. The pharmacological possessions of the nanoparticles augment due to the increased surface energy. The formulation of nanoparticles with a controlled size and magnitude at nano-scale could provide the better biological activities (Chandel et al. 2018). The applications of nanoparticles were reported in extensive fields like catalysts, biomedical, biosensors, antimicrobials, etc. (Thakur et al. 2018).

Primarily, zinc oxide nanoparticles (ZnONPs) were fabricated via a liquid-chemical reduction of zinc precursors like zinc nitrate and zinc acetate. To deal with the augmented demand of the technological textile markets and industries utilization, the nanoparticles were being formulated via inorganic chemical approaches to enhance the large-scale production. Numerous nanoparticle synthesis techniques, e.g., sol–gel, decomposition, vapor phase deposition, and precipitation were utilized but these techniques associated with the huge production cost along with the hazardous chemicals that combined with the nanoparticles (Bakayoko et al. 2020). So as to overcome these problems, scientists turned their interests on phytosynthesis approach using an herbal plant source with the minimal cost and less chemical utilizations. This approach particularly consumes bio-molecules to reduce zinc precursors and generate clean ZnONPs with non-toxic by-products that could be effortlessly administered and utilized (Thi et al. 2020). The incorporation of textile technology and medical science has emerged into a new field known as medical textiles. The applications of new fields in the medical textiles were recognized with progression of either new fiber technologies or the combination of functional biomedical materials e.g., nanoparticles (Pintaric et al. 2020).

It was reported that the phyto-synthesis of metal oxide nanoparticles is economically feasible and is a perfect eco-friendly substitute for well-recognized chemical fabrication techniques, which uses harsh chemicals for nanoparticles manufacture (Bandeira et al. 2020). The *Boswellia* species generally grows on the high-altitude hills. The gum resin was gathered by made an incision on the barks and utilized for many therapeutic purposes (Wichtl et al. 2004). *Boswellia serrata* resins also called as Indian frankincense were extensively utilized in Asian folklore medicine to treat the arthritis and many other complications (Pharmacoepia 2010). *B. serrata* gum resin has the therapeutic effects against numerous ailments like anticancer and antibacterial activity (Gaurea and Bapat 2016). There are several reports available for the synthesis of metal oxide NPs and biological activities (Bhardwaj et al. 2020; Zhang et al. 2020). Alluri et al. (2020) found that the *B. serrata* resin extract mitigated the pain and protected the cartilage in monoiodoacetate-induced osteoarthritis in rats. The gum resin of *B. serrata* alleviated the CCl_4-induced inflammation and fibrosis in the rat liver (Eltahir et al. 2020).

But limited literatures are available for the coating of metal oxide NPs on the cotton fabrics for the antimicrobial purposes (Roman et al. 2020). Nonetheless, the formulation of ZnONPs from *B. serrata* and its antibacterial and wound healing applications were not investigated yet. Therefore, here, we formulated and evaluated the ZnONPs from *B. serrata* resins (BS-ZnONPs) and its coated cotton fabrics for wound healing and antibacterial uses against the nosocomial pathogens.

Materials and methods

Chemicals

Zinc acetate dehydrate, MacConkey agar, vancomycin, gentamycin, chloramphenicol, tetracycline, Mueller Hinton agar (MHA), DMEM, FBS, and other chemicals were procured from the Sigma-Aldrich, USA. All other chemicals used in this study were of analytical grade.

Collection and preparation of *B. serrata* resin extract

The resins of *B. serrata* were collected from Kolli Hills of Namakkal district, Tamilm Nadu, India. It was identified by botanist Prof. C. Rajasekaran, FSEDI, FRSB (UK), FLS (Lon), FISPP Professor, School of BioSciences and Technology, VIT University, Vellore—632,014. India. Prof. C. Rajasekaran with the following credentials IUCN—Species Survival Commission—Member. IUCN—Commission on Environmental, Economic and Social Policy—Member. Zonal Secretary—Indian Society for Plant Physiology. The resin samples were washed thoroughly and then left to dehydrate at room temperature. After that, the dried resins were ground into fine powder using mechanical grinder and then 10 g of powdered resin sample was added to the 100 mL of deionized water and let it stand for 24 h at 37 °C. After 24 h, the supernatant was transferred to 50 mL Falcon tubes and centrifuged at 1000 rpm for 10 min. The solution was heat macerated at 60 °C for 30 min and resulting extract were cooled to 37 °C. After that, the resultant suspension was filtered and stored at 4 °C for nanoparticles synthesis.

Synthesis of ZnONPs from *B. serrata* resin

For green formulation of ZnONPs from the *B. serrata*, 60 mL of extract was heated to 60 °C and the 0.1 M of zinc acetate dihydrate and 1 M sodium hydroxide (NaOH) solution was added and stirred constantly until it was reduced to a deep yellow colored precipitate. Then, ZnONPs precipitate containing suspension was centrifuged at 6000 rpm for 15 min to attain the pellet. The pellet was cleaned many times via adding the Milli-Q Water. The resultant ZnONPs pellet was dehydrated at 80 °C for 6 h in an oven. Finally, the fabricated ZnONPs were employed for several characterization purposes (Fig. 1).

Characterization of formulated BS-ZnONPs

The generation of BS-ZnONPs in the reaction medium were confirmed through an UV–Vis Spectral study. The formulated BS-ZnONPs were determined for its maximum absorbance with the aid of UV–Vis Double beam spectrophotometer (Shimadzu-1700, Japan) at a wavelength ranging from 350 to 750 nm.

The functional molecules found on the *B. serrata* resin extract and fabricated BS-ZnONPs were studied through Fourier transforms infra-red spectroscopy (FT-IR). The spectrum of bio-formulated BS-ZnONPs was investigated in the range of 450–500 cm^{-1} using FT-IR spectrophotometer (JASCO INC 410, Japan).

The size and morphology of BS-ZnONPs were examined by scanning electron microscopy (SEM) (JEOL/EO JSM-5600 SEM, JEOL, Ltd., Akishima, Japan). The samples were prepared on copper grid covered with carbon via dropping of 30–60 µl of sample on the grid and then examined beneath the analyzer at 1000 × magnification.

The elemental compositions of formulated BS-ZnONPs were studied through energy-dispersive X-ray (EDX) spectroscopy. EDX study was executed with the aid of JEOL/EO JSM-5600 SEM analyzer equipped with EDX (JEOL, Ltd., Akishima, Japan).

The size and extents of the BS-ZnONPs were further characterized by transmission electron microscope (TEM, TECNAI F30, Oregon, USA). Briefly, the copper grid with the sample BS-ZnONPs was utilized and illuminated using electronic radiation under vacuum. Likewise, the microphotographs were taken via an electron beam transmitted through the sample.

The formulated BS-ZnONPs were examined using XRD (X'pert Pro PANalytical) system. The samples were studied at 45 mA and 40 kV voltage and scanned for 0.02–0.5 s.

Embedding of cotton fabrics and formulated BS-ZnONPs

The cotton fabrics were cut into pieces with 5 × 2 cm^2 dimensions, cleaned and sterilized at 121 °C for 15 min with 15 lbs of pressure in autoclave. Then, cotton fabrics were treated with 1% of SDS solution. Then they were desiccated for 5 min at 50 °C in an oven. The cloth was impregnated with BS-ZnONPs by soaking in a solution containing 1 mG of lyophilized BS-ZnONPs in 100 mL of distilled water. The soaked cotton fabrics were then subjected to ultra-sonication for 30 min (Petkova et al. 2016). BS-ZnONPs-impregnated cotton fabrics were then examined beneath the SEM equipped with EDX.

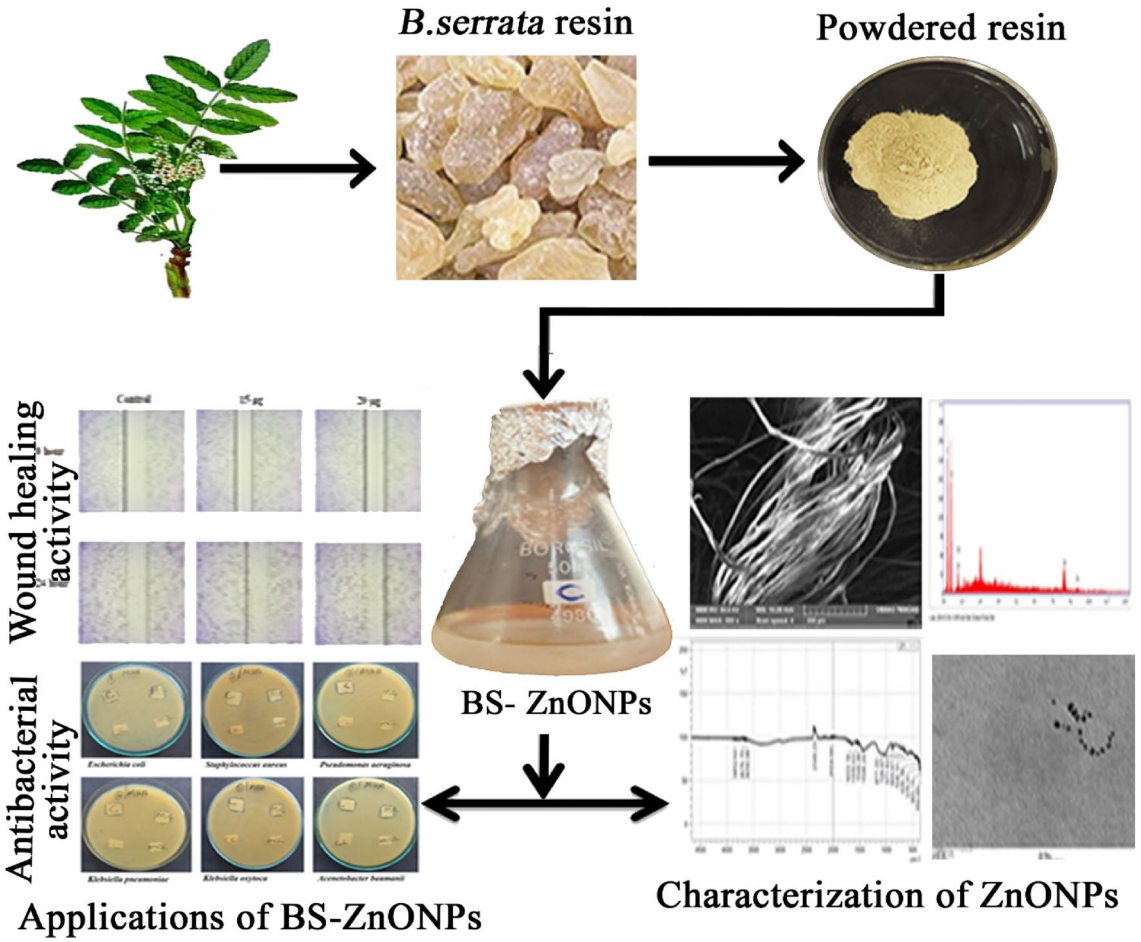

Fig. 1 Schematic diagram of the synthesis of BS-ZnONPs

Sample collection and isolation of nosocomial pathogens

Thirty wound and skin infection samples were collected aseptically with at most care using sterile cotton swabs by experienced laboratory personnel in the Department of Microbiology, Mohan Kumaramangalam Medical College hospital, Salem. The samples were then taken to the lab using Amies transport media. Swabs were streaked on the blood agar and MacConkey agar by sterile inoculation loop. The plates were then sustained at 37 °C for 24–48 h. Preliminary identification of bacteria was based on the colony characteristics of the organisms. The bacterial pathogens were identified based on their microscopy and biochemical characteristics and all the isolates were obtained in pure cultures using the methods described by Kirk et al. (1975), Stiles and Lai-King (1981), Collee et al. (1989), and Chessbrough (2000).

Antibiotic sensitivity test

The antibiotic sensitivity test was executed on every strain by the disc diffusion technique with reference to the previous method (CLSI 2016). The four different antibiotics, i.e., vancomycin (5 µG/mL), gentamycin (10 µG/mL), chloramphenicol (30 µG/mL), and tetracycline (30 µG/mL) was utilized for this assay. Briefly, the strains were streaked on the surface of the MHA plates in aseptic condition let it stand for 30 min for even distribution. Then the antibiotic discs were placed on the surface of the MHA plates. Then the plates were sustained for 24 h at 35 °C. After that, the results were monitored and interpreted according to CLSI guidelines.

Antibacterial effect of formulated BS-ZnONPs against nosocomial pathogens

The antibacterial actions of the formulated BS-ZnONPs were studied by the technique of well diffusion technique against the both gram-positive (*Staphylococcus aureus*) and gram-negative (*Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsilla oxytoca*) nosocomial pathogens. Initially, the strains were loaded on the MHA and streaked over the medium. Then, the well was made at 6 mm in size and impregnated with 20–60 µG/mL of formulated BS-ZnONPs. Then, plates were kept in incubation at 37 °C for 24 h. Finally, the antibacterial potential of BS-ZnONPs was examined by measuring the inhibition zone diameters (Rahimi-Nasrabadi et al. 2013).

Antibacterial effect of BS-ZnONPs-loaded cotton fabrics

The antibacterial activity of fabricated BS-ZnONPs-embedded textile fabrics were studied against the both gram-positive (*S.aureus*) and gram-negative (*K. pneumoniae, A. baumanii, P. aeruginosa, E. coli,* and *K. oxytoca*). Briefly, as mentioned for BS-ZnONPs, the strains were streaked on the surface of MHA medium and then the sterile cotton with 60 µG/mL of formulated BS-ZnONPs coated cotton pieces at 1 cm diameter were placed on the strains streaked MHA medium. Then, the plates were sustained for 24 h at 37 °C (Maghimaa and Alharbi 2020). Followed by the incubation, the inhibition zones were monitored and tabulated to determine the antibacterial property of BS-ZnONPs-embedded cotton fabrics.

MIC and MBC

Minimum inhibitory concentration (MIC) was assessed by the broth micro dilution method in a 96-well plate (Andrews 2001; Djahaniani et al. 2017). 100 µL of BS-ZnONPs was transferred to the first well, then serially diluted and the concentration of the BS-ZnONPs is sliding in the wells, and ultimately, the bacterial culture was transferred in each well and maintained overnight at 37 °C, followed by minimal bactericidal concentration (MBC), is a paired assay of MIC and it was done in LB agar plates.

Cell collection and maintenance

The mouse-derived fibroblast L929 cells were purchased from the American type culture collection (ATCC), USA. The cells were grown on the DMEM with FBS (10%) at 37 °C in the CO2 (5%) incubator. The cultured cells were used for the additional assays.

MTT cell viability assay

The effects of BS-ZnONPs on the normal fibroblast L929 cells were inspected by MTT technique (Mosmann 1983). Concisely, L929 cells were added onto the 96-well plate at 6×10^3 cells/well population and sustained at 37 °C for 24 h. Then, cells were supplemented with different dosages of formulated BS-ZnONPs (5–30 µG/mL) and again maintained for 24 h at 37 °C. Later 24 h of incubation, 100 µL of MTT suspension was added to each well and again sustained for additional 4 h. Then, the medium was removed and 100 µl of serum-free media was replenished and formed formazan crystals were dissolved by adding the DMSO. Finally, absorbance was taken using the microplate reader at 570 nm and viability was determined using formula: treated cells (O.D)/control (O.D) × 100 (%).

Wound scratch assay

As mentioned in viability assay, L929 cells were loaded onto the 6-well plate and maintained for 24 h at 37 °C. After the 80% of confluency, the wound scratch was made in every well with the aid of the 200 µl micro-tip. Then the detached cells were removed through cleansing the plate with saline. After that, cells were supplemented with the formulated BS-ZnONPs (15 and 20 µG/mL) and then again incubated for 24 h. Followed by incubation, the cells were cleaned with the buffered saline and then scratch closure status was investigated beneath the optical microscope and the microphotographs taken at 0- and 24-h time period.

The relative migration ratio (RMR) were determined by the formula:

$$\mathrm{RMR} = [(A0 - A1)] \times 100,$$

where $A0$—Area of scratch made initially, $A1$—Area of scratch after 24-h incubation.

Statistical analysis

Data were analyzed using the SPSS version 17.0 statistical software and data were portrayed as mean ± SD of triplicates. The statistical comparisons between the groups were determined by one-way ANOVA subsequently by Student–Newman–Keuls multiple comparison assay. $p < 0.05$ was deliberated as statistically significant.

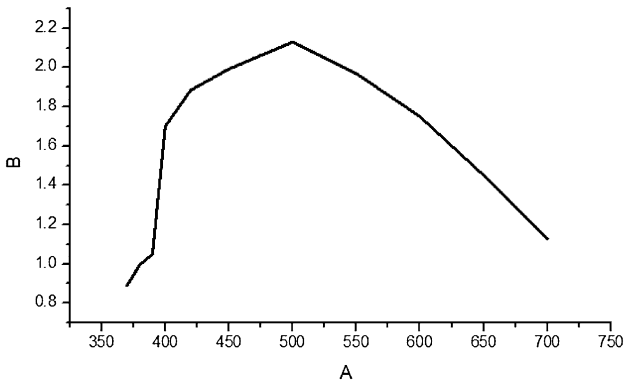

Fig. 2 UV–vis spectroscopic analysis of synthesized BS-ZnONPs UV–vis spectroscopic analysis revealed the maximum absorption peak at 500 nm, which indicates the formation of BS-ZnONPs in the solution. *Axis: **A**: Wavelength; **B**: Absorbance (nm)

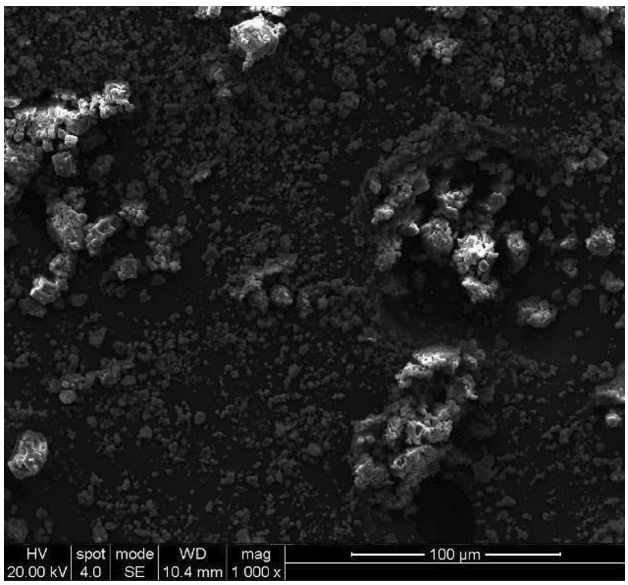

Fig. 3 SEM analysis of synthesized BS-ZnONPs The photomicrographs taken by SEM revealed that the BS-ZnONPs shows the uniform dispersion with defined shapes at size ranging from 40 to 90 nm

Results

Characterization of formulated BS-ZnONPs

The formation of BS-ZnONPs was confirmed by the UV-spectral study. As depicted in the Fig. 2, the formulated BS-ZnONPs were demonstrated by the surface Plasmon resonance spectrum at 500 nm. It evidenced the development of BS-ZnONPs in the solution.

The morphology of BS-ZnONPs and elemental compositions of BS-ZnONPs-coated cotton fabrics were examined through the SEM equipped with EDX and the outcomes are portrayed in Figs. 3 and 4. The SEM photomicrograph demonstrated that the formulated BS-ZnONPs have well-dispersed and sphere shapes (Fig. 3). The EDX pattern of BS-ZnONPs-coated fabrics exhibited that the fabricated BS-ZnONPs have higher percentage of zinc (Fig. 4). These outcomes proved the bio-formation of BS-ZnONPs and its binding and existence on the surface of cotton fabrics.

The morphology and magnitude of the fabricated BS-ZnONPs were studied via the TEM microphotographs and are depicted in the Fig. 5. The TEM microphotographs confirmed the existence of BS-ZnONPs in a uniformly dispersed state with oval shapes with the size ranging from 60 to 90 nm.

The functional groups that specially found on the formulated BS-ZnONPs were investigated through FT-IR. Fig. 6 demonstrated the existence of different functional groups. The peaks at 3650.08, 3675.75, 3852.62 cm^{-1} may be due to the O–H stretching and vibrations of alkane and hydroxyl groups (Fig. 6). The bands at 1506.24, 1540.48, 1559.02 cm^{-1} may be due to the hydroxyl groups of zinc and adsorbed water molecules. The bands at 420.78, 536.31 cm^{-1} were typically due to the vibrations of Zn–O bond. The peaks of nanoparticles were noted at 660.41, 671.82, 728.87, 877.22, 1653.16, 2034.00, and 2349.23 cm^{-1} (Fig. 6). These outcomes evidenced that the numerous functional groups were bound on the formulated BS-ZnONPs.

The synthesized BS-ZnONPs were assessed using XRD analysis to detect the crystallinity and purity, and the finding is represented in the Fig. 7. The fabricated BS-ZnONPs exhibited several peaks at different intensity, which is shown in Fig. 7. These peaks demonstrate the face-centered polygonal arrangements. The crystallinity and purity of the fabricated BS-ZnONPs were confirmed by the data of XRD study.

FT-IR analysis of the *B. serrata* resin extract

The functional groups present in the extract of the *B. serrata* resin were analyzed by the FT-IR. As represented in the Fig. 8, the presence of different functional groups was evidenced by various peaks. The occurrence of O–H stretching and vibrations of alkanes were evidenced by the peaks at 2931.19 and 1952.70 cm^{-1}. The presence of hydroxyl groups was evidenced by the peaks at 1701.66, 1452.76, and 1377.16 cm^{-1}. The peaks at 888.63 and 437.18 cm^{-1} demonstrate the presence of various functional groups (Fig. 8).

Result of antibiotic sensitivity test

The species of all the isolates were identified based on their biochemical characteristics and the detailed results

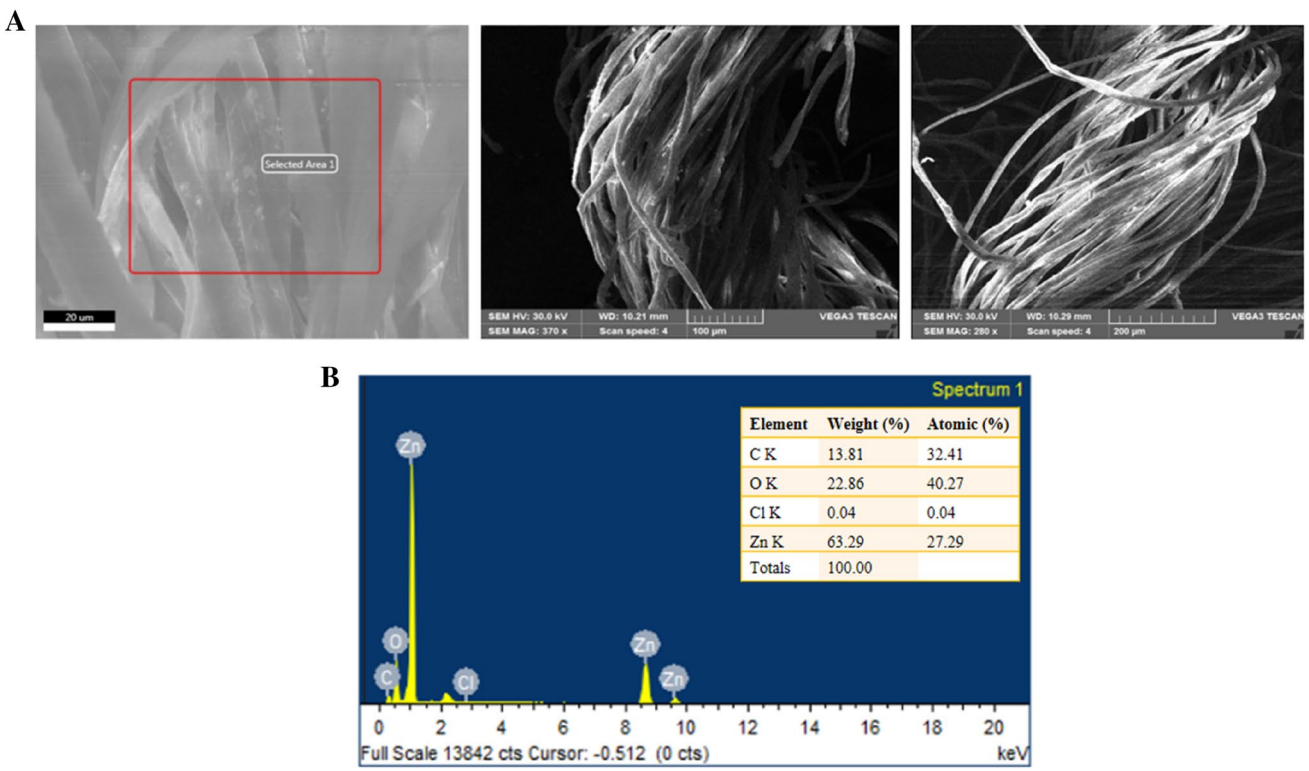

Fig. 4 **A** SEM and **B** EDX analysis of fabricated BS-ZnONPs-embedded cotton fabrics. The outcomes of SEM along with the EDX studies evidence the presence of formulated BS-ZnONPs on the cotton fabrics. **A**—SEM analysis; **B**—EDX analysis

are illustrated in the Table 1. The antibiotic sensitivity profile of the isolated pathogens revealed the major variations as depicted in the Table 2. Many strains demonstrated the resistance to the antibiotics and some showed the sensitivity. The *S. aureus* and *E. coli* exhibited the resistance to vancomycin and gentamycin but are sensitive to the chloramphenicol and tetracycline. The *K. pneumoniae* possessed the resistance to vancomycin, gentamycin, and chloramphenicol. All the other strains demonstrated the mixed outcomes, i.e., resistance, sensitivity, and intermediate based on the antibiotics.

Antibacterial effect of fabricated BS-ZnONPs

The fabricated BS-ZnONPs were examined for its antibacterial action towards nosocomial microbes like *K. pneumoniae*, *K. oxytoca*, *S. aureus*, *P. aeruginosa*, *A. baumanii*, and *E. coli*. Our findings clearly proved that BS-ZnONPs displayed noticeable antibacterial potential against the tested pathogens (Fig. 9). The maximum sensitivity was showed by the *K. pneumoniae* (18 mm) and *K. oxytoca* (18 mm) against the 60 µG/mL of fabricated BS-ZnONPs. The *A. baumanii* (17 mm) and *P. aeruginosa* (16 mm) also demonstrated the higher sensitivity against the 60 µG/mL of fabricated BS-ZnONPs (Table 3). The BS-ZnONPs effectively inhibited the growth of all the tested nosocomial pathogens.

Antibacterial effect of synthesized BS-ZnONPs-embedded cotton fabrics

BS-ZnONPs-coated fabrics were tested against the nosocomial pathogens to prove its antibacterial potential. The BS-ZnONPs-coated cotton fabrics appreciably prevented the growth of all the tested pathogens (Fig. 10). The maximum inhibition was observed in *A. baumanii* (22 mm) and *K. pneumoniae* (20 mm). The cotton fabrics with BS-ZnONPs also inhibited the growth of tested pathogens. All the tested nosocomial pathogens displayed sensitivity against the BS-ZnONPs-coated cotton fabrics (Table 4). This result proved that the BS-ZnONPs-coated fabrics were effective against the nosocomial pathogens.

MIC and MBC

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), of BS-ZnONPs against the bacterial isolates are shown in Table 5. The

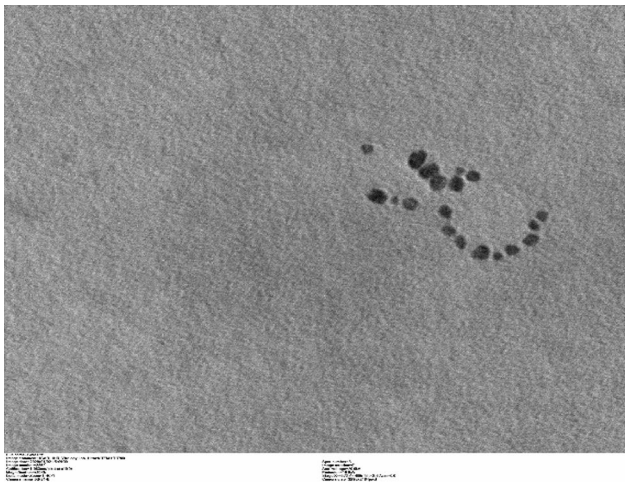

Fig. 5 TEM analysis of fabricated BS-ZnONPs The microphotographs taken by TEM demonstrated that the formulated BS-ZnONPs revealed a uniform spherical shaped morphology with average size ranging from 60 to 90 nm

bacterial growth was effectively inhibited by BS-ZnONPs in all the isolates tested.

Effect of BS-ZnONPs on cell viability

The effect of formulated BS-ZnONPs on the viability of L929 cells was investigated by MTT assay. The outcomes clearly showed that the formulated BS-ZnONPs did not possess cytotoxicity to the L929 cells. Cell viability of L929 did not reduce with the increasing dosages of BS-ZnONPs (Fig. 11). All the tested doses of fabricated BS-ZnONPs did not possess toxicity to the L929 cells. This result witnessed that BS-ZnONPs were non-toxic to the L929 cells.

Effect of BS-ZnONPs on the wound healing activity

The wound healing potential of formulated BS-ZnONPs was investigated by in vitro wound scratch assay using L929 cells. The outcomes of scratch assay revealed that the BS-ZnONPs enhanced the L929 cell migration and enhanced the rate of wound closure (Fig. 12). Both concentrations of formulated BS-ZnONPs (15 and 20 µG/mL) improved the cell migration and thereby closed the wound scratch in rapidly. This result disclosed the wound healing potential of formulated BS-ZnONPs.

Discussion

In the last few years, the increased advancements in the nanomedicine provide greater promise for the treatment of microbial diseases. The nanomaterials can act as a perfect antimicrobial agents and carriers for antimicrobials for the enhancement of potentials of antibiotics (Baptista et al. 2018; Supraja et al. 2016). The increased resistance developed by the pathogens restricted the quantity of drugs used in the current therapeutic procedures. The microbial strains which are isolated from the patients are showed higher resistance to more than one antibiotic. In this case, the multidrug-resistant microbes are the imperative threat to the health care workers (Magiorakos et al. 2012). The antibiotic resistance of pathogens was elevated rapidly in recent times because of their increased ability against the pathogens

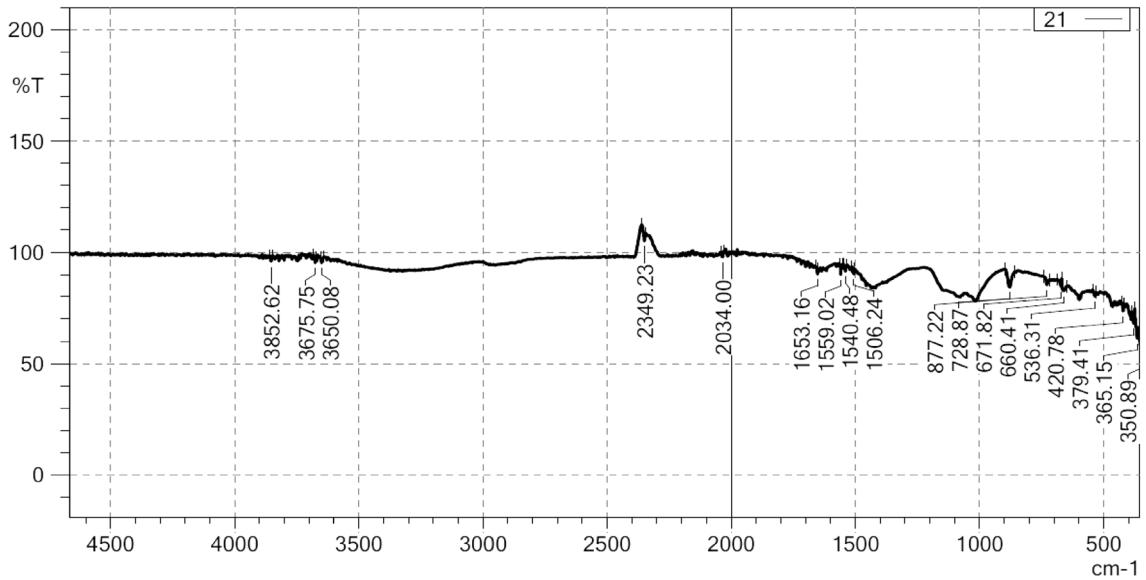

Fig. 6 FT-IR analysis of fabricated BS-ZnONPs The functional groups that specially found on the formulated BS-ZnONPs was investigated through FT-IR and the results indicated the occurrence of alkane, hydroxyl, and zinc molecules

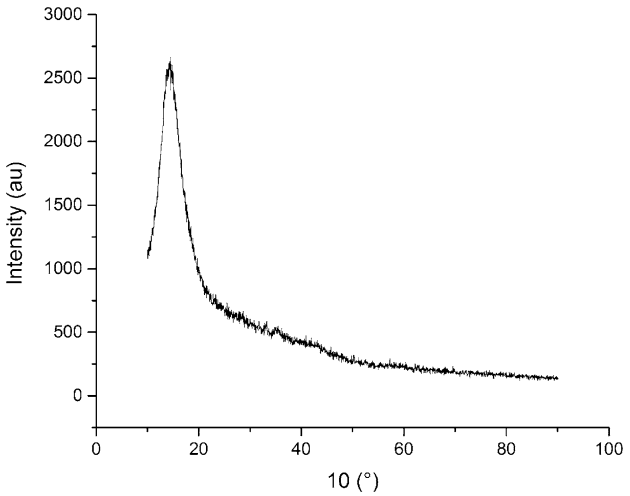

Fig. 7 XRD analysis of fabricated BS-ZnONPs XRD analysis demonstrated the different peaks, which confirms the crystalline nature of fabricated BS-ZnONPs

(Perelshtein et al. 2016). Similarly, in this study, we also found that isolated microbes possessed the resistance against the antibiotics. The outcomes of antibiotic sensitivity profile of the isolated pathogens demonstrated the resistance to the antibiotics and some showed the sensitivity. Particularly, the *S. aureus* and *E. coli* possessed the resistance to vancomycin and gentamycin and *K. pneumoniae* demonstrated the resistance to vancomycin, gentamycin, and chloramphenicol.

The existence of potential pathogens on the area of human healthcare centers becomes a major problem because of the increased chances of infections and spreading to others. The diseases due to the pathogenic microbes are the biggest health hazard to the human society (Beyth et al. 2015). To overcome these issues, the exploration of novel antimicrobial agents emerged as a prime target of current medicine (Tacconelli et al. 2018). The novel approaches were continuously explored for the development of potent antimicrobial agents using the inorganic metals and/or metal oxides-coated

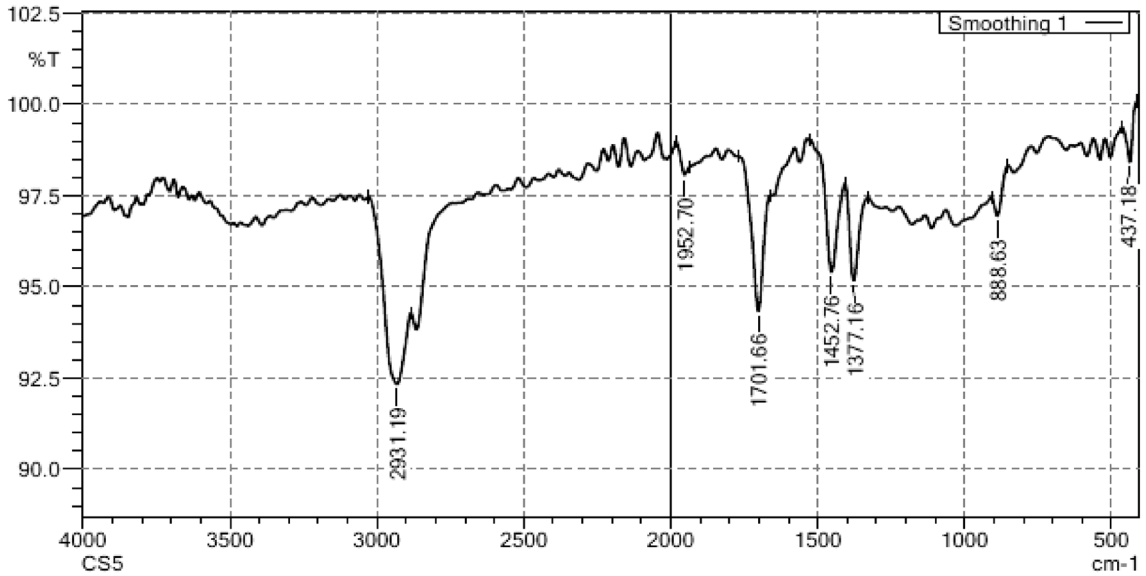

Fig. 8 FT-IR analysis of *B. serrata* resin extract FT-IR spectrum of formulated CSP-Cr-NCs exhibited the presence of several stretching and bonding such as O–H, hydroxyl groups of zinc, and Zn–O bond

Table 1 Biochemical characterization of the nosocomial pathogenic isolates

Isolates	Indole	Methyl red	Voges proskauer	Citrate test	Urease	Catalase	Coagulase	Oxidase
Pseudomonas aeruginosa	N	N	N	P	N	P	N	P
Klebsilla pneumoniae	N	N	P	P	P	P	N	N
Staphylococcus aureus	N	P	P	P	P	P	P	N
Escherichia coli	P	P	N	N	N	P	N	N
Acinetobacter baumanii	N	N	N	P	N	P	N	N
Klebsiella oxytoca	P	N	P	P	P	P	N	N

P Positive, *N* Negative

Table 2 Antibiotic sensitivity test against the pathogens (ZOI in mm)

Bacterial isolates	Vancomycin (5 µG/mL)		Gentamycin (10 µG/mL)		Chloramphenicol (30 µG/mL)		Tetracycline (30 µG/mL)	
	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf
Pseudomonas aeruginosa	7 ± 0.774	R	15 ± 1.732	R	19 ± 1.155	S	18 ± 1.732	S
Klebsilla pneumoniae	6 ± 0.577	R	11 ± 1.155	R	11 ± 0.577	R	13 ± 1.155	I
Staphylococcus aureus	14 ± 1.155	I	24 ± 1.155	S	14 ± 0.577	I	19 ± 1.155	S
Escherichia coli	7 ± 0.577	R	12 ± 0.577	R	18 ± 0.577	S	19 ± 0.577	S
Acinetobacter baumanii	7 ± 0.577	R	19 ± 1.155	S	7 ± 0.577	R	19 ± 1.732	S
Klebsiella oxytoca	8 ± 1.155	R	20 ± 1.732	S	20 ± 1.732	S	13 ± 1.155	I

Data were represented as mean ± SD for triplicates

R Resistance, *I* Intermediate, *S* Sensitivity, *ZOI* Zone of Inhibition, *Inf* Inference

Values do not share common superscripts and significantly differ at $p < 0.05$

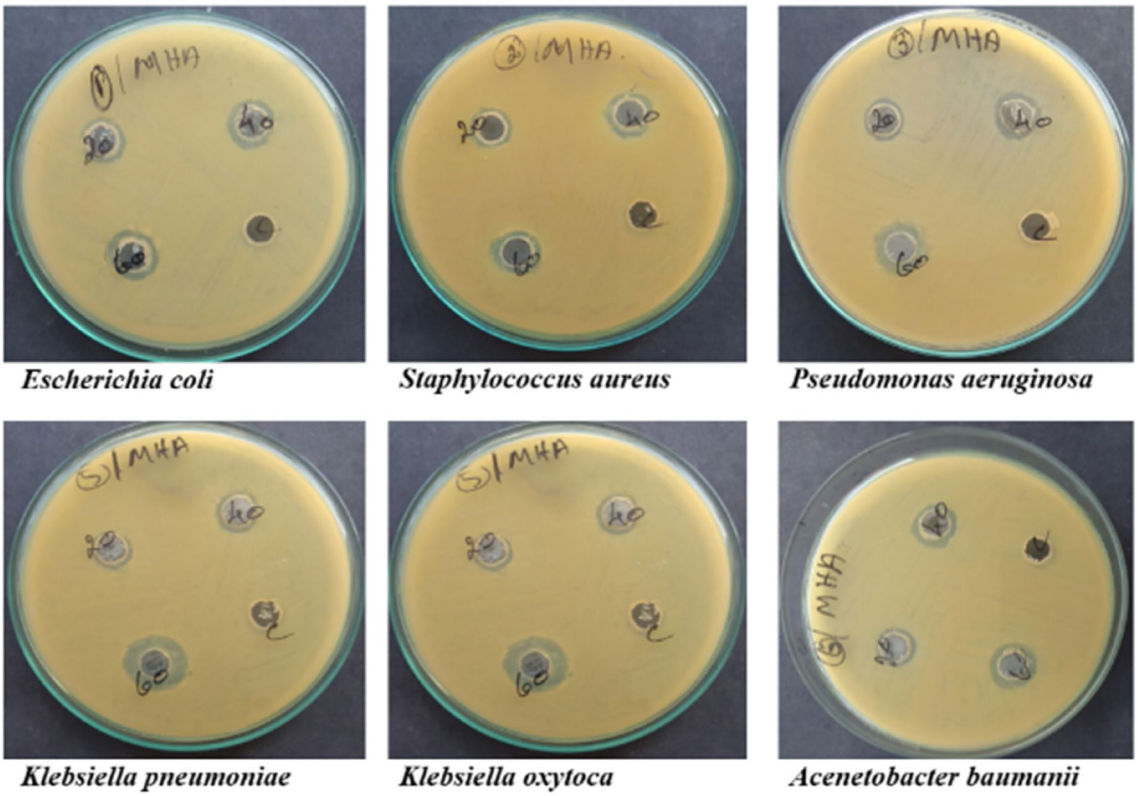

Fig. 9 Antibacterial effect of fabricated BS-ZnONPs The synthesized BS-ZnONPs at the dose of 20, 40, and 60 µG/mL inhibited the growth of tested pathogens. The maximum zone of inhibition was observed on the *K. pneumoniae* (18 mm) and *K. oxytoca* (18 mm) against the 60 µG/mL of synthesized fabricated BS-ZnONPs

fabrics to enhance their prevention against infection (Jones et al. 2015; Basri 2021). The novel nano-agents demonstrated more stability and antimicrobial potentials even after the wash (Salat et al. 2018). In recent times, some inorganic metal oxides (Abramov et al. 2009; Perelshtein et al. 2009) and NPs-embedded textiles have gained an much interests due to their remarkable antimicrobial effects with the durability against the pathogens (Xu et al. 2016; Li et al. 2017). The substitute inorganic metal oxide NPs have gained the much research focuses because of their safety and targeted toxicity towards pathogens (Singh et al. 2012; Imraish et al. 2021a, b). The unified research approaches were turned into the nano-materials-coated textiles like medical clothes to lessen the chances of nosocomial infections (Khosravian et al. 2015).

In recent times, the utilization of biological materials as the templates of green nanotechnology was elevated and the

Table 3 Antibacterial effect of fabricated BS-ZnONPs

S. No	Organisms	20 µG/mL	40 µG/mL	60 µG/mL
1	*Escherichia coli*	11 ± 0.577	13 ± 0.577	15 ± 0.577
2	*Staphylococcus aureus*	12 ± 1.155	13 ± 1.155	15 ± 1.155
3	*Pseudomonas aeruginosa*	10 ± 0.577	14 ± 0.577	16 ± 0.577
4	*Klebsiella pneumoniae*	11 ± 0.577	14 ± 1.155	18 ± 0.577
5	*Klebsiella oxytoca*	11 ± 1.155	13 ± 1.732	18 ± 1.155
6	*Acinetobacter baumanii*	12 ± 0.577	15 ± 0.577	17 ± 0.577

Data were represented as mean ± SD for triplicates

Values do not share common superscripts and significantly differ at $p < 0.05$

plant materials were extensively utilized for the formulation of metal oxide NPs (Garima et al. 2011; Imraish et al. 2021a, b). The plant extract was received a greater interest than other biological materials because of their plentiful sources and holding the wide variety of reducing agents, i.e., secondary metabolites. It has already been reported that the gum resins of Boswellia have several bioactive phyto-constituents with remarkable biological activities such as E-beta ocimene and Cembrene (Antimicrobial and antioxidant), Sabinene (antitumor and larvicidal), Beta elemene (anticancer and wound healing), Allo aromandendrene (antibacterial and antifungal), Alpha pinene (anticancer, antidiabetic, antioxidant, antimicrobial, and analgesic), Alpha boswellic acid (anticancer, antimicrobial, anti-inflammatory, Immunostimulator, and anti-arthritic), and acetyl-11-keto-boswellic acid (anticancer, antimicrobial, anti-inflammatory,

Table 4 Antibacterial effect of fabricated BS-ZnONPs-embedded cotton fabrics

S.No	Name of the organism	Cotton fabrics	Mixed cotton fabrics
1	*Escherichia coli*	11 ± 0.577	18 ± 0.577
2	*Staphylococcus aureus*	19 ± 0.577	18 ± 1.155
3	*Pseudomonas aeruginosa*	19 ± 0.577	17 ± 0.577
4	*Klebsiella pneumoniae*	21 ± 2.887	20 ± 0.577
5	*Klebsiella oxytoca*	20 ± 0.577	18 ± 1.155
6	*Acinetobacter baumanii*	19 ± 0.577	22 ± 1.155

Data were represented as mean ± SD for triplicates

Values do not share common superscripts and significantly differ at $p < 0.05$

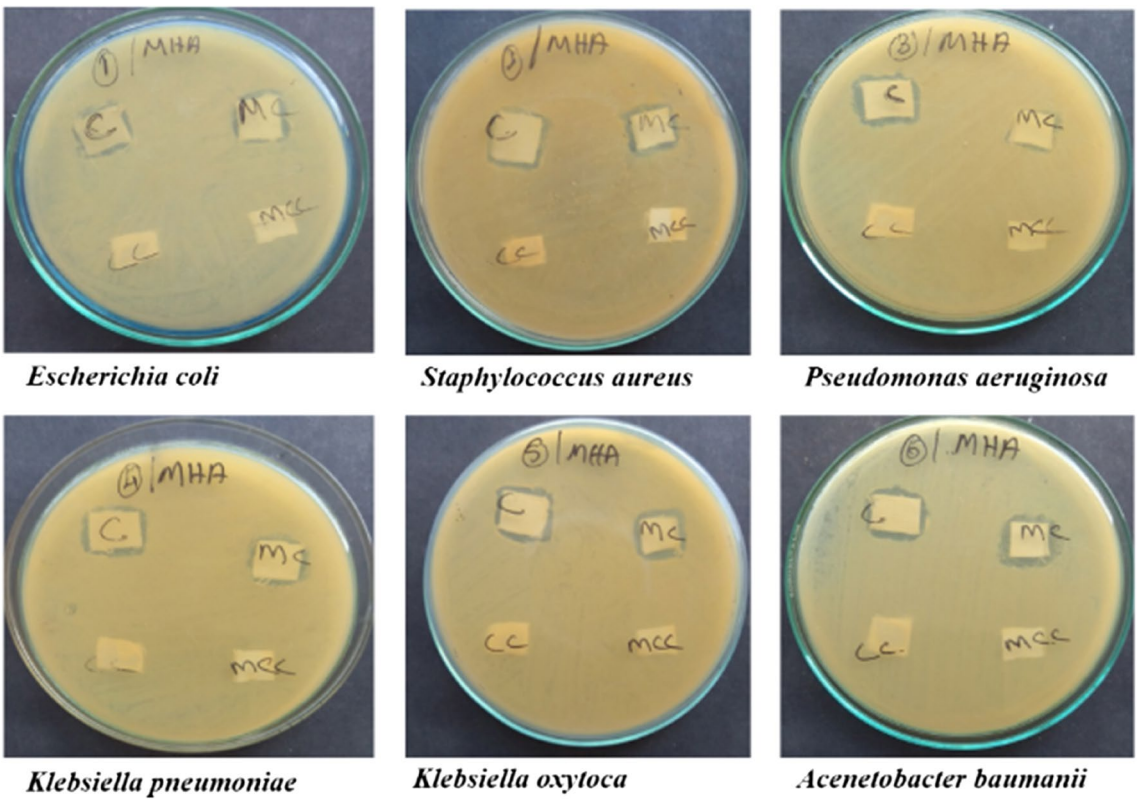

Fig. 10 Antibacterial effect of fabricated BS-ZnONPs-embedded cotton fabrics The 60 µG/mL of synthesized BS-ZnONPs-coated cotton fabrics showed the notable antibacterial activity against the tested pathogens. The highest zone of inhibition was observed against the *A. baumanii* (22 mm) and *K. pneumoniae* (20 mm)

Table 5 MIC and MBC of BS-ZnONPs against the isolates

S.No	Name of the organism	MIC (µG/100 µl)	MBC (µG/100 µl)
1	*Escherichia coli*	62.5	125
2	*Staphylococcus aureus*	125	125
3	*Pseudomonas aeruginosa*	62.5	62.5
4	*Klebsiella pneumoniae*	125	125
5	*Klebsiella oxytoca*	125	125
6	*Acinetobacter baumanii*	62.5	62.5

MIC Minimum inhibitory concentration, *MBC* Minimum bactericidal concentration

Immunostimulator, anti-arthritic, and anti-asthma properties). Hence, in this study, we utilized the *B. serrata* extract for the green formulation of ZnONPs. The nano-carriers could guard the drugs from the enzymatic attacks and withstand the drug deliverance to augment the bioavailability and half-life (Walvekar et al. 2019). The nano-medical approach for the enhancement of antibiotic deliverance for killing of pathogens denoted the lesser adverse effects and drug resistance. In this exploration, we noticed that the formulated BS-ZnONPs were displayed the potent antibacterial activity against the nosocomial pathogens. The maximum sensitivity was observed on *K.pneumoniae* (18 mm) and *K.oxytoca* (18 mm) against the 60 µG/mL of fabricated BS-ZnONPs. This outcomes witnessed the antibacterial actions of fabricated BS-ZnONPs against nosocomial pathogens.

The metal oxide NPs are primarily formulated by Au, Zn, Ag, or Cu and are seems to possess the potential antimicrobial effects. Nonetheless, the utilization of these NPs could be limited due to their toxicity to the normal mammalian cells. The NPs with extremely smaller size could generate or release the more radicals that the imperative task of killing the pathogenic microbes (Walvekar et al. 2019). Among the various metal oxide NPs, ZnONPs were exhibited the magnificent benefits in targeted drug delivery, molecular diagnostics, bioimaging probes, and micro-electronics. ZnONPs were extensively examined for their antimicrobial properties (Sharma et al. 2010). Furthermore, the antibacterial efficiency of ZnONPs was reported to be effective than the zinc oxide, principally categorized because of their smaller magnitude comprising a superior surface-dependent volume that illustrates noticeable antibacterial effects (Kumar et al. 2011). So as to be utilized in the therapeutic

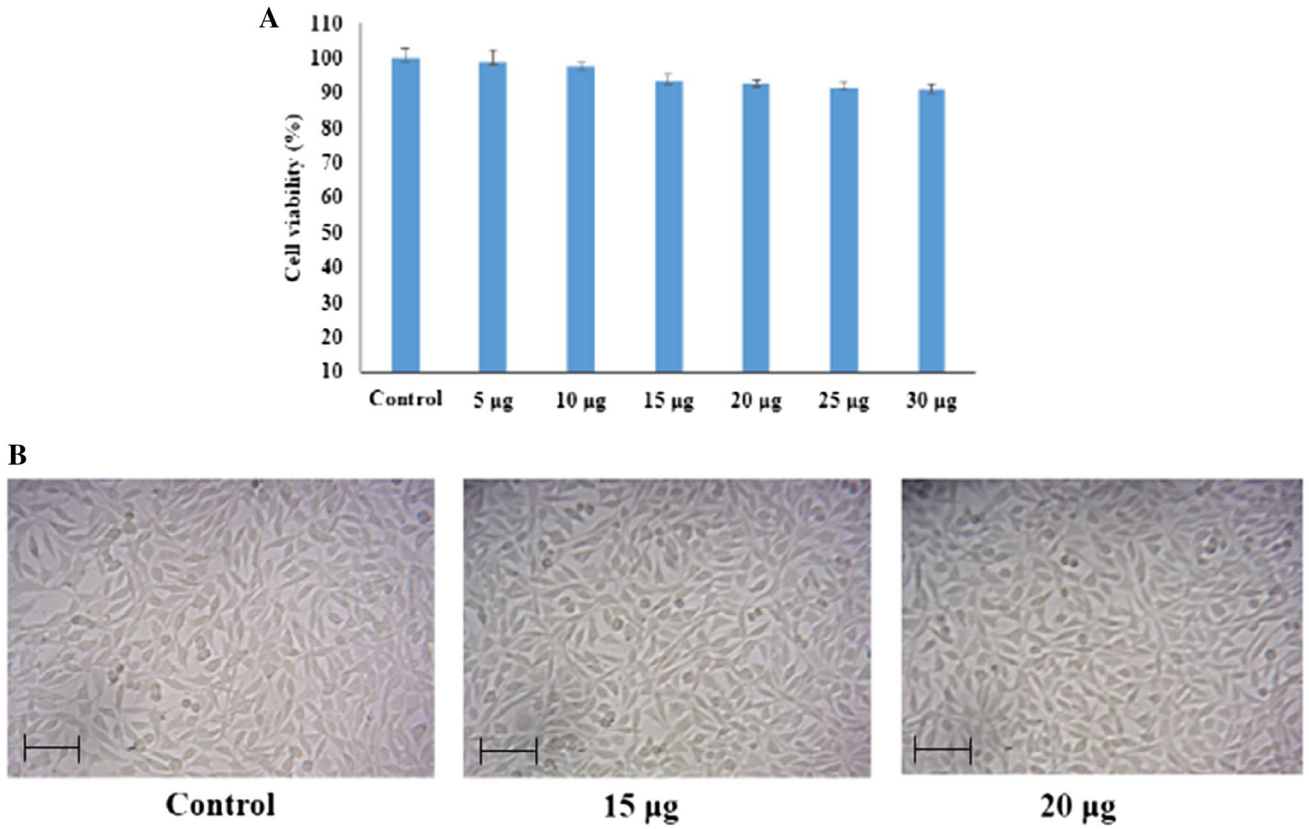

Fig. 11 Effect of synthesized BS-ZnONPs on the viability of L929 cells The MTT cell viability assay revealed that the formulated BS-ZnONPs did not showed any cytotoxicity to the L929 cells (**A**). Results were illustrated as mean ± SD of triplicate measurements. Data did not share mutual superscripts and vary significantly at $p < 0.05$. Microscopic images revealed the normal cell morphology that indicates the non-toxic nature of BS-ZnONPs to L929 cells (**B**). *Magnification: 40×; scale bar: 50 µm

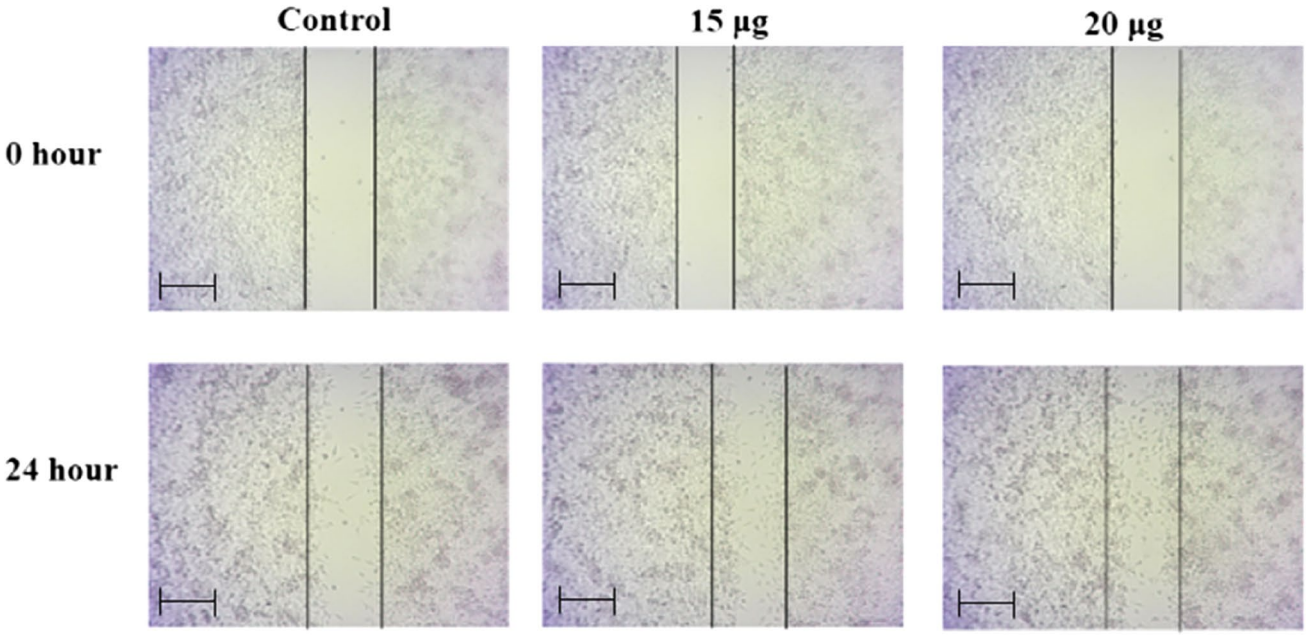

Fig. 12 In vitro wound healing activity of fabricated BS-ZnONPs. BS-ZnONPs demonstrated the remarkable wound healing activity. Photomicrographs revealed that BS-ZnONPs appreciably improved the cell migration of L929 cells and increased the wound closure. *Magnification: 20×; scale bar: 50 µm

purposes, the novel antimicrobial agents must demonstrate augmented antimicrobial effects and also did not show any toxicity to the normal human cells. It was reported that most of the existing antibiotics were more toxic to the patient. The gentamicin, an extensively utilized antibiotic, demonstrated the potent antibacterial activity to the aerobic bacteria and used to treat the urinary and respiratory tract infections, eye and skin infections. On the other hand, gentamicin also possessed the adverse effects on the patients (Hayward et al. 2018).

It was proved that the hospital clothes, linens, and scrubs did not provide an effective protection from the pathogens for healthcare workers. The survival and transmission of between patients and hospital workers were reported in numerous reports (Ferrer et al. 2014). Consequently, the currently utilized fabrics need an enhancement on their antibacterial effects to prevent the nosocomial infections. Similarly, the BS-ZnONPs-coated cotton fabrics also demonstrated the appreciable antibacterial potential against the nosocomial pathogens. The maximum sensitivity was showed by the *A.baumanii* (22 mm) and *K.pneumoniae* (20 mm). All the tested nosocomial pathogens were displayed the sensitivity against the BS-ZnONPs-coated cotton fabrics.

The antimicrobial materials have held many applications in the biomedical field, particularly when they are in direct contact with the human body. They must be demonstrating several requirements and fall under the guidelines of safe use within the body. Primarily, they must be biocompatible, not reactive to the body, and possess the good stability to the bodily fluids. The presence of more pathogens on the biofilms could result in severe infections. Hence, the selection of suitable materials toward pathogens is crucial in the biomedical fields (Fuchs and Tiller, 2006; Vakayil et al. 2021). Our findings disclosed that the formulated BS-ZnONPs did not possess toxicity to L929 cells. This result proved the non-toxic nature of BS-ZnONPs to normal cells. Similarly, the outcomes of scratch assay revealed that the BS-ZnONPs improved the L929 cell migration and speed up the wound closure rate. This result evidenced the wound healing potential of formulated BS-ZnONPs. In this research work, there are some limitations that we only provided the primary assay findings with positive findings that suggested the beneficial roles of BS-ZnONPs-embedded cotton fabrics. Though, the further studies on this context still needed in the future to make a clear conclusion about the beneficial roles of BS-ZnONPs-embedded fabrics for antimicrobial and wound healing applications.

Conclusion

The findings of this study revealed the antibacterial property of BS-ZnONPs and embedded cotton fabrics against nosocomial pathogens. The BS-ZnONPs and embedded fabrics effectively prevented the growth of nosocomial pathogens.

The BS-ZnONPs also demonstrated its non-toxic nature to the L929 cells. The finding of scratch assay also proved the wound healing potential of formulated BS-ZnONPs. Hence, it was clear that the BS-ZnONPs-coated cotton fabrics can be a possible alternative for the prevention of nosocomial infections and wound healing purposes. However, additional research is still required in the future to clearly understand the underlying mechanisms of antimicrobial and wound healing properties of BS-ZnONPs and embedded fabrics.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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