Biosynthesis and Antibacterial Activity of Silver and Gold nanoparticles using Liquorice root: A Green Chemistry Approach

Chandan Singh[1], Jatindra K Pradhan[1], Ritesh K Baboota[1], Pradeep K Naik[1], Anil Kant[1], Harvinder Singh[1]*

[1]Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan 173234, Himachal Pradesh, India.

*Address for correspondence: Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Distt.- Solan, Himachal Pradesh, India (Pin-173234).

Phone No. 91-1792-239240, Fax No. 91-1792-245362 Email: harvinder_07@yahoo.com

Abstract: Nanobiotehnology has gained a great impetus in modern world with potential application to human welfare. An important area of nanobiotechnology is the development of reliable and environmental friendly approach for synthesis of nanoscale particles through biological systems. This study focuses on biosynthesis of gold and silver nanoparticles using extract of liquorice root employing green chemistry approach. The treatment of aqueous solution of $AgNO_3$ and $HAuCl_4$ with liquorice root extract resulted in rapid formation of stable nanoparticles for both metals. The growth of nanoparticles is monitored by UV-vis spectrophotometer. The bionanoscale patricles were further characterized using Transmission Electron Microscopy, Atomic absorption spectroscopy, X-ray Diffraction and Fourier Transform Infrared Spectroscopy. Transmission Electron Microscopy revealed the presence of polydisperse gold and silver nanoparticles with an average size of 46.6nm and 30.2nm respectively. X-ray diffraction studies corroborated that the biosynthesized nanoparticles are crystalline gold and silver. Furthermore, liquorice root mediated gold and silver nanoparticles could act as an effective antibacterial agent and can prove as an alternative for the development of effective antibacterial agents to overcome the problem of resistance.

Keywords: Gold nanoparticles (AuNP), Silver nanoparticle (AgNP), antimicrobial assay

1. Introduction

Nanoscience is one of the potential areas of research and has opens up new frontiers in the field of material science and engineering. Research efforts in this area have resulted in innovative nanodevices and nanostructures for applications in diagnostics [1], biosensing [2], therapeutics [3], drug delivery and targeting [4] etc. Recent advances in the use of nanoparticles in medicine includes delivery of antigen for vaccination [5], gene delivery for treatment or prevention of genetic disorders [6], and in cardiac therapy [7] led to development of environmentally benign nanoparticle synthesis process to avoid adverse effects. Many biotechnological applications such as remediation of toxic metals employ microorganism such as bacteria [8] and yeast [9] but their application in synthesis as eco-friendly nanofactories is relatively new. Bacterium *Pseudomonas aeruginosa* was applied for the extracellular synthesis of gold nanoparticles (AuNPs) with diameter ranging in 15-30

nm [10], *Rhodococcus sp*. synthesized smaller (5-15 nm) AuNPs intracellularly [11], while fungus *V. luteoalbum* synthesized AuNPs intracellularly in diameter sparing few to 100 nm [12]. Synthesis of silver nanoparticles (AgNPs) is also obtained using *Aspergillus fumigates* with diameter 5-25 nm. Similarly *Fusarium oxysporum* was able to synthesize AgNPs in the range of 5-15 nm extracellularly [13], AgNPs with diameter few to 200 nm were produced using Bacterium *Pseudomonas stutzeri* [14], while the gungus, *Verticillium* synthesized both AuNPs and AgNPs with diameter 20 and 25 nm respectively [15,16].

Current modalities of synthesis of nanoparticles are mediated by plant extract (known as green synthesis). This has several advantages over the traditional methods because of its cost effectiveness, easy availability, maintenance of culture etc. As an example, leaf extracts of geranium (*Pelargonium graveolens*) resulted in synthesis of spherical rods, flat, sheets and triangular AuNPs [17]. Controlled syntheses of AuNPs were achieved using lemongrass (*Cymbopogon flexuosus*) to obtain anisotropic gold nanotriangles [18]. Alfalfa (*Medicago sativa*) biomass also efficiently reduce Au (III) ions to Au (0) nanoparticles of different types such as fcc tetrahedral, hexagonal platelet, and irregular shaped particles [19], while leaf extracts of *Capsicum annuum* reported for the crystalline AgNPs, which was a time dependent reaction since 5 hours reaction time led to spherical nanoparticles (10 ± 2 nm) and with increase in reaction time to 9 hours and 13 hours, the size of the nanoparticles was increased to 25 ± 3 nm and 40 ± 5 nm, respectively [20]. Recently it was reported that a plant polyphenol, quercetin (3,5,7,3',4'-pentahydroxyflavon,$C_{15}HgO_7O_2H_2O$) was involved in rapid biosynthesis of AgNPs [21]. Plants rich in polyphenol like *Cinnamomum camphora* was used for synthesis of both SNPs and GNPs in the range of 55-80 nm [22], similarly plant like amla (*Emblica officinalis*), showed potential for extracellular synthesis of gold (15-20 nm) and silver nanoparticles (10–20 nm) [23]. Neem (*Azadirachta indica*) leaf broth has also been used for the extracellular synthesis of pure metallic silver, gold and bimetallic Au/Ag nanoparticles [24]. Similarly extract of ginger (*Zingiber officinale*) rhizome was observed to be effective in production of spherical AgNPs and AuNPs [25].

In our attempt to synthesize AgNPs and AuNPs we have used extract of Liquorice root (LR) (*Glycyrrhiza glabra*, Fabaceae). It is a traditional medicinal plant consisting of glycyrrhizic acid as a major metabolite which has many pharmacological importance such as: glycyrrhizic acid disrupts latent Kaposi sarcoma, exhibit and also possesses a strong anti-viral effect [26]. Liquorice affects the body's endocrine system as it contains isoflavones (phytoestrogens) which might lower the amount of serum testosterone [27]. Liquorice can also be used to treat ileitis, leaky gut syndrome, irritable bowel syndrome and Crohn's disease as it is antispasmodic in the bowels [28]. To best of our knowledge this is the first report of synthesis of AgNPs and AuNPs using liquorice root extract,. Apart from the synthesis, we have also made an attempt to analyze the antibacterial activity of surface adsorbed liquorice biomolecules on both gold and silver nanoparticles.

2. Materials and methods

2.1 Synthesis of silver and gold nanoparticles

Salts of Au (III) ($HAuCl_4.3H_2O$, Sigma–Aldrich) and Ag (I) ($AgNO_3$, Fisher Scientific) were used for the biosynthesis of AgNPs and AuNPs using liquorice root (LR) extract. Before preparation of LR extract, it was washed properly to remove dust and other contaminants using deionized water.Briefly 5.0 g of LR powder was soaked with 20 mL deionized water for r 24 hours and t was centrifuged at 10,000 rpm for 10 min to obtain the root extract. It was then used for reduction of Au

(III) and Ag (I) salts-- 1.0×10^{-3} M of $AgNO_3$ and $HAuCl_4$ solutions (50.0 mL) each mized with 5.0 mL of LR extract at 37 °C temperature for complete reduction.

2.2 UV-Visible spectrum analysis

Bioreduction of aqueous Au (III) and Ag (I) ions during exposure to the LR extract were monitored by UV-Vis spectrophotometer. Periodic sampling of aliquots (2.0 mL) of aqueous components of both silver and gold solutions was recorded at room temperature using PerkinElmer spectrophotometer at a resolution of 1 nm.

2.3 Transmission Electron Microscopy (TEM) analysis

TEM analysis for both silver and gold nanoparticles was performed on the carbon coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min and the extra solution was removed using the blotting paper. TEM measurements were performed on a Morgagni 268(D) (Netherlands), which was operated at accelerating voltage of 100 kV.

2.4 FTIR (Fourier Transform Infrared Spectroscopy) analysis

For the FTIR analysis, lyophilized solutions of both silver and gold nanoparticles after 48 hours of reaction with LR were used. Before analysis, 10.0 mL of solution was centrifuged at 4000 rpm for 10 min and the resulted suspension was redispersed into 20.0 mL of deionized water. The process of centrifuging and redispersing was repeated three times to make nanoparticles free from proteins or other biogenic compounds present in the solution. There after the purified suspension was completely dried in lyphoilizer and analyzed by PerkinElmer-Spectrum RX-IFTIR.

2.5 AAS (Atomic Absorption Spectrophotometer) Analysis

After the complete reduction of silver and gold nanoparticles using liquorice root (LR) extract, the mixture were centrifuged at 4000 rpm for 10 min and filtered with 0.22µm filter (PALL-GF-A/E-I). The suspension free supernatant was analyzed with atomic absorption spectrophotometer (PerkinElmer Analyst 400) to determine the concentration of Ag and Au ions in their respective nanoparticle solutions.

2.6 XRD (X-ray Diffraction) analysis

Lyophilzed solutions were analyzed for crystalline nature of silver and gold nanoparticles by X-ray diffraction analysis using X'Pert Pro x-ray diffractometer operated at 40 mA current and 45 kV voltage with CuKα radiation.

2.7 Antibacterial Susceptibility Test

Antibacterial susceptibility test of AuNP and AgNP [29] were measured against *E. coli* bacterial strain by Kirby-Bauer method [30,38]. The bacterial strain was cultured in LB broth overnight at 37 °C shaking constantly at 150 rpm. The turbidity of the culture was adjusted to yield of uniform suspension containing 10^5-10^6 cells per mL. The culture was swabbed on to plates using sterile swab and whatman filter discs were placed on the plates and loaded with pure Ag (1 mM), Au (1mM), AgNP (68.78 ± 0.034mg/L), and AuNP (75.52±0.062 mg/L) solutions where as Chloramphenicol (C30) and Rifampicin (R10) antibiotics discs (commercial, Hi-Media) were taken as references. The plates were incubated overnight at 37 °C. The next day, the zones of inhibitions were analyzed to determine the effect of all solutions on bacterial growth.

3.Results and discussion

Unique optical properties of metal nanoparticles such as specific absorption/scattering frequency, makes them detectable even at very low concentration. It is well known that the purple-red color of colloidal dispersion of gold nanocrystals comes from specific absorption/scattering frequency or the excitation of Surface Plasmon Resonance (SPR) vibrations[31,32].The maximum absorption of gold nanoparticles was observed at 540 nm(fig.1a). The plasmon resonance frequency depends on the size and the shape of the particles. As an example in the case of spherical gold nanoparticles the plasmon frequency can be tuned from 510 to ~540 nm depending on the size [33]. Similarly the plasmon resonance frequency of oblate particles or nanorods is different.

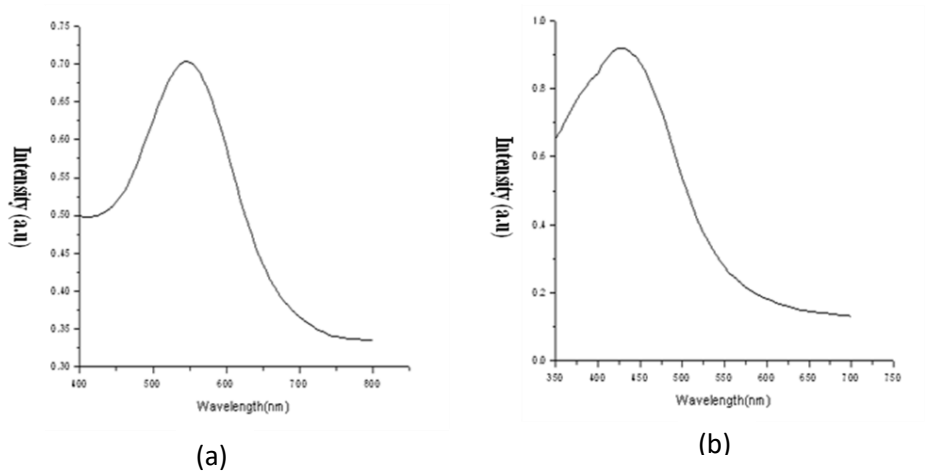

Fig: 1. UV-Vis spectra recorded for biosynthesized (a) gold and (b) silver nanoparticles with maximum absorption observed at 540nm and 430nm respectively.

and is depending on in which axis the electrons are oscillating. The resonance frequency along the short axis will be similar to the one of spherical nanocrystals (as in our case only spherical gold nanocrystals were reported), but the additional plasmon resonance appearing due to the long axis will be at larger wavelength. However, the frequency of this additional resonance can be widely changed by altering the length of gold nanocrystals [34, 35]. Silver nanocrystals exhibited yellowish brown color in the colloidal dispersion and this color is resulted due to the specific absorption/scattering frequency similar to the gold nanocrystals. UV-vis spectrum of these nanocrystals dispalyed the maximum absorption at 430 nm (fig.1b). The rate of reduction for biosynthesis of both AuNP and AgNP was observed to be different. The Gold nanocrystals were reduced at much faster rate than that of silver nanocrystals as demonstrated from their respective UV-vis spectrums-- Au(III) was completely reduced into Au(0) in just 120 min ,while Ag(I) took more than 12 hours for the complete bioreduction .The comparatively slow reduction rate of silver ions relative to the gold ions may be attributed to the difference in the reduction potential of the two metals.

It is equally important to understand the exact nature of the biosynthesized gold and silver particles. The nature of the biosynthesized gold and silver nanoparticles was deduced from the xrd spectrum of the sample. XRD pattern obtained for AuNP and AgNPs (fig.2a and 2b) depicted three intense peaks in the whole spectrum of $2\Theta^{\circ}$ values ranging from 30° to 70°. XRD spectra of pure crystalline gold and silver structures have been published by the Joint Committee on Powder Diffraction Standards. A comparison of our XRD spectrum with the standard confirmed that the gold and silver

particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2Θ values of 38.5◦, 46.25◦ and 64.69 for AuNP and 38.12◦, 44.25◦ and 64.91 for AgNP respectively [111, 200220] Moreover, in the case of silver nanoparticle one small insignificant impurity peak was observed at 66.5◦ which may be attributed to the presence of other organic substances in the supernatant.

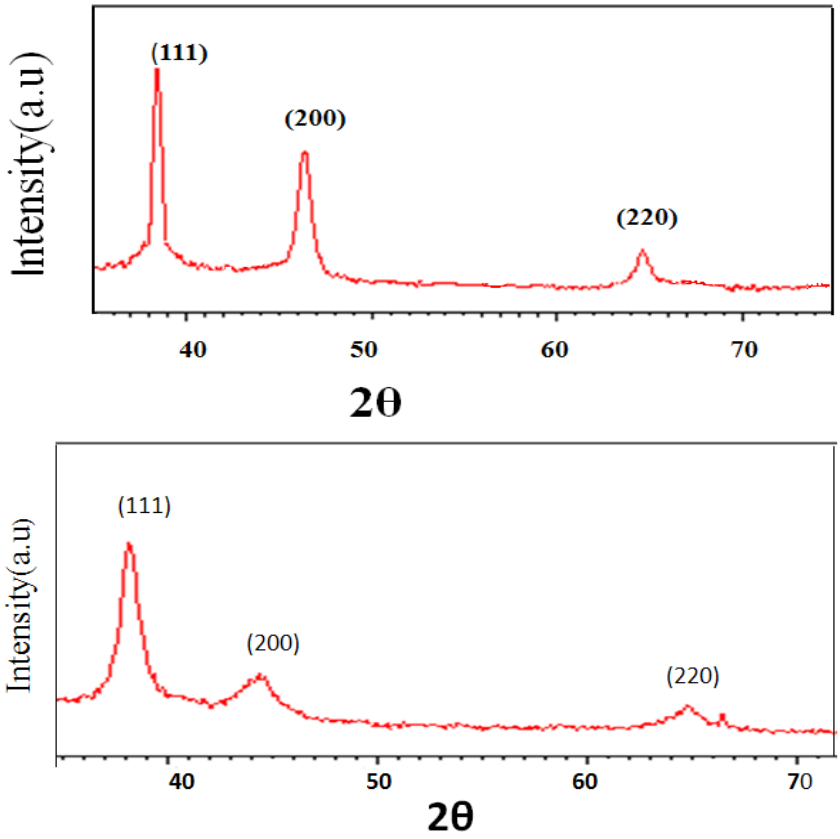

Fig: 2. X-ray diffraction Spectrum of [a] gold and [b] silver nanoparticles synthesized by reduction of $AuCl_4^-$ ions and Ag^+ ions using liquorice root extract. Labelled peaks correspond to characteristic diffaraction peaks of elemental Au(0) and Ag(0).

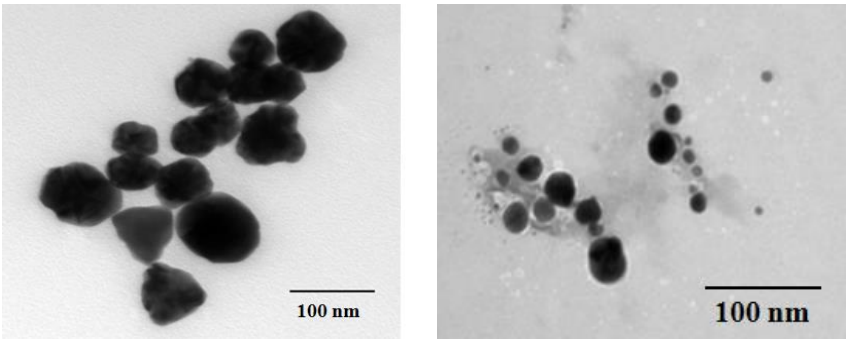

Fig: 3. Representative TEM images illustrating the formation of (a) gold and (b) silver nanoparticles, biologically synthesized by reduction of $AuCl_4^-$ and Ag^+ ions respectively using liquorice root extract.

TEM images of gold and silver nanocrystals were taken after the complete reduction of Au(III) and Ag(I) ions with the LR extract. It is shown from the TEM images of gold nanocrystals that most of the nanoparticles were spherical in nature having an average diameter of 46.6 nm (Fig. 3a), whereas very few of them were triangular or hexagonal in nature. Such a variation in shape and size is a common phenomenon in biosynthesized gold nanoparticles. The spherical nature of gold nanocrystals is also supported by UV-vis spectrum, where the maximum absorption is observed at 540 nm. In the case of deviation from the spherical geometry the maximum absorption could have been in UV-visible and the near IR region. Similarly fig.3b represents TEM images of silver nanocrystals which were very similar to the gold nanocrystals, the morphology of AgNP was predominantly spherical with an average diameter of 30.2 nm, and they appear to be monodisperse.

AAS (Atomic Absorption Spectrophotometer) analysis showed higher content of silver and gold in their respective silver and gold nanoparticle solutions. In the case of silver nanoparticles the concentration of silver in three repeated experiments were 68.41, 68.85 and 69.08mg/L and the average was observed to be 68.78±0.034mg/L. While in the case of gold nanoparticles readings of three replicates were 74.57, 75.56 and 75.52 mg/L with an average of 75.52±0.062 mg/L.

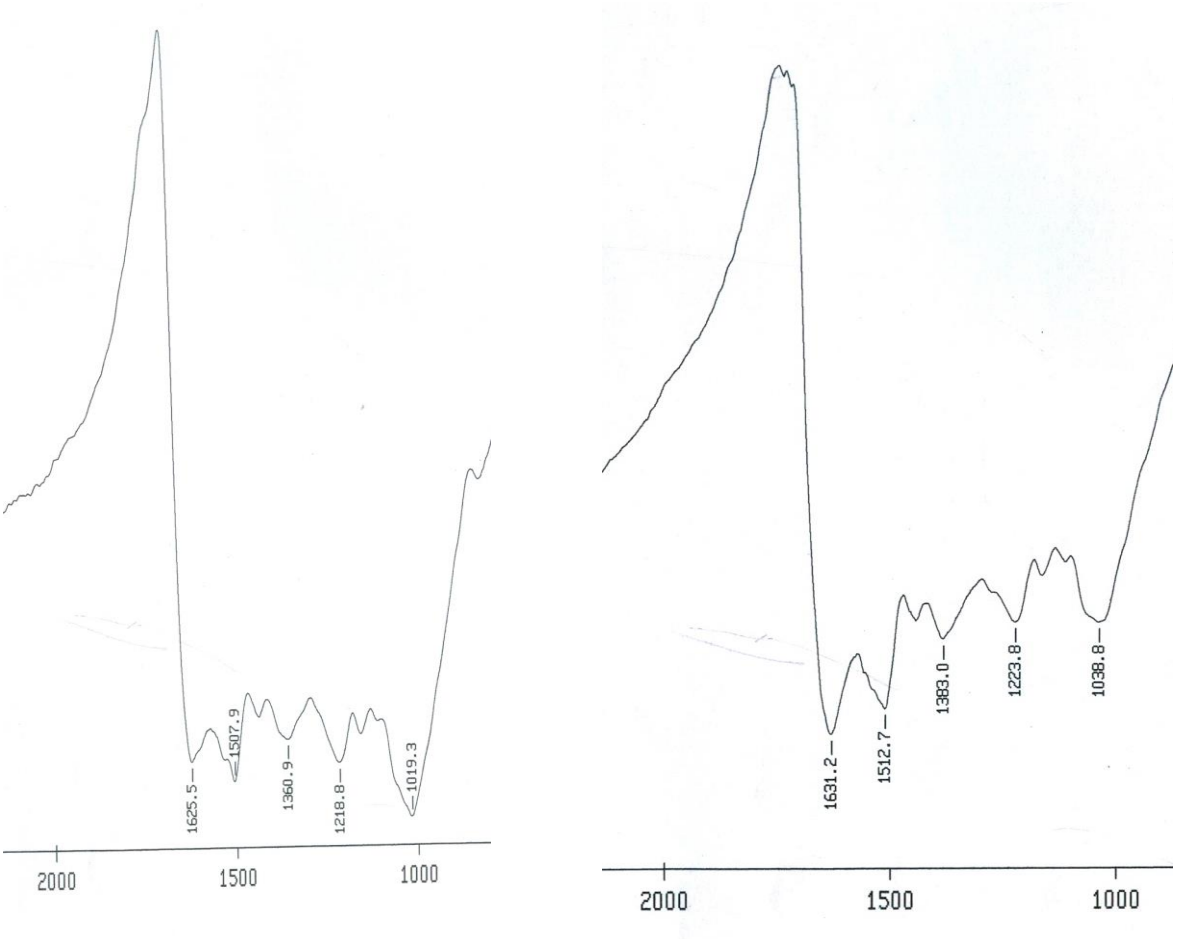

Fig: 4. FTIR absorption spectrum obtained from [a] gold nanoparticles biologically synthesized by reduction of $AuCl_4^-$ ions and [b] silver nanoparticles biologically synthesized by reduction of Ag^+ ions using rice root extract.

FTIR analysis was performed to identify the possibility of involvement of biomolecules in the LR extract for the synthesis and efficient stabilization of these metal nanoparticles. Representative FTIR spectrum of both gold and silver nanocrystals were shown in fig.4a and 4b, which manifests several absorption peaks 1625,1631,1507,1512,1360 ,1383,1218,1223,1019 and 1038 cm^{-1} in the region of 1000-2000 cm^{-1}.The two absorption peaks located around 1625 and 1631 cm^{-1} can be assigned as the absorption peaks of -C=C [36], whereas another two absorption peaks located at 1507 and 1512 cm^{-1} can be attributed to the stretching vibration of -C=C (aromatic ring). Wide absorption spectra at 1360 and 1383 cm^{-1} can be assigned to the nitro group, while in the case of silver nanoparticles this absorption peak refer to NO_3^- present in the residual solution[37]. Absorption peaks at 1218 and 1223 cm^{-1} may result from the stretching vibration of -C=O(acid) and absorption peaks located at 1019 and 1038 cm^{-1}can be attributed to –C-O-C or –C-O[36].

The antimicrobial activity of Liquorice root mediated gold and silver nanoparticles was performed against *E.coli*. The mean of three replicates of zone of inhibition (mm) around the disc with pure gold/silver solutions and LR mediated gold and silver nanoparticles is presented in Table 1(following the guidelines of antimicrobial susceptibility given by CLSI, 2008) [38]. Zone of inhibition with variable diameter was observed in all the treatments except pure gold solution.

Table 1. Comparative reproducibility of antibacterial assay with different solutions using the standard Kirby-Bauer method against *E. coli*.

	Chloramphenicol	Rifampicin	Pure Au Solution	AuNP Solution	Pure Ag Solution	AgNP Solution
Microorganism			*E. coli*			
Diameter of zone of inhibition (mm)	34.7	13.8	0	6.2	8.4	10.6

Results clearly demonstrate that newly synthesized AuNP and AgNP showed bactericidal effect, which can be attributed to the bimolecules adsorbed on the surface of nanoparticles in both the cases, which still remains to be examined. AgNP solution exhibited considerable amount of antimicrobial susceptibility quite comparable to the commercial antibiotics used and also with appropriate controls. Though the bactericidal property of AgNP is well known the mechanism of action is only partially understood. Previous reports suggests that ionic silver strongly interacts with thiol group of vital enzymes and even modulates the phosphotyrosine profile of bacterial proteins which resulted in inhibition of bacterial replication ability and arrests bacterial growth [39-40].

4. Conclusions: The green chemistry approach addressed in the biosynthesis of AgNPs and AuNPs using extract of Liquorice root seems to be excellent alternative of already existing various chemical based methods. It is rapid, cost effective and environment friendly approach. A key challenge in the application of these materials is prevention of agglomeration of nanomaterial, which was overcome in the present study and it may be due to the surface stabilization by Liquorice extract. The results also showed that surface functionalization of both AuNP and AgNP by biomolecule. Liquorice presented good antibacterial activity against model organism *E.coli*. Detailed investigations are still needed to understand the antibacterial effect of biomolecules adsorbed on the surface of nanoparticles.

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