Plant Archives Vol. 19, Supplement 2, 2019 pp. 762-768 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

GREEN SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *AJUGA PARVIFLORA* BENTH. IN WALL.

Anuj Kandwal[1*], M.C. Purohit[1], Arun K. Khajuria[2] and R.K. Joshi[3]

[1]Department of Chemistry, Hemvati Nandan Bahuguna Garhwal University, BGR Campus Pauri, Pauri (Garhwal) 246001, Uttarakhand, India

[2]Department of Botany, Government Degree College, Kathua 184101, Jammu and Kashmir, India

[3]Department of Chemistry, Govt. P.G. College Dakpathar, Dehradun 248125, Uttarakhand, India

*Corresponding author: Email: anujkandwal4@gmail.com

Abstract

In the present investigation, stable silver nanoparticles were synthesized via green approach. In this approach, silver nanoparticles were synthesized by the interaction of 3 mM silver nitrate solution with the aqueous leaf extract of *Ajuga parviflora*. Initial colour change and surface-plasmon-resonance (SPR) absorbance bands between 448-456 nm gave support to the synthesis of silver nanoparticles. These nanoparticles were further characterized by XRD, EDX, TEM and FTIR techniques. XRD analysis showed that nanoparticles are crystalline in nature with face centred cubic phase. TEM measurements showed that nanoparticles are spherically shaped with their average size less than 16 nm. Important FTIR peaks at 3394.9, 1601.8, 1499.4 and 1419.9 cm-1 were predicted for hydroxyl, carbonyls, unsaturated C-C bonds and phenolic groups respectively. Thus, the FTIR spectra confirms the presence of phytochemicals which were responsible for reducing, capping and stabilizing the nanoparticles. Further, synthesized nanoparticles showed significant antibacterial activity against gram negative *Pseudomonas aeruginosa* and *Escherichia coli* and gram positive *Staphylococcus aureus* and *Bacillus subtilis* bacterial strain.

Keywords: Silver nanoparticles, *Ajuga parviflora,* Surface plasmon resonance, crystalline.

Introduction

Over a past few decades, nanoscience has become one of the most encouraging field of research in modern science with tremendous interdisciplinary applications in biology, chemistry, physics, agricultural and material science (Shankar *et al.,* 2004; Abou El-Nour *et al.,* 2010). The term 'nanoparticles' is commonly used to describe those particles having size less than 100 nm in at least for one dimension. Nanoscale materials generally show exceptional difference in the biological, physical and chemical properties compared to same material at macro scale. Nanoparticles exhibits enhanced characteristics like high surface area to volume ratio, tunable pore size and high reactivity and therefore they possess novel magnetic, physicochemical and optoelectronic properties (Mohanpuria *et al.,* 2008; Poulose *et al.,* 2014; Khajuria *et al.,* 2019). At present, research on metallic nanoparticles is growing intensively as they provide better material properties with functional adaptability.

Recently, metallic nanoparticles (silver, gold, zinc oxide, iron oxide etc.) have drawn the attention of many researchers owing to their several applications to the electronics, communications, UV-protection cosmetics, catalysis, sensors, environmental protection, biological and medicinal industries. Metallic nanoparticles are developed by several methods including biological, physical, chemical and other electrochemical, photochemical, radiolytic and sonolytic methods. However, physical and chemical methods suffer some serious drawbacks as they are not only expensive but also require toxic chemicals, high radiations and high temperature and pressure, thus responsible for the environmental contaminations. Over the last few years, the bio-inspired synthesis of nanoparticles using plant extracts has emerged as a promising methodology for the synthesis of metallic nanoparticles (especially silver, copper, zinc oxide, iron oxide and gold nanoparticles), as this method is very cost effective, more stable, non-hazardous, environmentally benign and non-toxic (Khajuria *et al.,* 2017; Nasiriboroumand *et al.,* 2018). Therefore, nowadays, research has been shifted towards plant-mediated synthesis of nanoparticles. The synthesis of nanoparticles through the plant extracts is influenced by several parameters such as temperature, time of reaction, pH and the nature of biomolecules present in the plant extracts. Phytochemicals like alkaloids, flavones, polyphenolic acid, ketones, terpenoids, aldehydes, tannins, essential oils, saponins, resins etc., present in the extracts of various parts of plants (roots, leaf, fruits, seeds and bark) acts as reducing, capping and stabilizing agent of metallic nanoparticles. Various researchers have reported synthesis of silver nanoparticles using different plant materials (Dwivedi and Gopal 2010; Zargar *et al.,* 2011; Faramarzi and Sadighi 2013; Moghaddam *et al.,* 2014; Pawar and Chaudhari 2017). Due to large surface area to volume ratio and high catalytic activity of silver nanoparticles, they are used in several catalytic processes. Moreover, several pharmacological activities, such as antibacterial, antifungal, antiplasmodial and larvicidal activities of silver nanoparticles have also been reported (Sharma *et al.,* 2009; Ofakor *et al.,* 2013; Vijayakumar *et al.,* 2013; Kumar *et al.,* 2018; Morejon *et al.,* 2018).

After reviewing data, it has been found that no report has been texted regarding the green synthesis of silver nanoparticles by using the aqueous leaf extract of *Ajuga parviflora*. *Ajuga parviflora* is one of the potent medicinal herb of Himalaya belongs to Lamiaceae family commonly used against wide spectra of diseases from common body pains to chronic diseases such as diabetes. Plant is also used to cure hypertension and hepatitis (Hamayun *et al.,* 2006; Ahmad *et al.,* 2018). It exhibited several pharmacological activities, such as antibacterial, antifungal, antioxidant, insecticidal (Rahman *et al.,* 2013; Gulzar *et al.,* 2017; Kumar *et al.,* 2018). Several phtochemicals like alkaloids, withanolides, essential oils and terpenoids were isolated from the *Ajuga parviflora* plant (Nawaz *et al.,* 2000; Singh *et al.,* 2015; Kumar *et al.,* 2018). *Ajuga parviflora* is a perennial pubescent herb, 10 to 30 cm in height, commonly found in grassy slopes of Himalaya and sometimes also found in oak

forests, plant is distributed in entire Himalaya from Kashmir to Uttarakhand in India and mountains of Afghanistan, Nepal and Bhutan. Plant with its stem branched from the base, short flowers with corolla pink purple to blue and leaves, sessile, hairy, ovate and tinged with purple on lower surface (Collett, 1980; Gaur, 1999). In the previous work, we have successfully synthesized spherically shaped crystalline zinc oxide nanoparticles using leaf extract of *Ajuga parviflora* with their average particle size less than 20 nm (Kandwal *et al.,* 2019). Hence, present study aimed to designed a rapid, eco-friendly, novel route for the synthesis of silver nanoparticles through leaf extract of *Ajuga parviflora* and to investigate their antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Materials and Methods

Collection and authentication of plant materials

Fresh and healthy leaves of *Ajuga parviflora* were collected from Nagdev forest range, Pauri, Uttarakhand and authenticated from Herbarium Jammu University and its accession no. HBJU 16003 was collected.

Preparation of *Ajuga parviflora* leaf extract

Fresh and healthy leaves of *Ajuga parviflora* were entirely washed with double distilled water in order to take out any of adhesive dirt. Leaves were then shade dry for 14 days and the constant weight of leaves were achieved. Then, these dried leaves were mashed with mortar-pestle and 6 g of finely powdered *Ajuga parviflora* was taken with 120 ml double distilled water in 250 ml Erlenmeyer conical flask and heated at 70 °C for 20 minutes. Then, the extract was allowed to cool at room temperature and filtered in a separate conical flask using Whatman fiter paper no. 1.

Synthesis of silver nanoparticles

Ajuga parviflora leaf extract and 3 mM $AgNO_3$ solution (aqueous) was mixed in 1:9 in a 2 L Erlenmeyer flask. It was kept for 72 hours in the dark place. The colour of solution changed to dark red, pointing the formation of silver nanoparticles. Then the solution was centrifuged for 15 minutes at 7500 rpm and washed with distilled water followed by acetone to remove the impurities. The obtained material was dried at 50 °C for 20 hours in oven followed by mashing in mortar-pestle to get fine powdered greyish black silver nanoparticles and stored for the characterization of silver nanoparticles and anti-microbial activities.

Characterization

Initially, at regular interval formation of silver nanoparticles was preliminary monitored by using Elite-double beam UV-visible spectrophotometer. Then, silver nanoparticles was subjected to X-ray diffraction (XRD) analysis (X'PERT-PRO Diffractometer, PANalytical; CuKα radiation, λ_{max} = 1.54 Å) and its spectra was reported in the range of 2θ from 0º to 75º. Fourier transform infrared spectroscopy (Spectrophotometer Perkin Elmer Model RZX) analysis ranging from 4000-500 cm^{-1} was to recognize the bioactive components of plant extract accountable for capping and stabilizing the nanoparticles. The average size of silver nanoparticles was calculated by using Debye Scherrer's equation:

$$D = K\lambda / \beta \cos\theta$$

where D is average crystallite size, K is the Scherrer's constant, λ is the X-ray wavelength, β is the full width at half maxima (FWHM) and θ is the Bragg's diffraction angle. Energy Dispersive X-Ray (EDX) was performed for determining the elemental composition of synthesized nanoparticles. Transmission electron microscope (JEOL JEM 1400) analysis was used to determine the surface morphology of silver nanoparticles.

Antimicrobial activity

The silver nanoparticles synthesized from *Ajuga parviflora* leaf extract were tested for antimicrobial activity by Agar well diffusion method against four different bacterial strain i.e., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Muller Hinton Agar Medium (HI-Media) was used in bacterial assay and prepared by dissolving 33.9 g into 1000 ml of distilled water, this dissolved medium was then subjected to autoclave at 121^0C temperature and 15 Pascal pressure for 15 minutes. The autoclaved media, then poured into 20×90 mm petri plate (Borosil) under the laminar air flow chamber, precaution for vapor formation was in consideration during the work. Prepared plates were subjected to incubation for 24 hrs at 30^0C in bacteriological incubator to observe any contamination if present. Sterilized plates were then subjected for inoculation of bacterial strains. Suspension of bacterial strains were prepared by using sodium chloride (HI-Media) dissolved with required amount of distilled water by dissolving a loop of bacteria from prepared slant, 50µl of the suspension was then loaded on MHA plates, which further swapped gently over the surface of plate with the help of slider. Finally, wells of 8 mm were made by using sterilized cork borer, in which prepared sample solution (75 µL and 100 µL) was poured with the help of micropipette. Each plate was then incubated at 37^0C for 24 h and the diameter of zone of complete inhibition was measured with the help of scale (Perez and Anesini, 1994).

Result and Discussion

In the present work, aqueous leaf extract of *Ajuga parviflora* was used to synthesize silver nanoparticles. Bio-active components present in the leaf extract performed a key role in the synthesis of silver nanoparticles.

Change in the colour of solution from colourless to dark red may be due to the formation of silver nanoparticles. Fig. 1 shows the UV-visible spectrum of the solution having a broad absorption peak at λ_{max} = 448-456 nm which confirmed the presence of silver nanoparticles in the solution and its calculated energy band gap is between 2.72-2.78 eV.

XRD analysis

The XRD spectra showed the crystalline nature of ZnO nanoparticles and the average crystallite size was calculated using Debye-Scherrer's equation (Das *et al.,* 2010; Azizi *et al.,* 2013; Karthik *et al.,* 2014). The average calculated size of ZnO nanoparticles is found to be less than 15.8 nm. XRD spectra of synthesized nanoparticles showed sharp peaks at 2θ = 28.10°, 32.54°, 38.44°, 44.58°and 64.76° which are indexed to (210), (113), (111), (200) and (220) lattice planes respectively (Table-1) of face centred cubic nano crystals (Fig. 2)

TEM analysis

TEM analysis was used to study the surface morphology of silver nano structures (Fig. 3). These results confirmed that silver nanoparticles were spherical in shape with their average size less than 16 nm.

EDX analysis

EDX was carried out for the composition of nanoparticles. Fig. 4 shows a sharp signal at around 3 keV which confirms the presence of elemental silver. The other signals that were noticed in the spectra may be attributed due to the bioactive components present in plant extract.

FTIR analysis

FTIR spectra of nanoparticles showed several peaks which confirm the presence of different functional groups which were responsible for the reducing, capping and stabilizing of nanoparticles. FTIR peak in Fig. 5 at 3394.9 cm^{-1} corresponds to stretching vibrations of hydroxyl group. Further, peaks at 1601.8 cm^{-1}, 1499.4 cm^{-1}, and 1419.9 cm^{-1} may be attributed to C=O frequency of extensively conjugated systems, C=C aromatic stretching and C-O stretching of ArOH respectively. Peaks below 650 cm^{-1} region may be attributed to the silver nanoparticles.

Antimicrobial activity

Agar well diffusion method was used to perform the antimicrobial assay against four bacterial strains. The bio-synthesized silver nanoparticles of leaf extract of *Ajuga parviflora* was put in different concentrations i.e., 75 µL and 100 µL concentrations per well against gram positive (*Staphylococcus aureus* and *Bacillus subtilis*.) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria, sodium chloride solution was used as positive control. In the present study and it was found that saline have no effect on bacterial growth and gives no zone of inhibition, while the synthesized silver nanoparticles showed antimicrobial potential against all test organisms (Table-2). The maximum zone of inhibition was reported for *Staphylococcus aureus* (14.58±0.41mm) when 100 µL silver nanoparticles were put in the well and minimum zone of inhibition (13.96±0.62 mm) was reported in *Escherichia coli*. The possible mechanism for the antimicrobial activity of nanoparticles may involve the absorption of the nanoparticles by the host, inside the host organism these nanoparticles starts the production of free radical which interrupts the basic metabolism and some time may cause alteration or damage to genome of the host and results in the death of the host organism (Stoimenov *et al.,* 2002; Xie *et al.,* 2011).

Also, results of the present work are in accord with several other researchers, worked on synthesis of silver nanoparticles using different plant extract (Table-3).

Conclusion

It can be concluded that green synthesis of silver nanoparticles using *Ajuga parviflora* leaf extract is rapid, ecofriendly and cost effective. Spherically shaped, crystalline silver nanoparticles with their average size 16 nm are synthesized. It is observed that bioactive components of plant extract may be responsible for reducing, capping and stabilizing the silver nanoparticles. As the green route of synthesis doesn't require any toxic chemicals therefore it is a promising substitute of long-established chemical and physical methods.

Acknowledgements

Authors are thankful to Punjab University, Chandigarh for FTIR and CDRI Lucknow for providing TEM.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

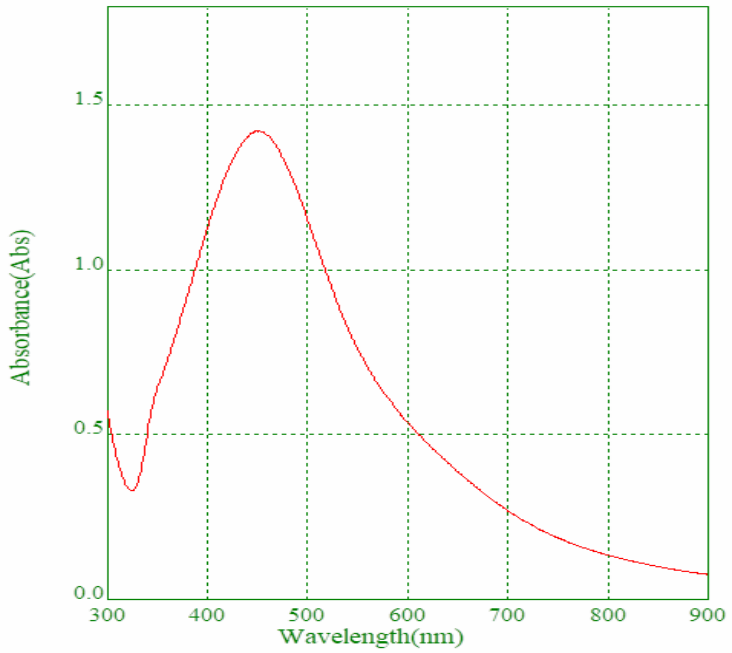

Fig. 1 : UV-visible spectrum of silver nanoparticles

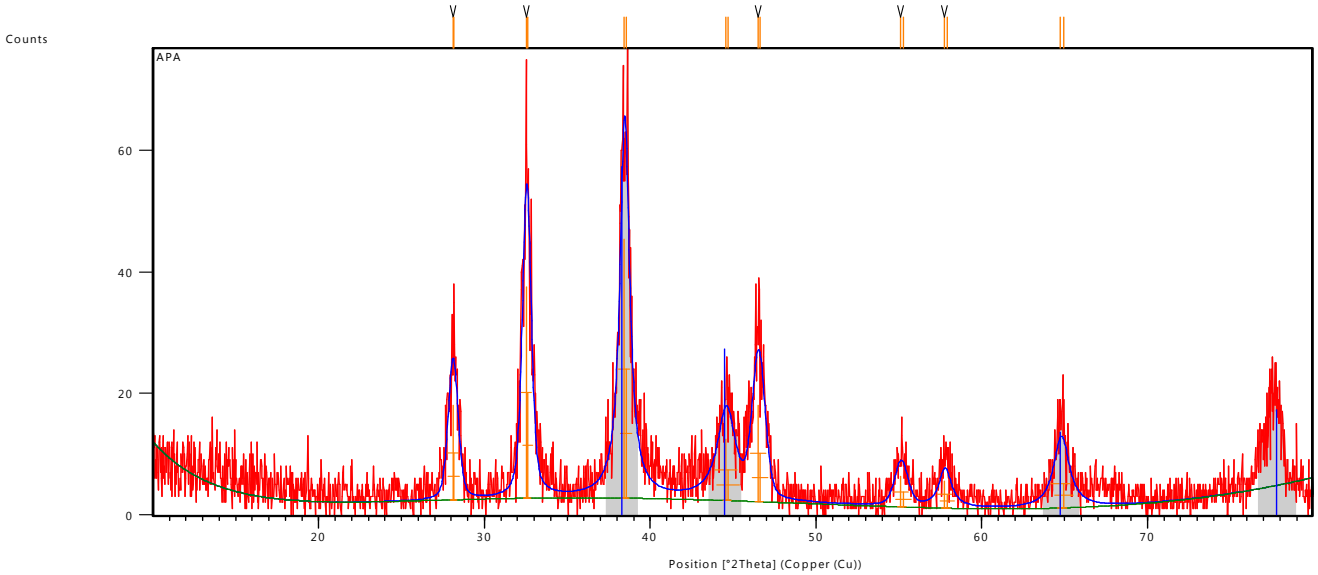

Fig. 2 : XRD spectra of silver nanoparticles

Table 1 : Peak list for average size calculation of nanoparticles

2θ (°)	**hkl**	**FWHM left**	***d*-spacing (Å)**	**Rel. int. (%)**
28.10	210	0.71	3.17	36.56
32.54	113	0.62	2.75	81.62
38.44	111	0.68	2.33	100
44.58	200	1.3	2.03	23.81
64.76	220	1.1	1.44	18.91

Where θ is the Bragg's diffraction angle, FWHM is full width at half maxima, (h,k,l) are miller indices and d-spacing is interplanar distance.

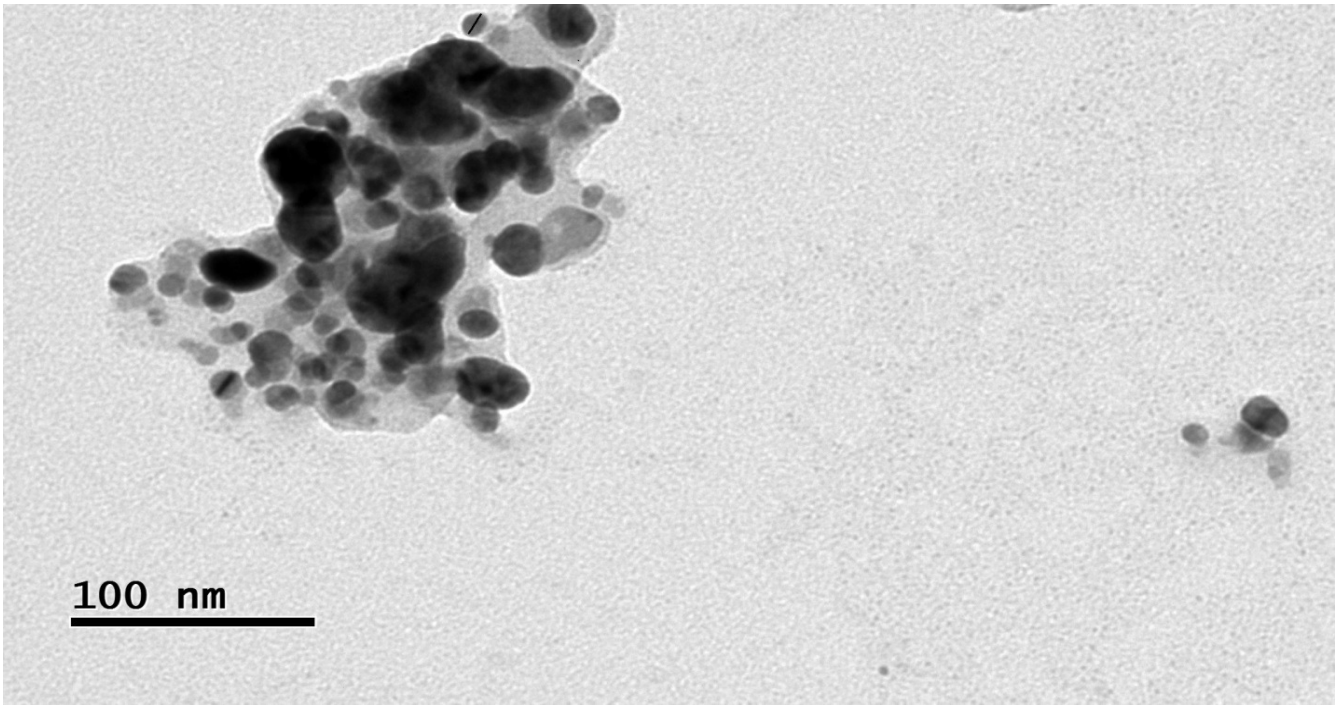

Fig. 3 : Transmission electron micro-image of silver nanoparticles

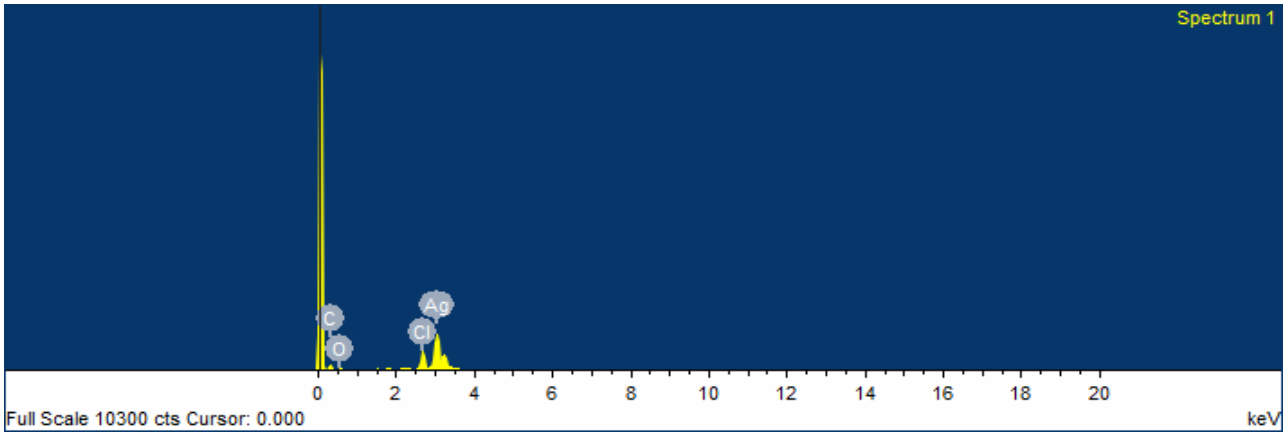

Fig. 4 : EDX pattern of silver nanoparticles

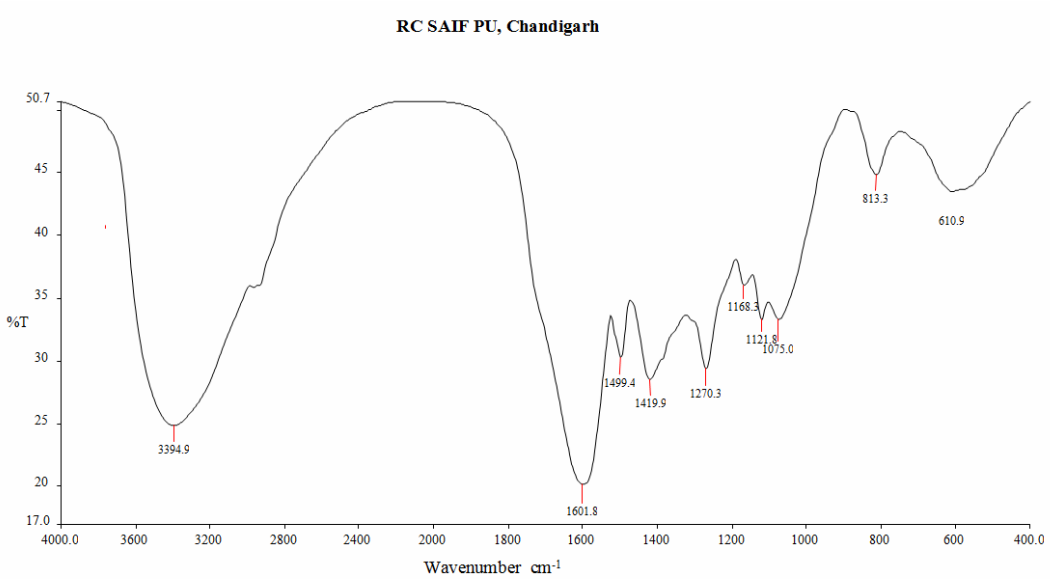

Fig. 5 : FTIR spectrum of silver nanoparticles

Table 2 : Values of Diameter of Zone of Inhibition Against Selected Microbial Strains

Diameter of zone of inhibition (mm)				
S.No.	**Test organisms**	**Control**	**75 µL**	**100 µL**
1.	*Staphylococcus aureus*	0.00 ± 00	13.21±0.64	14.58±0.41
2.	*Pseudomonas aeruginosa*	0.00 ± 00	12.89±0.44	14.33±0.78
3.	*Escherichia coli*	0.00 ± 00	12.91±0.64	13.96±0.62
4.	*Bacillus subtilis*	0.00 ± 00	12.82±0.42	14.02±0.72

± Standard deviation

Table 3 : Green synthesis of silver nanoparticles of some important medicinal plants

S.No.	Name of the plant	Family	Plant part	Shape	Size(nm)	Ref.
1.	*Geranium wallichianum*	Gereniaceae	Leaf	Spherical	12.5	(Badoni *et al.,* 2019)
2.	*Prunus persica*	Rosaceae	Leaf	Spherical	40-98	(Kumar *et al.,* 2017)
3.	*Moringa oleifera*	Moringaceae	Leaf	Spherical	9	(Moodley *et al.,* 2018)
4.	*Coleus forskohlii*	Lamiaceae	Roots	Needle Shaped	82.46	(Baskaran and bai, 2013)
5.	*Ocimum sanctum*	Lamiaceae	Leaf	Spherical	14.6	(Jain and Mehata, 2017)
6.	*Origanum vulgare*	Lamiaceae	Leaf	Spherical	2-25	(Carmona *et al.,* 2017)
7.	*Acalypha Indica*	Euphorbiaceae	Leaf	Spherical	20-40	(Menon *et al.,* 2017)
8.	*Coriandrum sativum*	Apiaceae	Leaf	Spherical	6.45	(Khan *et al.,* 2018)
9.	*Azadirachta indica*	Meliaceae	Leaf	Spherical	34	(Ahmed *et al.,* 2016)
10.	*Berberis vulgaris*	Berberidaceae	Leaf	Spherical	30-70	(Behravan *et al.,* 2019)
11.	*Euphorbia hirta*	Euphorbiaceae	Leaf	Spherical	40-50	(Elumalai *et al.,* 2010)
12.	*Ajuga parviflora*	Lamiaceae	Leaf	Spherical	15.8	Present Work

References

Abou El-Nour, K.M.M.; Eftaiha, A.; Al-Warthan, A. and Ammar, R.A.A. (2010). Synthesis and applications of silver nanoparticles. Arab. J. Chem., 3: 135-140.

Ahmad, L.; Semotiuk, A.J.; Liu, Q.R.; Rashid, W.; Mazari, P.; Rahim, K. and Sadia, S. (2018). Anti-hypertensive plants of rural Pakistan: Current use and future potential. Journal of Complementary Medicine Research, 7: 138-153.

Ahmed, S.; Saifullah, M.; Ahmad, B.L. and Swami, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 9: 1-7.

Azizi, S.; Namvar, F.; Mahdavi, M.; Ahmad, M. and Mohamad, R. (2013). Biosynthesis of Silver Nanoparticles Using Brown Marine macroalgae, *Sargassum muticum* Aqueous Extract. Materials, 6: 5942-5950.

Badoni, P.P.; Kumar, G.; Singh, M.; Singh, N.; Khajuria, A.K. and Dutt, S. (2019). Geranium wallichianum Leaf Extract Mediated Synthesis of Silver Nanoparticles: Characterization and its Antimicrobial Activity. Asian Journal of Chemistry, 31: 1128-1132.

Baskaran, C. and Bai, V.R. (2013). Green Synthesis of silver nanoparticles using Coleus forskohlii roots extract and its antimicrobial activity against Bacteria and Fungus. Int. J. Drug Dev. & Res., 5: 114-119.

Behravan, M.; Panahi, A.H.; Naghizadeh, A.; Ziaee, M.; Mahdavi, R. and Mirzapour, A. (2019). Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. International Journal of Biological Macromolecules, 124: 148-154.

Carmona, E.R.; Benito, N.; Plaza, T. and Sánchez, G.R. (2017). Green synthesis of silver nanoparticles by using leaf extracts from the endemic *Buddleja globosa* hope. Green Chemistry Letters And Reviews, 10: 250-256.

Collett, H. (1980). Flora Simlensis: a handbook of the flowering plants of Simla and neighbourhood, 2nd impression, Calcutta and Simla, Thacker, Spink & Co, 407.

Das, R.; Nath, S.S.; Chakdar, D.; Gope, G. and Bhattacharjee, R. (2010). Synthesis of silver nanoparticles and their optical properties J. Exp. Nanosci., 5: 357-362.

Dwivedi, A.D. and Gopal, K. (2010). Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 369: 27-33.

Elumalai, E.K.; Prasad, T.N.V.K.V.; Hemachandran, J.; Therasa, S.V.; Thirumalai, T. and David, E. (2010). Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities. J. Pharm. Sci. & Res., 2: 549-554.

Faramarzi, M.A. and Sadighi, A. (2013). Insights into biogenic and chemical production of inorganic nanomaterials and nanostructures. Adv. Colloid Interface Sci., 189-190: 1-20.

Gaur, R.D. (1999). Flora of the District Garhwal, North West, 1st edition, Transmedia, 459.

Gulzar, S.; Nawchoo, I.A.; Yaqoob, U. and Hassan, A. (2017). A Study on Variation of Phytochemical Constituents with Altitude and In vitro antimicrobial potential of two species of Ajuga L. Adv. Biomed. Pharma., 4: 19-30.

Hamayun, M.; Khan, A.; Afzal, S. and Khan, M.A. (2006). Study on traditional knowledge and utility of medicinal herbs of district Buner, NWFP, Pakistan. Indian journal of traditional knowledge, 5: 407-412.

Jain, S. and Mehata, M.S. (2017). Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. Scientific Reports, 7: 1-13.

Kandwal, A.; Purohit, P.; Khajuria, A.K.; Kumar, G.; Purohit, M.C. and Mandal, N.K. (2019). Synthesis of zinc oxide nanoparticles using leaf extract of *Ajuga parviflora* Benth. in Wall. and their characterization. Journal of Pharmacognosy and Phytochemistry, 8: 493-496.

Karthik, L.; Kumar, G.; Kirthi, A.V.; Rahuman, A.A. and Bhaskara Rao, K.V. (2014). *Streptomyces* sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application. Bioprocess Biosyst. Eng., 37: 261-267.

Khajuria, A.K.; Negi, A.; Bisht, N.S.; Maurya, V. and Kandwal, A. (2019). Green Synthesis, Characterization and Antimicrobial Activity of Zinc Oxide Nanoparticles Using Root Extract of *Viola canescens* Wall. ex. Roxb. Asian Journal of Chemistry, 31: 551-554.

Khajuria, A.K.; Bisht, N.S. and Kumar, G. (2017). Synthesis of Zinc oxide nanoparticles using leaf extract of *Viola canescens* Wall. ex, Roxb. and their antimicrobial activity. Journal of Pharmacognosy and Phytochemistry. 6: 1301-1304.

Khan, M.Z.H.; Tareq, F.K.; Hossen, M.A. and Roki, M.N.A.M. (2018). Green Synthesis and Characterization of silver Nanoparticles using *Coriandrum sativum* leaf extract. Journal of Engineering Science and Technology, 13: 158-166.

Kumar, G.; Badoni, P.P. and Singh, M. (2018). Bio-inspired synthesis of silver nanoparticles from *Cotoneaster acuminatus* leaf extract and their characterization. International Journal of Advance research in science and engineering, 7: 135-139.

Kumar, R.; Ghoshal, G.; Jain, A. and Goyal, M. (2017). Rapid Green Synthesis of Silver Nanoparticles (AgNPs) Using (*Prunus persica*) Plants extract: Exploring its Antimicrobial and Catalytic Activities. Journal of Nanomedicine & Nanotechnology, 8: 1-8.

Kumar, S.; Javed, M.S.; Kumar, P. and Kumar, R. (2018). Chemical Composition, In vitro antibacterial, and antioxidant activity of essential oil from leaves of *Ajuga parviflora* Benth. Asian J Pharm Clin Res., 11: 57-61.

Menon, S.; Agarwal, H.; Kumar, S.R. and Kumar, S.V. (2017). Green synthesis of silver nanoparticles using medicinal plant *Acalypha indica* leaf extracts and its application as an antioxidant and antimicrobial agent against foodborne pathogens. Int J App Pharm, 9: 42-50

Moghaddam, M.G.; Dabanlou, R.H.; Khajeh, M.; Rakhshanipour, M. and Shameli, K. (2014). Green synthesis of silver nanoparticles using plant extracts. Korean J. Chem. Eng., 31: 548-557.

Mohanpuria, P.; Rana, N.K. and Yadav, S.K. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanopart. Res., 10: 507-517.

Moodley, J.S.; Krishna, S.B.N.; Pillay, K.S. and Govender, P. (2018). Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. Adv. Nat. Sci.: Nanosci. Nanotechnol., 9: 1-10.

Morejon, B.; Pilaquinga, F.; Domenech, F.; Ganchala, D.; Debut, A. and Neira, M. (2018). Larvicidal Activity of Silver Nanoparticles Synthesized Using Extracts of *Ambrosia arborescens* (Asteraceae) to Control *Aedes aegypti* L. (Diptera: Culicidae). Journal of Nanotechnology, 6917938: 1-8.

Nasiriboroumand, M.; Montazer, M. and Barani, H. (2018). Preparation and characterization of biocompatible silver nanoparticles using pomegranate peel extract. Journal of Photochemistry & Photobiology, B: Biology, 179: 98-104.

Nawaz, H.R.; Malik, A.; Muhammad, P.; Ahmed, S. and Riaz, M. (2000). Chemical Constituents of *Ajuga parviflora*. Zeitschrift für Naturforschung, 55b: 100-103.

Okafor, F.; Janen, A.; Kukhtareva, T.; Edwards, V. and Curley, M. (2013). Green Synthesis of Silver Nanoparticles, Their Characterization, Application and Antibacterial Activity. Int. J. Environ. Res. Public Health, 10: 5221-5238.

Pawar, P.H. and S.R. Chaudhari (2017). Bio-directed synthesis of Ag Metal Nanoparticles using Tulsi (*Ocimum tenuiflorum*) leaves extract. Asian Journal of Research in Chemistry, 10: 646-650.

Perez, C. and C. Anesini (1994). In vitro antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. J. Ethnopharmacol., 44: 41-46.

Poulose, S.; Panda, T.; Nair, P.P. and Theodore, T. (2014). Biosynthesis of Silver Nanoparticles. J. Nanosci. Nanotechno., 14: 2038-2049.

Rahman, N.; Ahmad, M.; Riaz, M.; Mehjabeen, N. and Ahmad, R. (2013). Phytochemical, antimicrobial, insecticidal and brine shrimp lethality bioassay of the crude methanolic extract of *Ajuga parviflora* Benth. Pak. J. Pharm. Sci., 26: 751-756.

Shankar, S.S.; Rai, A.; Ahmad, A. and Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core—Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. Journal of Colloid and Interface Science, 275: 496-502.

Sharma, V.K.; Yngard, R.A. and Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci., 145: 83-96.

Singh, P.; Prakash, O. and Pant, A.K. (2015). Essential Oil Composition of *Ajuga parviflora* Benth. Growing in Western Himalayan Region of Uttarakhand (India). Journal of Essential Oil Bearing Plants, 18: 697-701.

Stoimenov, P.K.; Klinger, R.L.; Marchin, G.L. and Klabunde, K.J. (2002). Metal Oxide Nanoparticles as Bactericidal Agents. Langmuir, 18: 6679-6686.

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

Xie, Y.; He, Y.; Irwin, P.L.; Jin, T. and Shi, X. (2011). Antibacterial Activity and Mechanism of Action of Zinc Oxide Nanoparticles against Campylobacter jejuni. Appl. Environ. Microbiol., 77: 2325-2331.

Zargar, M.; Hamid, A.A.; Bakar, F.A.; Shamsudin, M.N.; Shameli, K.; Jahanshiri, F. and Farahani, F. (2011). Green Synthesis and Antibacterial Effect of Silver Nanoparticles Using *Vitex negundo* L. Molecules, 16: 6667-6676.