



## Silver nanoparticles of *Aloe vera* gel as strong therapeutic candidates

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**Key words:** *Aloe vera*, Antibacterial activity, Antifungal activity, Nanotechnology, Silver nanoparticles, Green synthesis, Eco-friendly

<http://dx.doi.org/10.12692/ijb/15.1.361-374>

Article published on July 18, 2019

### Abstract

The biological approach is the most preferred technique for preparation of nanoparticles being much easy, less time consuming, and more importantly eco-friendly. In the current study, green synthesis of silver nanoparticles (AgNPs) was carried out by using *Aloe vera* gel. Characterization of the biosynthesized AgNPs was conducted by UV-vis spectrophotometer which showed the absorbance peak at the 450 nm. The Scanning Electron Microscopic (SEM) analysis revealed further, the spherical shaped AgNPs with the average size of  $40\text{nm} \pm 4$ . Nonetheless, the elemental composition and phase centered cubic crystalline nature was confirmed by the Energy Dispersive X-ray (EDX) and X-ray Powder Diffraction (XRD) respectively. The involvement of the carboxyl group of lipids of *Aloe vera* essential oils in the reduction of Ag cations to AgNPs was confirmed by the Fourier-transform infrared spectroscopy (FTIR). Moreover, the bio-synthesized AgNPs when subjected to evaluation of bactericidal activity against both the Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*) and Gram-negative bacterial strains (*Escherichia coli*, *Enterobacter aerogenes* and *Agrobacterium tumefaciens*), showed higher activity against the Gram-negative bacterial strains. Furthermore, the particles under the study were also found to have potential to inhibit the growth of tested fungal strains (*Mucor species*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Fusarium solani*) in a concentration-dependent manner. Cytotoxicity against HePG2 and MCF7 cells was also found significant with  $\text{IC}_{50}$  of 13.89 and 19.29  $\mu\text{M}$ , respectively. Thus, it can be concluded that combination therapy of medicinal plants with metal nanoparticles might be one of the potential approaches to combat various diseases.

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## Introduction

Nanobiotechnology refers to the combination of nanotechnology and biology or application of the tools of nanotechnology in biology to solve problems at the atomic level (Bar *et al.*, 2009; Iravani, 2011). The products of nanotechnology such as nanomaterials or nanoparticles are the nanoscale entities having diverse applications in the biomedical research, by developing new equipment and tools (Deng *et al.*, 2008). Nanoparticles and nano devices have the potential to show a positive impact on human health and they can be used in the treatment of various serious diseases because of their activity, lower pore size and confined size. They are so technically designed that they can interact with sub-cellular levels of the body with high specificity (Bar *et al.*, 2009). The nanobiotechnology is at the interface of different multidisciplinary approaches where in chemistry, biology and biophysics, congregate (Ehlers *et al.*, 2007).

There are numerous methods of synthesizing nanoparticles but the most efficient method for synthesis of metal nanoparticles is the development of green chemistry approach, for its many advantages over the conventional synthesis of nanoparticles through chemical or physical methods. A green synthesis is an environment-friendly approach for manufacturing well-categorized nanoparticles. One of the most deliberated methods is the production of metal nanoparticles with the help of biological organisms. Plants appear to be the most influential and appropriate platform for large-scale biosynthesis of nanoparticles (Jain *et al.*, 2011).

This method is more suitable and with the help of this method, the level of synthesis is quicker as compared with the case of microbial's synthesis. Plants or plant-derivative resources for the biosynthesis of metal nanoparticles have guided researchers to examine contrivances of metal ions approval and bio reduction by plants, and to recognize the potential mechanism of metal nanoparticle development (Iravani, 2011). Thus, presently there is an extensive concern in developing uncontaminated, steadfast, biologically

compatible, benign, and eco-friendly green production of nanoparticles.

These medicinal plants are considered as a rich source of ingredients which can be utilized as a part of drug development and synthesis (Singh, 2015). Plants are an incredibly reasonable source of biologically active natural products which may fill in as financially significant elements in themselves or which may give lead structures to the advancement of modified derivatives having improved activity as well as reduced toxicity in the treatment of diseases (Sri *et al.*, 2010).

*Aloe vera* is a traditional medicinal plant which belongs to the family of Liliaceae (Saxena *et al.*, 2013). It is used for the treatment of several diseases, especially associated with stomach related problems and has been utilized for wounds, and skin issues (Choi *et al.*, 2001). Pharmacologically it detoxifies the system as aloes are immunity booster (Peng *et al.*, 1991). It is suggested in adjuvant treatment with anti-infection agents, to kill drug-induced gastritis and other antagonistic impacts in chemotherapy and Non-steroidal Anti-Inflammatory Drugs in different disorders, for example, type 2 diabetes, joint pain, eye ailment, tumour, liver protestations, retching, bronchitis, asthma, jaundice and ulcers. It also maintains a better gastric pH, helps in inflammatory bowel diseases, non-ulcer dyspepsia, gastric and duodenal ulcers (Vogler and Ernst, 1999). Several other medicinal effects of *Aloe vera* has been reported including anti-inflammatory, antitumor, laxative, antiseptic, antidiabetic, antioxidant, antibacterial and antiviral activity (Sahu *et al.*, 2013).

Thus, in the quest of exploring eco-friendly and biologically effective nanoparticles, the current study was designed with the objective of synthesizing silver nanoparticles using *Aloe vera* gel involving characterization and antimicrobial analysis.

## Materials and methods

### *Synthesis of silver nanoparticles*

Aqueous extracts of *A. vera* was prepared by the *Aloe*

*vera* gel. Plant stem was cleaned by washing with distilled water and 100 gram of thoroughly washed *Aloe vera* gel was finely cut and boiled in 100 mL of sterile distilled water. The resulting extract was filtered and stored at 4 °C for further experiments (Krishnaraj *et al.*, 2010). AgNO<sub>3</sub> (0.5 M) was prepared by taking 17g AgNO<sub>3</sub> dissolved in 200 ml of deionized water underneath magnetic stirring at room temperature for thirty minutes (Kwon *et al.*, 2005). Afterwards, 45 ml of 0.5M AgNO<sub>3</sub> was mixed with 5 ml aqueous plant extract and left until the color was changed to dark brown.

#### Characterization

The optical absorption features of AgNPs were recorded in the range of 300 to 600 nm wavelength by UV-visible spectrophotometer (UV 1602 BMS spectrophotometer, Spain)(Chandran *et al.*, 2006). Size and morphology of the samples were investigated using the Scanning Electron Microscopy (SEM) (TESCAN SEM, VEGA 3), operating at the voltage of 20kV (maximum) with the counting frequency of 2368 cps (maximum) (Kumar *et al.*, 2014). Chemical composition was confirmed by EDX (Oxford instruments) coupled with the SEM as plugin hardware. Magnified micrographs were taken up to the resolution of 10 µm in the scale bar.

The samples for X-ray diffraction (XRD) analysis were made by taking a small amount of solution from the bottle and drying it on a quartz plate (XRD D8 Advance, Bruker Germany). To determine the bioactive constituents of *Aloe vera* gel playing role in AgNPs synthesis, FTIR was performed (Naseem and Farrukh, 2015; Mohan and Renjanadevi, 2016, Mahendiran *et al.*, 2017).

#### Antimicrobial analysis

**Antibacterial activity:** The antibacterial activity of AgNPs was tested against five bacterial strains including two Gram-positive (*Micrococcus Luteus*, *Bacillus Subtilis*) and three Gram-negative (*Enterobacter*, *Agrobacterium tumefaciens* and *Escherichia coli*) using a disc diffusion method (Naseem and Farrukh, 2015; Mahendiran *et al.*,

2017). In a nutrient broth, the bacterial strains were sub cultured at 37°C and purified by incubation overnight. The specimen containing AgNPs were added to the discs on the agar surfaces swabbed with bacterial cultures and incubated at 37°C for 24-48 hours. The outcomes were noticed by calculating the zone of inhibition (mm) and the experiments were performed in triplicate. Distilled water and streptomycin were used as negative and positive control respectively.

**Antifungal activity:** The antifungal assay was performed by the agar tube dilution method as reported earlier (Ahmad *et al.*, 2008), against five fungal strains including *Mucor* species, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Solani*. Briefly, test tubes with screw caps were poured with 4 ml of Sabouraud dextrose agar (SDA) medium and subjected to the autoclave. After cooling down to 50 °C media was loaded with nanoparticles stock suspension to make 1000, 500 and 250 ppm final concentration. Tubes were subjected to slanted position and allowed to solidify at room temperature. Inoculation of fungal cultures was done in each tube from a 7-day-old fungal culture. Distilled water and terbinafine were added in the media to be used as negative and positive control, respectively. Incubation of the tubes was carried out at 28 °C for 7 days and results were recorded by measuring linear growth (cm) and growth inhibition was calculated with reference to the negative control with the given formula;

$$\text{Growth inhibition} = 100 \times \frac{\text{Fungal growth in the negative control} - \text{fungal growth in the tube containing sample}}{\text{fungal growth in the negative control}}$$

#### MTT assay

MTT assay was performed to test the cytotoxic effect of synthesized nanoparticles on breast cancer (MCF7) and liver cancer HePG2 cell line according to the reported procedure (Dilshad *et al.*, 2016). Briefly, cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (streptomycin & penicillin).

Cells at a conc. ~100000 were seeded in 96 well flat bottom plate (Corning, USA) and incubated for 24 hours at 37°C in an incubator (Shel Lab, USA) with 5% CO<sub>2</sub> supply. After 24 hours test material (in

triplicate) at different concentrations (50, 40, 30, 20, 10  $\mu$ M) was added to the wells containing the MCF7 and HePG2 cell lines along with untreated cells and the blank sample.

The plate was incubated again at 37°C with 5% CO<sub>2</sub> in the incubator for 24 hrs. Cell viability was then determined as follows: 10  $\mu$ l MTT (5mg/ml) was added to each treated sample, blank and untreated cells and incubated at 37°C for 3 hours. After that, 100  $\mu$ l of the solubilization solution (to dissolve formazan crystals) was added to the wells and incubation was done in dark at room temp for 2-4 hours. Finally, the absorbance of the samples was measured at 570 nm in plate reader FLUO star Omega (BMG Labtech).

#### Statistical analysis

All the experiments were run in triplicate, and the standard deviation was measured, furthermore, two way ANOVA was also applied using GraphPad Prism V.5.

### Results and discussion

#### Synthesis and Characterization of the biosynthesized AgNPs

The synthesis of AgNPs was visually confirmed by observing the dark brown colour of the reaction mixture placed in light overnight. Furthermore, the information about the size, aggregation and surface chemistry of *Aloe vera* nanoparticles were examined using UV- Vis spectrophotometer, which provided the confirmation of synthesis of AgNPs.

**Table 1.** Results of antibacterial assay of *Aloe vera* extract and AgNPs against different bacterial strains.

| AgNPs Conc (ppm)                              | Zone of inhibition (cm) $\pm$ S.E |                 |                    |                 |                       |                       |                     |                |                |                |
|---|-----------------------------------|-----------------|--------------------|-----------------|-----------------------|-----------------------|---------------------|----------------|----------------|----------------|
|   | Gram Positive Strains             |                 |                    |                 |                       | Gram Negative Strains |                     |                |                |                |
|   | <i>M. luteus</i>                  |                 | <i>B. subtilis</i> |                 | <i>A. tumefaciens</i> |                       | <i>E. aerogenes</i> |                | <i>E.Coli</i>  |                |
|   | <i>P.E</i>                        | <i>AgNPs</i>    | <i>P.E</i>         | <i>AgNPs</i>    | <i>P.E</i>            | <i>AgNPs</i>          | <i>P.E</i>          | <i>AgNPs</i>   | <i>P.E</i>     | <i>AgNPs</i>   |
| 100   | 0.75 $\pm$ 0.9                    | 1.1 $\pm$ 0.5   | 0.4 $\pm$ 0.23     | 0.75 $\pm$ 0.41 | 1 $\pm$ 1.33          | 1.6 $\pm$ 1.3         | 0.7 $\pm$ 0.34      | 1.4 $\pm$ 1.3  | 1.2 $\pm$ 1.2  | 1.9 $\pm$ 1.6  |
|   |                                   |                 | MIC 100            |                 |                       |                       |                     |                |                |                |
|   |                                   |                 | ppm                |                 |                       |                       |                     |                |                |                |
| 50  | 0.68 $\pm$ 1.1                    | 0.88 $\pm$ 1.21 | -                  | 0.5 $\pm$ 0.28  | 0.5 $\pm$ 1.5         | 0.75 $\pm$ 0.9        | 0.4 $\pm$ 1.3       | 0.62 $\pm$ 0.1 | 0.91 $\pm$ 0.9 | 1.2 $\pm$ 0.21 |
|   | MIC                               | MIC 50          |                    | MIC 50          | 5                     |                       | MIC 50              | 7              | 8              |                |
|   | 50ppm                             | ppm             |                    | ppm             | MIC 50                |                       | ppm                 |                |                |                |
|   |                                   |                 |                    |                 | ppm                   |                       |                     |                |                |                |
| 25  | -                                 | -               | -                  | -               | -                     | 0.51 $\pm$ 0.34       | -                   | 0.47 $\pm$ 0.2 | 0.53 $\pm$ 0.7 | 0.9 $\pm$ 1.1  |
|   |                                   |                 |                    |                 |                       | MIC 25                |                     | 3              | 8              |                |
|   |                                   |                 |                    |                 |                       | ppm                   |                     | MIC 25         | MIC 25         |                |
|   |                                   |                 |                    |                 |                       |                       |                     | ppm            | ppm            |                |
| 5   | -                                 | -               | -                  | -               | -                     | -                     | -                   | -              | -              | 0.5 $\pm$ 1.4  |
|   |                                   |                 |                    |                 |                       |                       |                     |                |                | MIC 5          |
|   |                                   |                 |                    |                 |                       |                       |                     |                |                | ppm            |
| AgNO <sub>3</sub>                             | -                                 | -               | -                  | -               | -                     | -                     | -                   | -              | -              | -              |
| Distilled H <sub>2</sub> O (Negative Control) | -                                 | -               | -                  | -               | -                     | -                     | -                   | -              | -              | -              |
| Streptomycin (Positive Control)               | 4                                 |                 | 2.2                |                 | 3.4                   |                       | 2                   |                | 3.6            |                |

The wavelength of spectrophotometer was set between 300 to 500 nm. In the UV-Visible spectrum a solid broad peak was perceived at 450 nm (Fig. 1a) which indicated the formation of AgNPs and spreading of the peak confers that particles are polydispersed (Van Dong *et al.*, 2012). AgNPs are recognized to exhibit a UV- Visible absorption extreme in the range between 400 to 500 nm because

of surface plasmon resonance (Van Dong *et al.*, 2012).

The elemental composition of the nanoparticles was examined by energy-dispersive X- ray spectroscopy (EDX) equipped on the SEM. The EDX profile showed a strong Ag signal indicating the Ag as a major component of synthesized nanoparticles. It was interpreted from the EDX spectrum (Fig. 1b) that

synthesized samples are purely Ag (with distinct Ag peaks) without adulterated elements. Peaks matching other than silver are due to the elements of the carbon-coated copper grid in the SEM measurement (Van Dong *et al.*, 2012).

The structural features regarding phase composition, the crystallographic orientation of the AgNPs were

further characterized by XRD (Fig. 2a) which has been used to outline and identify bulk materials and nanoparticles in different studies reported on the synthesis of nano particles (Zhang *et al.*, 2016).

The identified peaks [38.23 (1 1 1), 44.41 (2 0 0), 64.38 (2 2 0), 77.5 (3 1 1)] revealed the crystalline nature of synthesized AgNPs.

**Table 2.** Percentage inhibition of *Aloe vera* extract and AgNPs against different fungal strains.

| Conc.                     | Percentage Inhibition % ± SD |         |                  |            |                    |        |              |        |                 |          |
|---------------------------|------------------------------|---------|------------------|------------|--------------------|--------|--------------|--------|-----------------|----------|
|                           | <i>Solani</i>                |         | <i>A. Flavis</i> |            | <i>A.Fumigatus</i> |        | <i>Mucor</i> |        | <i>A. Niger</i> |          |
|                           | AgNPs                        | PE      | AgNPs            | PE         | AgNPs              | PE     | NPs          | PE     | NPs             | PE       |
| 1000ppm                   | 89.47±1.1                    | 40.70±2 | 73.9±0.67        | 37.24±0.14 | 83.74±2.1          | 43±0.4 | 60.64±1.4    | 15±1.2 | 81.87±1.1       | 37.6±0.4 |
| 500ppm                    | 78.94±0.3                    | 0       | 52.32±0.98       | 0          | 54.92±0.54         | 0      | 33.76±0.3    | 0      | 65.5±2          | 0        |
| 250ppm                    | 47.36±1.43                   | 0       | 46.54±1.7        | 0          | 28.92±1            | 0      | 13.33±0.1    | 0      | 41.81±0.76      | 0        |
| ED <sub>50</sub><br>(ppm) | 371                          | 948     | 698              | 951        | 500                | 948    | 700          | 951    | 357             | 951      |
| -ve control               | 0                            |         | 0                |            | 0                  |        | 0            |        | 0               |          |
| +ve control               | 100                          |         | 100              |            | 100                |        | 100          |        | 100             |          |

*Mucor species, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Fusarium solani*.

The XRD outcome was found in accordance with the standard ICSD No. 98-018-0878 (Martínez-Castañón *et al.*, 2009; Kumar Brajesh *et al.*, 2017). FTIR spectrometry was performed for the qualitative analysis of the expected to reduce and to cap agents/metabolites involved in the synthesis of nanoparticles (Fig. 2b). In the current study, FTIR spectra showed the disappearance of bands at 1400,

1300 and 1220 cm<sup>-1</sup>, could be the C–O stretching vibration of ethers and esters and also =C–O stretching of the carboxyl group of lipids of *Aloe Vera* essential oils, thus representing the involvement of their role in the synthesis of AgNPs. The electrostatic networks and hydrogen bond are the main reason for the synthesis of AgNPs by the plant extract because of their bioorganic capping molecules (Priya *et al.*, 2011).

**Table 3.** Analysis of variance for factors affecting the viability of HePG2 Cells.

| Source of Variation                      | Df | Sum-of- squares | Mean square | F-Value | P Value | Significant |
|--|----|-----------------|-------------|---------|---------|-------------|
| Interaction                              | 4  | 438.7           | 109.7       | 11.63   | <0.0001 | Yes         |
| Type of Sample (nanoparticles and Plant) | 1  | 1401            | 1401        | 148.5   | <0.0001 | Yes         |
| Concentration                            | 4  | 6583            | 1646        | 174.5   | <0.0001 | yes         |
| Residual                                 | 20 | 188.7           | 9.433       |         |         |             |

Further insight into the morphology and size of the synthesized AgNPs were observed by using SEM. The results of SEM analysis are indicated in Fig. 3, showing AgNPs to be spherical in shape with an average size of 40 nm ± 4. Our results have shown that the size range of synthesized AgNPs was

unobtrusively between the usual size ranges of nanoparticles which must be between 1 to 100 nm. Similar SEM results of the AgNPs within usual size range were also reported earlier (Singh A *et al.*, 2010). The experimental results displayed that SEM includes not only complete analysis of the

morphology of powdered silver, but also its topography. The fallouts we gained showed a narrow particle size dissemination which is fine designated by usual scattering (Puchalski *et al.*, 2007), although agglomerated at some points, as this potency might be due to the magnetic performance during SEM analysis of AgNPs and their greater surface area to

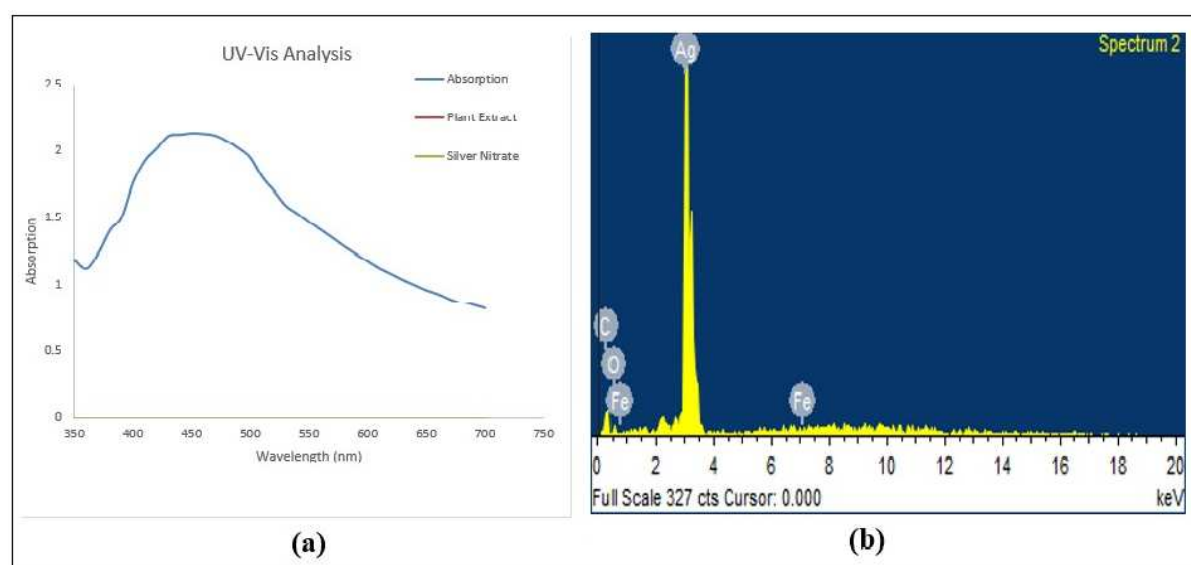
volume ratio have a tendency to be combined in order to decrease surface energy. Before measurement, the sample of AgNPs was filtered and derived in which aggregative formation is associated with the sample preparation in SEM analysis. SEM profiles of AgNPs with diverse interpretations have previously been described.

**Table 4.** Analysis of variance for factors affecting the viability of MCF7 Cells.

| Source of Variation                              | Df | Sum-of- squares | Mean square | F-Value | P Value | Significant |
|--|----|-----------------|-------------|---------|---------|-------------|
| Interaction                                      | 4  | 470.2           | 117.6       | 26.52   | <0.0001 | Yes         |
| Type of Sample (nanoparticles and Plant extract) | 1  | 2394            | 2394        | 540.0   | <0.0001 | Yes         |
| Concentration                                    | 4  | 470.2           | 117.6       | 26.52   | <0.0001 | yes         |
| Residual   | 20 | 88.67           | 4.433       |         |         |             |

The silver nanoparticles having spherical like formation shows the images presenting the aggregate of AgNPs (Ahmed *et al.*, 2016). Most of the time the

cluster of particles was is due to the weak capping agents, having a prominent role in the stabilization of nanoparticles (Nethradevi *et al.*, 2012).



**Fig. 1.** UV-Vis characterization of synthesized nanoparticles (a), EDX analysis (b).

#### Antimicrobial analysis of biosynthesized silver nanoparticles

**Antibacterial activity:** The antibacterial activity of prepared AgNPs (100, 50, 25, 5ppm) was tested against five different bacterial strains, two gram-positive, (*Micrococcus luteus*, *Bacillus subtiles*) and three gram-negative (*Enterobacter arogenes*, *Agrobacterium tumefaciens* and *Escherichia coli*) using disc diffusion method. Antibiotic streptomycin (100 ppm) was used as a positive control and distilled water as a negative control. *Aloe Vera* gel extract was

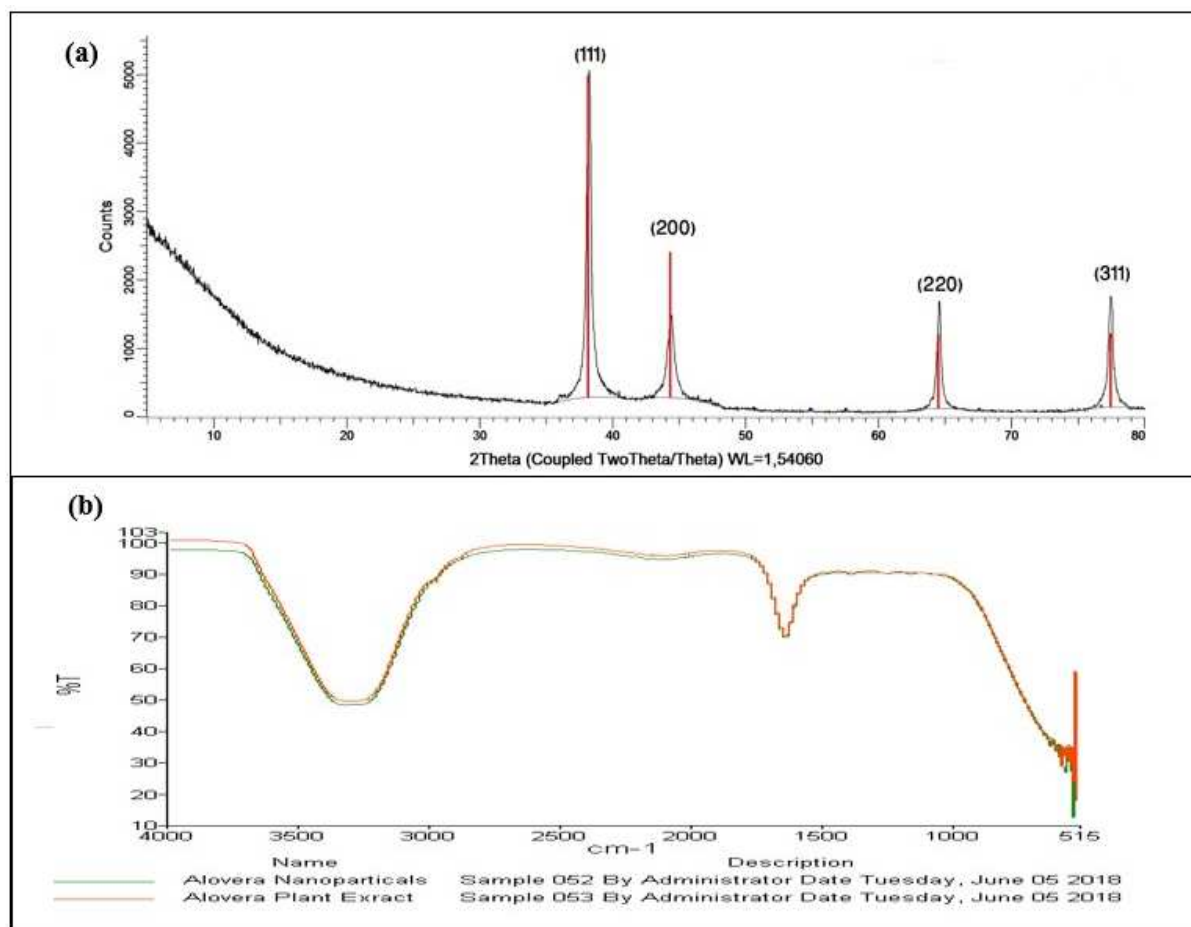
also tested for antibacterial activity in comparison with its AgNPs. It was observed that synthesized AgNPs inhibited the growth of all bacterial strains under study (Table 1, Fig. 4). Overall, the highest antibacterial activity of AgNPs was observed against *E.coli* 1.9 cm and the lowest was observed for *Bacillus subtilis* 0.75cm at 100 ppm. On the other hand plant extract showed relatively lower antibacterial activity as compared to AgNPs. The highest activity observed for plant extract was against *E.coli* (1.2 cm) and the lowest was against the *Bacillus subtilis* (0.4 cm) at



100 ppm. MIC was determined and are indicated in Table 1.

AgNPs were found more effective than that of the respective plant extract. The effect might be due to the small size of synthesized AgNPs as compared to the plant extract that enables them to penetrate into the thick walls of bacterial strains (Naveena and Prakash, 2013). Bactericidal activity of the synthesized nanoparticles was higher against gram-

negative bacteria than against gram-positive bacteria. It is mainly because of the fact that the cell wall of the gram-negative bacteria contains less peptidoglycan as compared to the gram-positive bacteria (Kumar *et al.*, 2016). Further reports also support the fact that nanoparticles have more bactericidal effects for gram-negative bacteria than gram-positive ones due to the thicker peptidoglycan cell wall (Naveena and Prakash, 2013).



**Fig. 2.** XRD (a) and FTIR (b) spectra of synthesized silver nanoparticles.

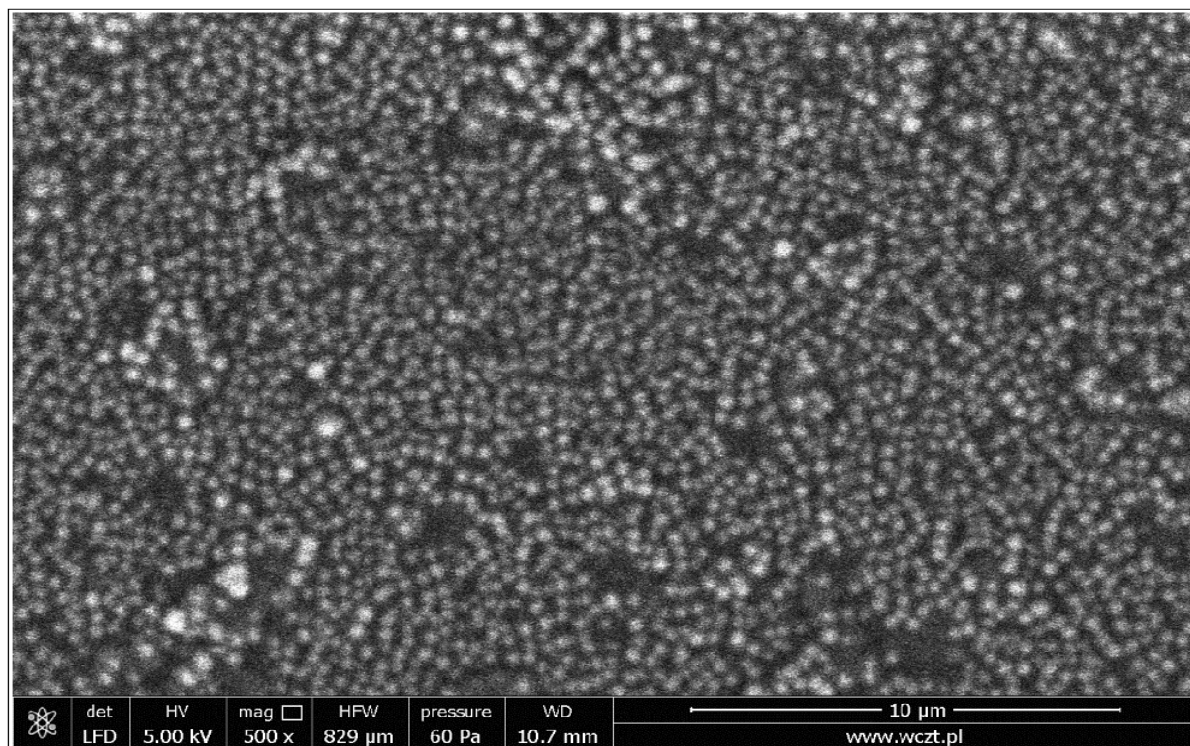
The size and shape of nanoparticles may affect antimicrobial activity. Even the stabilizing agent used may also affect the antibacterial activity, as well as hydrophobicity and charges of the particles, also can affect it. The smaller sized silver nanoparticles present the maximum antimicrobial activity. It was also observed that the smaller sized spherical shaped silver nanoparticles confirmed an improved antibacterial activity as compared to the larger and triangular shaped silver nanoparticles because they

have the greater surface area to volume ratio which makes them more reactive than other shaped particles (Pal *et al.*, 2007, Raza *et al.*, 2016). The shape and size dependent bactericidal efficacy could simplify a new pattern for making an allowance for the true role of silver nanoparticles as antimicrobial agents in drug design (Balaji *et al.*, 2015).

**Antifungal activity:** In the current study, the antifungal activity of the synthesized silver

nanoparticles utilizing *Aloe vera* gel has been evaluated against five fungal strains to determine their fungicidal potential. Nanoparticles were tested at different concentrations i.e. 1000, 500, 250 ppm and different percentage inhibition was observed

against different fungal strains. The results obtained are summarized in Table 2. Highest percentage inhibition was observed against *Solani sp.* i.e. 89%, whereas the lowest was observed against *Mucor sp.* i.e. 60% for AgNPs.



**Fig. 3.** SEM micrographs of synthesized silver nanoparticles.

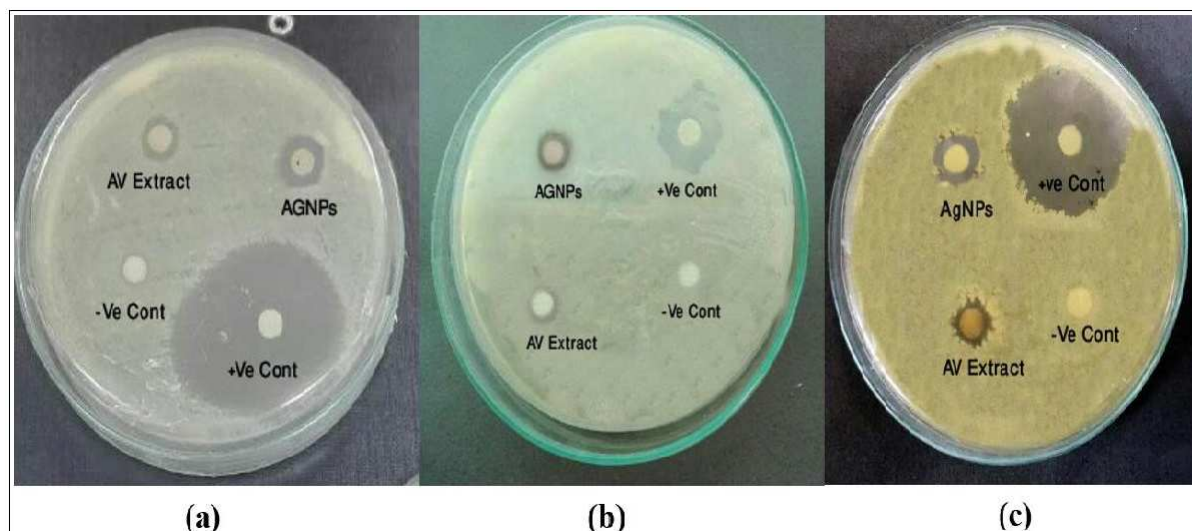
The plant extract was found less effective with highest growth inhibition against *Solani* (40%) and lowest against *Mucor* (15%). ED<sub>50</sub> of plant extract was also found higher i.e. around 950 ppm as compared to that of nanoparticles which ranged from 375-700 ppm. There was no growth observed in case of positive control which was antifungal drug terbinafine. Whereas, negative control (distilled water) showed 100% fungal growth. The impact of nanoparticles and their concentrations was also found statistically significant ( $p < 0.01$ ).

Above results show that “more the concentration of nanoparticles, less will be the growth of fungal strains.” Nanoparticles retarded the growth of the tested fungal strain in a concentration-dependent manner. In another report, the growth of fungi was reduced by using silver nanoparticles at different concentrations. The minimum growth was at

0.21mg/L using non stabilized silver nanoparticles (Panáček *et al.*, 2009). In another report, the antifungal activity of silver ions and nanoparticles was examined on the basis of colony formation by in vitro petri dish assays. As concentrations of the silver compound increased, colony formation decreased. Antifungal activity of silver to reduce the colony formation was apparent within 1 h (Jo *et al.*, 2009).

In fact, when AgNPs interact with microorganisms (bacteria, fungi, and viruses), silver ions (Ag<sup>+</sup>) are released and these ions may affect and damage the microorganism in different ways; for example, they attack the negatively-charged cell walls of the microbes to deactivate cellular enzymes and disrupt membrane permeability; consequently, cell lysis and cell death occurs (Feng *et al.*, 2000). Furthermore, a number of mechanisms have been attributed to the antimicrobial activity of silver NPs.

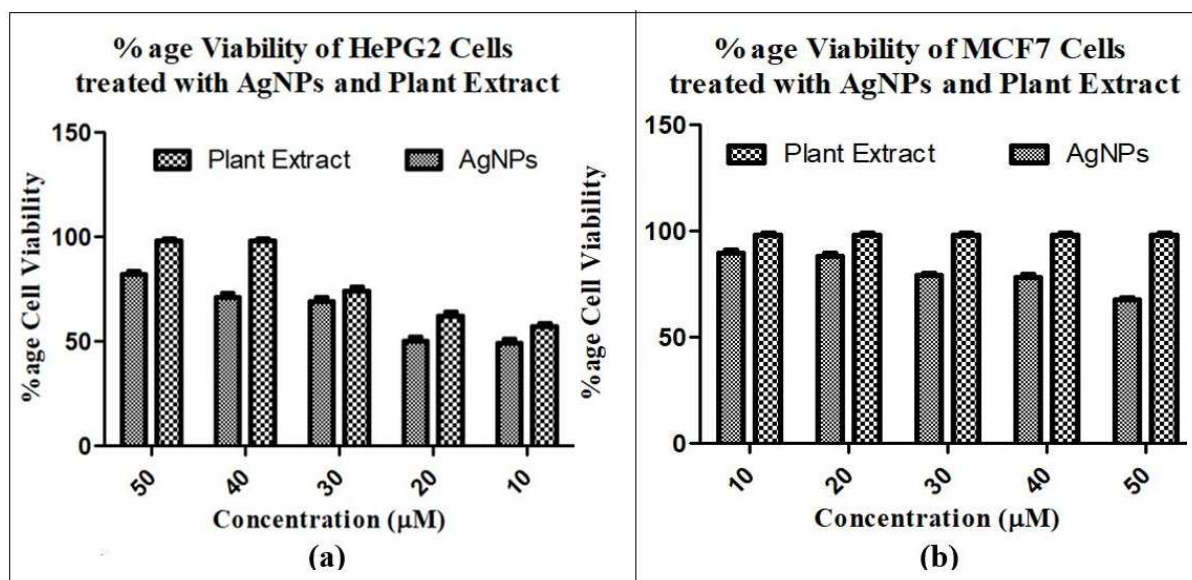




**Fig. 4.** Antibacterial activity of synthesized silver nanoparticles (AgNPs), *Aloe vera* plant extract (AV Extract) against *A. tumefaciens*(a), *E. aerogenes* (b) and *E.Coli*(c).

The AgNPs may alter the membrane permeability of the microbes allowing inflow of AgNPs into the cell. The interaction of the NPs with intracellular proteins, particularly sulphur containing membrane proteins and microbial DNA can interfere with cell division leading to cell death. Replication of microbes is compromised due to the release of Ag ions from the

AgNPs (Fernando *et al.*, 2018). Interestingly, the antimicrobial activity of nano-sized silver particles was found size- and shape-dependent, one of the reasons could be that different morphologies provide different areas to interact with microbes and thus results in different antibacterial efficiency (Van Dong *et al.*, 2012).



**Fig. 5.** Percentage viability of HePG2 (a) and MCF7 cells treated with AgNPs and plant extract.

As previously evaluated, these nanoparticles were found spherical in shape with an average size range of 40nm this can be related to their good antifungal activity. As reported earlier, isotropic geometries such as spherical particles also demonstrated high

antimicrobial effectiveness. Their main argument was a large surface to volume ratio of spherical shapes, which provided the maximum reactivity to obtain the highest antimicrobial activity. Therefore, the investigations to understand the influence of

nanoparticles with different geometry size, chemical functionality, and surface charge on biological systems is of great importance (Albanese *et al.*, 2012).

#### *Cytotoxicity evaluation by MTT assay*

To determine the cytotoxicity and cell viability, MTT assay was performed with HePG2 and MCF7 cancer cell lines. Viability of HePG2 cells decreased to 45% (Fig. 5a) and MCF7 cells to 67% (Figure 5b) when treated with AgNPs of *Aloe vera* at the concentration of 50  $\mu\text{M}$  with an  $\text{IC}_{50}$  value of 13.89 and 19.29  $\mu\text{M}$  respectively. *Aloe vera* plant extract did not show any activity against MCF7 cells though it was found effective against HePG2 cells with an  $\text{IC}_{50}$  value of 33.19  $\mu\text{M}$ . Impact of nanoparticles and concentration was also found statistically significant with  $p < 0.01$  for both cancer cell lines (Table 3 & 4). The proliferation of both cancer cell lines was found to be decreased in a concentration- dependent manner (Fig. 5). ISO standards confirm a drug or compound to be cytotoxic if it decreases the cell viability by more than 30% (Kurt *et al.*, 2017). Furthermore, It is reported that the cytotoxic effect of the silver nanoparticles is mainly because of the oxidative stress which generates ROS and that this activity is also size dependent (Carlson *et al.*, 2008, Kim *et al.*, 2009).

Previously, silver nanoparticles have been reported to induce apoptosis of various tumour cells, which mainly occurred because of silver translocation within the nucleus (Park *et al.*, 2010; Banerjee *et al.*, 2017). It was further elaborated that phagocytized silver nanoparticles totally block cell cycle in the S-phase and excites inflammatory signalling through ROS generation, which finally induces the secretion of TNF- $\alpha$  (Karunagaran *et al.*, 2017). Previously, silver oxide nanoparticles were also found cytotoxic to decrease the viability of HePG2 and Chang liver cells (Karunagaran *et al.*, 2017) and MCF7 cells as well (Franco-Molina *et al.*, 2010).

#### **Conclusion**

The fast and swift green synthesis of silver nanoparticles utilizing *Aloe vera* has been done which provided ecological well disposed of eco-friendly and

effective routes for the synthesis of considerable nanoparticles. Astonishingly silver nanoparticles have also displayed a strong powerful antimicrobial and cytotoxic potential. The antimicrobial and cytotoxic activity of synthesized silver nanoparticles was more than their corresponding plant extract. So, these stable metallic nanoparticles can be used in targeted drug delivery and treatment of various diseases.

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### Abbreviations

AgNPs; silver nanoparticles, conc; concentration, SE; standard error, P.E; Plant extract, M. luteus; Micrococcus luteus, S. aureus; Staphylococcus aureus, B. subtilis; Bacillus subtilis, A. tumefaciens; Agrobacterium tumefaciens, S. Setubal; Salmonella Setubal, E. aerogenes; Enterobacter aerogenes.