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Preparation and characterization of hybrid chitosan-silver nanoparticles (Chi-Ag NPs); A potential antibacterial agent

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ABSTRACT

In this study, a novel ecofriendly chitosan- silver nanoparticles hybrid was developed. Biological method using leaf extract of *T. portulacifolium* was used as reducing agent for its synthesis and the antibacterial efficiency of these hybrid nanoparticles were evaluated against the bacteria *E. coli* and *S. marcescens* organisms. The intense peak observed around 419 nm in the UV–Vis indicates the formation of silver nanoparticles. The XRD analysis showed that the hybrid chitosan-silver nanoparticles have a polycrystalline and face-centered cubic configuration. FTIR spectrum hybrid chitosan-silver nanoparticles indicated speaks vibration of N—H and O—H. The EDS analysis confirmed the presence of Ag, O, C and N elements in the prepared sample. The spherical shape was obtained from TEM analysis and it indicated that with average particles around 3.24 nm to 44.80 nm. The prepared hybrid chitosan-silver nanoparticles showed significant antibacterial activities against *E. coli* and *S. marcescens*. In addition, the surface membrane damages and surface morphology of test pathogens were visualized using FESEM analysis.

1. Introduction

Biopolymer coated nanoparticles have created a new insight towards medical, solar cells, sensors and biological applications [1–4]. The key methodology for the preparation of these nanoparticles is by physical [5,6] and chemical methods [7,8] which are expressively toxic and harmful to the environment [9,10]. Nanoparticles prepared using biological method is a rapid single step method, cost effective and ecofriendly, without any use of toxic chemicals and high-pressure equipment [11–14]. Currently, researches have shown that silver nanoparticles by biological method using floral extracts of medicinal plants results in an effective antibacterial activity due to their improved properties like size and shape of the particles [15]. Many polymers of biological source are preferred in various coating applications of nanomaterials of which chitosan are vastly used for its beneficial biological properties and polymeric capabilities [16–19]. A very few researches have reported the silver nanoparticles coated with chitosan prepared from green synthesis method [20–22]. Recently, prepared bovine serum albumin and chitosan coated silver nanoparticles exhibited strong antibacterial activity against oral bacterial and non-oral bacterial strains was reported by Cristobal et al. [23].

Identification of new effective drugs against pathogenic bacteria is a major challenge in modern medicine. New scientific approaches are combining materials with different mechanisms of antibacterial actions. Developing hybrid polymer coated nanomaterials deliver a new line against pathogenic bacteria [24]. Among the polymers the chitosan showed potential property with nanoparticles and Chitosan is a linear polymer of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine found from chitin. It showed low toxicity, biocompatibility, biodegradability, low immunogenicity and antibacterial activity [25]. As per our acquaintance, very few report on the hybrid chitosan-silver nanoparticles by green synthesis method. *Talinum portulacifolium* is a medicinal flora of the family Portulacaceae. It is used as the common leafy vegetable in many countries. The secondary metabolites screening of *T. portulacifolium* leaves extract exhibited the presence of active compounds (Crude proteins, lipids, flavonoids, phenols, and omega fatty acids) and pharmacological used as an analgesic and antioxidant [26–29]. Owing of the present study focus on preparation of hybrid chitosan-silver nanoparticles (Chi-Ag NPs) by biological method using *T. portulacifolium* leaf

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extract, its characterization and antibacterial properties against pathogenic bacteria (Graphical abstract 1) for the development of industrially important antibacterial nanomaterial.

2. Materials and methods

2.1. Materials

The *Talinum portulacifolium* (Forssk.) Asch. Ex Schweinf. Leaves were collected from Kavundampalayam (11.061562°N, 76.959414°E) north Coimbatore, Tamilnadu, India and taxonomic identification was done by Botanical Survey of India, TNAU (No. BSI/SRC/5/23/2019/Tech/3370). Silver nitrate ($AgNO_3$) and low molecular weight chitosan (degree of decetylation 75–58%) were purchased from HiMedia, India.

2.2. Phytochemical analysis

The preliminary phytochemical screening of *T. portulacifolium* leaf extract was done as per the standard methods of Goveas et al. [30].

2.3. Preparation of silver nanoparticles

The silver nanoparticles were synthesized by biological method using *T. portulacifolium* leaf extract as reducing agent. *T. portulacifolium* leaf extract and silver nitrate solution were used as precursors. In detail, 5% of *T. portulacifolium* leaves powder was added to 100 ml double distilled water taken in a 250 ml Erlenmeyer flask and was heated at 70 °C for 20 min. The obtained aqueous extract was filtered using Whatman No.1 filter paper and the filtrate was stored at 4 °C. About 10 ml of *T. portulacifolium* leaf extract was added into 90 ml of 1 mM silver nitrate solution and the reaction mixture was incubated at 37 °C for 2 h. This mixture solution resulted to form of dark brown coloured solution indicating the presence of silver nanoparticles.

2.4. Preparation of hybrid Chi-Ag NPs

The (50 mg) chitosan was added to a 20 ml of (0.1 M) acetic acid. The 20 ml of chitosan solution was taken in the burette and was added drop wise into the prepared silver nanoparticles solution. It was then kept in the magnetic stirrer for 20 mins for the development of hybrid chitosan silver nanoparticles (Chi-AgNPs). The obtained hybrid Chi-Ag NPs were washed to with double distilled water. The prepared material was centrifuged at 5000 rpm for 15 min and dried at 40 °C for 1 h to get the final product.

2.5. Characterization of hybrid Chi-Ag NPs

2.5.1. UV–Vis spectrum of silver nanoparticles

The bio reduction of silver ions in *T. portulacifolium* leaf extract solution was observed by periodic sampling of aliquots (0.5 ml) and subsequently measuring UV–Vis spectra of the solution in UV–Vis spectrophotometer (JASCO, V-670-Japan) at a range of 200 to 800 nm.

2.5.2. X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) measurement of the prepared hybrid Chi-AgNPs was carried out using X'Pert Pro X-ray diffractometer (PAN analytical BV, The Netherlands) equipped with Cu/Kα radiation source using Ni as filter at a setting of 30 kV/30 mA. All X-ray diffraction data were collected under the experimental conditions in the regular 2θ angular range.

2.5.3. Field emission scanning electron microscopy and energy dispersive X-ray diffraction spectroscopy microanalysis (FESEM-EDS)

The surface morphology of the prepared Chi-AgNPs was examined using Field Emission Scanning electron Microscopy (HITACHI SU6000 FESEM). The elemental composition analysis of the synthesized nanoparticles was analyzed using HITACHI SU6000 SEM equipped with an EDS attachment.

2.5.4. Transmission electron microscopy (TEM) analysis

Transmission electron microscopy analysis, (PHILIPS, CM 200, Operating voltages: 20200kv Resolution: 2.4 Å) was used to evaluate the individual size and shape of the hybrid Chi-AgNPs.

Table 1
Phytochemical screening of *T. portulacifolium* aqueous leaf extract.

Phytochemicals	GA-AE
Alkaloids	+
Phenolics	+
Saponins	+
Flavonoids	+
Steroids	+
Tannins	+

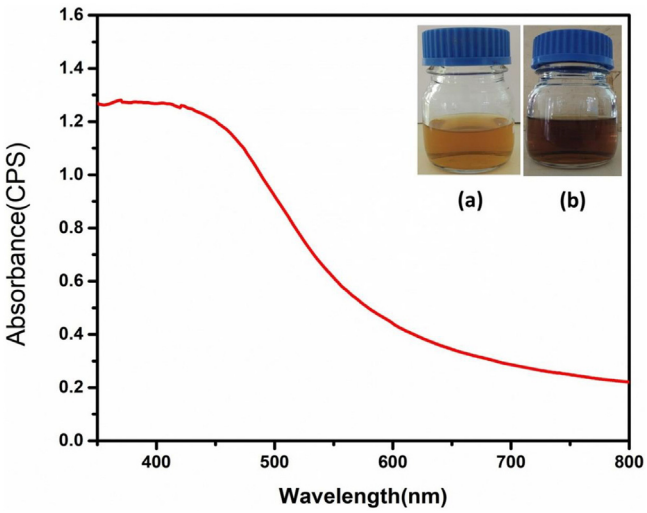


Fig. 1. UV–Vis spectrum of the *T. portulacifolium* assisted silver nanoparticles.

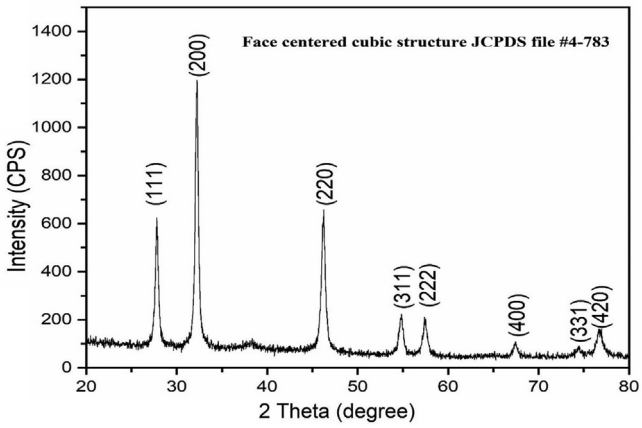


Fig. 2. XRD analysis of hybrid Chi-Ag NPs.

2.5.5. Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared (FTIR) spectroscopic analysis was done to analyze the bonding of synthesized nanoparticles with the functional groups of *T. portulacifolium* leaf extract through bridging linkage. FTIR spectra for synthesized nanoparticles was recorded on a Shimadzu FTIR spectrometer 8000 series, with a sample as KBr pellet method in the wavenumber region of 4000–400 cm^{-1}.

2.6. Antibacterial activity

The antibacterial activity of hybrid Chi-AgNPs were tested against *Escherichia coli* and *Serratia marcescens* by standard agar well diffusion method. The clinical isolated Gram-negative bacteria *E. coli* and *S. marcescens* were obtained from the Aravind eye hospital, Coimbatore, Tamilnadu, India. A swab of pathogenic *E. coli* and *S. marcescens* (1.5×10^8 CFU/ml) was done on an agar plate of MHA medium. The hybrid Chi-Ag NPs stock solution was prepared in sterile distilled water at the concentration of 1 mg/1 ml. Wells of 5 mm in diameter were made on agar plates using a well puncher and the wells were loaded with different concentrations of 10, 30and 50 μL of hybrid Chi-Ag NPs. The plates were incubated for 24 h at 37 °C and the antimicrobial activity was evaluated by measuring the zone of inhibition around the wells.

In addition, to analyze the surface membrane modifications of the bacterial strains, control (untreated) and hybrid Chi-Ag NPs treated bacterial cultures were observed using FESEM (SIGMA HV - Carl Zeiss with Bruker Quantax 200 - Z10 EDS Detector up to 1 nm). Overnight cultures of *E. coli* and *S. marcescens* were incubated with 50 μg/ml concentration of hybrid Chi-AgNPs for 3 h and untreated cultures served as control. After incubation the cells were collected by centrifugation, washed twice with 0.85% NaCl, and fixed with 2% glutaraldehyde at room temperature (~25 °C). The cells were resuspended in double distilled water and dehydrated on silicon substrate chips.

3. Results and discussion

3.1. Phytochemical analysis

The preliminary phytochemical analysis of *T. portulacifolium* leaf extract showed the presence of phytochemicals such as alkaloids, phenolics, saponins, flavonoids, tannins and steroids (Table 1). In agreement with our results the previous reports states that the phytocompounds from *T. portulacifolium* leaf extract showed the presence of phenolic content and it exhibits antioxidant and analgesic activity [31].

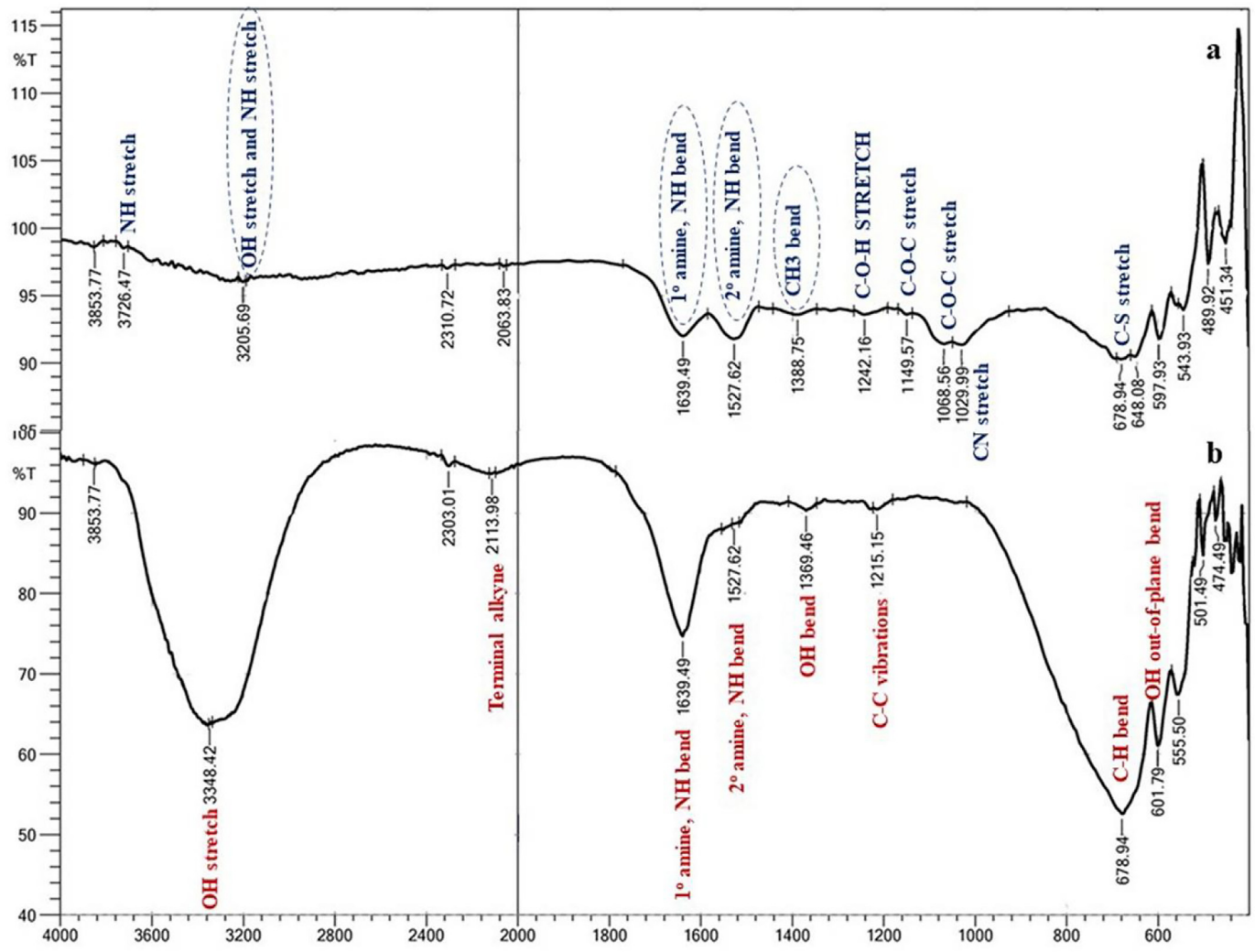


Fig. 3. (A) FTIR analysis of hybrid Chi-Ag NPs, (B) FTIR analysis of plant extract.

3.2. UV–Vis spectrum of silver nanoparticles

The absorbance spectrum of silver nanoparticles prepared by green synthesis method using *T. portulacifolium* leaf extract is shown in Fig. 1. The absorbance peak characteristic for silver nanoparticles was obtained at 419 nm [32]. The consequent colour changes were observed from a light yellow to a brown colour (Fig. 1a, b). The presence of various phytocompounds from *T. portulacifolium* leaf extract possess the ability to reduce silver ions (Ag^{+}) to silver nanoparticles (Ag°). The previous study supports our results of bioreduction of silver nanoparticles from plant leaf extracts [33,34].

3.3. XRD analysis of hybrid Chi-AgNPs

The crystalline structure of the prepared hybrid Chi-AgNPs were studied using X-ray diffraction profile (Fig. 2). The major characterization peaks were found at 27, 32, 46, 54, 57, 67, 74 and 76° respectively corresponding in to (111), (200), (220), (311), (222), (400), (331) orientation planes. The XRD pattern showed that the hybrid Chi-AgNPs have a crystalline and face-centered cubic (FCC) configuration in nature [35,36]. The observed diffraction pattern of hybrid Chi-AgNPs is consistent with standard JCPDS file #4-783. The crystallite size of the hybrid Chi-AgNPs was calculated by the Scherrer equation [37].

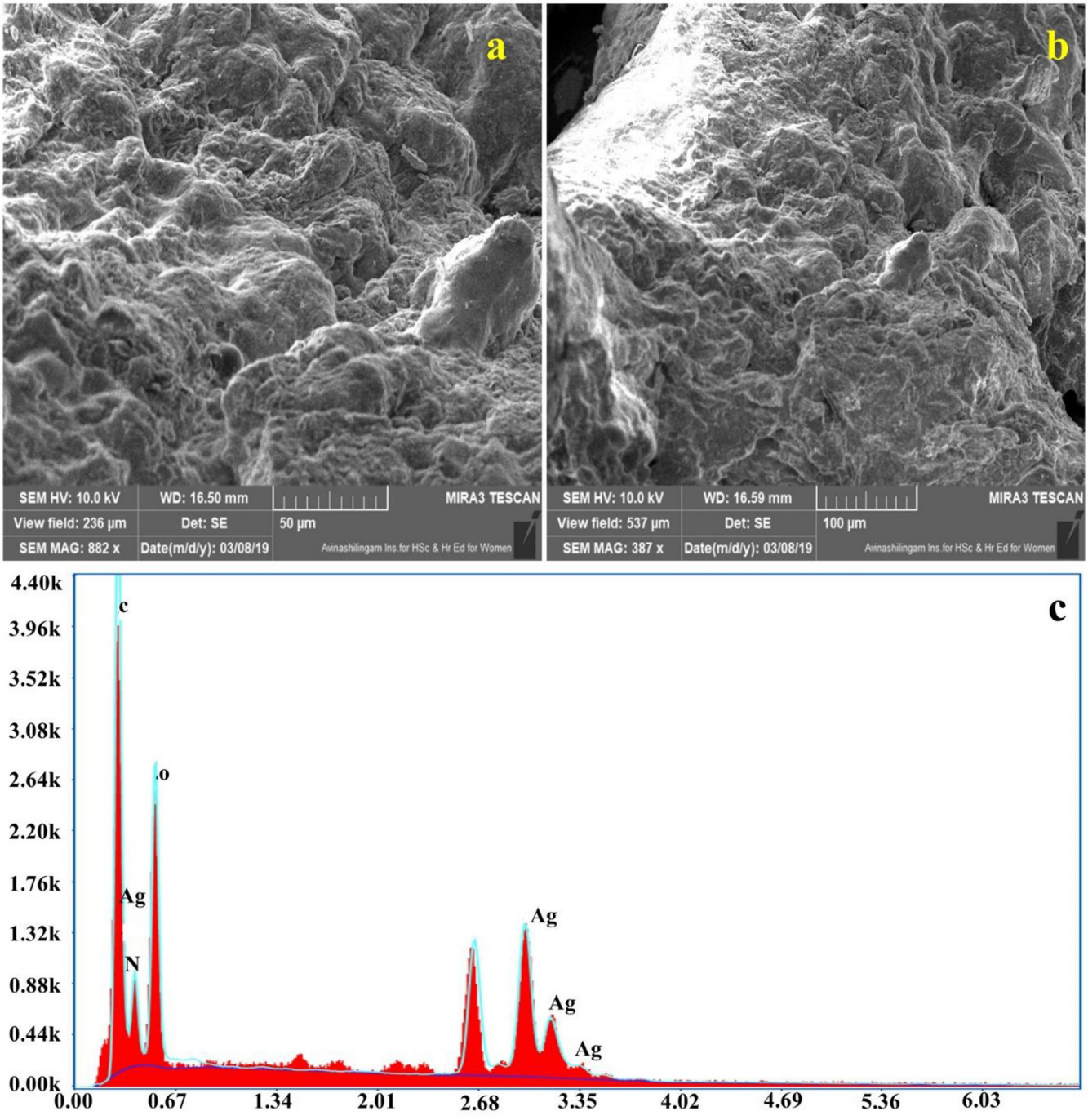


Fig. 4. (a-b) FESEM analysis of hybrid Chi-Ag NPs and (c) EDS analysis of hybrid Chi-Ag NPs.

$$D = \frac{K\lambda}{cos\theta}$$

where D = average crystallite size, K - shape constant, λ - wavelength of X-ray, β - Full Width Half Maximum (FWHM) of refection (in radians) located at 2θ and θ - angle of reflection (in degrees) was used to relate the crystallite size to the line broadening. The average crystallite size was found to be about 3 to 44 nm.

3.4. FT-IR spectral analysis of hybrid Chi-AgNPs

FTIR spectrum of *T. portulacifolium* leaf extract and hybrid Chi-AgNPs were showed in Fig. 3(a, b). The *T. portulacifolium* leaf extract exhibited the several peaks as an indication of extract in complex nature. The peaks 3348.42 cm^{-1} (O—H stretch), 2113.98 cm^{-1} (Terminal alkyne [monosubstituted]), 1639.49 cm^{-1} (Primary amine, N—H bend), 1527.62 cm^{-1} (Secondary amine, N—H bend), 1369.46 cm^{-1} (O—H bend), 1215.15 cm^{-1} (Skeletal C—C vibrations [Special methyl $-CH_3$ frequencies]), 678.94 cm^{-1} (Alkyne C—H bend), 601.79 (O—H out-of-plane bend), 555.50 cm^{-1} (Aliphatic iodo compounds, C—I stretch), 501.49 (Aliphatic iodo compounds, C—I stretch). The observed IR bands at 474.49 cm^{-1} are characteristic bands of phenolics and flavonoids that are abundant in dried *T. portulacifolium* leaf extract. This suggests the presence of phenolics and flavonoids may be that of acting as a reducing agent of silver nanoparticles.

The hybrid Chi-AgNPs showed absorbance peaks at 3723.47 cm^{-1} (N—H stretch intramolecular hydrogen bonds), 3205.69 cm^{-1} (H-bonded O—H stretch and N—H stretch intramolecular hydrogen bonds) [38], 2063.83 (metal carbonyls, S—H thiols), 1639.49 cm^{-1}(Primary amine, N—H bend) [39], 1527.62 cm^{-1} (Secondary amine, N—H bend), 1388.75 cm^{-1} (Methylene = CH_2 CH_3 bend) 1242.16 cm^{-1} (C-O-H stretch), 1149.57 cm^{-1} (asymmetric stretch C-O-C), 1068.56 cm^{-1} (symmetric stretch C-O-C), 1029.99 cm^{-1} (CN stretch Secondary amino), 678.94 cm^{-1} (C—S stretch), 648.08 cm^{-1} (C—S stretch), 597.93 cm^{-1}(Alcohol, OH out-of-plane bend), 543.93 cm^{-1}C-I stretch, 489.92 cm^{-1} (S—S stretch) and 451.34 cm^{-1} (S—S stretch).

The peaks at 1639.49 cm^{-1} correspond to N—H stretch, which are characteristic to polysaccharides proving the presence of chi-

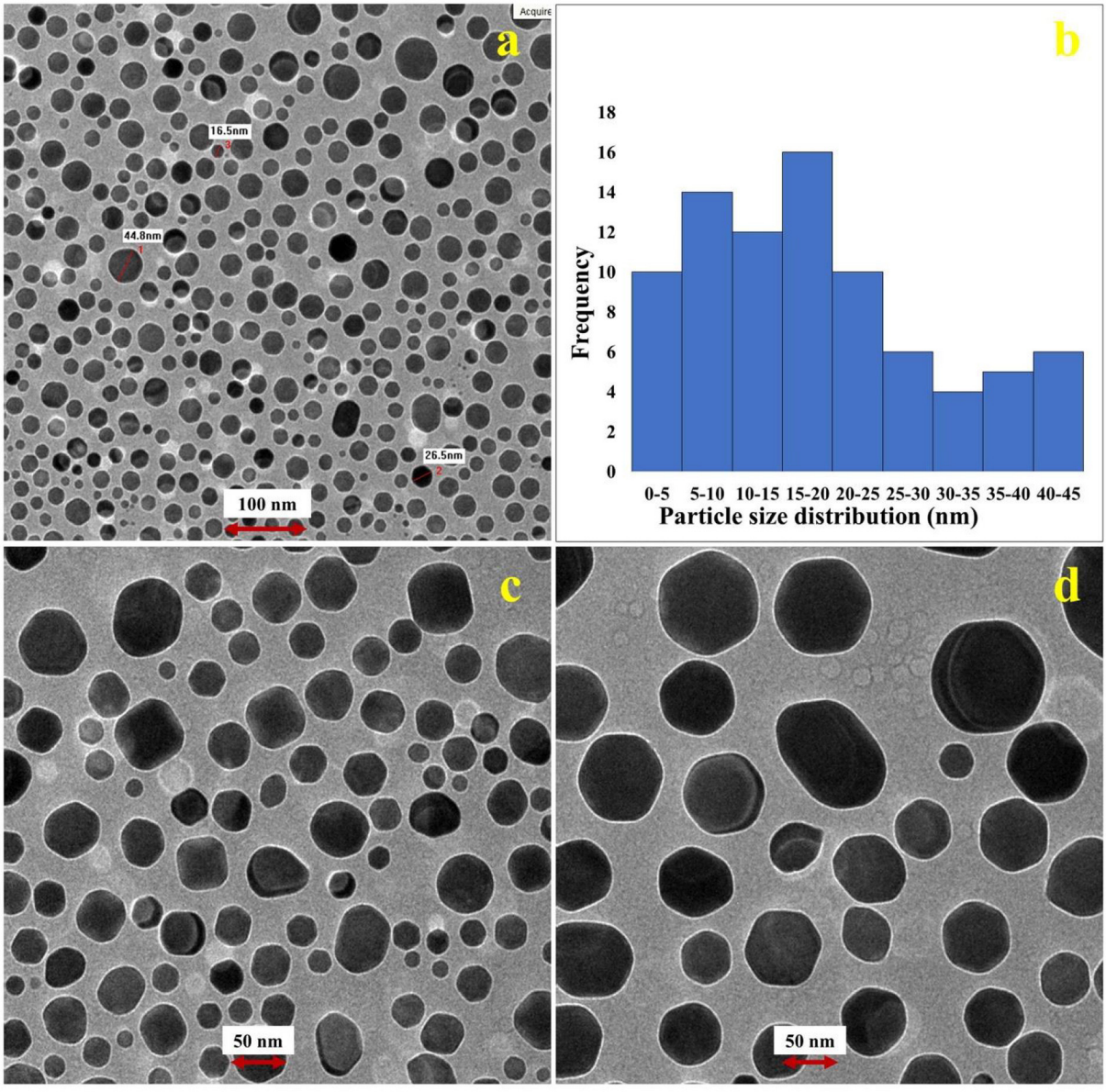


Fig. 5. TEM analysis of hybrid Chi-Ag NPs.

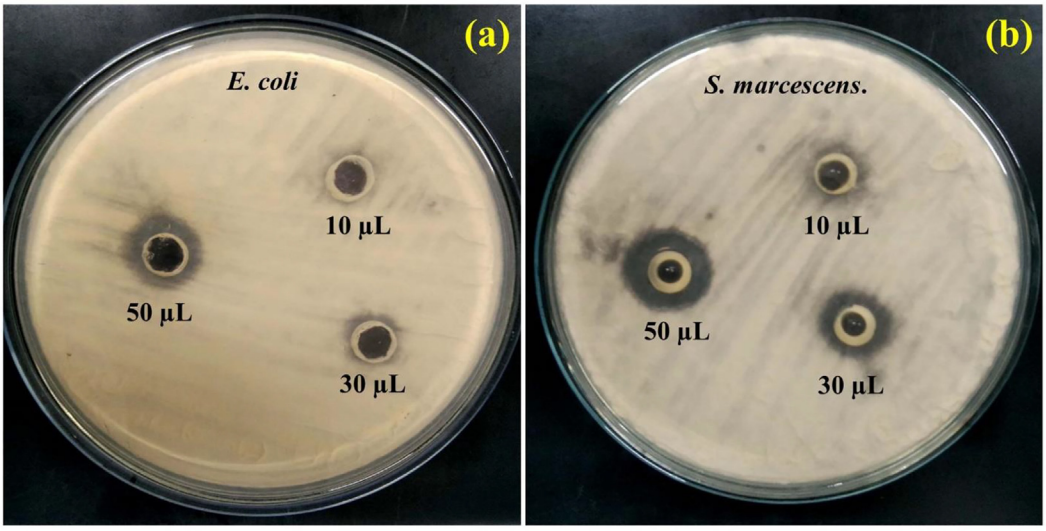


Fig. 6. Antibacterial activity of hybrid Chi-Ag NPs against (a) E. coli and (b) S. marcescens.

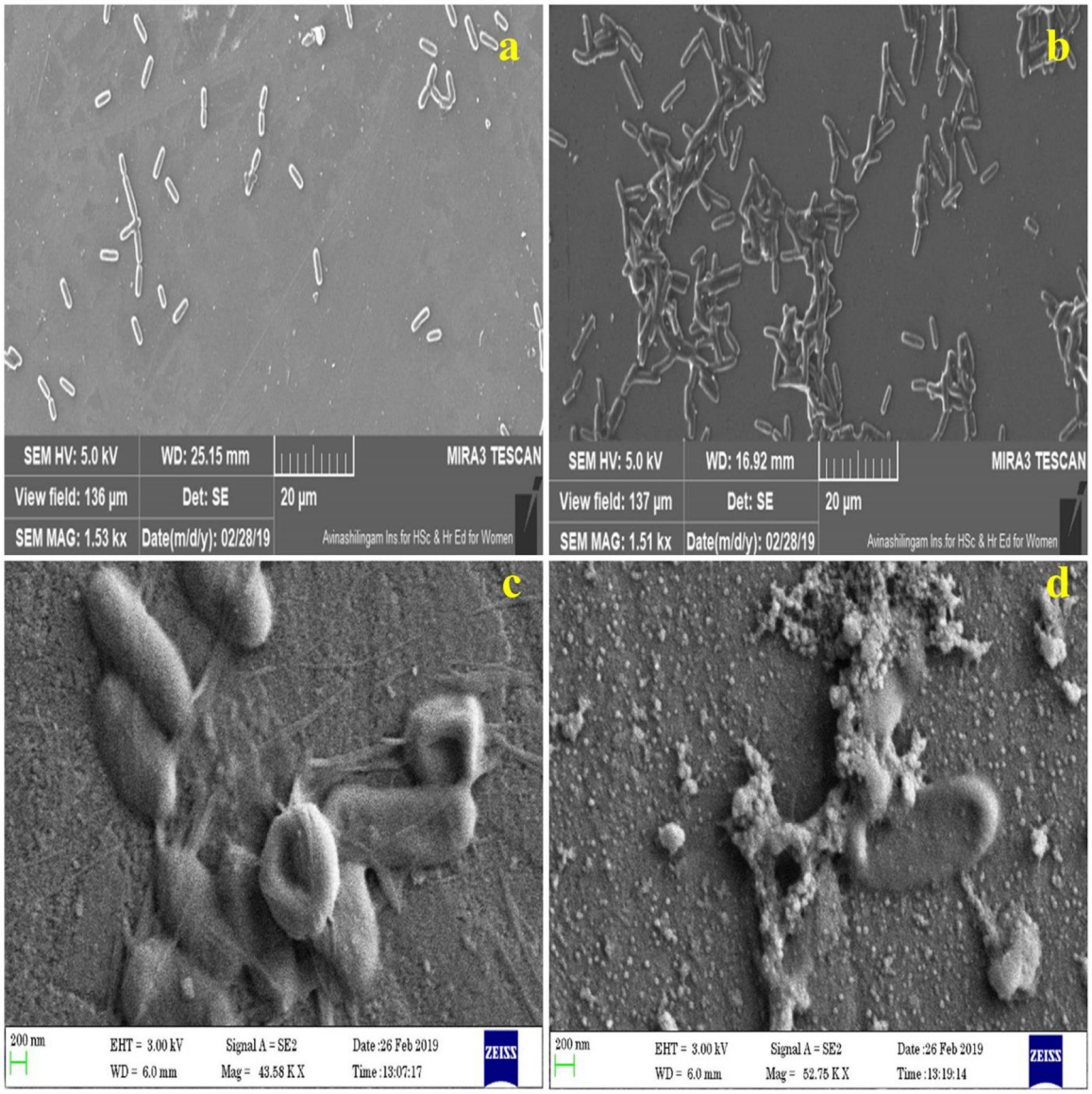


Fig. 7. FESEM image of hybrid Chi-Ag NPs treated and untreated pathogens. (a) E. coli and (c) S. marcescens control untreated pathogens. (b) E. coli and (d) S. marcescens hybrid Chi-Ag NPs treated pathogen.

tosan [40]. They are further confirmed using the characteristic peaks corresponding to the primary amine, secondary amine and Methylene (=CH_2). Similar peaks were obtained in the previous work of Solmaz Akmazet al. [41].

3.5. FESEM-EDS analysis of hybrid Chi-AgNPs

The surface morphology of prepared nanoparticles was determined by field emission scanning electron microscopy and elemental composition of prepared nanoparticles were determined by energy dispersive spectroscopy. The prepared hybrid Chi-AgNPs shows predominantly spherical shape with high aggregation, this may be due to the different quantity and nature of capping agents present in the prepared *T. portulacifolium* leaf extract [42]. The chitosan was strongly bound on the surface of the prepared silvernanoparticles (Fig. 4a, b). Similar observations were noticed in silver based chitosan bionanocomposite synthesized by using *Saccharum officinarum* extract [22].

Furthermore, analysis also confirmed the presence of silver nanoparticles on the chitosan suspension (Fig. 4c). The EDS spectrum of prepared silver nanoparticles and chitosan-silver nanoparticles showed maximum peaks around 3.29 keV corresponding to the binding energies of silver ions. In addition, Fig. 4c prepared hybrid Chi-AgNPs showed the absorbance peak of nitrogen (N) in the EDS spectrum due to the excitation of X-ray from chitosan biopolymer. The presence of nitrogen was supposed to arise from the amine (-NH_2) group of chitosan [44]. Similar findings were stated by Bharathi et al. [45] who have reported the presence of chitosan/iron oxide nanocomposite.

3.6. TEM analysis of hybrid Chi-AgNPs

The size and shape of the prepared hybrid Chi-AgNPs were determined by the TEM measurement. The obtained TEM image revealed that the prepared hybrid Chi-AgNPs are predominantly uniform in size and most of the particles are predominantly spherical in shape and few hexagonal shape with less agglomeration. The synthesized particles size ranged from 3.24 nm to 44.80 nm (Fig. 5). This is reasonable to conclude that the *T. portulacifolium* extract contains phytocompounds that prevent the agglomeration of the nanoparticles [46].

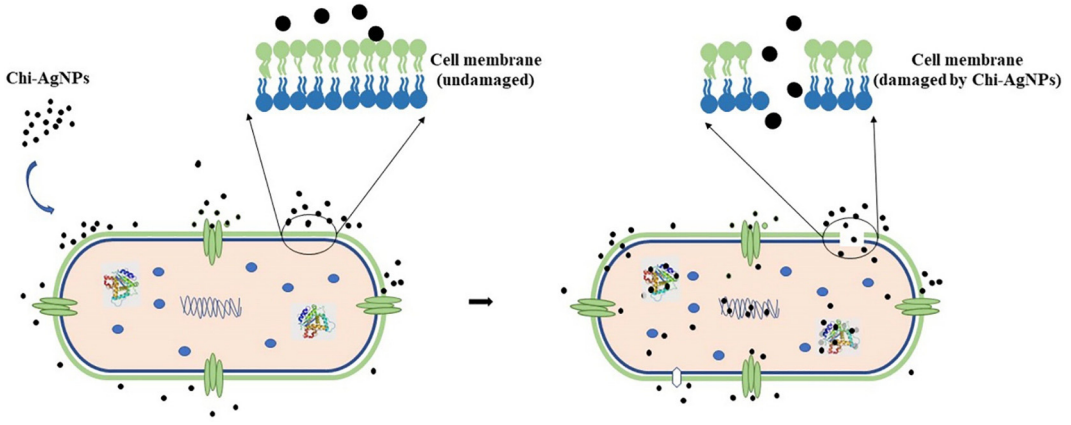
3.7. Antibacterial activity of hybrid Chi-AgNPs

The antibacterial activity of hybrid Chi-AgNPs against *E. coli* and *S. marcescens* were evaluated using by standard agar well diffusion method. In this present study, we observed the maximum activity of prepared hybrid Chi-AgNPs shown antibacterial activity around 20 mm against *S. marcescens*, followed by *E. coli* around 15 mm at the concentration of 50 μL. Whereas 10 and 30 μL concentration showed moderate activity (0 and 4 mm) against *E. coli* and 6 and 9 mm against *S. marcescens* respectively (Fig. 6). The antibacterial activity of hybrid Chi- Chi-AgNPs against *E. coli* and *S. marcescens* is concentration dependent manner.

Additionally, the morphological changes of hybrid Chi-AgNPs treated and untreated *E. coli* and *S. marcescens* were observed under FESEM analysis (Fig. 7). Fig. 7 a no significant morphological changes were observed in untreated *E. coli* and *S. marcescens*, where hybrid Chi-AgNPs treated at the concentration of 50 μL exhibited significant morphological changes against both tested organisms (Fig. 7b). This is due to the disruption by prepared hybrid Chi-AgNPs on the membrane surface of tested organisms by cell membrane damage (Scheme 1) leading to cell death [47,48]. This is evident to conclude that the prepared hybrid Chi-AgNPs exhibits a strong antibacterial activity which can be used as antimicrobial agent for biomedical and food industrial applications.

4. Conclusion

In the present study hybrid Chi-AgNPs were prepared effectively by ecofriendly and green synthesis method. The *T. portulacifolium* leaf extract was used as a reducing agent for the synthesis of silver nanoparticles. The absorbance peak at 419 nm in the UV–Vis spectrum specified the formation of silver nanoparticles. The FTIR spectrum of hybrid Chi-AgNPs showed the presence of silver and chitosan main chains. The EDS composition analysis confirmed the presence of Ag, O, C and N elements in the prepared sample. XRD and TEM analysis revealed that the hybrid Chi-AgNPs are spherical in shape having cubic fluorite structure with average particles size between 3.24 nm to 44.80 nm. The prepared hybrid Chi-AgNPs at the concentration of 50 μL showed significant morphological changes against *E. coli* and *S. marcescens* bacteria by the direct contact of hybrid Chi-AgNPs on the membrane surface of the organisms.



Scheme 1. A Schematic model representing the mechanism of hybrid Chi-Ag NPs inhibition of bacterial growth (A) E.coli (B) S. marcescens. Transport of hybrid Chi-Ag NPs inside the bacterial cell. Initial deposition of hybrid Chi-Ag NPs onto the bacterial cell surface, interact directly with bacterial cell surfaces. Finally, the hybrid Chi-Ag NPs were damage the bacterial cell wall.

In conclusion experimental results showed that the hybrid Chi-AgNPs prepared by green synthesis has gaining demand in the field of nanotechnology. These chitosan hybrid silver nanoparticles with antimicrobial potency may be exploited for several biomedical applications including development of catheters, antimicrobial gel formulations, food packaging ingredients, water purifications and so on. In future, chitosan combined with silver nanoparticles will be used as a possible tool to fight against rapidly spreading antibiotic resistance.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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