Synthesis and bio-physical characterization of Silver nanoparticle and Ag-mesoporous MnO₂ nanocomposite for anti-microbial and anti-cancer activity

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A B S T R A C T

In the present study, a unique precursor source and plant extract have been developed for biogenic synthesis of silver nanoparticles (AgNPs) using different silver precursor solutions. Mesoporous-MnO₂ nanocomposites (before doping AgNPs in meso-MnO₂) were prepared using anion-ionic surfactant (Triton X-100) and the antibacterial activity of the prepared sample was analyzed against various bacterial pathogens. The plant leaf extract of Alternanthera bettzickiana was used as a reducing agent. A fast ultra-sonication-assisted process facilitated the incorporation of AgNPs on mesoporous MnO₂. Different type of silver precursor has been used to prepare the bio- genic AgNPs for the first time. Particle sizes of AgNPs were obtained below 5–10 nm. Surface area, morphology, crystalline phase, and particle size of the synthesized nanoparticles were characterized by X-ray diffraction (XRD), Brunauer, Emmett, Teller (BET)/Barrett, Joyner, Halenda (BJH) method, Scanning Electron Microscopy (SEM), and high-resolution transmission electron microscopy (HR-TEM) techniques. The antibacterial activity of biogenic AgNPs and AgNPs@meso-MnO₂ were tested against gram-positive (Staphylococcus aureus and Streptococcus mutans) and gram-negative (Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa) bacteria using the well diffusion assay. All samples including biogenic AgNPs and AgNPs@meso-MnO₂ showed effective inhibition zones against all pathogens and no activity was observed for control and bulk meso-MnO₂. Preliminary studies related to the anti-cancer activity of the prepared AgNPs have also been studied and compared for different route prepared silver nanoparticles.

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1. Introduction

Toxin-free and biogenic methods of synthesizing silver nanoparticles (AgNPs) using plant leaf extracts is a healthy and economical way for the mass production of AgNPs [1]. Alternanthera bettzickiana (Regel) G. Nicholson is a plant that belongs to the Amaranthaceae family. It is used as an edible vegetable in Southeast Asia and India [2a,b]. The present study aims at evaluating the antibacterial activity of biogenic synthesized AgNPs using the leaf extract of A. bettzickiana. Methicillin-resistant pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium tuberculosis, and vancomycin-resistant pathogens such as Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter sp., Acinetobacter and Escherichia coli are referred to as superbugs due to their resistance to multiple antibiotics [3–6]. Mobile genetic elements like plasmids, integrons, and transposons carry antibiotic resistance genes and their transfer results in immediate multidrug resistance in recipient strains [5–8]. This allows certain pathogenic bacteria to thrive in hostile, antibiotic-laden environments. AgNPs are promising because of their unique antimicrobial property and hence, their synthesis is of great interest for the development of novel pharmaceutical products [9,10]. Though several NPs are used as antimicrobial agents, AgNP is one of the most powerful, natural, antimicrobial agents that prevent serious infections [11]. Synthesis of AgNPs from leaf extracts has been studied and their antibacterial activity along with the mechanism of action have been investigated in different types of pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, and Staphylococcus epidermidis [12,13]. Recent reports have shown that controlled-size AgNPs and their doped forms possess high antibacterial and photo-catalytic activities. Accordingly, AgNPs have attracted the attention of medical researchers and microbiologists [14–16]. AgNPs have been shown to exhibit interesting electro catalytic and biosensing properties [14,16–19]. High surface-to-volume ratio and particle size of AgNPs allow them to interact with microbial membranes of different pathogens. The antibacterial property of AgNPs is exploited for many applications in a wide range of fields, including medicine and...
dentistry [20–23]. NPs are capable of penetrating bacterial cells and by interacting with the thiol group of proteins, act as catalysts to inactivate enzymes that are needed for their metabolism [16] and affect DNA replication [24–26]. Metal NP or AgNP in its free ionic form is highly toxic to human cells. It has been demonstrated that doping of silver (Ag) with metal oxides decreases the toxic property of free Ag in human cells. It is therefore advantageous to use Ag-doped metal oxide NPs in antibacterial applications [12]. Porous, nanostructured, doped and undoped manganese oxide (MnO2) is reported to be effective in catalysis, energy storage battery materials, and is prepared from materials by low-cost techniques [14,27]. Cerium (III) doped MnO2 with a tunnel structure and made of materials with porous morphology are reported to be effective in removing phenolic compounds from waste water [28]. Hence, in the present study, we prepared mesoporous-MnO2 by a non-ionic surfactant assisted method and used it as a support to incorporate biogenic AgNPs. Leaf extract from the edible plant A. bettzickiana was used for the biogenic synthesis AgNPs. Additionally, the comparative antimicrobial activity of all the samples along with preliminary studies assessing the anti-cancer activity was studied using the biogenic synthesized AgNPs. The enhanced antibacterial activity observed for both gram positive and gram negative type of bacterial pathogens. Promising anticancer activity observed for trace level concentration of silver nanoparticle in human cell lines.

2. Experimental

2.1. Materials

Silver nitrate (AgNO3) A.R. grade was purchased from Sigma Aldrich and silver sulphate (AgSO4) from Riedel-de Haen AG (made in Germany). Chemicals for the syntheses of mesoporous MnO2 were purchased and used without further purification. Manganese sulphate (MnSO4), Triton X-100, potassium persulfate, and ammonia were used without further purification. Shimadzu UV-1601 PC scanning double beams ultraviolet (UV)-visible (vis) spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used for the study. The physicochemical characterization of the catalysts was carried out by X-ray diffraction (XRD) (Miniflex 600), scanning electron microscopy (SEM) (JSM-T220A, JEOL), transmission electron microscope (TEM) (JEOL-JEM-2100F) and N2-sorption isotherms (NOVA 2200e).

2.2. Plant collection and identification

The leaves of the plant A. bettzickiana were collected from the campus of King Saud University, Riyadh, Saudi Arabia. The collected plant leaves were submitted to Dr. Jacop Thamas (Curator Herbarium Unit, Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia), who confirmed the identity of the same (Fig. 1).

2.3. Aqueous extract preparation

The collected leaves were washed with de-ionized water for 5 min and 10 g of washed and finely chopped leaves were added into a 300 mL Erlenmeyer flask filled with 100 mL of sterilized double distilled water (1:10 ratio). The mixture was then boiled for 5 min and decanted. The solution was filtered with Whatman filter paper. The extracts were stored at −4 °C for further use. The collected leaves were washed with running water for 5 min. For preparing the aqueous leaf extract, 10 g of washed and finely chopped leaves were added into a 300 mL.

Fig. 1. X-ray diffraction patterns of AgNPs (a) Nitrate precursor (b) Sulphate precursor (c) AgNPs@MnOx (d) UV–Vis spectrometry analysis AgNPs prepared from nitrate and source.
Erlenmeyer flask filled with 100 mL of sterilized double distilled water (1:10 ratio). The mixture was then boiled for 5 min and decanted. The aqueous extract was filtered with Whatman filter paper (grade 1) and stored at −4 °C for further study.

2.4. Biogenic synthesis of AgNPs

For preparing 0.01 M AgNO₃ solution, 17 mg of AgNO₃ was weighed and added to a beaker containing 100 mL of deionized distilled water. Under optimized conditions, one part of the leaf extract was mixed with nine parts of Ag precursor solution (ratio between extract and AgNO₃ was maintained at 1:9) in an Erlenmeyer flask and allowed to react at 75 °C without any disruption. After 15 min, the color of the solution changes into brown representing the formation of AgNPs. The absorbance of this brown colored solution was measured by UV–vis spectrometric analysis. The solution containing the synthesized AgNPs from different precursor sources, such as 0.01 M AgNO₃ and 0.01 M AgNO₃, were slowly dried by evaporation and used for further studies. The same procedure was used to synthesize biogenic AgNPs from different precursor sources, such as 0.01 M AgNO₃ and 0.01 M AgNO₃.

2.5. Synthesis of Ag@meso-MnO₂ by ultrasonication

AgNP-doped mesoporous MnO₂ was prepared by a novel route not reported previously. Firstly, precipitation was carried out using MnSO₄·H₂O, Triton X-100 as a non-ionic surfactant, ammonium persulphate (NH₄HSO₅) as the oxidizing agent, and ammonia as a directing agent. Triton X-100 (2 mL) was dissolved in a minimum amount of deionized water (240 mL) and stirred continuously for 60 min, followed by the addition of 0.1 M MnSO₄ dissolved in 50 mL of deionized water. Then, 0.1 M NH₄HSO₅ was added to MnSO₄ solution and stirred vigorously for 60 min. After complete mixing, 40 mL of ammonia solution was added drop wise and stirred vigorously until the completion of precipitation. After 12 h of continuous stirring, the solution was filtered and dried at 120 °C to remove volatile impurities. The dried meso-MnO₂ was calcined at 400 °C for 3 h, for the complete removal of the surfactant and was used as a support for Ag incorporation. After 10 min of ultrasonication, the prepared meso-MnO₂ was poured in water and mixed with biogenic prepared AgNPs dispersed solution. Ultrasonication was continued for another 10 min. The solvent was then evaporated under infrared light for further analysis. The material prepared by the above method is designated as Ag@meso-MnO₂.

2.6. Bacterial strains

For assessing the antibacterial activity of the doped AgNPs, three gram-negative bacteria (Salmonella typhi, Escherichia coli and P. aeruginosa) and two gram-positive bacteria (Staphylococcus aureus and Streptococcus mutans) were used. All cultures were obtained from King Abdul-Aziz medical hospital, Riyadh, Saudi Arabia. The cultures were stored at 4 °C for further study. For determining the antibacterial activity, bacterial young cultures (18 h) were prepared from this stock culture.

2.7. Determination of antibacterial activity

An agar well diffusion method was used to assay the effect of the synthesized doped nanoparticle (NP) samples with non-doped materials against the above-mentioned five bacteria. Mueller-Hinton agar plates were prepared and inoculated with 1 mL (1.0 × 10⁷ colony-forming units) of 18 h young bacterial cultures by spread plate method using a sterile swab [29a]. In each plate, three wells (6 mm) were made using a sterile borer. Test solution at a concentration of 1 mg/mL was prepared. Various concentrations of doped NPs, 5 μL, 10 μL, 25 μL, 50 μL, and 100 μL (25–100 μg/mL⁻¹) were added into well number 1, 2, and 3 for each bacteria. Six wells were made in a culture plate for AgNPs prepared from nitrate and sulphate sources. In the case of Ag doped meso-MnO₂, only three different concentrations were tested. All the plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibitory zones around the wells in each plate was measured by scale in millimeters. The results were tabulated and compared with crude plant extract, AgNO₃ solution and biogenic synthesized AgNPs. Each test was performed three times for confirmation of results.

2.8. Determination of colorectal cancer activity of biogenic synthesized AgNPs

2.8.1. Cell culture

Human HT-29 and SW620 colon cancer cell lines were obtained from the American Type Culture Collection (ATCC). The cells were grown in RPMI (Invitrogen) containing 10% heat-inactivated fetal bovine serum, 100 μg/mL streptomycin, 100 units/mL penicillin, and 2 mM L-glutamine and were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

2.8.2. Cell viability assay

Cell viability was assessed by MTT assay [29b]. Briefly, HT-29 and SW620 cells, 5 × 10³ cells/well were seeded in a 96-well plate for 24 h. AgNPs solved by sonication in ethanol (1 mg/mL) at varying concentrations (0, 2.5 μM, 5 μM, 7.5 μM, and 10 μM) was added to the cells and incubated for 24 h. After incubation, 10 μL of MTT (5 mg/mL) was added into each well for 2–4 h. The insoluble formazan crystals formed were dissolved in dimethylsulfoxide (DMSO) and quantified with a microplate reader (ELX800, BioTek, USA) at 540 nm. Survival rate percentage was measured using the formula: (Absorbance of treated sample)/(Absorbance of control) × 100.

3. Results and discussion

3.1. Physico-chemical characterization of AgNPs prepared by different precursors and Ag@meso-MnO₂

XRD pattern of the different precursor route prepared AgNPs and Ag@meso-MnO₂ are shown in Fig. 1a–c. A number of Bragg reflection peaks were observed at 20 values of 27.81°, 32.16°, 38.12°, 44.3°, 46.21°, 54.83°, 57.39°, 64.42° and 77.45° which are indexed to (210), (122), (111), (200), (231), (142), (241), (220) and (311) planes of biogenic method produced AgNPs and it forms based on the face-centered cubic structure and was matched with the JCPDS, file No. 04-0783 [30]. The XRD results confirmed that the AgNPs synthesized by the plant extract are pure and crystalline in nature. The size of AgNPs was calculated using Debye-Scherer’s equation $D = 0.9λ/β\cosθ$, where $D$ is the

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample name</th>
<th>Crystallite size (nm)</th>
<th>BET (m²/g)</th>
<th>Pore volume (cc/g)</th>
<th>Pore size (nm)</th>
<th>Elemental composition (at%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesoporous MnO₂</td>
<td>17.3</td>
<td>65</td>
<td>3.36 × 10⁻²</td>
<td>2.2</td>
<td>22.56</td>
</tr>
<tr>
<td>2</td>
<td>AgNPs@ MesoMnO₂ nanocomposite</td>
<td>14.4</td>
<td>55</td>
<td>2.93 × 10⁻²</td>
<td>2.8</td>
<td>21.29</td>
</tr>
</tbody>
</table>

Table 1

The BET surface and pore size, average crystallite size values of the meso-MnO₂ and AgNPs@mesoMnOx nanocomposite materials calculated by Scherer equation.
crystalline size. The crystallite size of AgNPs were calculated using the above mentioned formula with respect to the most intense peak (hkl value of (111) in Fig. 1a–b) and was estimated to be around 13 nm and 15 nm, respectively, for AgNPs prepared from the nitrate and sulphate sources. Pure mesoporous-MnO2 and Ag incorporated meso-MnO2 were confirmed by the respective XRD patterns and the d space values were in accordance with JCPDS file number 24–0508 where MnO3 phase of MnOx formation occurred [31]. The average crystallite size of the prepared meso-MnO2 nanocomposite materials was calculated by Scherer equation and is listed as a tabular form in Table 1. Elemental composition of Ag@meso-MnOx and meso-MnO2 confirmed the presence of silver nanoparticle existence and all other elements with respect to the atomic percentage.

The UV–vis spectra of biogenic synthesized AgNPs are shown in Fig. 1. The absorbance maximum (λmax) of the prepared samples was obtained within the range of 400–420 nm (Fig. 1d). Fig. 2 shows the N2 sorption-desorption behavior of the mesoporous-MnO2 and its composite form was studied by BET and BJH analysis and the active surface area of Ag@meso-MnO2 was analyzed. Pore size values and respective hysteresis are shown in Fig. 2 and Table 1. Isotherm curves for Ag loaded meso-MnO2 and pure meso-MnO2 displayed type IV isotherm, wherein, the H3 hysteresis loop mostly agreed to the presence of aggregated tubular shaped nanofibers-like particles [32]. The BJH pore size distribution of meso-MnO2 samples specified a mesoporous nature with the size ranging from 2 nm to 15 nm.

Fig. 3 shows the SEM-EDX data for Ag@meso-MnO2 and its elemental content mapping is shown Fig. 3b. Fig. 3b shows SEM-EDX images of Ag@meso-MnO2 at 100 nm scale, indicating fibrous nanowires/tubular morphology. Fig. 3a represents a SEM image at 100 nm scale indicating the occurrence of a spherical shaped nucleation particle on top of nanotubes of the individual meso-MnO2 phase.

3.2. TEM characterization of biogenic synthesized AgNPs and Ag@meso-MnO2

Powder of AgNP (1 μg) was dispersed in distilled water. Few drops of this solution were placed on a copper-coated grid and dried at 70 °C before TEM analysis. The TEM micrographs of AgNPs prepared from different precursor sources are shown in Figs. 4(a,b) and 5(a,b). Very fine NP of Ag was obtained in the AgNPs prepared using nitrate precursor and the particle size obtained was below 5 nm and ranged upto 15 nm. Very fine metal rich NPs (black dot type particles in Fig. 4a) are visible in the images and no agglomeration of particles was observed. Fig. 5(a and b) shows the AgNPs prepared using the sulphate source of Ag
forming little dense and metal rich NPs with size varying from 5 nm–30 nm. The particle size measurement is scaled in red line in Fig. 5b. Comparative conclusion obtained from TEM studies confirm the presence of very small particles of Ag obtained from the nitrate source of Ag compared to the AgNPs prepared using the sulphate precursor. HR-TEM images of Ag@meso-MnO₂ is shown in Fig. 6(a,b), the AgNPs (black dots) are clearly visible and is deposited in nanotubes of meso-MnO₂ matrix with porous MnOₓ carbon composite. Fig. 6b is a closer view of NPs present on the lattice of meso-MnO₂.

3.3. Determination of antibacterial activity of biogenic method prepared AgNPs and Ag-meso-MnO₂

Bacterial strains

The assessment of antibacterial activity of doped AgNPs was carried out using three gram-negative bacteria (Salmonella typhi, Escherichia coli and P. aeruginosa) and two gram-positive bacteria (Staphylococcus aureus and Streptococcus mutans).

Tables 2, 3 represents the antibacterial activity of AgNPs and AgNP doped meso-MnO₂ against representative gram-positive and gram-negative bacteria using the well diffusion method. Pictures of plates representing results of antibacterial activity, discs of AgNPs prepared from nitrate/sulphate source and Ag doped meso-MnO₂ are shown in Figs. 7, 8. AgNPs synthesized from the sulphate source exhibited significant antibacterial activity, since 5 μM concentration of AgNPs was effective against 100 μM concentration of both gram-positive and gram-negative bacteria when compared to Ag doped meso-MnO₂.

The exact concentration or atomic % of AgNPs in doped meso-MnO₂ was smaller compared to the concentration of AgNPs used for antibacterial testing. Results from the antibacterial study confirm that a concentration of 50–100 μg/plate of AgNPs incorporated meso-MnO₂ could inhibit the growth of all five bacterial cultures (Salmonella typhi, Escherichia coli, P. aeruginosa, Staphylococcus aureus, and Streptococcus mutans) (Table 2 and Fig. 7).

The amount of doped Ag present in meso-MnO₂ is responsible for the antibacterial activity and the quantity of AgNPs present in 25 μg/disc, 75 μg/disc, and 100 μg of Ag-meso MnO₂ is between 5 and 10 μg AgNPs, and pristine meso-MnO₂ did not show any antibacterial activity. Earlier reports have indicated that AgNPs synthesized using the leaf extract of Mimusops elengi L. showed antibacterial activity against K. pneumoniae, Micrococcus luteus and Staphylococcus aureus at a concentration of 15 μg/disc [33]. Furthermore, reports indicate that AgNPs synthesized using the aqueous extract of Solanum torvum exhibited antibacterial activity against B. subtilis, P. aeruginosa and Escherichia coli at concentrations ranging from 10 to 100 μg/disc [34]. AgNPs modified with titanium, which inhibits the growth of Staphylococcus aureus.
and *Escherichia coli*, contains a higher concentration of Ag [35]. Compared to the above studies, the amount of Ag present in Ag@meso-MnO2 required for antibacterial activity is very less. Thus, this preparation could be economical to treat bacterial infectious diseases. Most of the AgNPs reported till date showed antibacterial activity either towards gram-positive or gram-negative bacteria and in some cases *Staphylococcus aureus* and *Escherichia coli* were tested [36,37]. In the present study, Ag@meso-MnO2 showed two times higher antibacterial activity against both gram-positive and gram-negative bacteria compared to AgNP-doped metal oxides prepared from other plant sources [33].

The activity of doped Ag is highly dispersed in meso-MnO2 matrix as Ag is more exposed to bacterial cells and shows higher activity compared to bulk Ag particles [38]. The prepared Ag@meso-MnO2 are highly stable, resistant to heat up to 400 °C as revealed by thermal analysis, and can be reused compared to AgNP polymer composites [39,40]. Another advantage of doping AgNPs into MnO2 lattice is that it can decrease the toxicity of free Ag ions to human cells. AgNPs based materials have been found to be more effective against all pathogenic bacteria especially gram-negative bacteria *Salmonella typhi* followed by *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus* and *P. aeruginosa* respectively. Zone of inhibition was absent in the control sample against all bacteria. These results confirm that Ag doped materials suppressed bacterial growth and exhibited significant antibacterial activity. AgNPs-doped meso-MnO2 showed an effective zone of inhibition against both gram-positive and gram-negative bacteria at concentration ranging from 50 μg to 100 μg. Highest activity was observed for *Salmonella typhi* and *Streptococcus mutans* compared to other pathogens. An interesting aspect of the present study is the finding that the prepared AgNPs and Ag@meso-MnO2 exhibited antibacterial activity against both gram-positive and gram-negative bacteria. AgNPs prepared from the nitrate source was effective as an antibacterial agent from 25 μg and AgNPs prepared from the sulphate source was effective from 5–100 μg (Table 3 and bar diagram Fig. 9). Table 3 shows the antibacterial activity of AgNPs prepared from the sulphate source exhibiting highest antibacterial activity towards all the tested bacterial pathogens compared to AgNPs prepared from the nitrate source. Fig. 8 shows the clear zone of inhibition occurred for all bacterial pathogens from lower concentration to higher concentration. Tables 2 and 3 indicates a similar trend in antibacterial activity upon addition of 50 μg and 100 μg of AgNPs in the cultured plate. The overall order of antibacterial activity demonstrated by AgNPs and Ag@meso-MnO2 are as follows: *Salmonella typhi* (−) > *Streptococcus mutans* (+) > *Escherichia coli* (−) > *Staphylococcus aureus* (+) > *P. aeruginosa* (−).

AgNPs-bacterial interaction can be broadly explained based on three approaches: (i) the electrostatic attraction between negatively charged AgNPs which is initially stabilized by organics present and positively charged residues of the integral membrane proteins present on the surface of pathogenic bacteria, (ii) alternation in physicochemical characteristics or structural integrity of the bacterial cell wall, leading to alterations in osmoregulation of the bacterial cell causing extrusion of intracellular material and cell death [41], (iii) AgNPs tend to penetrate through bacterial membranes, thereby facilitating their internalization into the cell. The formation of pits/holes and disruption of the bacterial cell wall indicate evidence of internalization of AgNPs. After internalization, AgNPs may exhibit antibacterial activity through multiple pathways.

**Table 2**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Name of the bacteria</th>
<th>Zone of the inhibition (in mm) of AgNPs (μg)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>5 (μg)</td>
</tr>
<tr>
<td>1</td>
<td><em>S. typhi</em></td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td><em>S. mutans</em></td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td><em>S. aureus</em></td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td><em>E. coli</em></td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td><em>P. aeruginosa</em></td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Name of the organisms</th>
<th>Zone of the inhibition in μg level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant extract 100 μg</td>
<td>AgNO3 1 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td><em>S. typhi</em></td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td><em>S. mutans</em></td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td><em>E. coli</em></td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td><em>S. aureus</em></td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td><em>P. aeruginosa</em></td>
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namely, inhibition of DNA replication [41], and by blocking cellular respiration [42]. These mechanisms may occur in parallel and might contribute towards a rapid antibacterial effect.

Both gram-positive and gram-negative microorganisms such as Salmonella abony, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, K. pneumoniae, and P. aeruginosa cause hospital infections. These pathogenic bacterial strains are resistant to most classical antibiotics as they acquire resistance mechanism through horizontal gene transfer of drug resistant genes [43]. Hence, there exists a need for novel antibacterial agents and AgNPs are promising because of their high antibacterial activity. In the present report, we showed that biogenic synthesized AgNPs and its doped meso-MnO$_2$ exhibited enhanced antibacterial activity against both gram-negative and gram-positive bacteria. In the future, we can employ these materials along with common antibiotics to treat bacterial infections caused by multidrug-resistant ‘superbugs’. The methodology of NP synthesis presented here is greener and uses less toxic agents (AgNPs and Ag@meso-MnO$_2$), and can be adopted in wider applications towards a cleaner and infection-free house and hospital development. Fig. 9 shows the three-dimensional bar diagram representation of the antibacterial activity...
of biogenic AgNPs prepared from different routes. It is very clear that AgNPs prepared from sulphate source has shown very effective antibacterial activity from very low concentration. Fig. 10 shows the images of plant leaf and its extract form, AgNPs formed immediately after mixing the extract with silver nitrate. The overall characterization and outcome results are shown in the form of pictorial representation.

Preliminary anti-cancer activity was carried out for AgNPs prepared from different precursors. Treatment of human colon cancer cell lines, HT-29 and SW620 with different concentration of AgNPs inhibited the increase in cell number as compared to the control as determined by MTT assay (Figs. 11–14).

In an MTT assay, treatment of human colon cancer cell line HT-29 with different concentration of AgNPs inhibited the increase in cell number as compared to the control (Fig. 11). Similar effects were observed in another colon cancer cell line (Fig. 12).

Anticancer activity of cell viability for HT-29 & SW620 cell lines on Ag nanoparticles prepared from nitrate puncture are showing a gradual increase in anti-cancer activity with respect to AgNPs concentration. Figs. 13, 14 shown the anticancer activity of AgNPs prepared from sulphate precursor. At higher concentration (20–30 μM) of AgNPs addition causes the effective damage of cancer cell by silver nanoparticle.

Fig. 9. A 3D bar diagram of Antimicrobial activity of AgNPs prepared from various precursor (a) Ag-meso MnOx (b) AgSO\textsubscript{4} (c) AgNO\textsubscript{3}.

Fig. 10. Schematic of *Alternanthera bettzickiana* plant and extract provide the AgNPs with good crystalline quality. The comparative results of biogenic route prepared AgNPs by different precursor.
The mechanism of the anticancer activity of the biogenic synthesized silver nanoparticles reported earlier suggests that the accumulation of more number of AgNPs inside cells resulting in enhanced stress, ultimately leading to cell death, through generation of intracellular oxidative stress by the nanoparticles and decreasing of ATP production from mitochondrial and is a causative factor for apoptosis due to lack of cellular energy [44].

In earlier, a few in vitro cancer cell lines studies have been previously reported with biogenic silver nanoparticles. Chandrasekaran et al. reported that the significant cytotoxic activity was noted in latex extract biosynthesized AgNPs than crude latex extract with LC50 value 91.3 μg and 311 μg respectively [45–47]. Our present study the human colorectal cancer cell line HT-29, SW620 was inhibited at very low concentrations (10–15 μg) using biogenic synthesized AgNPs may potentially prove to be a chemotherapeutic agent in in vitro and in future study plan to carry out on in vivo by biogenic method prepared silver nanoparticles and doped nanocomposites. In conclusion, AgNPs prepared from nitrate source is showing higher anti-cancer activity compared to AgNPs prepared by sulphate precursor. Ag nanoparticles prepared from different starting precursors such as nitrate and sulphate precursor was studied for both anti-microbial and anti-cancer activity. The synthesized AgNPs were further doped on mesoporous MnO2 for comparison purpose with pure AgNPs and pristine MnO2. TEM micrographs confirmed the formation of fine particle of AgNPs of different sizes ranging from 5–15 nm (made using nitrate precursor), while little large particle size was obtained for AgNPs made using sulphate precursor. AgNPs synthesized using nitrate precursor showed enhanced and effective anticancer activity against both gram-positive and gram-negative bacteria. AgNPs prepared from sulphate precursor exhibited amazing antibacterial activity against the tested bacterial pathogens compared to all other samples. AgNPs synthesized by A. bettzickiana extracts confirmed that trace levels of NPs could act against colorectal cancer cell lines. The AgNPs synthesized in the present study was developed by toxic free plant extract and hence could be used as a direct target against multidrug resistant superbugs and finds application in the field of cancer research and mass production of antibacterial agents by economic route.

4. Conclusion

Facile synthesis process was developed to produce biogenic synthesized AgNPs in large quantities, using the newly identified vegetable plant extract of A. bettzickiana for the first time. Silver nanoparticle prepared from different starting precursors such as nitrate and sulphate precursor was studied for both anti-microbial and anti-cancer activity. The synthesized AgNPs were further doped on mesoporous MnO2 for comparison purpose with pure AgNPs and pristine MnO2. TEM micrographs confirmed the formation of fine particle of AgNPs of different sizes ranging from 5–15 nm (made using nitrate precursor), while little large particle size was obtained for AgNPs made using sulphate precursor. AgNPs synthesized using nitrate precursor showed enhanced and effective anticancer activity against both gram-positive and gram-negative bacteria. AgNPs prepared from sulphate precursor exhibited amazing antibacterial activity against the tested bacterial pathogens compared to all other samples. AgNPs synthesized by A. bettzickiana extracts confirmed that trace levels of NPs could act against colorectal cancer cell lines. The AgNPs synthesized in the present study was developed by toxic free plant extract and hence could be used as a direct target against multidrug resistant superbugs and finds application in the field of cancer research and mass production of antibacterial agents by economic route.

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