



Study of antimicrobial properties of *Piper betel* coated nanozirconium on cotton gauze

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Abstract

Metal oxide nanoparticles attract huge attention nowadays. In the current study, zirconium nanoparticles were prepared and impregnated on cotton gauze fabrics by the reduction of zirconium oxychloride. The synthesized zirconium nanoparticles (ZrNPs) were analyzed using UV–Vis absorption, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and Transmission electron microscopy (TEM). The results revealed that the ZrNPs were spherical in shape and crystalline in nature and ranged in size from 10 to 80 nm. Cotton gauze fabrics containing ZrNPs were studied for antimicrobial properties. ZrNPs were uniformly distributed and a high deposition density was observed on the surface of the cotton. Cotton fabrics loaded with the synthesized ZrNPs showed remarkable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Therefore, the current work may aid to further investigate the zirconium nanoparticles synthesized using *Piper betel* for their potential applications in wound dressings.

Keywords *Piper betel* · Zirconium nanoparticles · Antimicrobial activity · Cotton gauze fabric · Bacterial strain

Introduction

In concern with the environmental problems, ecotoxicological importance has been introduced in various fields. The textile industry has given a remarkable importance for the development of antimicrobial textile garments because of their direct human body contact. The carbohydrates in fabrics serve as a nutrient for the growth of microbes, which produce volatile odor, dullness to the color and reduction in

fabric strength. Fabrics with antiviral, antifungal and antibacterial properties give protection to the human skin and prevent dermatitis (Rathinamoorthy et al. 2011; Narayanan et al. 2021). There is a budding awareness concerning the usage of antibacterial fabrics as medical garments, protective clothes, and bed spreads to abate the chances of nosocomial infections. Plants are the greatest sources of different phytochemicals and can be used as natural antimicrobial agents with safety and efficacy than the chemical antimicrobial agents, because they are non-toxic and non-allergic to the human skin and do not produce resistance among the microbes (Oves et al. 2018). Recently, few studies have studied the antimicrobial activity of plant extracts on textile clothes and especially, in the field of medical clothes.

Piper betel is an evergreen perennial plant, which is used as a remedy for different types of diseases. In Ayurvedic medicine, the extract of betel leaf is used as an adjuvant and to produce better effects. It is used with other medicines irrespective of its independent use (Pradhan et al. 2013). The phytochemical analysis of betel leaf showed the presence of proteins, carbohydrates, minerals, fats, fibres, essential oils, alkaloids and vitamins such as A, B and C (Guha 2006). Among these, phenolic content of the betel leaf acts the active ingredient in medicine (Bissa et al. 2007). Recent research works on betel leaves also showed the presence

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of starch, diastases, sugars and essential oils as their major components (Chopra and Chopra 1958; Kanjwani et al. 2008). According to Sarkar et al. (2000), the fresh leaves of betel possess antifungal, antibacterial, antiseptic and anti-helminthic properties and they also possess a better wound healing property (Abdul Rahman 2005). Many natural compounds have shown antimicrobial properties and have been used for the treatment of wound infections. The betel leaf extract produced better antimicrobial activity against *Streptococcus pyogenes*, *E. coli* and *Pseudomonas* species and especially, against *Enterococcus faecalis* and *Klebsiella pneumonia* causing urinary tract infections (Agarwal et al. 2012; Chakraborty and Shah 2011).

Moreover, nanotechnology is an emerging field involving the synthesis of novel materials at a nanoscale level for science and technology applications. Plant extracts are used for nanoparticle fabrication and have grabbed attention because of their economical and ecofriendly nature (Parashar et al. 2009). The green synthesis of nanoparticles from plant sources is influenced by different factors such as the source of the plant, phytochemicals and pigments in the crude extracts of roots, stem, bark, leaves, latex and seeds (Leela and Vivekanandan 2008). Different nanoparticles are synthesized using plant parts such as stem, bark, leaf and root because of the huge availability, easy handling and the presence of alkaloids, flavanoids, phenols, tannins and terpenoids, which mediate the nanoparticle synthesis (Kavitha et al. 2013). The textile industry is involved in improving the textile products by immobilizing the nanoparticles onto the fibers and those products are used for healing wounds and other applications in hospitals against infections and contamination produced by bacteria (Lee et al. 2003). Among the various nanoparticles synthesized, the usage of ZrNPs in the biological field is hugely growing in the recent years. It has been used as a carrier for penicillin and alendronate in drug delivery system (Tharani 2016). Therefore, the present work has involved the study of effective antimicrobial property of *Piper betel* extract coated nano zirconium on cotton gauze fabrics, which would help in preventing wound infections.

Materials and methods

Chemicals and preparation of *Piper betel* leaf extract

All the reagents purchased were of analytical grade and used without any further purification. Zirconium oxide was purchased from Sigma-Aldrich. Fresh leaves of *P. betel* were collected and shade-dried after washing with distilled water. The dried leaves (10 g) underwent extraction with 100 mL of water, in a soxhlet apparatus at 60–70 °C for 10–12 h. Water was removed from the extract under vacuum and a

semisolid mass (1.435 g) was obtained. The yield of extract was 14.35% w/w for the aqueous extraction of *P. betel*. The solid extract was stored in a sterile storage container and refrigerated until used for further experiments.

Green synthesis of zirconium nanoparticles

Synthesis zirconium nanoparticles of *P. betel* was prepared by green synthesis method. 80 mL of Zirconyl chloride (1 mM) was taken and added with 20 mL of *P. betel* leaf extract and stirred for 2 h at 80 °C. The mixture was incubated overnight to allow the formation of the nanoparticles. The synthesized particles were dried by vacuum evaporation in a hot air oven at 200 °C for 1–2 h.

Characterization of zirconium nanoparticles

The fabricated nanoparticles were characterized by various techniques UV–Visible spectroscopy, transmission electron microscopy (TEM), X-Ray Diffraction (XRD) and Fourier-transform infrared (FTIR) spectroscopy. UV–Vis absorption spectra were recorded using a digital spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 200–800 nm. The absorbance readings were obtained every 1 nm at the scan rate of 600 nm/min. The size and shape of the Zr nanoparticles were observed by the transmission electron microscopic (TEM) analysis (JEM – 1230, JEOL, Akishima, Japan). The crystallinity of the nanoparticles was studied by X-Ray Diffraction (XRD) with an X-ray diffractometer (Rigaku Corporation, Tokyo, Japan). The chemical structure and functional groups of the Zr nanoparticles were determined by Fourier-transform infrared (FTIR) spectroscopy (TIR-Vertex 8 V, Bruker, Germany). Potassium bromide pellet method was used for the preparation of the sample. FTIR spectrum was obtained in the wavenumber range of 4000–400 cm⁻¹ with the resolution of 2 cm⁻¹.

Antimicrobial activity of betel leaf extract coated nano-zirconium treated and untreated fabrics

The bacterial strains such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as test microorganisms. All bacteria were inoculated in Mueller Hinton (MH) broth at 37 °C for 24 h. Cotton gauze fabrics treated with synthesized ZrNPs and untreated fabrics were used. The antimicrobial activity of ZrNPs was studied by well diffusion method on cotton gauze fabrics. Nutrient agar Petri plates were prepared and allowed to solidify. Then, the plates were inoculated with microbial inoculums of selected strains and allowed to dry. Finally, cotton gauze fabrics treated with ZrNP and untreated cotton gauze fabrics were placed on the medium and kept in incubator at 37 °C for 24 h. After incubation period, the

zone of inhibition is measured in millimeters and results were recorded.

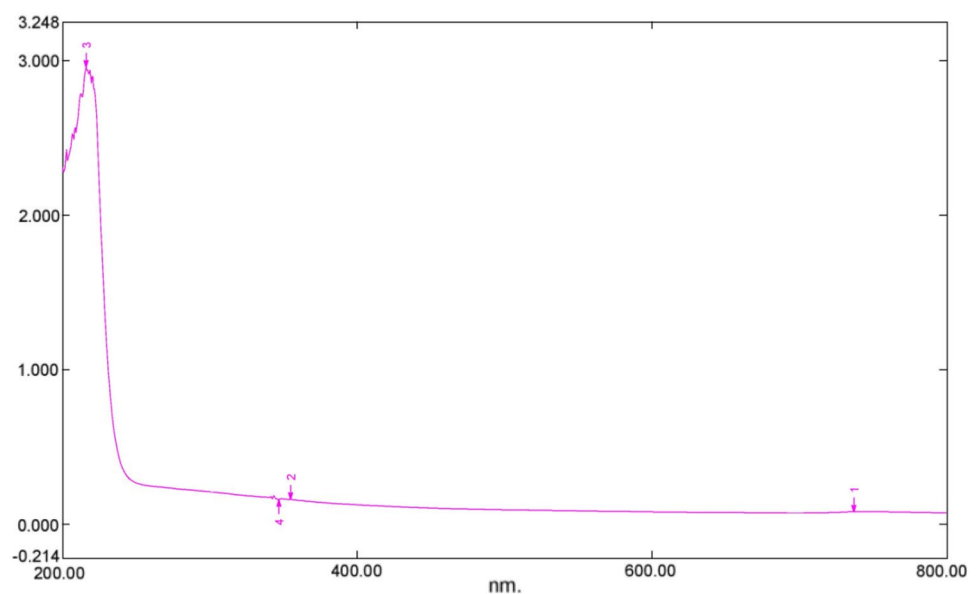
Results and discussion

Nowadays, the development of antimicrobial clothes and their usage have been greatly increased and appreciated by the medical field. *Piper betel* is reported to possess antimicrobial, antifungal and antiviral properties (Ramya and Maheshwari 2015) and was used to synthesize ZrNPs. The fabricated nanoparticles were subjected to various characterization studies and tested for antimicrobial activity on cotton fabrics.

UV–Visible spectroscopy

UV–Visible absorption spectroscopy is a simple, sensitive technique to confirm the successful synthesis of nanoparticles (Chellapandian et al. 2019). UV–Visible absorption spectrum was recorded for the ZrNPs between 200 and 800 nm. There were no peaks between 400 and 800 nm demonstrating the absence of particle aggregation. Synthesis of ZrNPs was confirmed with the spectral peak at 240 nm (Fig. 1). The obtained peak displayed a minor blue shift in comparison with the bulk. The peaks signified the shift of an inner shell electron to conduction. Several forms of Zr comprising ions, atoms or clusters intensely interacted with the water molecules to generate ZrO_2 , which was rapidly quenched in solution to develop nanoparticles (Bai et al. 2016).

Fig. 1 UV–Visible spectrum of Zirconium nanoparticles synthesized using *Piper betel* leaf extract



FTIR analysis

The synthesis of ZrNPs and their functional groups were confirmed by FTIR spectroscopy. The spectrum most probably indicated the presence of macromolecules such as lipids, sugars, carbohydrates, nucleic acids and, especially, proteins that ensured the stability of green ZrNPs as compared to the chemically synthesized NPs (Guerrini et al. 2018). The peaks were obtained between 4000 and 400 cm^{-1} . The band peaks at 3013 cm^{-1} , 2853.54 cm^{-1} and 1739.21 cm^{-1} to 702.97 cm^{-1} were the O–H stretching vibrations. The band at 492 cm^{-1} was due to Zr–O stretching. The weak bands between 2500 and 1739.21 cm^{-1} might have been due to the presence of C–H and C–O groups. The strong peaks between 700 and 400 cm^{-1} were attributed to the stretching of O–H group indicating the stretching and bending of water absorbed by the ZrNPs (Velazquez-Jimenez et al. 2014). Based on the fingerprint characters of the peak positions, the shapes and intensities along with the essential components in the material are observed in Fig. 2.

XRD analysis

To study the crystalline nature of the nanoparticles, XRD pattern of the sample was studied by an X-ray diffractometer. The XRD pattern demonstrated that the product had a cubic phase (JCPDS card No. 27–0997). All the diverse peaks in Fig. 3 were related to the ZrNPs and conformed to the Joint Committee for Powder Diffraction Studies (JCPDS). The results showed the diffraction peaks at 27.52°, 28.47°, 31.74°, 31.84°, 40.61°, 45.58°, 56.58°, 66.32° and 75.38° (Fig. 3). The diffraction peaks were witnessed at 2θ values

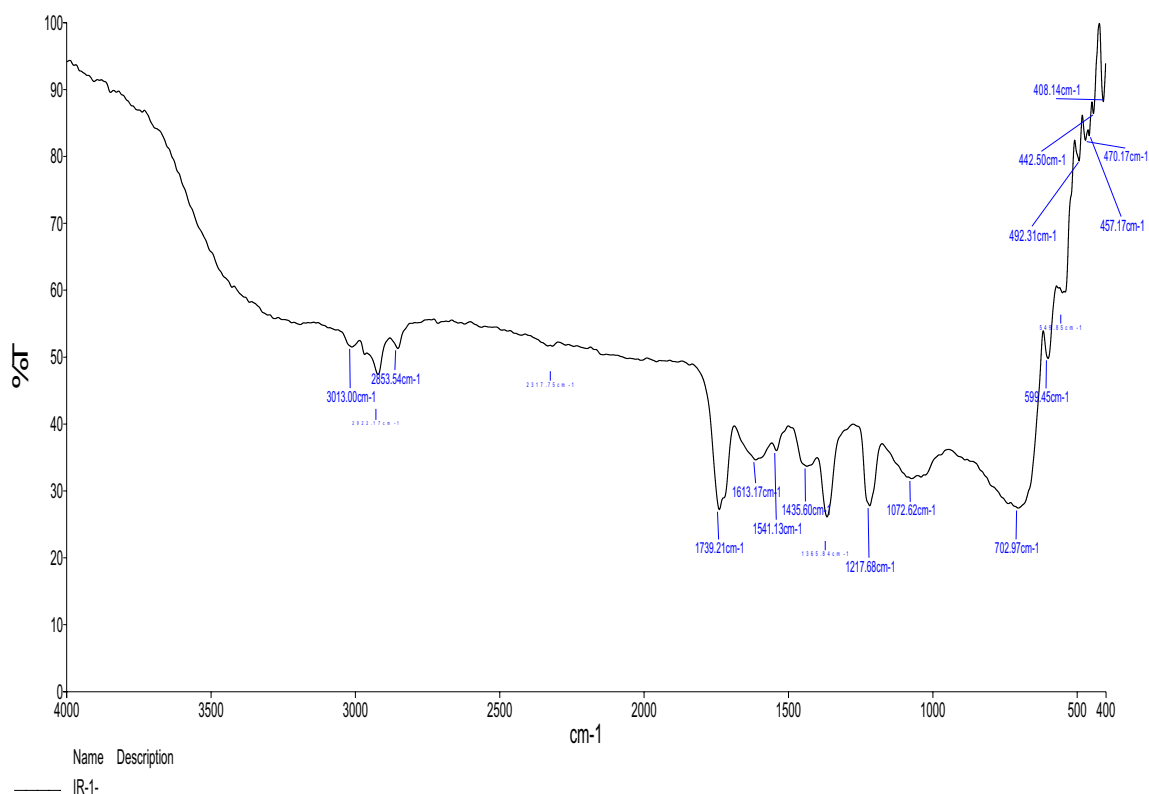
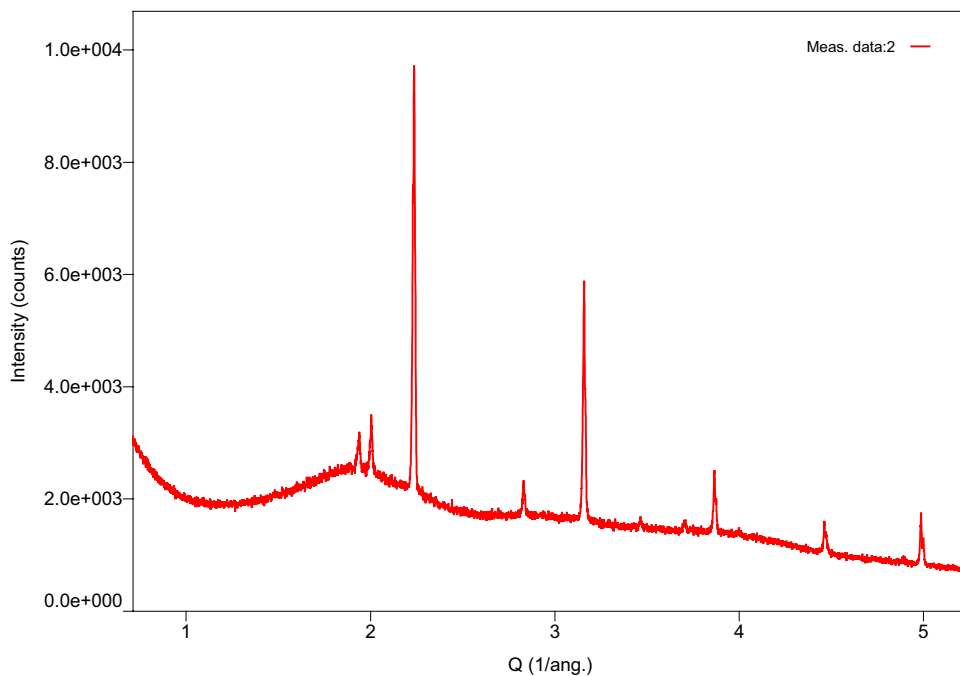


Fig. 2 FTIR spectrum of Zirconium nanoparticles synthesized using *Piper betel* leaf extract

Fig. 3 XRD pattern of Zirconium nanoparticles synthesized using *Piper betel* leaf extract



of 28.47° and 66.32° pertinent to the diffraction planes, (111) and (311) of ZrO₂, respectively. These diffraction peaks matched with the previously established JCPDS card

number 79–1769. The grain size evaluated by the relative intensity peak for ZrNPs was about 20 nm. The sharp peaks indicated the crystalline structure and crystallinity of ZrNPs

as confirmed by the XRD pattern (Fig. 3). The results were concordant with the earlier reports (Majedi et al. 2016).

TEM analysis

TEM analysis was employed for the morphological examination of ZrNPs. The results revealed that the as prepared nanoparticles were spherical in shape. The size of the particles ranged from 10 to 35 nm. This could be corroborated with the particle size obtained from the XRD analysis. There was no aggregation of particles (Fig. 4). Similar results have been reported earlier, which confirmed the results obtained in the present study (Ahmed et al. 2021).

Antimicrobial activity

The antibacterial activity of the betel leaf extract coated zirconium nanoparticles was assessed using well diffusion by the measurement of zones of inhibition against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Cotton fabrics treated with ZrNPs and untreated cotton fabrics were tested. The untreated cotton revealed no antibacterial activity. Conversely, ZrNPs treated cotton fabrics exhibited significant inhibition on the bacterial pathogens. The maximum zone of inhibition was exhibited against *E. coli* than the other pathogens selected (Fig. 5). The zones of inhibition exhibited by ZrNPs treated

cotton against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 21, 8, 18 and 7 mm, respectively. Methicillin-resistant *S. aureus* (MRSA) among the Gram-positive and *Pseudomonas aeruginosa* (*P. aeruginosa*) among the Gram-negative bacteria were the predominant pathogens in burn wound infections (Shariati et al. 2020). These bacterial species were susceptible to the synthesized nanoparticles, which was in accordance with the earlier reports (Gouda 2012). The antibacterial potential of the metal nanoparticles has been well documented by numerous scientists. The nanoparticles of TiO₂, MnO and ZrO possess superior antimicrobial activity because of their safety, durability and heat resistance while comparing with other antibacterial agents from organic sources. Nanoparticle attack the microbes by affecting the cell membrane and thereby, inhibit cell respiration leading to death of the cells (Gouda 2012). Similarly, in the current study, zirconium nanoparticles synthesized using the betel leaf extract exhibited antimicrobial activity against the tested pathogens. Similar results reported earlier were supporting these results (Jangra et al. 2012). The phytochemicals present in the betel leaf extract have contributed for the antimicrobial property and the spherical shape of the nanoparticles were also responsible for the enhanced activity. The observed antimicrobial activity strongly suggested that the synthesized nanoparticles had the potential of preventing wound infections.

Fig. 4 TEM images of Zirconium nanoparticles synthesized using *Piper betel* leaf extract

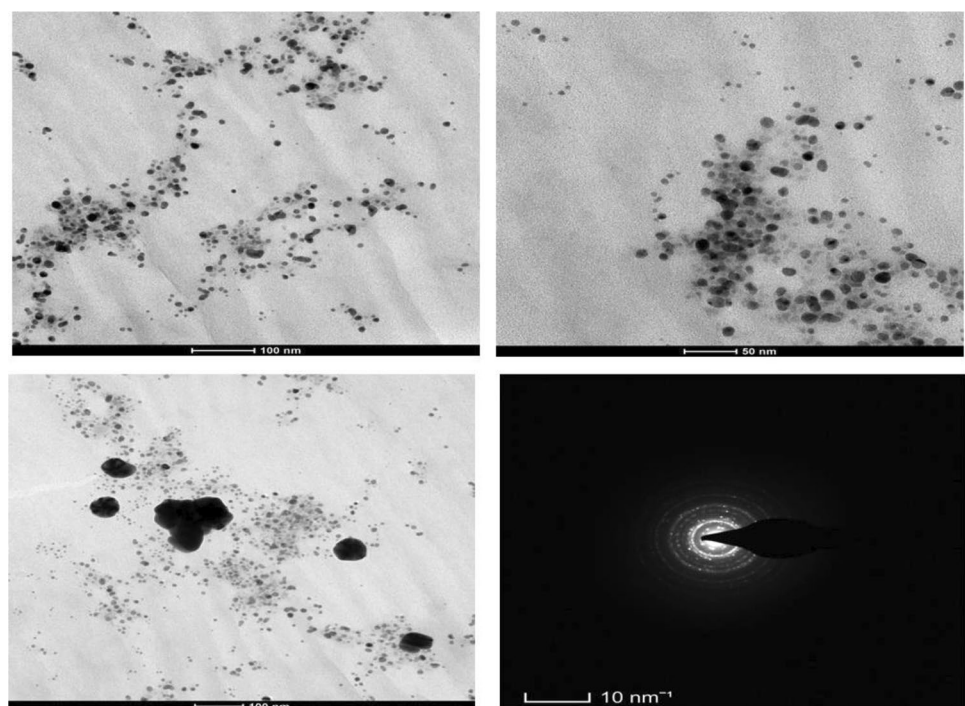
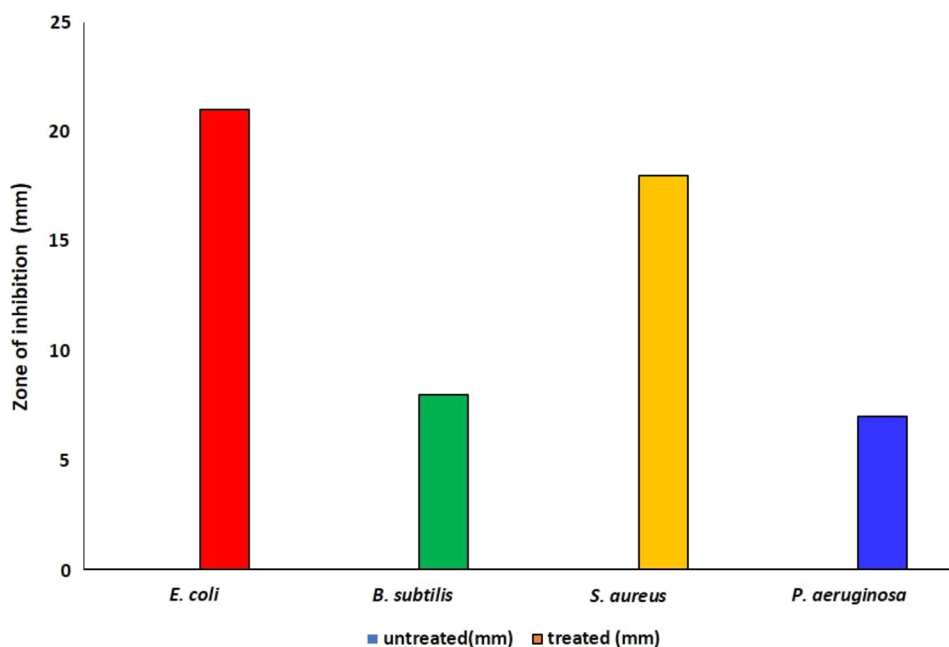


Fig. 5 Antimicrobial activity of cotton fabrics treated with and without Zirconium nanoparticles



Conclusion

Cotton fabrics with versatile features were effectively synthesized by coating cotton with ZrNPs. Importantly, ZrNPs treated cotton fabrics deemed to possess a robust antibacterial activity against both Gram-negative and Gram-positive bacteria. This can be implemented for coating textile fabrics to produce medically useful fabrics such as bandage gauze cloths, masks, gloves, surgical cloths, sanitary napkins, diapers, socks and other hospital fabric items. The functionalized cotton fabrics made ready in this study are effective to thwart and treat infections in the wound bed. Despite demanding further studies for clinical implementation, it portrays an immense scope for the development of expedient medical textiles for wound healing.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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