Journal of
Interdisciplinary Nanomedicine

BRITISH SOCIETY FOR NANOMEDICINE

Open Access

Journal of Interdisciplinary Nanomedicine ISSN 2058-3273

ORIGINAL ARTICLE

Green synthesis of silver nanoparticles using *Alysicarpus monilifer* leaf extract and its antibacterial activity against MRSA and CoNS isolates in HIV patients

Muthupandi Kasithevar,[1] Muthupandian Saravanan,[2*] Periyakaruppan Prakash,[1**] Hema Kumar,[1] Muhammad Ovais,[3] Hamed Barabadi[4] & Zabta Khan Shinwari[3]

[1] *Department of Chemistry, Thiagarajar College, Madurai625009, India*

[2] *Department of Microbiology and Immunology, Institute of Biomedical Sciences, College of Health Science, Mekelle University, Mekelle1871, Ethiopia*

[3] *Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad45320, Pakistan*

[4] *Department of Pharmaceutical Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran19166, Iran*

Keywords
Alysicarpus monilifer, antibacterial, CoNS, green nanomaterials, green synthesis, MRSA, silver nanoparticles.

Correspondence
Muthupandian Saravanan, Department of Medical Microbiology and Immunology, Institute of Biomedical Sciences, College of Health Sciences, Mekelle University, 1871 Mekelle, Ethiopia. Email: saravanan.muthupandian@mu.edu.et
Periyakaruppan Prakash, Post Graduate and Research Department of Chemistry, Thiagarajar College, Madurai-625009, Tamil Nadu, India. Email: kmpprakash@gmail.com

Received: 18 March 2017;
Revised: 13 April 2017;
Accepted: 06 May 2017

Journal of Interdisciplinary Nanomedicine,
2017; 2(2), doi: 10.1002/jin2.26

Abstract

The emergence of multi-drug-resistant microorganisms in hospital environments is a global public health problem and threat to everyone, especially HIV-infected patients. Methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococci* (CoNS) are the major causative agents associated with morbidity and mortality in HIV patients. Therefore, control of MRSA and CoNS-related infections in HIV patients is a worldwide concern. To investigate novel, potent, and cost-effective therapeutic approaches, the current study reports a simple and rapid synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Alysicarpus monilifer* and its antibacterial efficacy against multi-drug-resistant MRSA and CoNS isolates from HIV patients. The green-synthesized AgNPs were characterized using ultraviolet-visible spectroscopy, transmission electron microscopy, energy dispersive X-ray analysis, selected area electron diffraction pattern, X-ray diffraction patterns, and Fourier transform infrared spectroscopy. Stable, well-defined AgNPs, mostly spherical in shape with mean size of 15 ± 2 nm, were obtained within an hour. Moreover, green synthesized AgNPs revealed significant dose-dependent antibacterial action against MRSA and CoNS isolates. This study concludes that biogenic AgNPs have demonstrated to be potent antibacterial agents in comparison with conventional antibiotics.

© 2017 The Authors. Journal of Interdisciplinary Nanomedicine published by John Wiley & Sons Ltd and the British Society for Nanomedicine
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococci* (CoNS) can cause chronic and lethal infections, such as infective endocarditis, bacteremia, pneumonia, and complicated skin and soft tissue infections. In persons with HIV infection, both colonization and infection with MRSA and CoNS have become increasingly common, which is due to weak immune system, behavioural risk factors, and extended vulnerability to the health care system (Wertheim et al., 2005; Hidron et al., 2010; Couto et al., 2016). Infection with methicillin-resistant *Staphylococci* leads to a raised mortality rate, prolonged antibiotic therapy, longer hospitalization, and higher expenses as compared with methicillin-sensitive *Staphylococci* infection (Filice et al., 2010; Lin et al., 2016).

In recent times, the role of nano-biotechnology has become progressively more significant in countering emerging drug-resistant clinical pathogens (Dizaj et al., 2014; Singh et al., 2014). Green synthesis of nanoparticles using plant extracts is a fascinating area in the field of nanobiotechnology, which is simple, eco-friendly, and cost-effective compared with the physico-chemical approach (Sun et al., 2014; Ovais et al., 2017). Nanochemistry includes the fundamental studies on the preparation of nanoparticles with different sizes, chemical compositions, and morphology-dependent optical, magnetic and electrical properties (Ahmad et al., 2010; Ovais et al., 2016). Silver nanoparticles have been used efficiently against multidrug-resistant pathogens (Ingle et al., 2008), in antimicrobial preparations (Gade et al., 2008), wound dressings, textile fabrics (Duran et al., 2007), and surgical masks (Li et al., 2006).The surface plasmon resonance (SPR) and high cross sections of scattering through intensification of electromagnetic field make nanoparticles the best choice for bioimaging and bio-labelling applications, where technique such as surface enhance Raman scattering can be used (Gan et al., 2012; Vijayakumar et al., 2013). Earlier studies on synthesized Ag nanoparticles have been demonstrated to acquire effective activity against the HIV (Lara et al., 2010).

In the synthesis of metal nanoparticles, control over the shape and size has been one of the challenging tasks. Different methods are reported for the synthesis of metallic nanoparticles, viz. solvothermal, sonochemical, and reverse microemulsion (Rao et al., 2006). These nanoparticles exhibit excellent antimicrobial activity; however, starting materials used are mostly toxic (Sathyavathi et al., 2010). To develop biocompatible and biodegradable nanoparticulate system, an environmentally benign procedure that avoids the use of hazardous chemicals for nanoparticle synthesis is required. Thus, research has turned to utilize biological systems (Basavaraja et al., 2008; Mukherjee et al., 2012; Nethi et al., 2014; Patra et al., 2015; Arokiyaraj et al., 2017). Hence, the current study emphasized a simple and novel approach for green synthesis of silver nanoparticles (AgNPs) as an effective bactericidal agent against MRSA and CoNS isolates.

Experimental Section

Materials

Silver nitrate, media components, and other analytical reagents were obtained from Merck, Germany, and Oxoid, Hampshire, UK. The medicinal plant *Alysicarpus monilifer* used for the AgNPs synthesis was obtained from Thiagarajar College campus, Madurai, India.

Isolation of bacterial pathogens (MRSA and CoNS)

Nasal and throat swab samples were collected from HIV patients using sterile cotton swabs (pre-moistened with normal sterile saline) (Kumar et al., 2013). Following inoculation into Stuart's Transport media, swabs were transported to the Microbiology laboratory of Ayder Referral Hospital within 3–4 h. Nasal and throat swabs were cultured selectively onto mannitol salt agar and blood agar (Oxoid, Hampshire, UK), and culture was made by using the standard guidelines. The media was incubated in aerobic state at 37°C. Following incubation for 24 h, the isolated strains were subcultured onto Nutrient Agar (Oxoid, Hampshire, UK) for further biochemical characterization. Bacterial identification was carried out based on colony morphology and tube coagulase test. MRSA isolates were identified using Cefoxitin disc by the Kirby-Bauer disk diffusion method. This research study was morally accepted by Ethical Review Committee, College of Health Science Mekelle University, Mekelle, Northern Ethiopia (Ref. No-ERC0453/2014). These strains were sub-cultured for various time intervals to maintain their viability and were maintained on Muller–Hinton agar (MHA) slants (stored at 4°C). Additionally, subculturing of bacterial strains was performed at 37°C on MHA medium (pH 7.3 ± 0.2; Oxoid, Hampshire, UK) with mixing at 150 rpm. Bacterial colonies were suspended

in a sterile saline to obtain bacterial suspensions with a final concentration of 10^7 Colony Forming Unit (CFU)/mL by making a comparison for turbidity with 0.5 McFarland standards for further antimicrobial efficacy study using AgNPs.

Instrumentation

The particle size and surface morphology of the AgNPs were determined by transmission electron microscopy (TEM) (JEOL JEM 2100) with energy dispersive X-ray (EDX) spectrometer for elemental mapping. Fourier transformed infrared (FTIR) Spectroscopy was performed using the FTIR-8201 (Shimadzu). A V-560 double-beam spectrophotometer (JASCO) was employed for analysis of ultraviolet-visible spectra, and X-ray diffraction (XRD) was executed using PW3050/60 on the purified AgNPs with XPERT-PRO.

Preparation of leaf extract

The leaves of *Alysicarpus monilifer* were rinsed to remove fine clay particles as well as possible impurities. Afterward, the leaves were placed on the filter paper to remove any wetness and left for 1 h at room temperature to air-dry. Six grams of leaves were cut into small pieces, poured in an Erlenmeyer flask boiled with distilled water for 15 min, and filtered via Whatman No-1 filter paper.

Green synthesis of AgNPs

For the preparation of AgNPs, 150 mL of 1 mM silver nitrate aqueous solution was transferred to the Erlenmeyer flask holding 150 mL of extract of *Alysicarpus monilifer* leaves. After 10 min, the colour of the extract turned brown indicating the preparations of AgNPs. The brownish colour becomes denser, and after 1 h, no significant colour change was observed. The colour change is due to coherent excitation of the SPR in the metallic nanoparticles. Afterward, the AgNPs were rinsed and centrifuged with deionized water and then lyophilized. The lyophilized AgNPs were stored in an amber-coloured bottle to be used for further characterization and potential application.

Disc diffusion assay

Silver nanoparticles (AgNPs) antibacterial efficacy against MRSA and CoNS isolates was evaluated by performing disc diffusion assay. Inoculum of both bacterial isolates was prepared by aseptically, and cell density was adjusted to 0.5 McFarland standard (10^6 CFU/mL), and then the inoculum was transferred and spread evenly on an MHA plates, and two discs were utilized (Whatman No-1 filter paper) to impregnate the AgNPs solution of various dilutions (50 and 100 μg/mL). Negative control consisted of the leaf extract (100 μg/mL) while positive control was Erythromycin (30 μg/mL) and Amoxicillin calvunic acid (30 μg/mL) (Oxoid, Hampshire, UK). The primed plates were then subjected to further incubation (24 h, 37°C).

Bactericidal activity of AgNPs against MRSA and CoNS

To determine the bactericidal potential of AgNPs, 10^7 CFU of MRSA and CoNS isolates were treated individually with various concentrations of AgNPs (100, 80, 60, 40, and 20 μg/mL) and 100 μg/mL of lyophilized plant extract as a control. The resulting preparations were initially incubated (1 h, 37 °C) and then transferred to MHA plates. These plates with test samples were then incubated (24 h, 37 °C), and the bacterial colonies were enumerated by using an automated colony counter.

Bacterial growth kinetics assay

The growth kinetics of MRSA and CoNS was determined to check the effect of AgNPs in various concentrations of AgNPs (100, 80, 60, 40, and 20 μg/mL) with 10^7 cells of each bacterial isolate cultured in Lysogeny broth medium in 100 mL conical flask and incubated overnight on a rotary shaker at about 200 rpm. A control (in the absence of AgNPs) was kept under the similar conditions. The samples were then subjected to incubation at 37°C, and the growth kinetics was determined by measuring optical density (OD) at 600 nm at time points of 3 to 24 h of incubation.

Cytotoxicity (MTT) assay of AgNPs

The cytotoxicity assay of the purified and lyophilized AgNPs was performed against non-transformed Vero cell line (ATCC® CCL-81™) (derived from the kidney cell of African green monkey). The cells were cultured in a 25 cm^3 flask containing 10% fetal bovine serum supplemented with Dulbecco's Modified Eagle's Medium, 100 IU/mL Penicillin, 100 μg/mL Streptomycin, and 50 μg/mL of Gentamycin (Sigma-Aldrich, USA). Cells were harvested and cultured as monolayers at 37ºC in 5 % CO_2 incubator under a humidified atmosphere. The experiment was performed using cells from less than or equal to passage 20. In the experimental study, the serum containing culture media was substituted by serum-free media containing 1.5–200 μg/mL AgNPs (lyophilized) in dimethyl sulfoxide (DMSO), and the

stock culture was properly stored at −20°C. The final DMSO volume was less than 1%.

The fully grown monolayer of Vero cell culture was dispensed in culture plate of 96 wells (adjusted the density of viable cell to 1 * 10^5 cells/well) and incubated for 6 h to allow the cells for attachment. The cells were tested with different concentrations (1.5–200 μg/mL) of lyophilized AgNPs in serial dilution for each well, and less than 1% of DMSO was used as control, then 100 μl/well of MTT (50 μg/mL) was transferred to every one of the well that incubated the plate for 12, 24, 48, and 72 h in CO_2 incubator. The cytotoxic effect of the AgNPs on Vero cell was evaluated by rinsing the dye crystals DMSO (100 μl), and OD was determined at 570 nm by a 96-well model 680 micro plate readers (BIO-RAD, USA).

Results and discussion

The biosynthesis of metallic nanoparticle exploiting plant extracts in comparison with other bioreductants is more fruitful in terms of its ease (Bhadra et al., 2014). It is well known that the phytochemicals not only reduce the Ag^{1+} into Ag^0 but also are responsible for its capping to make these nanoparticles highly stable (Arokiyaraj et al., 2017; Ovais et al., 2016). In the current study, leaf extract of *Alysicarpus monilifer* is exploited for the green synthesis of AgNPs followed by its detailed bactericidal and cytotoxic activities.

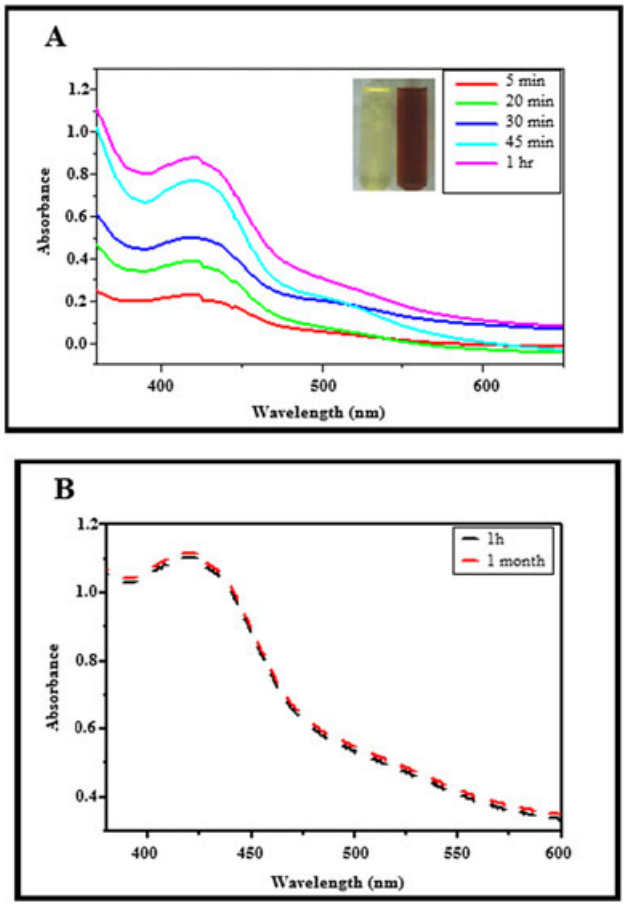


Figure 1. (A and B) Ultraviolet-visible spectra of silver nanoparticles recorded at different time intervals.

Physicochemical Characterization of AgNPs

Ultraviolet-visible spectroscopy

To determine the development of metallic nanoparticles and exploit their optical properties, ultraviolet-visible spectroscopy was utilized. Plasmon bands were recorded from aliquots isolated at specific intervals of the bio-reduction. The preparation of the AgNPs was primarily identified by appearance of brownish colour within 10 min. The reaction was completed and confirmed by Plasmon absorption peak, reaching a constant value after an hour. The spectrum (Fig. 1A) illustrates the SPR band of the spherical AgNPs at 422 nm, indicating that the formation of AgNPs in the reaction mixture is in agreement with the AgNPs synthesized so far (Wani et al., 2013; Ovais et al., 2016). The stability was evaluated over time (30 days), but no change was observed in absorption peak value, showing (Fig. 1B) that the nanoparticles are highly stable, after 1 month under ambient conditions (28°C).

TEM, SAED, EDX, and XRD analysis

Transmission electron microscopy (TEM) measurements put through the particle size and morphology of the synthesized AgNPs. Synthesized AgNPs were monodispersed, spherical in shape and a few hexagonal, with size range between 5 and 45 nm with a mean particle size 15 ± 2 nm (Fig. 2A). The selected area electron diffraction pattern displays four circular bright rings correlated to the (111), (200), (220), and (311) planes, which verify that the synthesized AgNPs are crystalline. The spots are indexed to face centred cubic structure of Ag (Fig. 2B). The EDX pattern clearly indicates that the AgNPs are crystalline in nature. The EDX was also used for the elemental detection of the AgNPs. It can be observed from the EDX pattern that a sharp peak of silver appears at 3 KeV, which confirms the preparation of AgNPs (Fig. 2C). Several Bragg reflections with 2θ values of 37.9°, 44.2°, 64.8°, and 77° associated to the different set of (111), (200), (220), and (311) planes are obtained, which may be

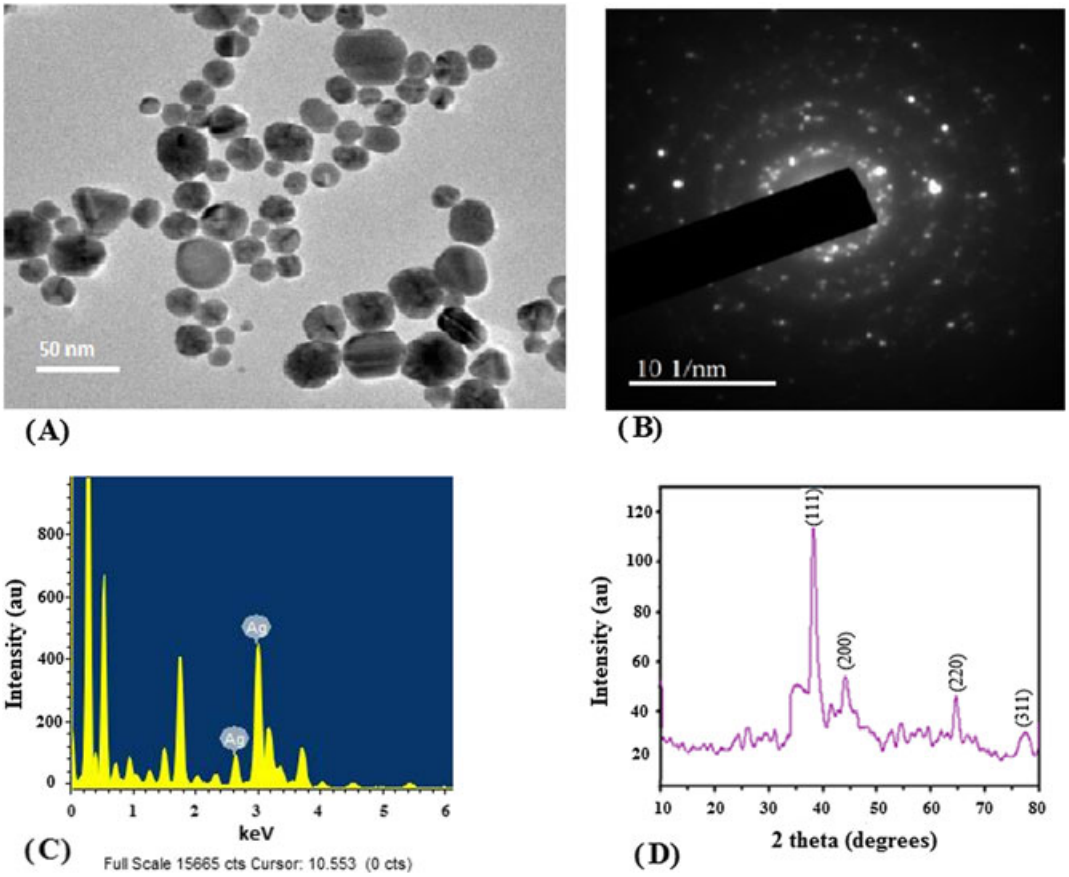


Figure 2. (A) Transmission electron microscopy image (scale = 50 nm) of the green synthesized silver nanoparticles (AgNPs). Corresponding selected area electron diffraction (B) patterns and energy dispersive X-ray (C) of AgNPs. (D) X-ray diffraction patterns of AgNPs.

recorded as the band for face centred cubic (JCPDS file no. 89-3722) structure of silver (Fig. 2D). Therefore, XRD confirms that the samples are the pure AgNPs with highly crystalline nature. The mean particle size of AgNPs can be determined by Debye–Scherrer equation: $D=k\lambda/\beta\cos\theta$ (where D is the thickness of the nanocrystal, k is a constant; λ is the Bragg's angle 2θ). The mean particle size calculated from the XRD patterns is 15 ± 2 nm, which is confirmed by the TEM study as well.

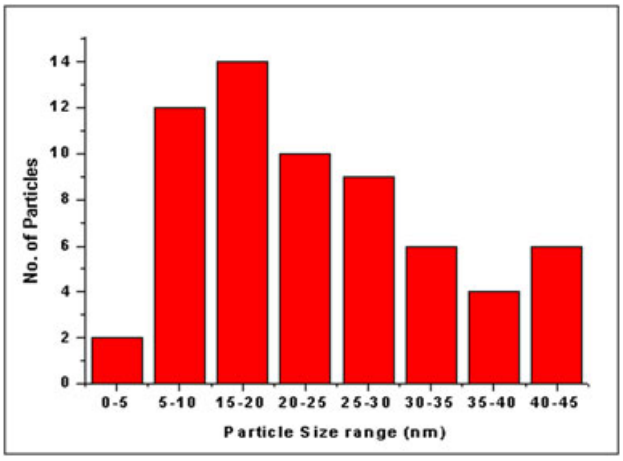


Figure 3. Histogram of the percent frequency distribution of silver nanoparticles.

The percent frequency distribution histogram of AgNPs is shown in (Fig. 3). The spherical particles possess high surface tension and go through a shrinkage process to minimize the surface tension, resulting in formation of blunt shape nanoprisms. The particle size histograms of silver particles showed that the particles size ranges from 5 to 45 nm.

FTIR analysis

Fourier transformed infrared spectroscopy is a useful technique for detecting the contribution of different functional groups in the nanoparticle synthesis. The FTIR spectra were recorded for both *Alysicarpus monilifer* leaf extract (a) and synthesized AgNPs (b) (Fig. 4A and B). Many phyto-chemicals have been

obtained from the species of *Alysicarpus*, mainly carbohydrates, steroids, glycosides, alkaloids, tannins, and phenolic compounds. Secondary metabolites such as carbohydrates, glycosides, phytosterols, saponins, proteins, alkaloids, and flavonoids are also present (Kakrani et al., 2011a,b). It is observed from FTIR spectra that there is a clear change in the spectra vis-à-vis shifting of characteristic peaks after encapsulation. The band intensities in different regions of the spectrum for the control and test samples after the reaction AgNPs were analysed as shown in Figure 4 (A and B). The peak at 3395/cm is due to the -NH stretching vibrations of the secondary amines, and the peak at 3693/cm is attributed to the -OH stretching vibration of the hydroxyl functional groups of the polyphenols and alcohols (Vellaichamy and Periakaruppan, 2016a). The peaks at 2914 and 2845/cm are characteristic of stretching vibrations of methyl groups or C–H of aldehydic amine groups, and the peak observed at 1737/cm is attributed to a carbonyl stretching vibration of ester groups. The peak at 2365/cm is assigned to the N-H or C=O stretching of proteins present in the leaf extract. The strong peak appearing at 1643/cm is assigned to the -OH bending mode or C=O stretching vibration of carbonyl and carboxylic group of amide I (Vellaichamy and Periakaruppan, 2016b). The weak band observed at 1540/cm is due to C–H deformation vibrations, and the peak at 1196/cm is the bending vibration of C–N group of amide II and III or C–O stretching vibration of

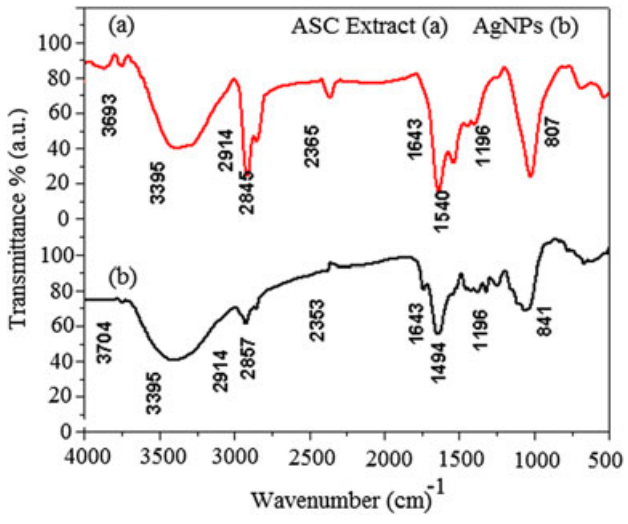


Figure 4. Fourier transformed infrared spectrum of *Alysicarpus* monilifer leaf extract (A) and leaf extract mediated synthesized silver nanoparticles (AgNPs) (B).

polyols. The peak appearing at 807/cm is the plane bending vibration of N–H groups in the proteins (Balakumar et al., 2017).

Flavonoids, as antioxidants, may inhibit the progressive damage of pancreatic beta-cell caused by oxidative stress (Karthikeyan et al., 2014). Saponins are used in anti-inflammatory, anti-oxidant, weight loss, hyperglycaemia, hypercholesterolemia, anticancer, etc. (Mandal et al., 2005). Moreover, lignans are the main component in the extracts of leaf, which contains metabolites, such as (+)- ethyl acetate extract, (+)- petroleum ether extract, (+)- methanolic extract, important for the bioreduction process of metallic ions and stabilization of nanoparticles (Bhadra et al., 2014; Patra et al., 2015). Hydroxyl groups (OH^-) are high in lignans and may be responsible for the bio-reduction process during AgNPs synthesis (Khan et al., 2015; Ahmed et al., 2016). Figure 5 illustrates the plausible mechanism of AgNPs formation via leaf extract of *Alysicarpus monilifer*.

Antibacterial efficacy of green synthesized AgNPs

The antibacterial efficacy of AgNPs against MRSA and CoNS clinical isolates was performed by modified Kirby-Bauer disc diffusion method. It was observed that MRSA and CoNS are highly sensitive to the antimicrobial activity of AgNPs with a mean zone of inhibition (ZOI) in diameter of 19 and 17.5 mm, respectively, at 100 μg/mL concentration of AgNPs. With 50 μg/mL AgNPs concentration, the ZOI was 14 and 16 mm, respectively, for MRSA and CoNS (Fig. 6). It is assumed from the results that the ZOI increases linearly with the amount of the AgNPs. Therefore, many researchers support the concept that AgNPs inhibits bacterial growth in a dose-dependent manner (Suganya et al., 2015; Różalska et al., 2016). In addition, the synthesized AgNPs have remarkable antibacterial efficacy as compared with antibiotics erythromycin and amoxicillin. When clinical bacterial pathogens are exposed to AgNPs, they are found to affect the membrane permeability, causing cellular leakage that consequently inhibits cell growth and replication ability. AgNPs can affect some bacterial macromolecules, leading to disintegration and ultimately resulting in cell death (Singh et al., 2014). Green synthesized AgNPs are more biocompatible and exhibit higher antibacterial effect in comparison with chemically synthesized AgNPs (Bhadra et al., 2014).

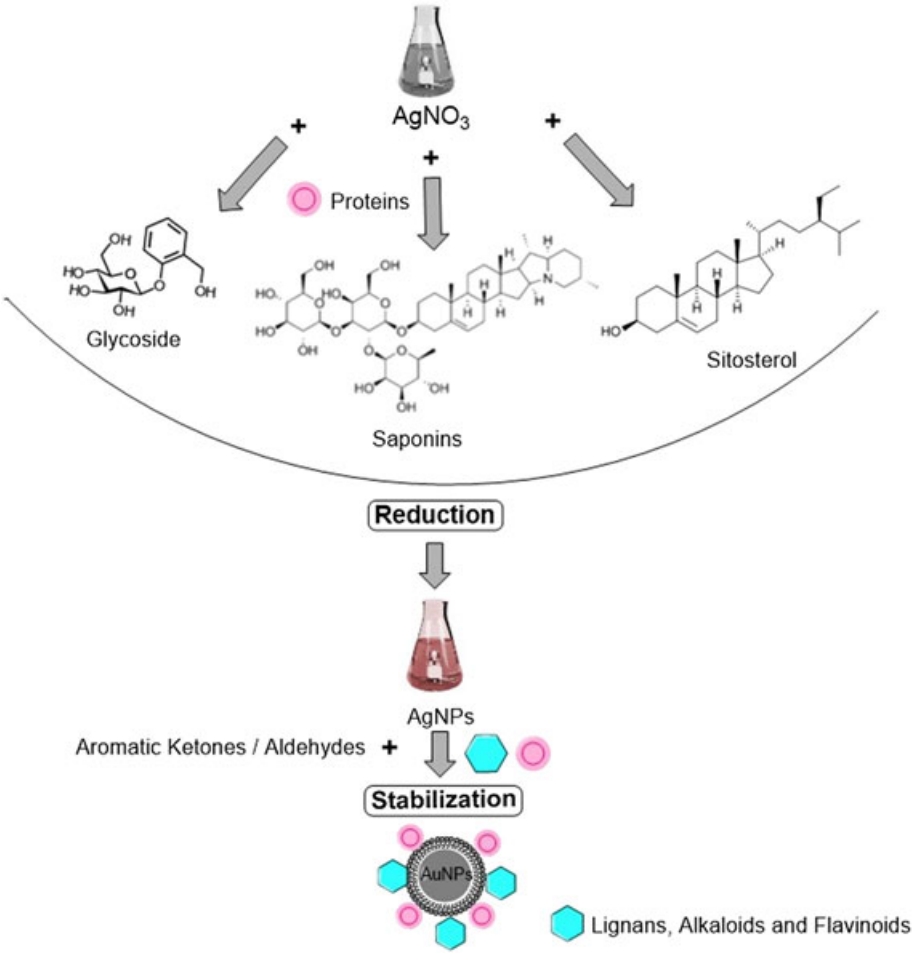


Figure 5. Plausible mechanism of silver nanoparticles (AgNPs) biosynthesis from leaf extract of *Alysicarpus* monilifer.

Bactericidal activity of AgNPs against MRSA and CoNS

Bactericidal activity of AgNPs was performed against MRSA and CoNS clinical isolates. Around 10^7 CFU of bacterial isolates were treated with various concentrations of Ag-NPs (100, 80, 60, 40, and 20 μg/mL) grown on MHA plates. After treatment, a higher reduction in number of colonies is observed

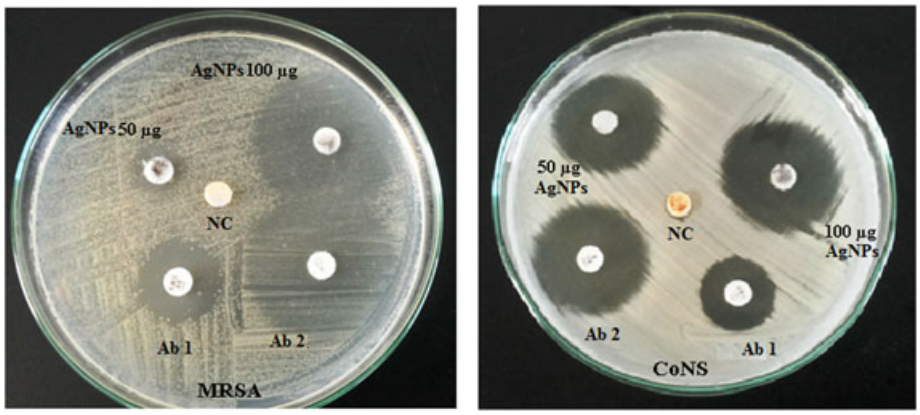


Figure 6. Determination of antibacterial activity of green synthesized silver nanoparticles (AgNPs) (50 and 100 μg) against methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococci* (CoNS) isolates of HIV patients. "NC" represents the negative control consisted of the leaf extract (100 μg/mL), while positive controls are "Ab 1" erythromycin (30 μg/mL) and "Ab 2" amoxicillin calvunic acid (30 μg/mL).

respectively in MRSA and CoNS after 24 h with 80 and 60 μg/mL of AgNPs. The AgNPs primarily anchor the cell membrane at various sites and then readily penetrate it, causing structural changes thereby perforations that result in the leakage of substances from intracellular stores (Dizaj et al., 2014). When it reaches the interior, AgNPs releases silver ions, which leads to produce reactive oxygen species that have an influence on the membrane proteins disturbing the electron transport chain (Ahmed et al., 2016).

Growth kinetics of AgNPs-treated MRSA and CoNS isolates

Bacterial growth kinetics for MRSA and CoNS isolates was determined upon treatment with green synthesized AgNPs. Around 10^7 CFU of isolated strains grown on MHA plates were treated with increasing concentrations of AgNPs (20, 40, 60, 80, and 100 μg/mL). In MRSA and CoNS, after treatment with 80 and 60 μg/mL of AgNPs, respectively, a higher fall in quantity of colonies was observed (Fig. 7). The growth kinetics was measured at OD 600 nm, on regular intervals of 3 to 24 h. It is noticeable from the curve that the quantity of bacterial cells decreases, with higher amount of AgNPs and with time of exposure. Furthermore, there is no growth inhibition in bacterial culture alone without AgNPs treatment, and the cells survive until they reach the decline phase. Researchers found from the investigations that AgNPs, owing to their nano-size, can pass readily through the cell membrane, resulting in better antibacterial effect. Mukherjee et al., 2012 suggested that the antibacterial activity of AgNPs may be attributed to the electrostatic attraction forces between the weak positive charge of

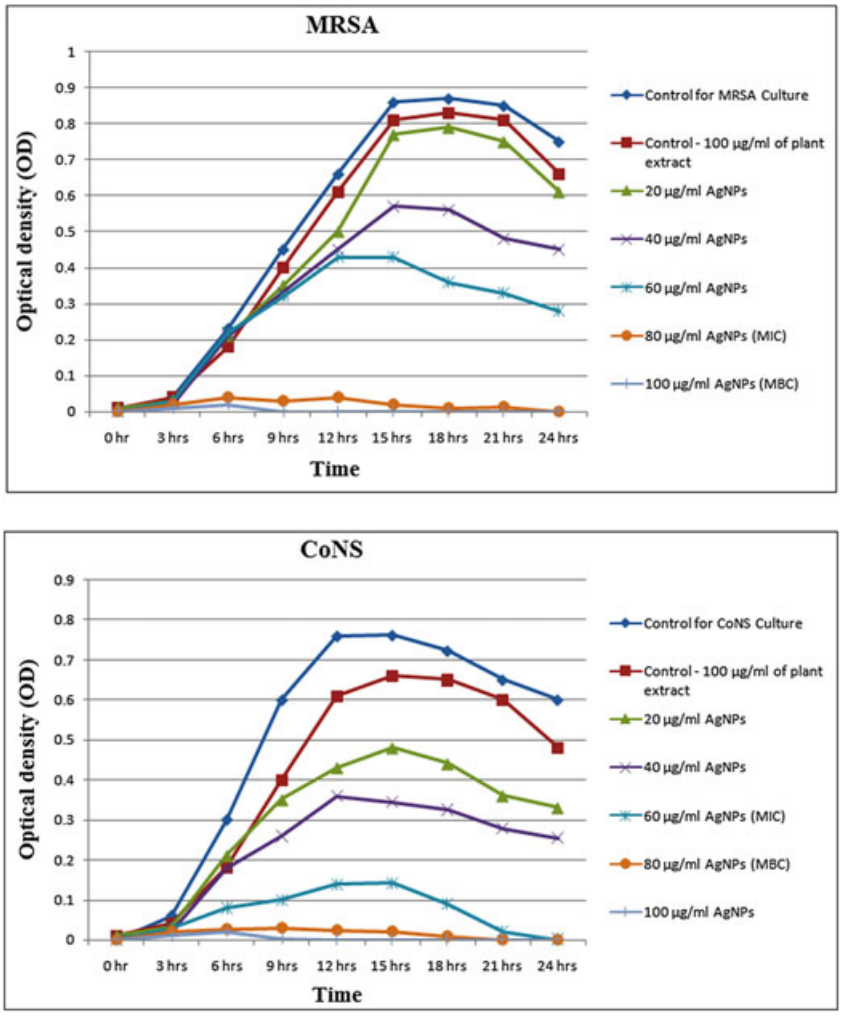


Figure 7. The growth kinetics of methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococci* (CoNS) in the presence of green silver nanoparticles (AgNPs) following isolation from HIV infected patients.

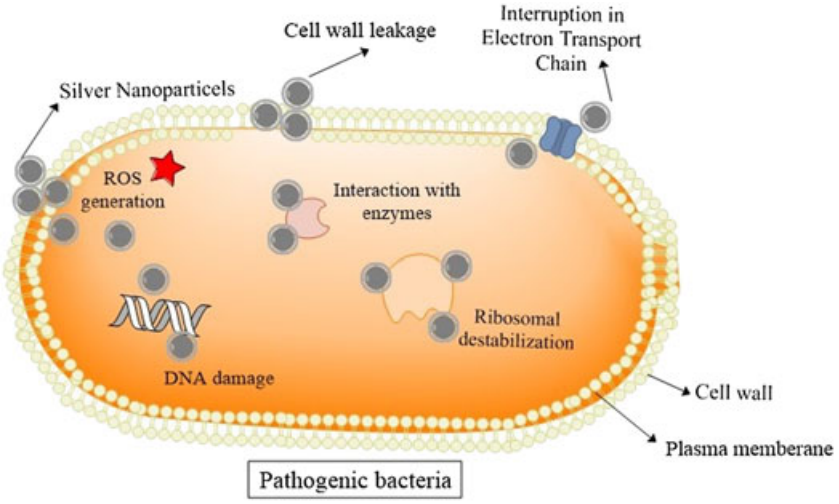


Figure 8. The proposed mechanism of bactericidal activity shown by biogenic silver nanoparticles.

the Ag ion and negative charge on the cell membrane (Bhadra et al., 2014). AgNPs may also act as carrier to transport Ag^+ more efficiently to bacteria cell whose proton motive force would consequently reduce the local pH and increase Ag^+ release (Ovais et al., 2016). In comparison with the antibacterial activity of other metallic nanoparticles, AgNPs have gained overriding significance with their unique mode of action. They are capable of affecting integrity of cell membrane and interact with disulfide bonds of intracellular enzymes, disturbing metabolic processes and inhibiting the major functions of bacterial cell like, cellular uptake and respiration, whereas other nanoparticles induce antibacterial activity by generating oxidative stress thereby affecting bacterial functions (Bhadra et al., 2014). The plausible anti-bacterial mechanism of biogenic AgNPs is extensively demonstrated in (Fig. 8) (Dizaj et al., 2014; Karthikeyan et al., 2014).

Cytotoxicity (MTT) assay

It is clear from the MTT assay results given in Table 1 that AgNPs acquire negligible cytotoxicity against Vero cell line until the prolong incubation time. MTT assay on Vero cell line was performed in duplicate. AgNPs exhibit no toxicity at the entire concentrations range until subjected to incubations for 48 h. However, toxicity of 18.34 ± 1.24%, 14.1 ± 1.00%, 5.69 ± 0.36%, and 1.0±0.08% was observed at concentrations 200, 100, 50, and 25 μg/mL, respectively, at 72 h of incubation. No cytotoxicity was produced at concentration of 3.125, 6.25, and 12.5 μg/mL at 72 h of incubation, and limited toxicity was observed at 25 μg/mL. IC_{50} values could not be calculated for AgNP-treated Vero cells across the concentration range and incubation time investigated as shown in Table 1.

Previously, several studies have been focused on the use of cell culture methods to perform cytotoxicity

Table 1. In vitro cytotoxicity effects of green silver nanoparticles (AgNPs) synthesized from *Alysicarpus monilifer* against Vero cell line.

AgNPs (μg/mL)	Vero cell line (10,000 cells/well) (% of toxicity)					IC_{50} concentration of AgNPs
	6 h	12 h	24 h	48 h	72 h	
200	nt†*	nt	nt	nt	18.34 ± 1.24	-
100	nt	nt	nt	nt	14.1 ± 1.00	-
50	nt	nt	nt	nt	5.69 ± 0.36	-
25	nt	nt	nt	nt	1.0 ± 0.08	-
12.5	nt	nt	nt	nt	nt	-
6.25	nt	nt	nt	nt	nt	-
3.125	nt	nt	nt	nt	nt	-
1.5	nt	nt	nt	nt	nt	-

*No toxicity.

assay of AgNPs (Gaiser et al., 2012; Albers et al., 2013). Before proceeding to in vivo trials, it is a fundamental procedure to determine the cytotoxicity of any natural and synthetic product on an established cell line (Gopinath et al., 2008). Our biogenic AgNPs show no significant cytotoxicity against Vero cells.

Conclusion

In the current work reported, AgNPs have been synthesized using *Alysicarpus monilifer* leaf extract. The synthesized AgNPs are stable even up to a month. The EDX analysis and XRD study confirm the existence of elemental silver and its crystalline structure. AgNPs have been proven to show efficient activity against both MRSA and CoNS isolates from HIV patients. Also, green synthesized AgNPs are biocompatible with normal Vero cell line and have high antibacterial activity. It is found in growth kinetics study that the growth of bacteria cells decreases in a dose-dependent manner while synthesized AgNPs also show high activity against bacteria even at low concentrations. Moreover, it is clear from the bactericidal activity that with higher concentrations of AgNPs, nearly absolute reduction in quantity of colonies has been obtained. While comparing with commercially available antimicrobial agents, the present work is a simple, rapid, environmental friendly and economical protocol for green (biological) synthesis of potent antibacterial silver nanomaterials against MRSA and CoNS, which may act as a successful alternative approach to the conventional antibiotics.

Acknowledgments

The authors gratefully acknowledge Management, Thiagarajar College, Madurai, Sophisticated Analytical Instrument Facility (SAIF), IIT- Madras and Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan, for the characterization studies. The authors also acknowledge the Department of Microbiology, College of Health Science, Mekelle University, for providing facilities for microbiological studies.

Conflict of Interest

The authors report no conflicts of interest.

REFERENCES

Ahmad, N., Sharma, S., Alam, M. K., Singh, V., Shamsi, S., Mehta, B., and Fatma, A. **2010**. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B Biointerfaces *81*:81–86.

Ahmed, S., Ahmad, M., Swami, B. L., and Ikram, S. **2016**. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res *7*:17–28.

Albers, C. E., Hofstetter, W., Siebenrock, K. A., Landmann, R., and Klenke, F. M. **2013**. *In vitro* cytotoxicity of silver nanoparticles on osteoblasts and osteoclasts at antibacterial concentrations. Nanotoxicology *7*:30–36.

Arokiyaraj, S., Vincent, S., Saravanan, M., Lee, Y., Oh, Y. K., and Kim, K. H. **2017**. Green synthesis of silver nanoparticles using *Rheum palmatum* root extract and their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Artif Cells Nanomed Biotechnol *45*:372–379.

Balakumar, V., Prakash, P., Muthupandi, K., and Rajan, A. **2017**. Nanosilver for selective and sensitive sensing of saturnism. Sens Actuators B *241*:814–820.

Basavaraja, S., Balaji, S., Lagashetty, A., Rajasab, A., and Venkataraman, A. **2008**. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. Mater Res Bull *43*(5):1164–1170.

Bhadra, M. P., Sreedhar, B., and Patra, C. R. **2014**. Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system). Theranostics *4*:316–335.

Couto, N., Monchique, C., Belas, A., Marques, C., Gama, L. T., and Pomba, C. **2016**. Trends and molecular mechanisms of antimicrobial resistance in clinical *Staphylococci* isolated from companion animals over a 16 year period. J Antimicrob Chemother *71*(6):1479–1487.

Dizaj, S. M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M. H., and Adibkia, K. **2014**. Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C *44*:278–284.

Duran, N., Marcato, P. D., De Souza, G. I., Alves, O. L., and Esposito, E. **2007**. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol *3*(2):203–208.

Filice, G. A., Nyman, J. A., Lexau, C., Lees, C. H., Bockstedt, L. A., Como-Sabetti, K., Lesher, L. J., and Lynfield, R. **2010**. Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. Infect Control Hosp Epidemiol *31*:365–373.

Gade, A., Bonde, P., Ingle, A., Marcato, P., Duran, N., and Rai, M. **2008**. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Mater Bio *2*:243–247.

Gaiser, B. K., Hirn, S., Kermanizadeh, A., Kanase, N., Fytianos, K., Wenk, A., Haberl, N., Brunelli, A., Kreyling, W., and Stone, V. **2012**. Effects of silver nanoparticles on the liver and hepatocytes in vitro. Toxicol Sci *13*:537–547.

Gan, P. P., Ng, S. H., Huang, Y., and Li, S. F. Y. **2012**. Green synthesis of gold nanoparticles using palm oil mill effluent (POME): a low-cost and eco-friendly viable approach. Bioresour Technol *113*:132–135.

Gopinath, P., Gogoi, S. K., Chattopadhyay, A., and Ghosh, S. S. **2008**. Implications of silver nanoparticle induced cell apoptosis for in vitro gene therapy. Nanotechnology *19* 075104.

Hidron, A. I., Kempker, R., Moanna, A., and Rimland, D. **2010**. Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. Infect Drug Resist *3*:73–86.

Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., and Rai, M. **2008**. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Curr Nanosc *4*:141–144.

Kakrani, P. H., Kakrani, H. N., and Saluja, A. K. **2011a**. Evaluation of anti-inflammatory activity of methanolic extract of the aerial parts of *Alysicarpus monilifer* L. (DC.). J Pharm Res *4*:3529–3520.

Kakrani, P. H., Kakrani, H. N., and Saluja, A. K. **2011b**. Pharmacognostic evaluation of aerial parts of *Alysicarpus monilifer* L.(DC.). Int J Pharm Pharm Sci *3*:128–134.

Karthikeyan, K., Dhanapal, C., and Gopalakrishnan, G. **2014**. A review on medicinal importance of Alysicarpus monilifer. Int J Chem pharm Sci *5*:1–5.

Khan, T., Khan, M. A., and Nadhman, A. **2015**. Synthesis in plants and plant extracts of silver nanoparticles with potent antimicrobial properties: current status and future prospects. Appl Microbiol Biotechnol *99*:9923–9934.

Kumar, S., Bandopadhyay, M., Banerjee, P., and Laskar, S. **2013**. Nasal methicillin-resistant *Staphylococcus aureus* colonization in HIV-infected patients from eastern India. Saudi J Health Sci *2*:14–17.

Lara, H. H., Ayala-Nuñez, N. V., Ixtepan-Turrent, L., and Rodriguez-Padilla, C. **2010**. Mode of antiviral action of silver nanoparticles against HIV-1. J Nanobiotechnology *8*:1.

Li, Y., Leung, P., Yao, L., Song, Q., and Newton, E. **2006**. Antimicrobial effect of surgical masks coated with nanoparticles. J Hosp Infect *62*:58–63.

Lin, J., Peng, Y., Ou, Q., Lin, D., Li, Y., Ye, X., Zhou, J., and Yao, Z. **2016**. A molecular epidemiological study of methicillin-resistant *Staphylococci* environmental contamination in railway stations and coach stations in Guangzhou of China. Lett Appl Microbiol *4*:131–137.

Mandal, P., Babu, S. S., and Mandal, N. **2005**. Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterapia *76*:462–465.

Mukherjee, S., Sushma, V., Patra, S., Barui, A. K., Bhadra, M. P., Sreedhar, B., and Patra, C. R. **2012**. Green chemistry approach for the synthesis and stabilization of biocompatible gold nanoparticles and their potential applications in cancer therapy. Nanotechnology *23*:455103.

Nethi, S. K., Mukherjee, S., Veeriah, V., Barui, A. K., Chatterjee, S., and Patra, C. R. **2014**. Bioconjugated gold nanoparticles accelerate the growth of new blood vessels through redox signaling. Chem Commun *50*:14367–14370.

Ovais, M., Khalil, A. T., Raza, A., Khan, M. A., Ahmad, I., Islam, N. U., Saravanan, M., Ubaid, M. F., Ali, M., and Shinwari, Z. K. **2016**. Green synthesis of silver nanoparticles via plant extracts: beginning a new era in cancer theranostics. Nanomedicine *12*:3157–3177.

Ovais, M., Raza, A., Naz, S., Islam, N. U., Khalil, A. T., Ali, S., Khan, M. A., and Shinwari, Z. K. **2017**. Current state and prospects of the phytosynthesized colloidal gold nanoparticles and their applications in cancer theranostics. Appl Microbiol Biotechnol *101*:1–15.

Patra, S., Mukherjee, S., Barui, A. K., Ganguly, A., Sreedhar, B., and Patra, C. R. **2015**. Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics. Mater Sci Eng C *53*:298–309.

Rao, C. N. R., Müller, A., and Cheetham, A. K. **2006**. The Chemistry of Nanomaterials: Synthesis, Properties and Applications. Sons, John Wiley &.

Różalska, S., Soliwoda, K., and Długoński, J. **2016**. Synthesis of silver nanoparticles from Metarhizium robertsii waste biomass extract after nonylphenol degradation, and their antimicrobial and catalytic potential. RSC Adv *6*:21475–21485.

Sathyavathi, R., Krishna, M. B., Rao, S. V., Saritha, R., and Rao, D. N. **2010**. Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. Adv Sci Lett *3*:138–143.

Singh, K., Panghal, M., Kadyan, S., Chaudhary, U., and Yadav, J. P. **2014**. Green silver nanoparticles of *Phyllanthus amarus*: as an antibacterial agent against multi drug resistant clinical isolates of *Pseudomonas aeruginosa*. J Nanobiotechnology *12*:40.

Suganya, K. U., Govindaraju, K., Kumar, V. G., Dhas, T. S., Karthick, V., Singaravelu, G., and Elanchezhiyan, M. **2015**. Size controlled biogenic silver nanoparticles as antibacterial agent against isolates from HIV infected patients. Spectrochim Acta A Mol Biomol Spectrosc *144*:266–272.

Sun, Q., Cai, X., Li, J., Zheng, M., Chen, Z., and Yu, C.-P. **2014**. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. Colloids Surf A Physicochem Eng Asp *444*:226–231.

Vellaichamy, B., and Periakaruppan, P. **2016a**. Ag nanoshell catalyzed dedying of industrial effluents. RSC Adv *6*:31653–31660.

Vellaichamy, B., and Periakaruppan, P. **2016b**. A facile, one-pot and eco-friendly synthesis of gold/silver nanobimetallics smartened rGO for enhanced catalytic reduction of hexavalent chromium. RSC Adv *6*:57380–57388.

Vijayakumar, M., Priya, K., Nancy, F., Noorlidah, A., and Ahmed, A. **2013**. Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. Ind Crop Prod *41*:235–240.

Wani, I. A., Khatoon, S., Ganguly, A., Ahmed, J., and Ahmad, T. **2013**. Structural characterization and antimicrobial properties of silver nanoparticles prepared by inverse microemulsion method. Colloids Surf B Biointerfaces *101*:243–250.

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., and Nouwen, J. L. **2005**. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis *5*:751–762.