Annals of Agri-Bio Research **27** (1) : 1-7, 2022

Impact Score : 0.28 (Scopus)

Bacterial Inhibition and Cytotoxicity of AgNPs Synthesized via Alkaloids Rich Leaf Extract of *Berberis lycium* R. and *Mentha piperata* L.

MAMTA SHARMA*, DUSHYANT KUMAR, PANKAJ THAKUR[1] AND ENZA TORINO[2]

School of Biological and Environmental Sciences, Shoolini University, Solan-173 229 (H. P.), India
**(e-mail : mamta131526@gmail.com; Mobile : 70185 78162)*

(Received : July 13, 2021; Accepted : September 05, 2021)

ABSTRACT

Current focus of nanotechnology is to develop environmentally safe methodologies for formulation of nanoparticles. The presented study reports a rapid, simplistic green synthesis of environmentally safe AgNPs capped and stabilized with leaf extracts of *Mentha piperita* and *Berberis lysium*. The formation and stability of reduced AgNPs were monitored by TEM and UV Spectroscopy. The stability was evaluated at varying pH and different concentration of NaCl at temperature range of 20-80°C. Antimicrobial activity of the AgNPs was accomplished by disc diffusion method against both *S. typhi* and *E. coli*. The highest antimicrobial activity of AgNPs synthesized by *M. piperita* and *B. lyceum* was found against *S. typhi* (700 µg) and *E. coli* (700 µg), respectively, which could be due to high content of berberin and menthol in extract. It was inferencing that leaf extract of *M. piperita* and *B. lycium* proved good bio-reductant for bio-synthesis of AgNPs which may prove versatile for assorted biomedical and pharmaceutical applications. Novelty of this present study was that plant extracts were very cost-effective, eco-friendly, and effective substitute for bio-synthesis of AgNPs for antibacterial activity and cytotoxic activity.

Key words : AgNPs, UV-visible spectroscopy, FT-IR, TEM, antimicrobial, *Escherichia coli, Salmonella typhi*

INTRODUCTION

Metal nanoparticles have established global attention due to their extensive applications in the biomedical and physio-chemical industries and likewise fields. Nanoparticles are special group of small sized materials with unique features and extensive applications in diverse fields. Nanoparticles possess completely distinctive properties in comparison with their large-sized counterparts (Cheng *et al.,* 2018). AgNPs fabricated using indigenous medicinal plants of India are one of the leading areas of research in nanotechnology. *Mentha piperita* L., a medicinally imperative plant, belongs to family Lamiaceae. The phenolic constituents of the leaves include several flavonoids, alkaloida and other specific compounds of that species (Karatoprak *et al.,* 2019). The main volatile components of essential oil are menthol and menthone. Peppermint has been reported to have significant antimicrobial and antiviral activities, strong antioxidant, antitumor actions, and some antiallergenic potential. *Berberis lycium* R. belongs to family Berberidaceae is an evergreen shrub growing in Himalayan region and is extensively used for the treatment of several diseases (Hussain *et al.,* 2019).

Silver is an effective antimicrobial agent against bacteria, viruses and microfungi, although its antimicrobial mechanism has not been fully understood yet (Naik, 2020). Nanoparticles directly attack at the cellular level at targeted site in order to cure diseases (Armand *et al.,* 2016) as shown in Fig. 1. In medicines and drug industries, silver nanoparticles have a wide applications including skin ointments, creams containing silver to prevent infection of burns and open wounds (Li *et al.,* 2015), medical devices and material prepared with silver-impregnated polymers (Polivkova *et al.,* 2017). *B. lycium* contains berberine, an isoquinoline alkaloid. Both clinical trials and animal research have indicated that berberine administration prevented ischemia-induced ventricular

[1]Himalayan Center of Excellence in Nanotechnology, Shoolini University, Solan, (H. P.), India; Jawahar Lal Nehru University, New Delhi; Center for Advanced Biomaterials for Healthcare, Italian Institute of Technology, Largo Barsantie Matteucci 53, Naples, Italy 80125.
[2]Center for Advanced Biomaterials for Healthcare, Italian Institute of Technology, Largo Barsantie Matteucci 53, Naples, Italy 80125.

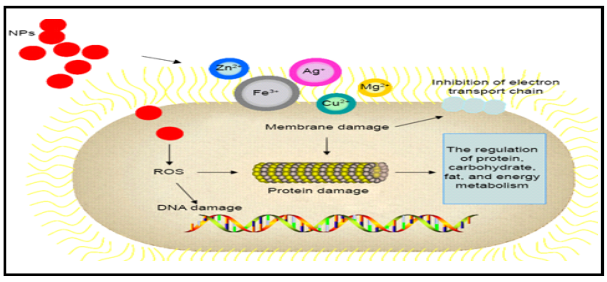

Fig. 1. Nanoparticles (NPs) can attack bacteria cell through multiple mechanisms involving formation of ROS leading to membrane, nucleus, protein and DNA damage.

tachyarrhythmia, stimulated cardiac contractility, lowered peripheral vascular resistance and blood pressure (Zhao and Ashraf, 2015). Extremely small dimensions of NPs are useful for accomplishing antimicrobial actions and fighting intracellular bacteria (Gayathri *et al.,* 2015). In general, small-sized silver (5, 9, 10, 12 and 13.5 nm) NPs have high antimicrobial activities (Berrak *et al.,* 2018).

MATERIALS AND METHODS

Fresh green, undamaged leaves of *B. lycium* and *M. piperita* were harvested from the vicinity of Shoolini University, Solan. Species identification was confirmed by Botanical Survey of India, Dehradun (Uttarakhand, India). Fresh leaves were dried at ambient temperature and grounded into fine powder by using pestle-mortar. Fifty g of fine grounded powder was subjected to extraction with 100 ml methanol in Soxhlet apparatus at temperature of 65°C for 72 h. The crude methanolic extract was obtained and stored at 4°C for further use. The methanolic extracts of both plants were studied for the presence of various phytochemical constituents like carbohydrate, alkaloids, flavanoids, glycosides, saponins, phenols, tannins and terpenoids using standard protocols (Oikeh *et al.,* 2020).

The Ag precursors, silver nitrate ($AgNO_3$) and trisodium citrate (Na_3Cit) were used as reagents (Sharma *et al.,* 2019). For synthesis of the AgNPs, 50 ml aqueous solution of $AgNO_3$ (0.25 mm) was boiled for 30 min at 110°C. Freshly prepared aqueous sodium citrate (5.0 ml, 2.5 mm) containing plant extract of *B. lycium* and *M. piperita* plant (separately) of concentration 0.10-5 mm was added in the silver salt solution and kept under vigorous stirring at 110°C. Subsequently, the colour of the solution transformed directly from blue to pale yellow within 30 seconds. The reaction was allowed to proceed further for 30 min in order to produce a stable solution. The solution was then left undisturbed at room temperature for 2 h. The colour of the Ag solution prepared firstly appeared blue and then transformed from blue to pale yellow within 15 min as shown in Fig. 2. The prepared AgNPs were washed with distilled water and separated using a centrifuge at 5000 rpm, in order to remove supernatant and then re-dispersed in distilled water (Sharma *et al.,* 2020).

A UV/visible Spectrophotometer was used for the spectro-photometric analysis. The reduction of pure silver ions was monitored by measuring the UV-vis spectrum of the colloidal solution obtained after 10 min of adding 300 µl of sample solution to 3 ml of deionized water. FT-IR spectroscopic studies were carried out to investigate and find possible bio-reducing agents present in the extract. The spectra of

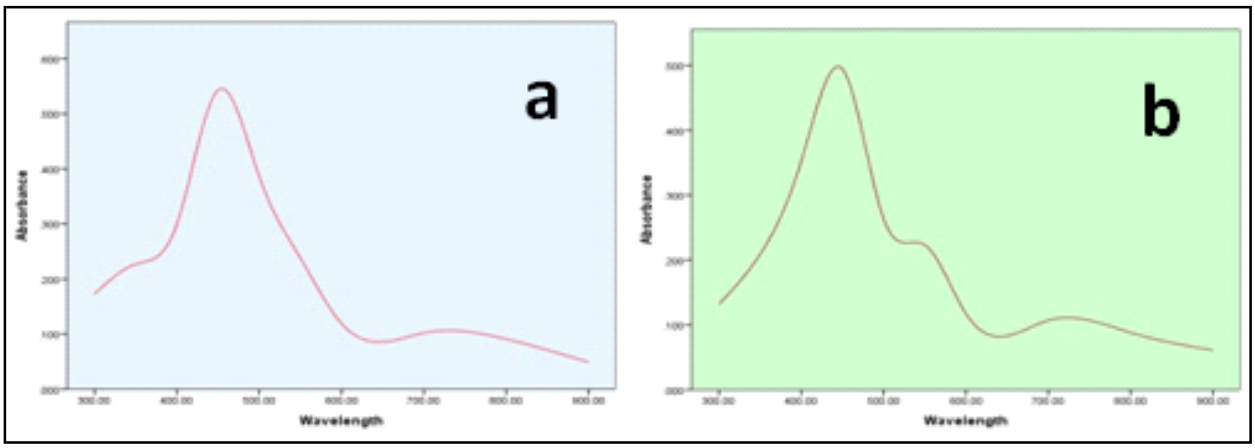

Fig. 2. The absorbance spectrum of silver nanoparticles of *B. lycium* and *M. piperita* showing maximum absorbance near 450 nm.

Ag were recorded after adding silver nitrate solution, respectively. The morphology of AgNPs was characterized by transmission electron microscopy (TEM). The samples were prepared by mounting a drop of solution on a carbon coated Cu grid and allowing it to dry in air. The samples were observed with the help of a Philips CM10 transmission electron microscope operating at 120kV. The system was fitted with an intensified video camera to assist the alignment and a slow scan CCD (charge-coupled device) camera.

The silver nanoparticles were synthesized from the leaf extract of *B. lycium* and *M. piperita*. The extract was analyzed for antimicrobial activity by agar disc diffusion method (Bindhu and Umadevi, 2015) against pathogenic bacteria. Test microorganisms selected for antimicrobial activity were *Salmonella typhi* and *Escherichia coli*. A sterile cotton swab was used to make a lawn on agar plates and the discs which had been impregnated with Ag nanoparticles of *B. lycium* and *M. piperita* leaf extracts (silver nanoparticles synthesized) in different concentrations (400 µg/ml, 500 µg/ml, 600 µg/ml and 700 µg/ml) were subjected to antibacterial screening. The plates were then incubated at 37°C for 18-24 h. After the incubation, the diameter of inhibitory zones formed around each disc was measured in mm and recorded.

The effects of biosynthesized different AgNPs on the L929 fibroblast cell (mouse) viability were examined by using XTT cell viability assay (Riss *et al.,* 2016). DMEM-F12 medium supplemented with 10% fetal bovine serum, penicillium-streptomycin was used for culturing of L929 cells. Cells were incubated at 37°C with 5% CO_2. Fully confluent cells were trypsinizationed. Trypan blue stained cells were counted with Thoma slides and viable cell number was adjusted to 150 viable cells in 1 ml medium. One hundred microliters from cell suspension were seeded in each well of 96-well plate. Increasing concentrations of (0, 6, 18, 42, 60, 180 and 300 µg/ml) BAgNPs and MtAgNPs were separately added to wells and incubated for 24 h. Then, medium containing AgNPs was aspirated and 100 µL of 0.5 mg/ml 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2 H-tetrazolium-5-carboxanilide (XTT) solution (with 7.5 µg/ml phenazine methosulfate) in fresh medium was added. Plates were incubated for further 4 h at 37°C and optical density was measured at 450 nm with multiplate reader. The viable cell percentage was calculated, taking into account the 100% viability of untreated cells. Fifty per cent of the inhibitory concentration (IC50) was used for the cytomorphological observation. After the treatment, the cells (control and treated) were washed and fixed at 1:1 ratio of methanol and glacial acetic acid for 1 h at room temperature.

$$\text{Viability \%} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100$$

All the experiments were done in triplicates. Data were expressed as mean± standard error of the mean (SEM). Significance of difference among mean values was evaluated by ANOVA followed by Tukey's multiple comparison tests (Graph Pad Software Inc. San Diego CA, USA and IBM SPSS Statistics).

RESULTS AND DISCUSSION

Phytochemical screening of leaf extracts of both the plants revealed the presence of phenols, alkaloids and tannins (Table 1).

The standard graph of AgNPs was prepared from the leaf extract of *B. lycium* and *M. piperita* at different wave lengths i. e. 300-900 nm. As demonstrated in Fig. 1, the colloidal solutions exhibited a strong absorption between 350-460 nm divulging the formation of AgNPs. The effect of $AgNO_3$ concentration, temperature

Table 1. Quantity determination of various phytochemicals

S. No.	Test performed	*Berberis lycium*	*Mentha piperita*
1.	Carbohydrates (mg/g FW)	0.619±0.098	0.682±0.066
2.	Proteins (mg/g FW)	0.669±0.081	0.805±0.047
3.	Alkaloids (mg/g FW)	0.742±0.083	0.770±0.080
4.	Flavanoids (mg/g FW)	0.446±0.068	0.464±0.079
5.	Tannins (mg/g FW)	0.427±0.093	0.452±0.079
6.	Phenols (mg/g FW)	0.445±0.078	0.742±0.083
7.	Saponins (mg/g FW)	0.552±0.089	0.552±0.089
8.	Chlorophylls (mg/g FW)	0.494±0.052	0.446±0.068

and one hour's contact time was also investigated on the synthesis of AgNPs in 5 ml of aqueous extract. Spectrum survey showed that by increasing the concentration of $AgNO_3$ peak intensity increased; therefore, it can be inferenced that by increasing the $AgNO_3$ concentration, the silver ions (Ag+) got turned into Ag (Fig. 2). By increasing $AgNO_3$ concentration, the brown colour of solution became thick.

The FTIR- spectra were recorded for functional group characterization of AgNPs synthesized by leaf extract of *B. lycium* and *M. piperita* (Fig. 3). The graphs at 3383 and 1640/cm in AgNPs of *B. lycium* showed the presences of O-H (Alcohol and phenol) stretch and C = C (Alkenyl) stretch, respectively. The characteristic absorptions/cm of 713-665 showed the aromatic C-H bending in Fig. 4. The broads at 3398, 3376 and 1637/cm in AgNPs of *M. piperita* showed the presences of O-H.

The structural characteristics of biosynthesized AgNPs were studied by Transmission Electron Microscope (TEM). The TEM micrographs of synthesized AgNPs divulged mono-dispersity with the dimensions of average size from 10-30 nm with predominantly spherical shapes (Fig. 4). The aggregation of the nanoparticles indicated that they were in the direct contact but stabilized by a capping agent. In agreement with the UV-Visible Spectroscopic observations, the TEM images revealed that Agnano crystals were spherical in shape and in the range of 10-30 nm with considerable agglomeration (Fig. 4).

Different concentrations of silver nanoparticles prepared from leaves of plants were used for antimicrobial activity assay. Both the tested bacterial pathogens were found susceptible to the leaf extracts (Table 2). The *M. piperita* and *B. lycium* showed highest zone of inhibition against *E. coli* i.e. 14.67±0.05 mm and 13.46±0.88, respectively, at concentration of 700 µg/ml (Table 2; Fig. 5).

The principle of XTT test revealed conversion of XTT to orange-coloured water-soluble dye resulted due to mitochondrial activity. Only living cells were capable of converting XTT reactant, so the optical absorbance value was directly related to cell viability. The cell viability

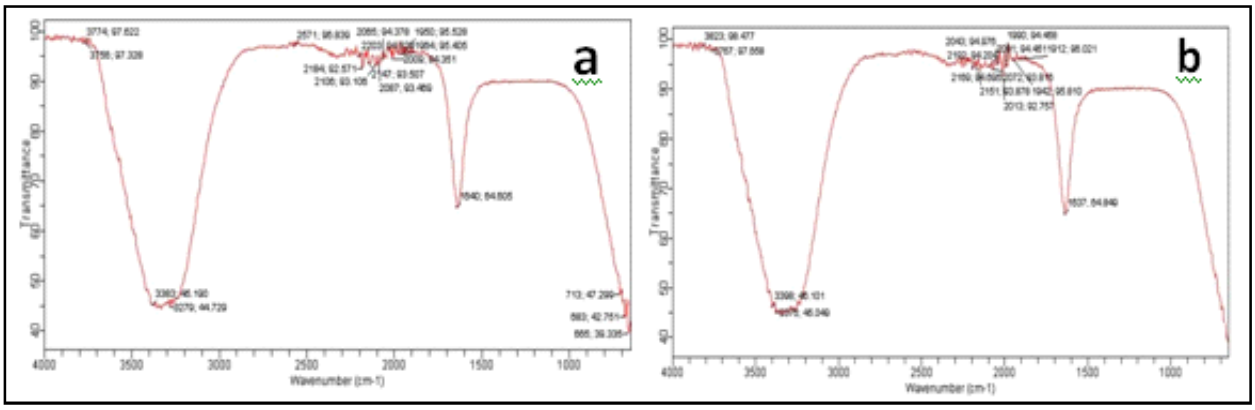

Fig. 3. FT-IR pattern of AgNPs synthesized with the leaf extract of *B. lycium* and *M. piperita*.

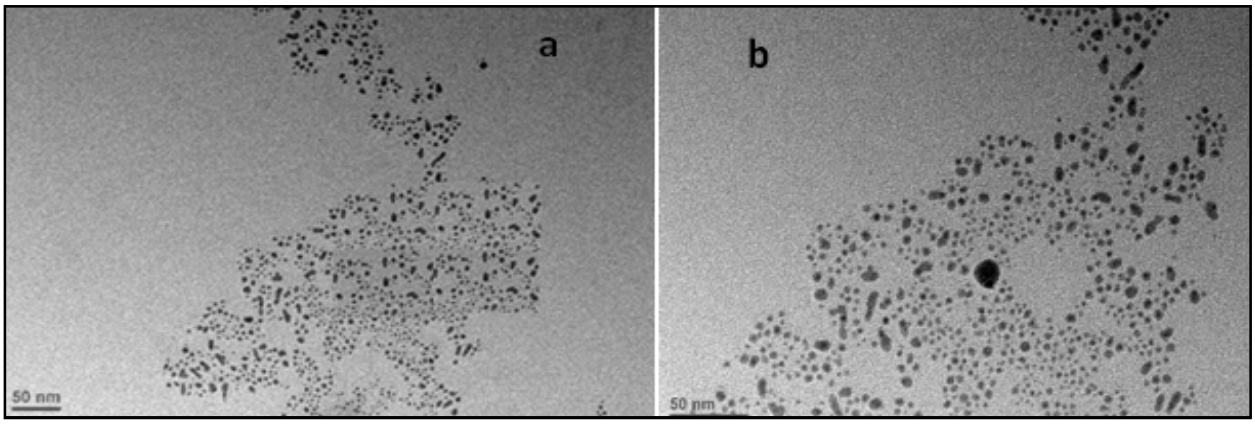

Fig. 4. TEM micrographs of Ag-NPs synthesized with the leaf extract of *B. lycium* and *M. piperita*.

Table 2. Antimicrobial activities of Ag-NPs with *B. lycium* and *M. piperita* on clinical bacterial strains

S. No.	Plant used	Type of bacterial strains	Inhibition zones (mm)			
			400 µg/ml	500 µg/ml	600 µg/ml	700 µg/ml
1.	*B. lycium*	*E. coli*	10.21±0.3	10.93±0.33	11.67±0.33	13.46±0.88
2.		*S. typhi*	10.33±0.33	11.33±0.33	11.67±0.33	13.33±0.66
3.	*M. piperita*	E. coli	10.27±0.06	11.67±0.04	12.33±0.88	14.67±0.05
4.		*S. typhi*	10.98±0.39	12.00±0.57	13.33±0.66	15.00±0.57

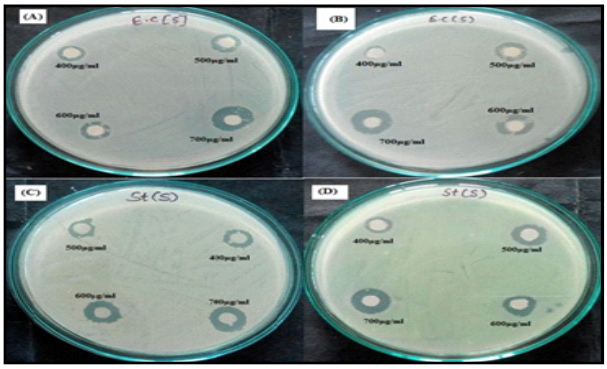

Fig. 5. Zone inhibition formed by the Ag-NPs of (A) *B. lycium*, (B) *M. piperita* leaf extract on *E. coli* bacteria, (C) *B. lycium* and (D) *M. piperita* leaf extract on *S. typhi.*

was importantly affected with increased concentrations of NPs; cells completely lost their viability near the concentration of 150 µg/ml. CsAgNPs were most toxic nanoparticles among others (Fig. 6). Cell viability decreased in minimum examined concentrations of NPs (Erci *et al.*, 2018). The toxicity profile of MpAgNPs was similar to Ts2AgNPs and they had the least toxic effect among examined nanoparticles. The cells lost their viability concentration of approximately 150 µg/ml. Toxicity amount of NPs was considered as higher in BAgNPs than MtAgNPs.

Nanoparticles are one of the most attractive nanomaterials for various applications like antimicrobial, electronic, catalytic and various biomedical applications as silver has a resonance of strong surface (Chatzimitakos and Stalikas, 2016). *M. piperita* oil and menthol had moderate antibacterial effects against both gram positive and gram-negative bacteria (Kamalakannan *et al.*, 2014). Berberine is also known to have antitumor effect. Nanoparticles synthesized from the plant extract could be used for better drug delivery (Sharma and Puri, 2018). Inhibitory activity was shown by the components present in the extracts of plants and not by the solvents used for extraction (Poongunran *et al.*, 2015; Tu *et al.*, 2019). The antibacterial effect of nanoparticles synthesized by the plant *Azadirachta indica* leaf extract on *Staphylococcus aureus* and *Escherichia* coli was reported as inhibition zone diameter of 9 mm in both bacteria (Velusamy *et al.*, 2015; Shriya *et al.*, 2019). Thus, AgNPs of *B. lycium* and *M. piperita* can be used in future to inhibit the growth of bacterial pathogens.

CONCLUSION

Plants derived medicines are widely used

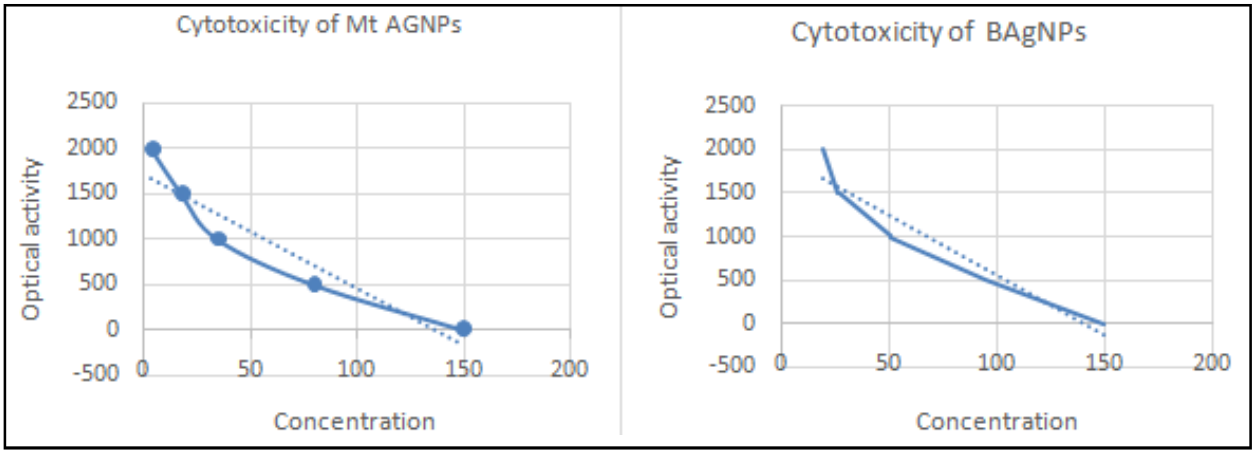

Fig. 6. Cytotoxicity of the silver nanoparticles on L929 cells. (A) BAgNPs and (B) MtAgNPs.

because they are safer than synthetic alternatives. Medicinal plants such as *B. lycium* R. and *M. piperita* L. had medicinal value. The crude plant extracts of both plants were used to analyze the phytoconstituents such as alkaloids, flavanoids, phenolic compounds and saponins. Results showed that the maximum amounts of phytochemicals were found in *M. piperita* leaf extract. The present study proved the use of medicinal plant for biosynthesis of silver nanoparticles which was rapid, cost effective and environmentally-safe with potential use against microbes. The results showed the silver nanoparticles of *M. piperita* and *B. lycium* to be effective against the *E. coli* and *S. typhi* bacterial isolates at 20, 25, 30 and 35 µl concentrations. These nanoparticles in a small amount for drug development will be helpful to cure many diseases. The synthesis of nanoparticles in large scale using these plant extracts may have commercial viability and to develop studies in the interface between biology and material science.

ACKNOWLEDGEMENTS

The authors are thankful to the faculty and management of Shoolini University, Solan, H. P., India and NIPER, Chandigarh for support and facility.

REFERENCES

Armand, L., Tarantini, A., Beal, D., Biola-Clier, M., Bobyk, L., Sorieul, S., Pernet-Gallay, K., Marie-Desvergne, C., Lynch, I., Herlin-Boime, N. and Carriere, M. (2016). Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents. *Nanotoxicology* **10** : 913-923.

Berrak, A., Ozlem, O., Burcak, G., Pervin, R., Ismail, O. and Umran, S.G. (2018). Green synthesis and characterization of silver nanoparticles using the fungus A. niger and bioactive potential against microorganisms and cancer cells. *Lat. Am. J. Pharm.* **37** : 979-986.

Bindhu, M. R. and Umadevi, M. (2015). Antibacterial and catalytic activities of green synthesized silver nanoparticles. Spectrochim. Acta A : Mol. *Biomol. Spectrosc* **135** : 373-378.

Chatzimitakos, T. G. and Stalikas C. D. (2016). Qualitative alterations of bacterial metabolome after exposure to metal nanoparticles with bactericidal properties : A comprehensive workflow based on 1 H NMR, UHPLC-HRMS and metabolic databases. *J. Proteome Res.* **15** : 3322-3330.

Cheng, H., Yang, N., Lu, Q., Zhang, Z. and Zhang, H. (2018). Syntheses and properties of metal nanomaterials with novel crystal phases. *Advanced Materials* **30.** *doi : org/ 10.1002/adma. 201707189.*

Erci, F., Cakir-Koc, R. and Isildak, I. (2018). Green synthesis of silver nanoparticles using *Thymbra spicata* L. var. spicata (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity. *Artificial cells, Nanomedicine Biotechnology* **46(S)** : 150-158.

Gayathri, A., Sekar, D. S. and Sakthi, R. (2015). Wound healing activity of *Curcuma longa* with *Oleum olivae. JAIR* **3** : 479-480.

Hussain, S., Hamid, A., Ahmad, K. S., Mehmood, A., Nawaz, F. and Ahmed, H. (2019). Quantitative ethnopharmacological profiling of medicinal shrubs used by indigenous communities of Rawalakot, District Poonch, Azad Jammu and Kashmir, Pakistan. *Revista Brasileira de Farmacognosia* **29** : 665-676.

Kamalakannan, S., Gobinath, C. and Ananth, S. (2014). Synthesis and characterization of fungus mediated silver nanoparticle for toxicity on filarial vector, *Culex quinquefasciatus. Int. J. Pharm. Sci. Rev. Res.* **24** : 124-132.

Karatoprak, G. S, Yücel, Ç., Kaytan, H. Ç., Ilgün, S., Safak, E. K. and Kosar, M. (2019). Antioxidant and cytotoxic activities of aerial and underground parts of *Hypericum scabrum* L. *Iranian J. Sci. Tech. Transactions A : Sci.* **43** : 2107-2113.

Li, H., Chen, Q., Zhao, J. and Urmila, K. (2015). Enhancing the antimicrobial activity of natural extraction using the synthetic ultrasmall metal nanoparticles. *Sci. Rep.* **5** : 1-13.

Naik, B. S. (2020). Biosynthesis of silver nanoparticles from endophytic fungi and their role in plant disease management. *Microbial Endophytes* **1** : 307-321.

Oikeh, E. I., Oviasogie, F. E. and Omoregie, E. S. (2020). Quantitative phytochemical analysis and antimicrobial activities of fresh and dry ethanol extracts of *Citrus sinensis* (L.) Osbeck (sweet orange) peels. *Clinical Phytoscience* **6** : 1-6.

Polivkova, M., Hubacek, T., Staszek, M., Svorcik, V. and Siegel, J. (2017). Antimicrobial treatment of polymeric medical devices by silver nanomaterials and related

technology. *Int. J. Mol. Sci.* **18** : 419. *doi : 10.3390/ijms18020419.*

Poongunran, J., Perera, H. K., Fernando, W. I., Jayasinghe, L. and Sivakanesan, R. (2015). α-Glucosidase and α-amylase inhibitory activities of nine Sri Lankan antidiabetic plants. *J. Pharmaceutical Res. Int.* **6** : 365-374.

Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A. and Worzella, T. J. (2016). Minor L. cell viability assays. *Assay Guidance Manual* [Internet].

Sharma, M. and Puri, S. (2018). Plant Tolerance and photooxidative effect of light stress on withnolides in *Withania somnifera*. *Int. J. Pharm. Res*. **10** : 148-155.

Sharma, M., Thakur, P., Saini, R. V., Kumar, R. and Torino, E. (2020). Unveiling antimicrobial and anticancerous behaviour of AuNPs and AgNPs moderated by rhizome extracts of *Curcuma longa* from diverse altitudes of Himalaya. *Scientific Reports. doi.org/10.1038/s41598-020-67673-4.*

Sharma, V., Kaushik, S., Pandit, P., Dhull, D., Yadav, J. P. and Kaushik S. (2019). Green synthesis of silver nanoparticles from medicinal plants and evaluation of their antiviral potential against chikungunya virus. *App. Microbiol. Biotechnol.* **103** : 881-891.

Shriya, Sharma, M., Kumari, A. and Choudhary, S. (2019). Temperature stress mediated consequences on physiology and secondary metabolites of *Datura stramonium* L.. *Int. J. Pharm. Sci. Res.* **10** : 3085-3091.

Tu, T. H., Cam, P. T. N., Phong, M. T., Nam, H. M. and Hieu, N. H. (2019). Synthesis and application of graphene oxide aerogel as an adsorbent for removal of dyes from water. *Materials Letters* **238** : 134-137.

Velusamy, P., Das, J., Pachaiappan, R., Vaseeharan, B. and Pandian, K. (2015). Greener approach for synthesis of antibacterial silver nanoparticles using aqueous solution of neem gum (*Azadirachta indica* L.). *Ind. Crops Prod.* **66**. 103-109.

Zhao, L. and Ashraf, M. A. (2015). Influence of silver-hydroxyapatite nanocomposite coating on biofilm formation of joint prosthesis and its mechanism. *West Indian Med. J.* **64** : 506-513.