ISSN: 2349 - 4891

International
Journal of Recent Research and Applied Studies
(Multidisciplinary Open Access Refereed e-Journal)

Pharmacological Potential of Silver Nanoparticles (AgNPs) derived from *Evolvulus Alsinoides*

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Received 4th April 2016, Accepted 1st June 2016

Abstract

This study investigates an efficient and sustainable route of AgNPs preparation from 1 mM aqueous $AgNO_3$ using leaf extracts of Evolvulus alsinoides. The AgNPs were characterized by UV–vis spectrophotometer, scanning electron microscope (SEM), DLS-Size and zeta potential analysis showed that the synthesized silver nanoparticles are varied from 50 - 75 nm and have the spherical shape. Further the possibility of protein as a stabilizing material in silver nanoparticles is revealed by FTIR analysis and the XRD investigation confirms monocrystalline phase of silver with FCC crystal structure. The antimicrobial activity of Ag nanoparticles was investigated against some human pathogens. In these tests, silver nanoparticles (AgNP) can inhibit microbial growth and even kill microbes, from now confirmed their antimicrobial significance.

Keywords: Evolvulus alsinoides, Silver nanoparticles, Antimicrobial activity, Green synthesis.

Introduction

In the case of silver nanoparticles, the antibacterial effect is greatly enhanced and because of their tiny size. Nanoparticles have immense surface area relative to volume. Therefore, minuscule amounts of silver nanoparticles can lend antimicrobial effects to hundreds of square meters of its host material. Among inorganic antibacterial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage (Sinthiya and Koperuncholan, 2015 and Koperuncholan and Ahmed John, 2011). The antibacterial and antiviral actions of silver, silver ion, and silver compounds have been thoroughly investigated. With the arrival and growth of microbial organism's resistant to multiple antibiotics, and the continuing importance on health-care costs, many researchers have tried to change new, effective anti-microbial reagents free of resistance and cost. Such problems and desires have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Ahmed John and Koperuncholan, 2012a).

Therefore, antimicrobial silver is now used extensively to combat organisms in wounds and burns. It works because pathogens cannot mutate to avoid the antimicrobial effect of silver (Anitha et al., 2011 and Fazal Mohamed et al., 2011). The importance of silver ions has been also found in the treatment of burn wound by various researchers who studied the antimicrobial properties of silver nanoparticles against virulent pathogens (Ahmed John and Koperuncholan, 2012) The effect of the nanoparticles was found to be significantly more pronounced on MDR strains. For these reason, we investigated the *Evolvulus alsinoides* derived silver nanoparticles against for some human pathogens for their antimicrobial properties.

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Materials and Methods

Aqueous extraction

Freshly collected *E. alsinoides* leaves were shad dried and powdered. The 10 g of sterilized fine powder was taken and mixed with 100 ml of Milli Q water and kept in boiling water bath at 60 °C for 10 min. The extracts were filtered with Whatman No. 1 filter paper. The filtrate was further filtered through 0.6 µm sized filter paper. The filtrate was used for the present study. The filtered extracts were stored in a refrigerator at 4°C for further studies to avoid microbial pollution.

Biosynthesis of silver nanoparticles

The 1mM of silver nitrate was reduced using 100 ml of 5% leaf extract at room temperature for the synthesis of silver nanoparticles. Silver nitrate has taken in similar quantities without adding plant extracts to main respective controls. It was resulting in the dark brown solutions indicating the formation of silver nanoparticles. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of silver ions, incubated at room temperature under dark condition and observations were recorded

Characterization of silver nanoparticles

UV-Vis spectroscopy

Synthesis of silver nanoparticles by reducing, the respective metal ion solution with leaves extract may be easily observed by UV- Vis spectroscopy. The absorption spectra of leaves extract quantities and metal concentration was measured using a Perkin- Elmer Lambda- 45 spectrophotometer in 300-1000 nm range.

Fourier transform infrared spectroscopy (FTIR)

Samples were measured by Shimadzu 8400s and using spectral range of 4000- 400 cm-1with resolution of 4 cm^{-1}. The FTIR spectra of leaf extract taken before and after synthesis of silver nanoparticles were analysed to study the possible functional groups for the formation of silver nanoparticles.

Scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDX)

The freeze dried sample of Ag NP solution was sonicated with distilled water, small drop of this sample was placed on glass slide allowed to dry. The accelerating voltage of the microscope was kept in the range 10-20 kV. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDX analysis of Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDX normally reveals the presence of phases.

Dynamic light scattering (DLS)

A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie-scattering theory. In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor (Vibronics, model: VPLP1). Then experiment was carried out in computer controlled particle size analyzer [ZETA Sizers Nanoseries (Malvern Instruments Nano ZS)] to find out the particles size distribution.

X-ray diffraction (XRD)

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherland) using Cu Ká radiation. The generator voltage and current was set at 35 KV and 25 mA respectively. The ZnO and Ag samples were scanned in the 2è ranges 15 to 700C range in continuous scan mode. The scan rate was 0.04/sec. Phases present in the sample has been identified with the search match facility available with Philips Expert high score software. The crystallite size of the calcined powders was determined from X-ray line.

Antimicrobial activity

The antimicrobial assays were also performed by standard disc diffusion method (Vignesh et al 2012a, 2012b, 2013). The media was autoclaved and cooled. The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), Salmonella *typhimurium* NCIM 2501 (B5), *Vibrio cholarae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1) and *Cryptococcus* sp. MTCC 7076 (F2). *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The media was poured in the petri discs and kept for 30 minutes for solidification. A sterile cotton swab was used to inoculate the bacterial and fungal suspension on surface of MHA and PDA agar plates (Pandiyarajan et al. 2013, Vignesh et al., 2015a, 2015b). The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used (Beevi et al., 2012). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus) (Lakshmi praba et al., 2013). After incubation, the zone of inhibition was measured with ruler. All the trial was performed thrice and mean values were presented.

Results and discussion

Biosynthesis of AgNPs

Ag nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon resonance (Koperuncholan and Ahmed John, 2011a). On mixing the extract with aqueous solution of the $AgNO_3$ complex, a change in the colour from colourless to yellowish brown was observed. It was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles.

Characterization of silver nanoparticles

UV–Vis spectral analysis

The UV-Vis absorption spectra of the Ag NP were shown in Figure 1. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 420 nm. A remarkable broadening of peak at around 350 nm to 480 nm indicates that the particles are polydispersed (Koperuncholan and Manogaran, 2015). It was observed that the peak was blue shifted in the absorption spectrum from 350nm to 480 nm with increasing reaction time.

Scanning electron microscope (SEM)

The SEM image of Silver nanoparticles synthesized by green synthesis process by using 10 % *E. alsinoides* extract and 1mM AgNO3 concentration was shown in Figure 2. It gave a clear image of highly dense silver nanoparticles. The SEM image showing silver nanoparticles synthesized using *E. alsinoides* extract confirmed the development of silver nanostructures.

Energy dispersive spectroscopy (EDS)

EDX characterization has shown absorption of strong silver signal along with other elements, which may be originate from the biomolecules that are bound to

the surface of nano silver particles. From EDX spectra, shown in Figure 3, it is clear that silver nanoparticles reduced by *E. alsinoides*.

Dynamic light scattering of particle size and zeta potential analyses

The Figure 4 shows the particle size of the Ag nanoparticles samples. After analysing data, it was found that Ag nanoparticle size were in the range of 80-120 nm. However, beyond 100 nm range the percentage of nanoparticles present is very less. The highest fraction of AgNPs present in the solution was of 85nm. From the plot it was evident that the solution was consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis. Zeta potential measures the potential stability of the particles in the colloidal suspension. Silver nanoparticles generally carry a negative charge. The synthesized silver nanoparticles from the plant showed negative charge and were stable at room temperature. DLS-zeta potential showed negative charge (-35.8) which indicated that the sample is moderately stable at room temperature (Figure 5).

Fourier transform infra-red spectroscopy

Different functional groups absorb characteristic frequencies of IR radiation. Thus, IR spectroscope is an important and popular tool for structural elucidation and compound identification. Our observation confirms the presence of such compounds in the sample which coat covering the silver nanoparticles known as capping agents. FTIR analysis of *E. alsinoides* raw leaves and their plant derived AgNPs has been shown in Figure 6. FTIR showed the presence of bands due to O-H stretching due to (3434cm-1) vibration of the alcoholic compounds, aldehydic C–H stretch (2371,2123 and 2076cm-1), C-O stretch (1636cm-1) arises from carbonyl group, N-O, C-C (687 cm-1) and C-O stretch

X-ray diffraction

The *E. alsinoides* extract-mediated synthesized Ag nanostructure was confirmed by the characteristic peaks observed in the XRD image which was shown in Figure 7. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines. These diffraction lines observed at 2θ angle 32.20 and 38.10 respectively, have been indexed as (111) and (200) respectively. The typical XRD pattern revealed that the sample contains a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 70 nm derived from the FWHM of peak corresponding to 111 plane with cubic and hexagonal shape.

Antimicrobial studies

The bioactive compounds were naturally available in many sources and were used for many medicinal purposes. But it need some sort of treatment for marketable (Vignesh et al., 2011). In the case of nanoparticles, synthesis and application is very easy. In this study, inhibitory activities were recorded for both gram-positive and gram-negative bacteria. Gram-positive were highly inhibitory than gram-negative bacteria. The test concentrations (15 and 30 µL/disc) produce zone on MHA and PDA plates for bacteria and fungi, respectively. The antimicrobial activity of AgNPs samples were challenged against various NCIM and MTCC microbes using the disc diffusion method. In AgNPs sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from Micrococcus luteus NCIM 2871 (B4) in the bacterial division. But in fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus sp*. MTCC 7076 (F2).

In nanoparticles study, the higher (30 µL/disc) concentration got larger zone effect than the small (15 µL/disc) concentrations against certain microorganisms. All the microbial strains depict higher sensitivity to the higher concentration for the test sample (Vignesh et al., 2014; Muthukumar et al., 2015). There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. Koperuncholan (2015a) reported the best antimicrobial activity of *piper nigrum*, which showed maximum activity against *E. coli* and *Enterobacter aerogenes.* Several phytoconstituents such as terpenoids (Koperuncholan et al. 2010), flavonoids and tannins (Ramesh et al., 2014), are effective against certain microorganisms. The results of the present investigation clearly demonstrate the antibacterial and antifungal activities of the aqueous extracts of the leaves.

Table 1. Antimicrobial activity of *Evolvulus alsinoides* leaves extract derived AgNPs

S.No	Test Microorganisms		AuNPs µL/disc		PC	Diseases	Route of Transmission
	Bacteria		15	30	10 mcg		
1.	*Aeromonas liquefaciens*	B1	11	12	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis*	B2	13	16	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae*	B3	12	14	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus*	B4	11	13	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium*	B5	14	18	0	Typhoid	Water / Food
6.	*Vibrio cholarae*	B6	13	14	16	Cholera	Water / Food
	Fungi						
7.	*Candida albicans*	F1	12	13	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp.	F2	9	10	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis*	F3	10	10	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* F4		13	16	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)
Samples – 15, 30 mg/ml (well)

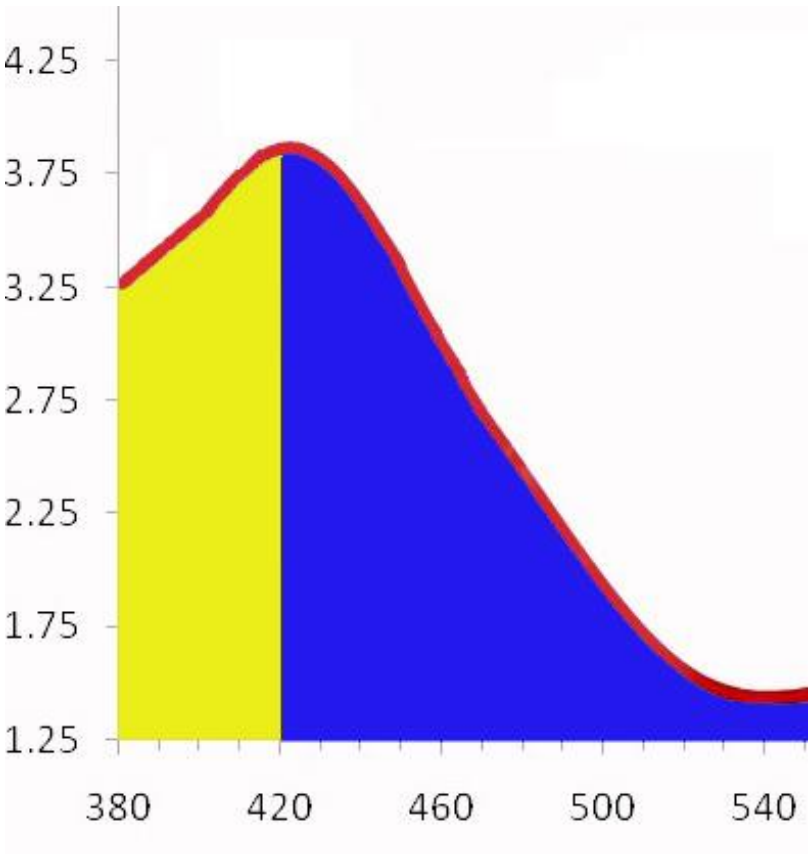

Figure 1. UV characterization of AgNPs.

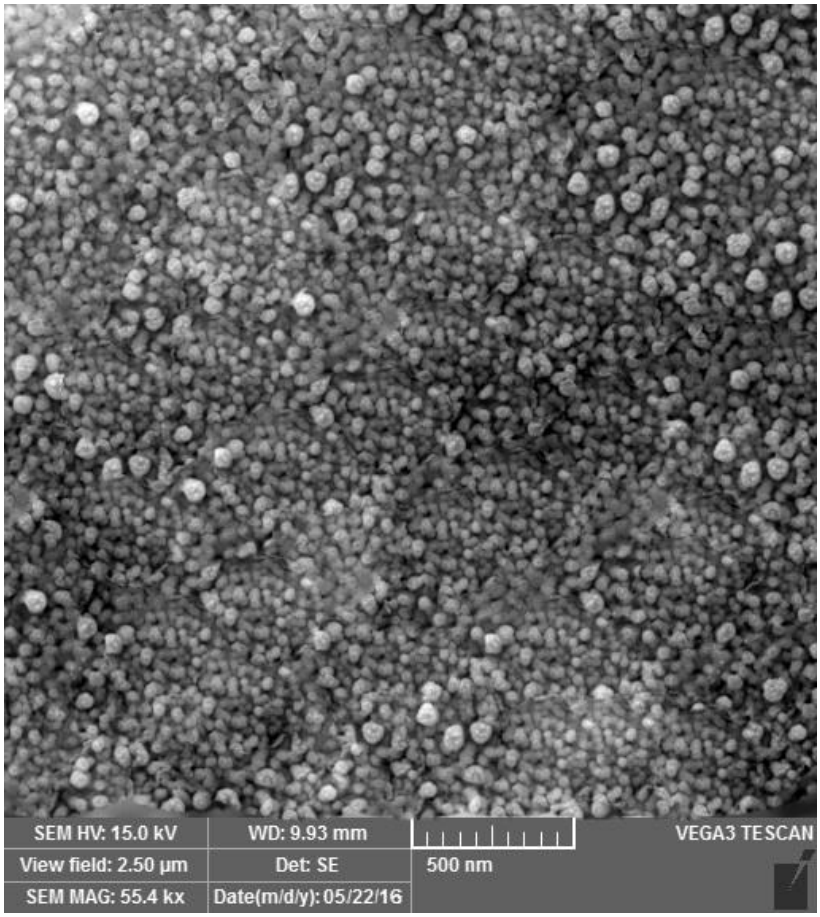

Figure 2. SEM characterization of AuNPs

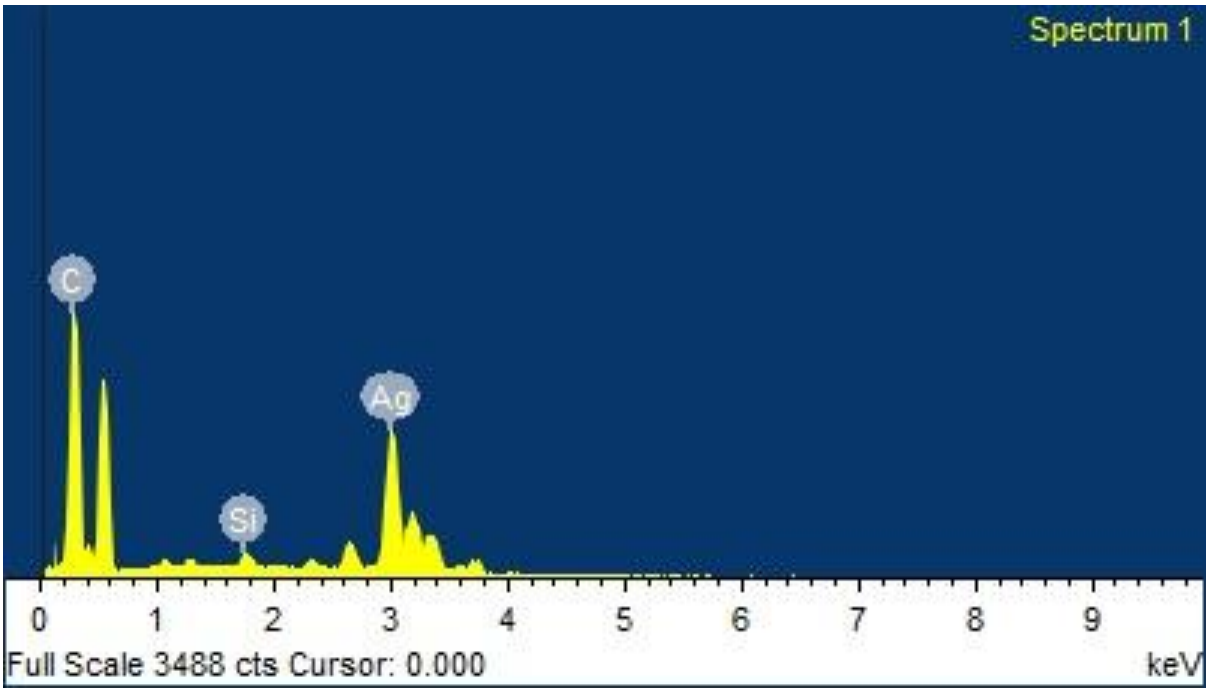

Figure 3. EDAX characterization of AgNPs.

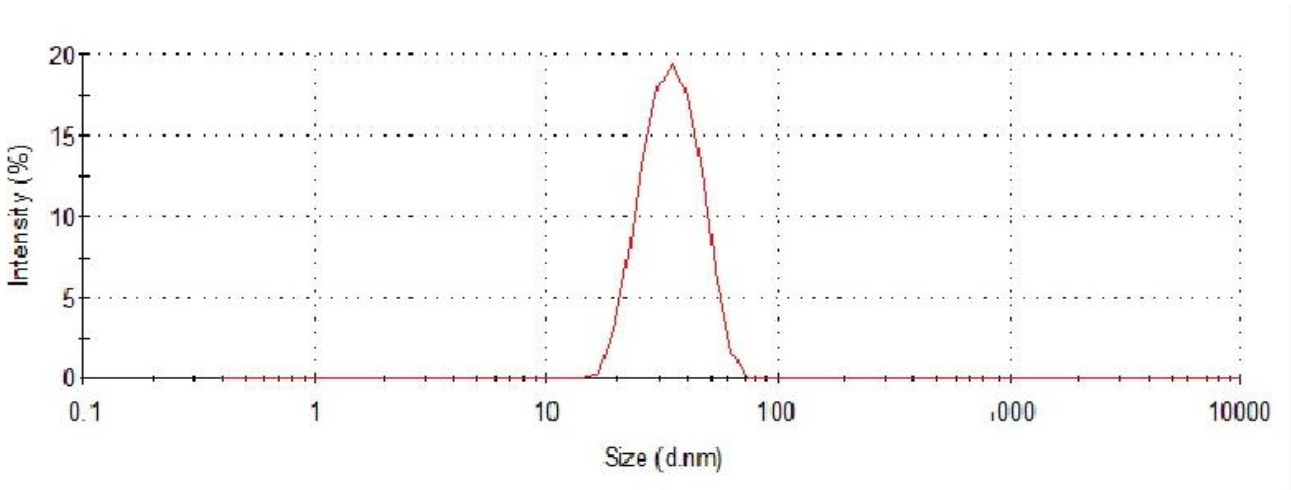

Figure 4. DLS Size distribution characterization of AgNPs.

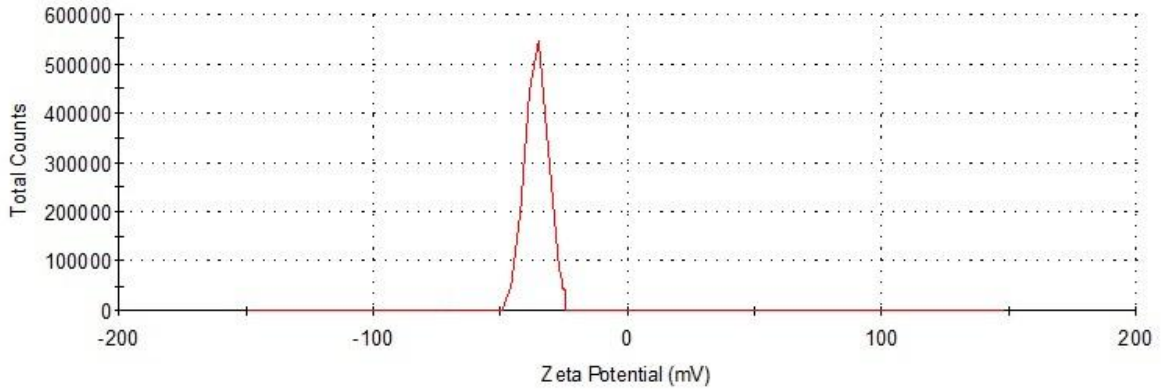

Figure 5. DLS Zeta potential characterization of AgNPs.

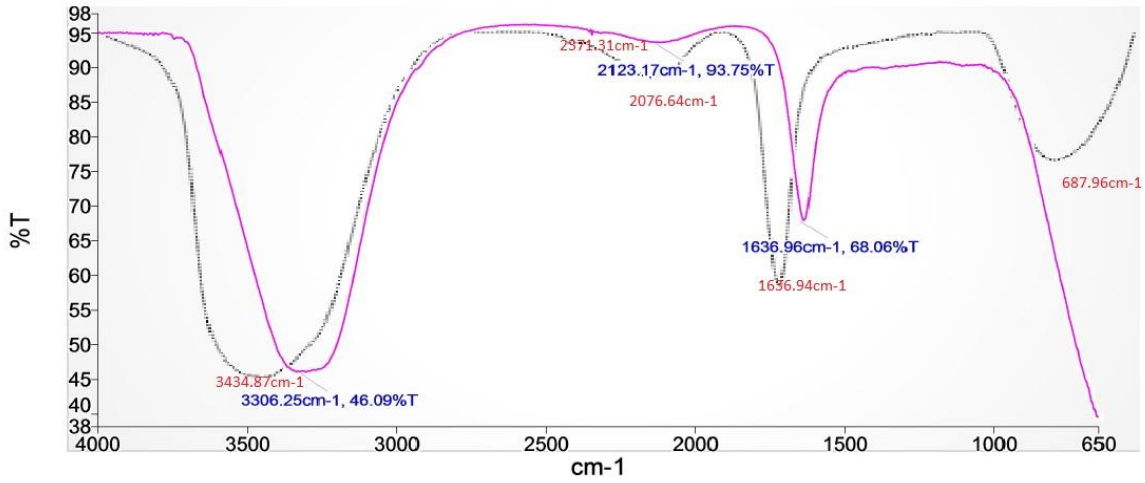

Figure 6. FTIR characterization of plant broth and AgNPs.

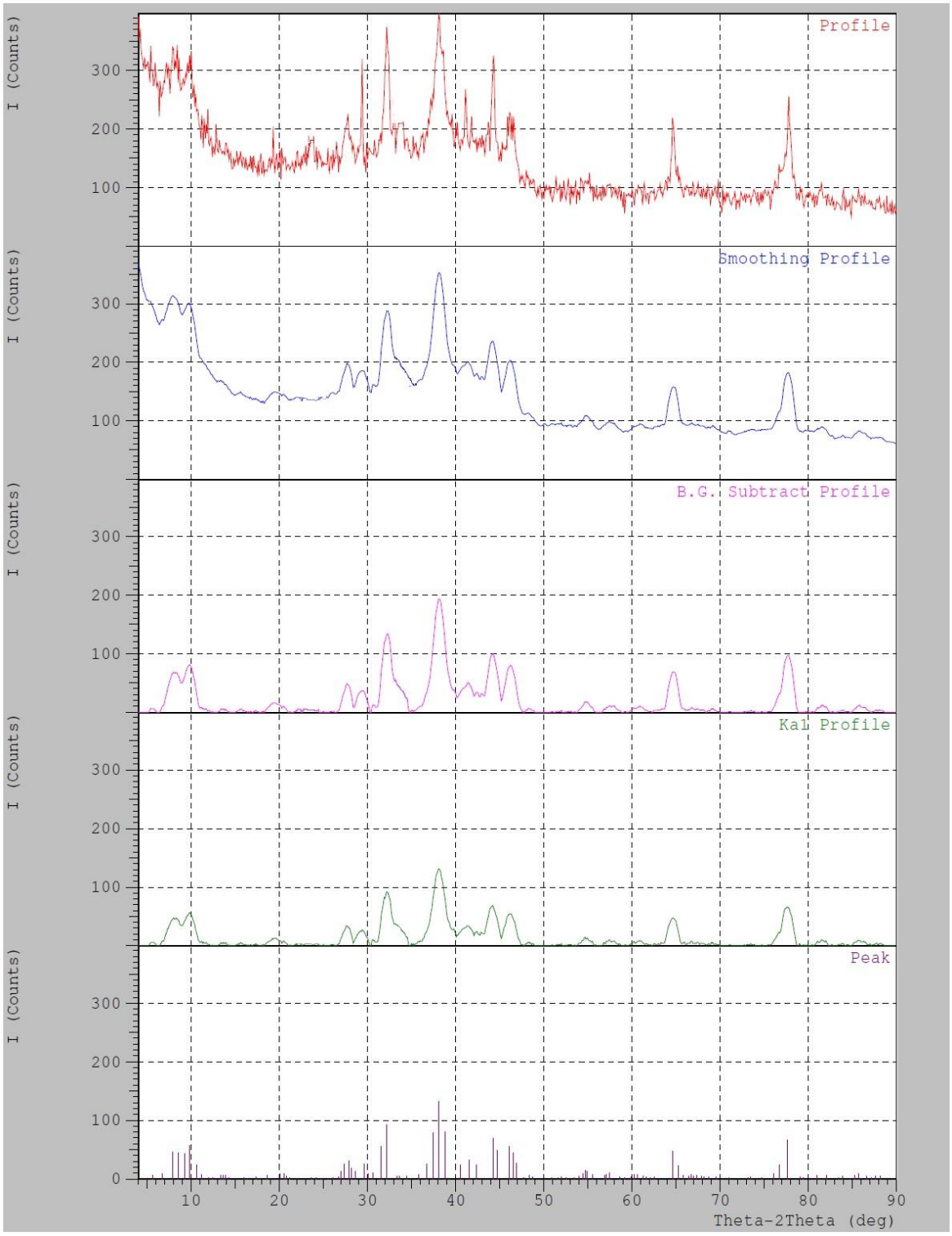

Figure 7. XRD characterization of AgNPs.

Conclusion

The potential benefits of nanotechnology in biomedical and industrial applications have become widely accepted and are the most promising sector for the generation of new applications in medicine. From the present study even at very small concentration (in µg/ml) *E. alsinoides* derived AgNPs possess very good antimicrobial activity which makes them a potent source of antimicrobial agent against some human pathogens. Due to the structural difference in the composition of the cell walls of Gram-positive and Gram-negative AgNPs have significantly less effect on the growth of Gram-positive bacteria. Also, green synthesis of AgNPs can potentially eliminate the problem of chemical agents that

may have adverse effects, thus making nanoparticles more compatible with the eco-friendly approach. Moreover, the synthesized AgNPs enhance the therapeutic efficacy and strengthen the medicinal values of *E. alsinoides*.

Acknowledgement

The authors thank the Biospark Biotechnological Research Center (BBRC), Tiruchirapalli, Tamil Nadu, India for characterizations of nanoparticles.

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