Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN **0976-9595**

Research Article

GREEN SYNTHESIS AND CHARACTERIZATION OF *ALCHEMILLA VULGARIS* SILVER NANOPARTICLES

Shilpee Jain*[1], A S Yadav[2], Ragini Gothalwal[1]

[1]*Biotechnology Department, Barkatullah University, Habib Ganj, Bhopal, Madhya Pradesh, India*

[2]*OSD in Higher Education, M.P. Govt. Bhopal, Madhya Pradesh, India*

**Corresponding author: jain.shilu620@gmail.com*

Received: 09-09-2022; Accepted: 10-10-2022; Published: 31-10-2022

 https://doi.org/10.55218/JASR.202213909

ABSTRACT

Among all the nanoparticles synthesized; silver nanoparticles have attained special place in the area of nano technology because of their antimicrobial, nontoxic, environmentally safe and biomedical applications. In general; their syntheses involves the use of hazardous chemicals or costly physical methods. However, the biological processes are making their ways in between and proving their advantages over them. The use of plants and their extracts is one of the most valuable methods which are gaining concerns due to their imperative biological benefits. Plants are not only beautiful but majestic because they are rich sources of various medicinally important substances. They explore the huge diversity which can be utilized towards rapid and single step protocol preparatory method for various nanoparticles keeping intact "the green principles" over the conventional ones and proving their dominance for medicinal importance. Here, in the presented work "one pot synthesis of silver nanoparticles" is described. Therefore; a simple, cost effective bio-reduction on the principle of "green synthesis" of silver nanoparticles using the *Alchemilla vulgaris* plant extract is reported. The beauty of the synthesis is: no involvement of any surfactant, catalyst or template. The aqueous silver ions are reduced to silver nanoparticles when exposed to plant extract. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry, FTIR spectroscopy, SEM, particle size and zeta potential.

Keywords: Green synthesis, *Alchemilla vulgaris,* Silver Nanoparticles, One pot synthesis.

1. INTRODUCTION

Nanomaterials have got a special place and have various applications in nanotechnology [1]. The metallic nanoparticles having larger surface area can be used as antibacterial agent [2]. Various physical, chemical, and biological methods are employed in the development of Nanoparticles [3]. The toxic compounds used in chemical and physical methods limits the biomedical applications, therefore, use of biological materials i.e. plants, plant components, bacteria for synthesis of nanoparticles are safe, stable, biocompatible, cost effective and eco-friendly [4]. Plant based synthesis has some advantages such as it is faster and stable. It is possible to get different sizes and shapes of NPs comparable to microorganisms. Many investigations showed that silver and zinc nanoparticles exhibited antibacterial, antioxidant and photo catalytic properties [5, 6], also they are superior product from the field of nanotechnology because of their exclusive properties such as antibacterial, anti-viral, antifungal and anti-inflammatory activities and due to their good stability [7, 8]. Silver and zinc nanoparticles have been used most widely in the different industries i.e. health, food packaging, textile and other. There are various reports of using green, i.e., natural and environment friendly reducing and capping agents for nanomaterial synthesis [9, 10]. Leaves of different plants such as *Azadirachta indica* (neem) [11], *Ocimum tenuiflorum* (black Tulsi) [12], *Ficus benghalensis* (Banyan tree) [13] etc. have been used for the synthesis of AgNPs [14]. One of the plant, that have medicinal quality to provide the rational means for the treatment of many diseases, is *Alchemilla vulgaris L.* syn. *Alchemilla xanthochlora* Rotham., commonly known as lady's mantle and bear's foot, well known species from the genus *Alchemilla* (Rosaceae) [15]. It has an anti-aging, anti-inflammatory, antimicrobial, antioxidant, astringent, coagulant, diuretic, emmenagogue, hepatic, hypo-glycemic, hypotensive, lithotriptic, pro-collagen, vasodilator, vulnerary actions [15]. Aerial parts of the plant are more useful medicinally and cosmetically. It contains tannins such as pedunculagin, salicylic acid, flavonoids, triterpenes mainly. The extract is light to medium amber liquid with a characteristic odor, prepared from leaves. It has flavonoids and phenolic acids in its extract, those are actually powerful antioxidants internally as well as externally on the skin [16]. Lady's Mantle has traditionally been used for issues associated with the reproductive system such as painful periods, irregularity, lack of bleeding, excessive bleeding, headache, uterine prolapse, abnormal and excessive vaginal discharge, vaginal infections, fibroids, endometriosis, infertility, post-partum disorders [17]. Hence, the aim of present study is to synthesize silver nanoparticles by using aqueous *Alchemilla vulgaris* extract and characterization of theses silver nanoparticles with UV-VIS spectrophotometry, FTIR, FE-SEM and zeta potential measurements.

2. EXPERIMENTAL

2.1. Collection and authentication of plant

The plant *Alchemilla vulgaris* was collected from Bristol Botanicals Ltd. The plant was washed, shade dried and powdered. The authentication was carried out by submitting the specimen in the Saifia Science College, Bhopal. Voucher no. is 159/Saif./Sci./Clg./Bpl.

2.2. Extraction of plant *Alchemilla vulgaris*

Extraction of *Alchemilla vulgaris* was carried out by the maceration method. The crushed plant of *Alchemilla vulgaris* was used for the extraction process. The powdered *Alchemilla vulgaris* was successively extracted with solvents of increasing polarity; petroleum ether, ethyl acetate and methanol [18]. Following the extraction processes, solvent was evaporated to dryness under reduced pressure using rotary evaporator and excessive moisture was removed. The dried extract were kept and labelled in air tight container.

2.3. Green synthesis of silver nanoparticles

To synthesize AgNPs, firstly, different concentrations (0.2, 0.5 and 1 mM) of silver nitrate solution were prepared. For 0.2, 0.5 and 1 mM AgNO3 solution 0.003, 0.008 and 0.016 g of silver nitrate was dissolved in 100 ml de-ionized water respectively. λmax of all the three solutions were estimated by scanning them 200-600nm in UV-Vis spectrophotometer. (Systronics PC based double beam spectrophotometer 2202) The absorptions of 0.2 mM, 0.5 mM, and 1 mM of $AgNO_3$ solution were found to be 208, 214, and 217nm respectively. Among all the three, 1mM $AgNO_3$ solution has shown the highest peak and thus it was chosen for silver nanoparticle preparation. 100 mL (1mM) aqueous solution of silver nitrate was prepared. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of plant extract were added separately to 10mL aqueous silver nitrate solution kept in separate beakers at room temperature (their notation shown in Table 1). The solution was kept in dark chamber until solution colour changed to yellow to dark yellow. After, 15 min, the solution turned yellow to yellow-red or dark brown indicating the formation of silver nanoparticles. The bioreduction of silver ions was monitored by periodic sampling by the UV spectrophotometer [19].

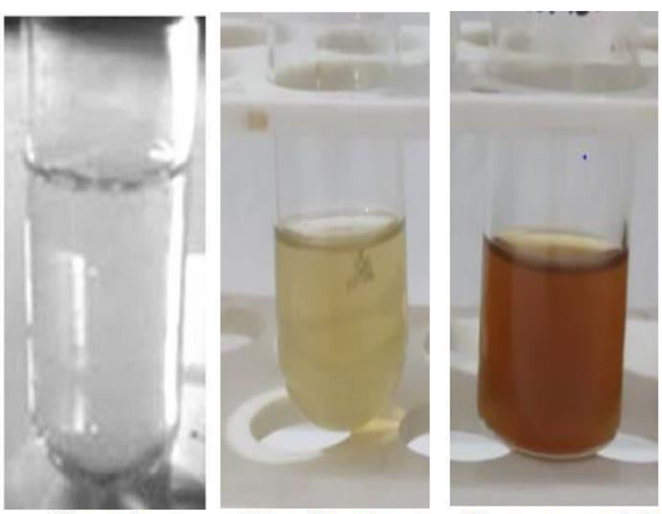
2.4. Characterization of silver nanoparticles

The nanoparticles were characterized using UV-VIS spectrophotometry, FTIR, FE-SEM, and zeta potential measurements. Functional groups attached to the metallic nanoparticle surface show different FT-IR pattern than those of free groups. FE-SEM is a powerful technique for imaging any material surface with a resolution down to about 1 nm. FESEM can give information about the purity of nanoparticle sample. The shape, morphology, and elemental mapping of AgNPs were studied using the High resolution field emission scanning electron microscope (HR FESEM Zeiss ULTRA Plus). For this purpose, the lyophilized sample was sonicated for a sufficient amount of time; the smear was made on a platinum grid, and allowed to dry overnight under vacuum. The grid was then coated with a thin film of palladium and finally subjected to FESEM. The value of particle size distribution of silver nanoparticles was determined using a Particle Size Analyser (PSA). Particle size characterization is of particular significance to nanomedicine. The size equality of nanoparticles to biological moieties is considered to impart many of their unique medical character. Also, zeta potential is an essential characteristic of nanoparticles, which is to predict the stability of the colloidal solution. The interaction among particles is critical to the stabilityof the colloidal solution. Zeta potential is a value that indicates repulsion force between particles. The colloidal solution stabilized by the presence of electrostatic repulsion rejected. The higher the repulsive force among particles rejected will cause the particles to be close to each other and will form aggregates. Zeta potential was measured using Horiba SZ-100. Zeta potential indicated the surface charge on the particles and was measured to determine the stability of nanoparticles.

3. RESULTS AND DISCUSSION

3.1. Visual observations

The synthesis of nanoparticles was initiated once the plant extract of *Alchemilla vulgaris* extract was introduced into 1mM $AgNO_3$ solution. A preliminary visual observation showed that the initial colour of the reaction mixture after the addition of plant extract to the aqueous silver nitrate solution was nearly colourless. Interestingly, the colour of the reaction mixture changed from pale-yellow then light brown to dark brown exponentially with reaction-time as aggregation proceeds depicting the silver nitrate salt got reduced to silver as represented in fig. 1. The *Alchemilla vulgaris* plant extract is depicted to contain important phytochemicals such as flavones, tannins, and other polyphenols which act as a reducing agent to give the reduced silver ions from silver nitrate. The obtained colour change confirming the synthesis of Ag NPs was compared to the similar type of colour inference was observed during the synthesis of AgNPs from *Ocimum sanctum, Moringa oleifera* and *Carica papaya* leaf extract [20, 21].



Silver nitrate Soln. (colorless) | ***Alchemilla vulgaris* plant extract soln.** | **Silver nanoparticle (Brown colour)**

Fig. 1: Visual observation of synthesized silver nanoparticles

There was a colour change from light brown or golden yellowish to dark brown which depicted that the silver salt got reduced to silver. A similar colour change was also reported when silver nitrate got reduced by aqueous neem leaf extract and walnut leaf extract [22]. The process of oxidation and the reduction reaction is responsible for the colour change, which is the indicator for Ag NPs synthesis. Appearance of different colours at different time intervals indicated that the morphology (shape, size and the size distribution) of silver nanoparticles alters with the reaction time.

3.2. Measurement of Ag nanoparticle by UV-Visible spectrophotometry

Formation of the nanoparticles in the aqueous solution was further confirmed by the UV-visible spectroscopy. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspensions. The UV-Vis spectra of silver nanoparticle after 4 h, 24 h, 48 h, 72 h and 96h of the reaction were documented, indicating the formation of silver nanoparticles due to excitation of surface plasmon vibrations in silver nanoparticles [23]. Silver nanoparticles are analyzed by UV spectra of Surface Plasmon Resonance (SPR) band observed at 436-411 nm [24]. If we increase the plant extract concentration up to 4 mL and 5ml, there is a decrease in the absorbance up to 424. The variation in values of absorbance confirms to the changes in the particle size. Fig.2 shows UV-Vis spectra recorded at different time intervals from solution of silver nitrate with extract. The sample 1ml displays an optical absorption band peak at about 436 nm, typical of absorption for metallic Ag nanoparticles due to the SPR. The sample 1ml, 2ml, 3ml, 4ml and 5ml typically show absorption band of synthesized silver nanoparticles at 436, 433, 430, 424 and 424 nm respectively. Effect of the reaction time on silver nanoparticles syntheses was also evaluated with UV-Visible spectra and it is noted that with an increase in time the peak becomes sharper and intense. The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution [25]. The spectra of silver nitrate solution and plant extract did not show any SPR band. The colloidal solution of silver nanoparticles was kept as such in closed sample tubes and absorbance was noted for 96 hrs at regular 24 hrs. interval and were found to be stable after 24 hrs. The 1ml concentration of nanoparticles was selected for further characterizations. This particular analysis was confirmed and justified when similar type of absorption spectra was observed within the range of 400-430nm., which is at higher energy as that obtained by with aqueous extract of neem leaf [26]. The similar type of spectral analysis was done during synthesis of Ag NPs using aqueous olive leaf extract [27]. The spectral analysis done for the synthesized nanoparticles with aqueous leaf extract of *Saccharina japonica* was in the same range that is within the range of 400-450nm. The peak occurring in this range confirms the synthesis and the presence of AgNPs [28].

3.3. Particle size and shape analysed by SEM

SEM analysis shows high-density AgNPs synthesized by *Alchemilla vulgaris* extract. It was shown that relatively spherical AgNPs were formed with the range of 96 to 113 nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [23]. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. The same type of SEM analysis is done during the green synthesis of Ag NPs from pomegranate peel extract. Upon SEM analysis the particle size obtained for the synthesized Ag NPs were in the range of 40-80nm [29]. So the nanoparticle which comes in the range of 0-100nm is considered to be the silver nanoparticle.

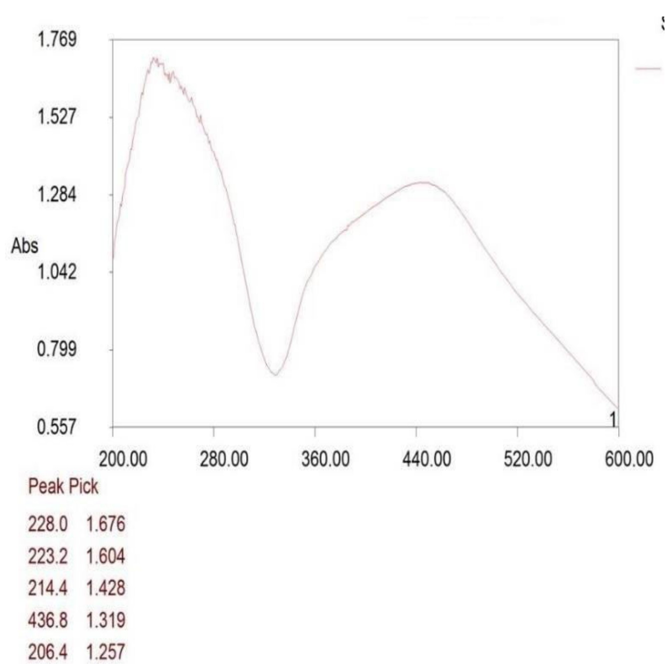


Fig. 2(A): Absorption spectra of *A. vulgaris* (1ml concentration) in range of 200-600nm with peak optical at 436nm

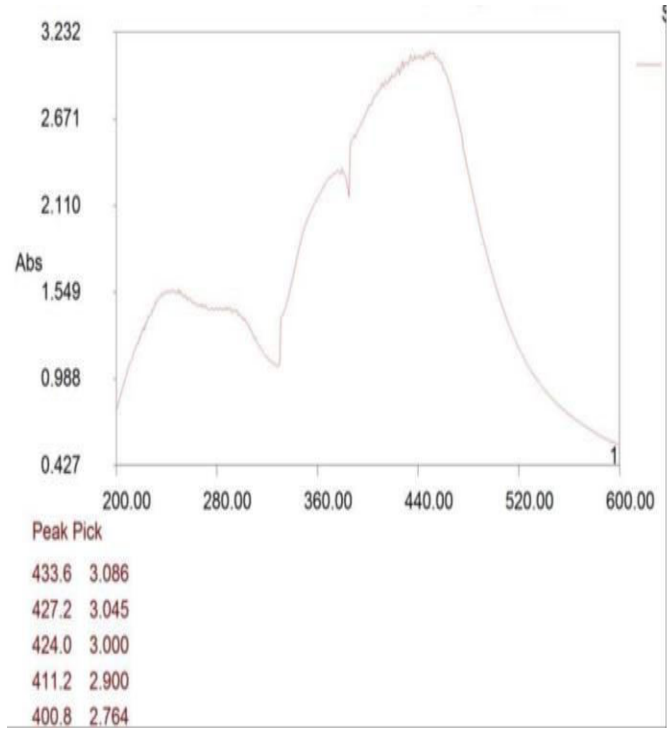


Fig. 2(B): Absorption spectra of *A. vulgaris* (2ml concentration) in range of 200-600nm with peak optical at 433nm

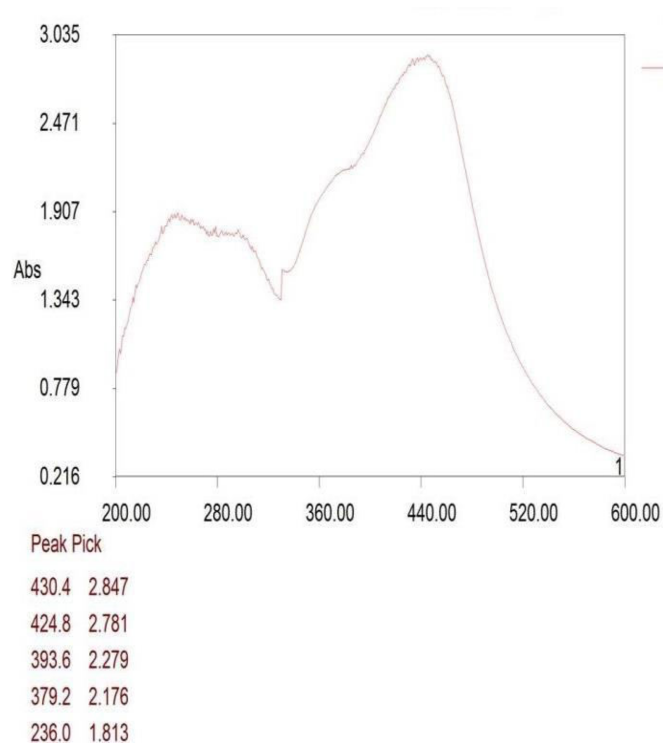


Fig. 2(C): Absorption spectra of *A. vulgaris* (3ml concentration) in range of 200-600nm with peak optical at 430nm

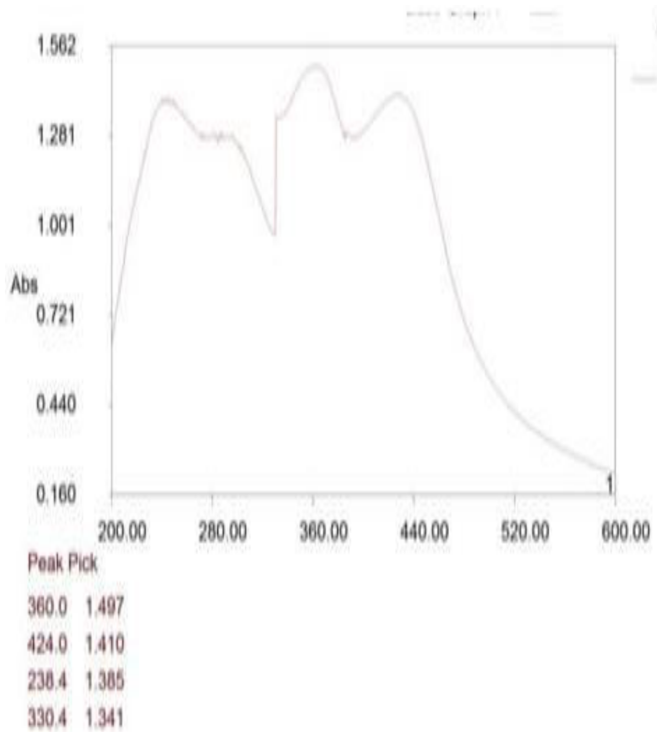


Fig. 2(D): Absorption spectra of *A. vulgaris* (4ml concentration) in range of 200-600nm with peak optical at 424nm

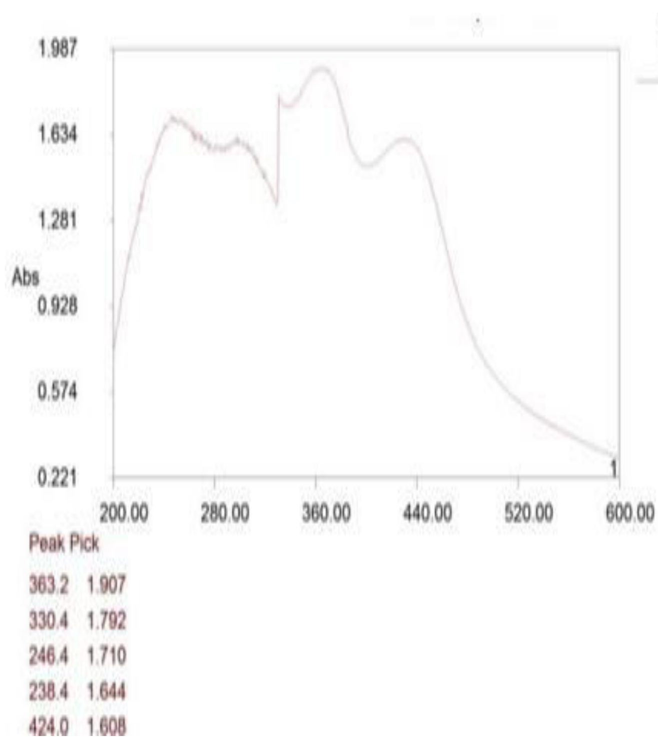
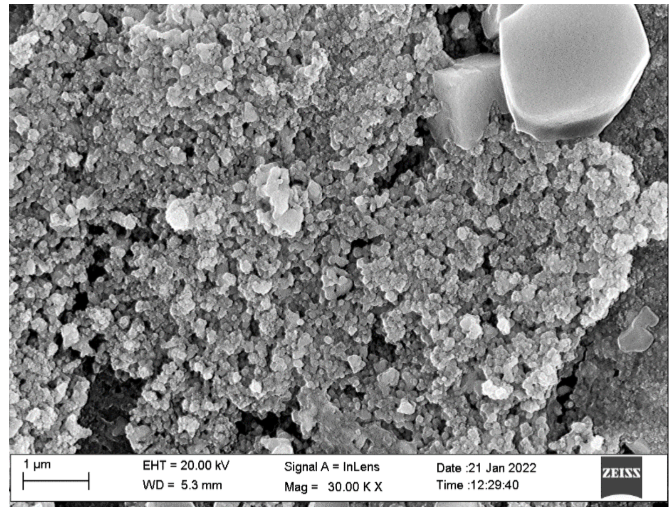
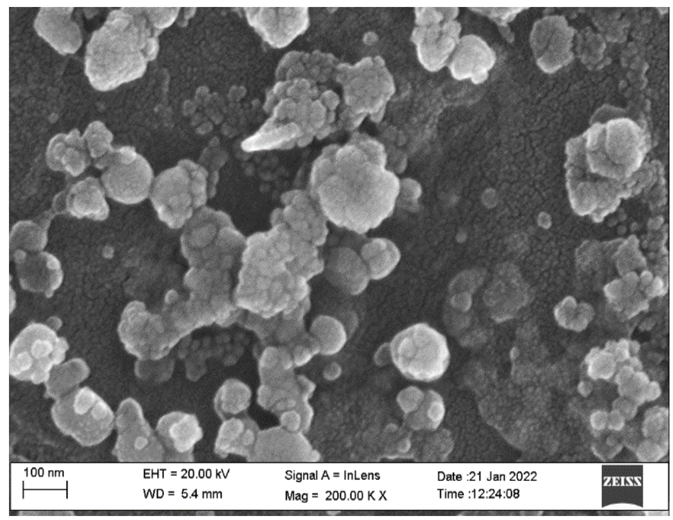


Fig. 2(E): Absorption spectra of *A. vulgaris* (5ml concentration) in range of 200-600nm with peak optical at 424nm



A



B

(A) Magnification 30kx and scale bar used 1000nm (B) Magnification 200kx and scale bar used100nm

Fig. 3: SEM microphotographs of synthesized silver nanoparticles

3.4. Analysis of Ag nanoparticle by FTIR

IR spectra of *Alchemilla vulgaris* extract were interpreted and the functional groups were identified. The first peak was found at 3385.60 cm^{-1}which confirmed the presence of amine group. Alkene (C=C) stretching peak was identified at 1617.76 cm^{-1}. Alkane (C-H) rock peak was found at 1355.22 cm^{-1}. Alkanes (C-H) stretching peak was found at 2928.58 cm^{-1}.Stretching peak at 1719.07 cm^{-1} indicated the presence of carbonyls group (C=O). Aliphatic amines (C-N) stretching peak was found at 1193.87 cm^{-1}. Alkenes (=C-H) bending peak was found at 1046.87 cm-1. Primary and secondary amines wagging peak (N-H) was found at 922.03 cm^{-1}. Bending peak at 757.67 cm^{-1} indicated the presence of alkynes. Alkyl halide (C-Br) stretching peak was found at 555.70 cm^{-1}. The FTIR of extract showing the same peak of the identified functional groups as the reference FTIR which confirmed the *Alchemilla vulgaris* extract. IR spectra of *Alchemilla vulgaris* nanoparticle were interpreted and the functional groups were identified. The first peak was found at 3412.48 cm^{-1} which confirmed the presence of amine group. Alkene (C=C) stretching peak was identified at 1628.84 cm^{-1}. Alkane (C-H) bending peak was found at 1384.62 cm^{-1}. Primary alcohol (C-O) stretching peak was obtained at 1049cm^{-1}. Halo compound (C-Cl) stretching was identified at 580.48cm^{-1}. The FTIR of extract showing the same peak of the identified functional groups as the reference FTIR and extract FTIR, which confirmed the *Alchemilla vulgaris* (Fig. 4 &5). A similar type of FTIR peaks were observed during the analysis of chemical composition of Ag NPs synthesized using *Saccharina japonica* leaf extract [28]. The *Memecylon edule* aqueous leaf extract used for the Ag NPs synthesis had shown similar type of peaks [30]. A similar output of FTIR peaks were observed during the synthesis of Ag NPs using aqueous neem leaf extract [26].These bands infers the presence of polyphenols and flavonoids. Similar types of peaks were observed during the presence of flavonoid and polyphenol compounds in *Viscum album* and *Allium sativum* leaf extracts [31].

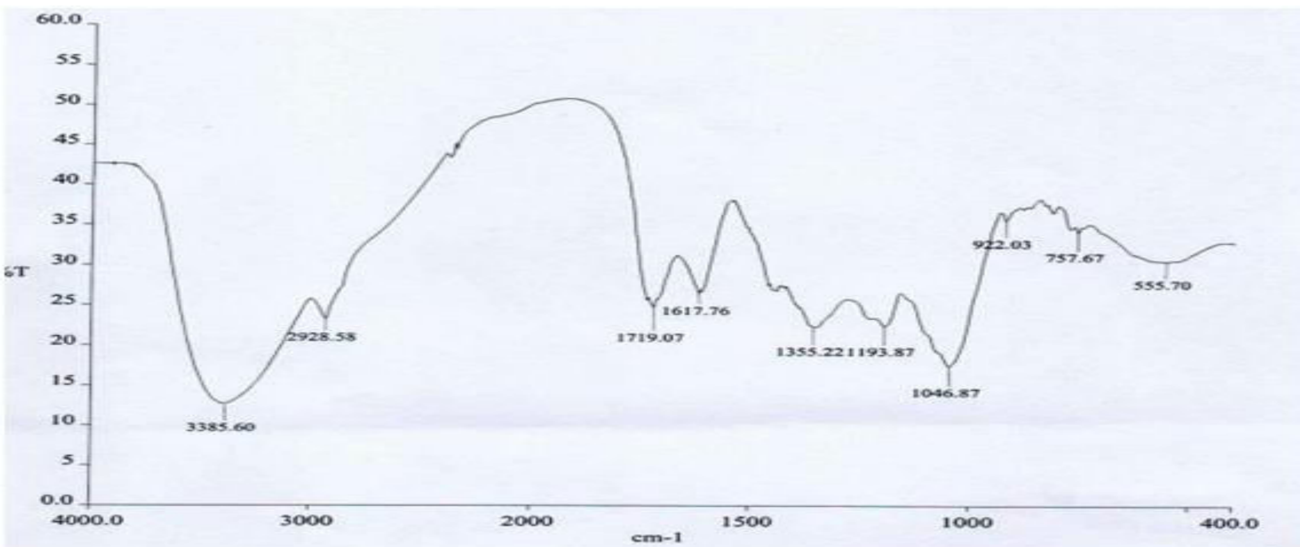


Fig. 4: FTIR of *Alchemilla vulgaris* extract

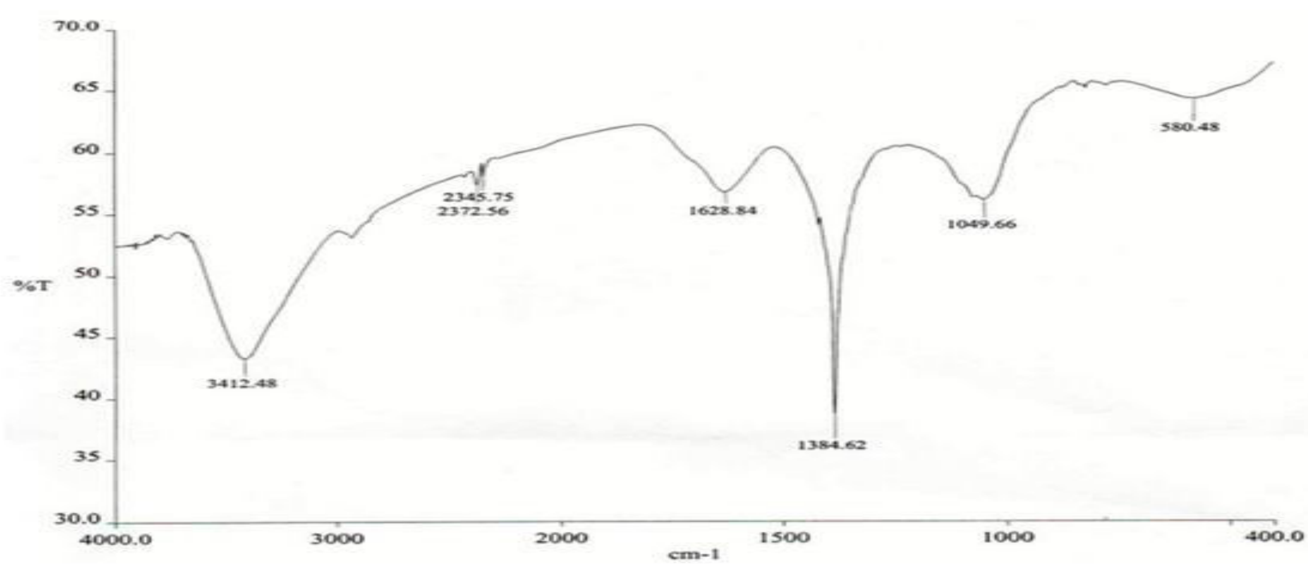


Fig. 5: FTIR of *Alchemilla vulgaris* nanoparticle

3.5. Determination of particle Size of Ag nanoparticle and their analysis through Zeta Potential

For the determination of the particle size and the stability of Ag NPs, the zeta potential analysis was done as shown in figs. 6 and 7, the structural characteristic of nanoparticles get altered due to a number of factors including incubation time, temperature, pH and the method used for nanoparticle preparation. Particle size was determined and the average particle size was foundto be 93.1nm. Knowledge of the zeta potential for nanoparticles preparation could help to predict the fate of the nanoparticles *in vivo* and to assess the stability of colloidal systems. Surface charge on the particles could control the particles stability of the nanoparticulate formulation through strong electrostatic repulsion of particles with each other. The zeta potential value can either be negative or positive. The negative potential value depicted from the synthesized Ag NPs due to the entrapment of the bioactive compound present in the extract. Zeta potential values were found to be -23.6 mV, which is below +/-30 mV that means the formulation of nanoparticle is stable. The similar type of analysis was made during the synthesis of Ag NPs using aqueous extract of *Urtica dioca* [32]. The aqueous *Pedalium murex* leaf extract used for the synthesis of Ag NPs had shown the similar type analysis [33].

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-23.6 mV	-0.000183 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -23.6 mV
Electrophoretic Mobility Mean : -0.000183 cm²/Vs

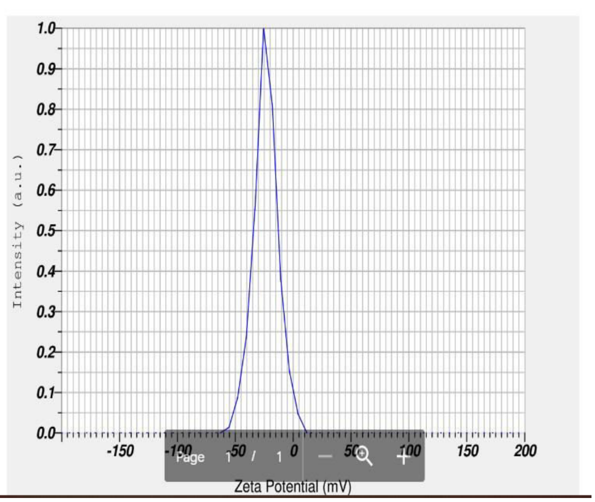


Fig. 6: Zeta potential of nanoparticle

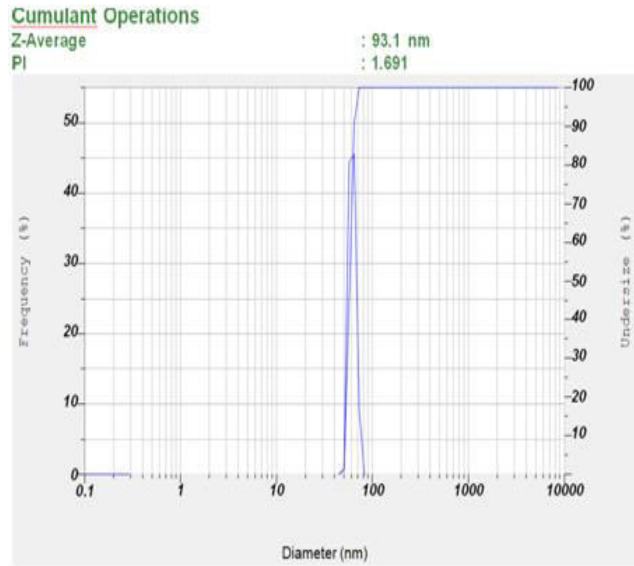


Fig. 7: Particle size of nanoparticle

4. CONCLUSION

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. In the present study, we have demonstrated that use of a natural, low cost biological reducing agent *Alchemilla vulgaris* plant extracts can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of toxic solvents and waste. The present study showed a simple, rapid, and economical route to synthesize silver nanoparticles. The use of *Alchemilla vulgaris* has the added advantage that this plant can be used by nanotechnology processing industries. Prepared nanoparticles can be used as bactericidal, in wound healing, water purification, and also in the field of medicine due to these applications, this method is potentially exciting for the large- scale synthesis of nanoparticles, could be used for drug delivery and further this can be used asa therapeutic agent in medical field.

Conflict of interest
None declared

Source of funding
None declared

5. REFERENCES

1. Demirezen-Yilmaz D, Koca F, Ertaş-Onmaz N. *Asian J App Chem Res.,* 2019; **2(2)**:1-11.
2. Khalil KA, Fouad H, Elsarnagawy T, Almajhdi FN. *Int J Electrochem Sci.,* 2013; **8**:3483-93.

3. Mahdavi S, Jalali M, Afkhami A. *Chem Eng Commun.,* 2013; **200**:448-470.
4. Arockiya F, Parthiban C, Ganesh-Kumar V, Anantharaman P. *Spectrochim Acta- A: Mol Biomol* Spectrosc., 2012; **99**:166-173.
5. Shriniwas P, Subhash KT. *Biochem and Biophysics Reports.*, 2017; 1076-1081.
6. Logeswari P, Silambarasan S, Abraham J. *Scientia Iranica.,* 2013; **20 (3)**:1049-1054.
7. Klaus-Joerger T, Joerger R, Olsson E, Granqvist C. *Trends Biotechnol.,* 2001; **19**:15-20.
8. Ahmed S, Ahmad M, Swami B, Ikram S. *J Adv Res.,* 2016; **7:**17-28.
9. Tavakoli F. *J Mat Res Bull.,* 2014; **49:**14-20.
10. Gholami T, Salavati-Niasari M, Varshoy S. *Int J Hydro Ener.,* 2016; **41**:9418-9426.
11. Verma A, Mehata MS. *J Rad Res Appl Sci.,* 2016; **9**:109-115.
12. Banerjee P, Satapathy M, Mukhopahayay A, Das P. *Biores Bioproc.,* 2014; **1**:3.
13. Saware K, Venkataraman A. *J Clust Sci.*, 2014; **25**:1157-1171.
14. Pathak AK, Jain DK, Gupta S, Raguwansi MB, Chandel HS. *Asian J Pharm Clin Res.,* 2008 **1(3)**:67-69.
15. Jain S, Yadav A, Gothalwal R. *J Adv Scienti Res.,* 2021; **12(04 Suppl 1)**:205-209.
16. Shilova IV, Suslov NI, Samylina IA, Baeva VM, Lazareva NB, Mazin EV. *Pharm Chem J.,* 2020; **53(11)**:1059-1062.
17. Pathak AK, Jain DK, Gupta S, Raguwansi MB, Chandel HS. *Plant Archives.* 2008; **8(2)**:783-785.
18. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Phytochem Anal., 2002; **13**:8-17.
19. Ahmed S, Ikram S. *J Nanomed Nanotechnol.,* 2015; **6**:309.
20. Prasad TNVKV, Elumalai EK. *Asian Pac J Trop Biomed.,* 2011; **1**:439.
21. Banala RR, Nagati VB, Karnati PR. *Saudi J Biol Sci.,* 2015; **22**:637.
22. Korbekandi H, Asghari G, Jalayer SS, Jalayer MS, Bandegani M. *J Nat Pharm Prod.,* 2013; **8**:23.
23. Ramar M, Manikandan B, Raman T, Arunagirinathan K, Palanisamy S, et al. *Spectrochim Acta- A: Mol Biomol Spectrosc.,* 2015; **138**:120-129.
24. Ulug B, Turkdemir MH, Cicek A, Mete A. *Spectrochim Acta- A: Mol Biomol Spectrosc.,* 2015; **135**:153-161.
25. Khalil MMH. *Arab J Chem.,* 2013; **7**:1131-1139.
26. Verma A, Mehata MS. *J Radiat Res Appl Sci.,* 2015; **9**:109.
27. Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D. *Arab J Chem.,* 2014; **7**:1131.
28. Sreekanth TVM, Pandurangan M, Kim DH. *J Clust Sci.,* 2016; **27**:671.
29. Phongtongpasuk S, Poadang S. *Adv Mat Res.,* 2016; **1131**:227.
30. Tamizhamudu E, Kantha DA. Int J Nanomed., 2011; **6**:1265.
31. Trifunschi S, Munteanu MF, Agotici V, Pintea S, Gligor R. *Int Curr Pharm J.,* 2015; **4**:382.
32. Jyoti K, Baunthiyal M, Singh A. *J Radiat Res Appl Sci.,* 2016; **9**:17.
33. Anandalakshmi K, Venugobal J, Ramasamy V. *Appl Nanosci.*, 2016; **6**:399.