GREEN SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ANTIANGIOGENIC ACTIVITY OF *AERVA LANATA* AND *MOMORDICA CHARANTIA* SILVER NANOPARTICLES (AGNPS)

Karra Geetha *1, Pamu Sandhya2

[1]Department of Pharmaceutics, CMR College of Pharmacy, Medchal, Hyderabad- 501401, Telangana, India.

[2]Department of Pharmaceutics, Shadan Women's College of Pharmacy, Hyderabad- 500004, Telangana, India

***Corresponding author :** Karra Geetha, Mail id: geetabiokarra@gmail.com

ABSTRACT

Green synthesis of silver nanoparticles using the aqueous leaf extract of *Aerva lanata* and *Momordica charantia* were screened for antimicrobial activity by chick chorioallantoic membrane assay (CAM) (*in-ovo* method). Green synthesis of silver nanoparticles using the aqueous leaf extract of *Aerva lanata* and *Momordica charantia* were screened for antimicrobial activity using the cup plate method against gram positive and gram-negativereference organisms (*Bacillus Subtilis* and *Escherichia coli)*. The extracts were incubated with $AgNO_3$ solution showed gradual change in colour to yellowish brown, with intensity increasing during the period of incubation. The nanoparticles were characterized for particle size, zeta potential and Transmission Electron Microscope (TEM). The silver nanoparticles prepared were found to be spherical in shape with nano size and stable. The biosynthesized silver nanoparticles (AgNPs) showed good antibacterial activity against clinical strains of two bacteria. The antiangiogenic activity of extract was screened by chick chorioallantoic membrane assay (CAM) (*in-ovo* method). In CAM assay, number of branches and angiogenic-score were evaluated and significant results were observed at 10^{-4}M. Green synthesis of silver nanoparticles using the aqueous leaf extract of *Aerva lanata* and *Momordica charantia* were screened for antimicrobial activity and showed good antibacterial activity against these two clinically active strains. The nano extracts also showed significant antiangiogenic property in the CAM assay.

KEYWORDS: *Aerva lanata, Momordica charantia,* silver nanoparticles, antimicrobial activity, antiangiogenic activity.

DOI Number: 10.14704/nq.2022.20.9.NQ44441 **Neuro Quantology 2022; 20(9):3838-3845**

I. INTRODUCTION

Nanotechnology is the study based on atomic, molecular and macromolecular scale that helps in controlled manipulation and understanding of structure in nanometric size range (1–100 nm) (Velusamy, et al, 2016; Li, et al, 2011; Wang, et al, 2012; Yamasaki, et al, 2013). Metal nanoparticles (MNPs) are engineered nanoparticles that are made up of silver, gold, and copper and find their application as electronic and catalytic fields. The unique optical properties make them highly useful in fields of particularly sensing, bio-conjugation, and surface enhancement Raman spectroscopy. Recently, various synthetic methods were evolved for the preparation of MNPs through chemical, photochemical, and thermal methods (Zare, et al, 2013; Mohammadi, et al, 2019). Among the MNPs, silver nanoparticles play a significant role in the field of biology and medicine. Also, silver nanoparticles are of great interest these days because of their surface Plasmon resonance, (Hu, et al,2005) optical properties, (Kim, et al, 2007) catalytic action, (Lu, et al, 2007) excellent antimicrobial activity, (Qasim Nasar, et al, 2019) *etc*. In the past few years, researchers have demonstrated a significant antimicrobial effect of silver nanoparticles to fight against infections and diseases (Soleimani, et al, 2017; Vivek, et al, 2011; Vanaja, et al, 2013; Kim, et al, 2007; Shrivastava, et al, 2007).

The traditional medicinal plants with antimicrobial properties are used in treating various infections while few of them are used in market as raw products (Bajpai, et al, 2007; Guari, et al, 2003). *Momordica charantia* (Family *Cucurbitaceae*) and *Aerva lanata (Amaranthaceae)* are used in many parts of Asia, amid its use for skin infections. Tea of these plants are in use for diabetes, to force out intestinal gases, in menstruation, and like antiviral for the treatment of measles and hepatitis (Mayya, et al, 2003; Ohno, et al, 2003).

So, in this study, an effort has been shown to screen the antimicrobial and antiangiogenic actions of extracts of the selected medicinal plants.

II. MATERIALS AND METHODS

Materials

Chemicals*:* Silver nitrate was purchased from Merck and used as received. Distilled deionized water was used throughout the reactions. Leaves of *Aerva lanata* and *Momordica charantia* were collected from Siddipet district of Telangana state. *Aerva lanata (Amaranthaceae)* was authenticated by Dr. K. Madhav Shetty at Osmania University (Botany department); (Vocher No. 288) *Momordica charantia* (Family *Cucurbitaceae*) was authenticated by Dr. Baba Shankar, Department of pharmacognosy, School of pharmacy, Anurag group of institutions. Specimen access No: AG/LCP/MC-155.

Bacterial strains: The assessment ofantibacterial activity was carried out using two different strains. The following microorganisms were used: Standard strains of *Bacillus Subtilis* (ATCC 25923), and *Escherichia coli* (ATCC 9763).

Methods

Preparation of plant aqueous extracts

Aqueous extracts were prepared by mixing 5 g of dried fresh leaves powder of plants with 100 mL of water with constant stirring on a magnetic stirrer. The mixture was boiled for 15 min followed by decantation, contents cooled and filtered through what man No. 1 filter paper. The boiled extract stored in refrigerator for further experimental procedures (Zhao, et al, 2004).

Synthesis of silver nanoparticles

Silver nitrate solution (1 mM) was prepared by adding $AgNO_3$ in Millipore water. The mixture of silver nitrate and plant extracts were subjected to agitation in incubator and the nanoparticles formed were subjected to characterization. The effect of the plant extracts was determined by using various concentration of plant extract mixed with constant $AgNO_3$ solution. To study the effect of temperature on nanoparticle synthesis, reaction mixtures containing plant extract, and 1.0 mM $AgNO_3$ were incubated at 30, 50, and 70 °C (Tan, et al, 2005; Ahmed, et al, 2016; Nasrollahzadeh, et al, 2019; Priyadarshini, et al, 2018).

Characterization of silver nanoparticles

The samples evaluated spectrophotometrically between 300–700 nm in Perkin Elmer precisely Lambda 25 spectrophotometer. The solution mixture was subjected to centrifugation at 12,000 rpm for 20 min; resulting pellet was dissolved in deionized water and filtered through 0.22 μm Millipore filter (Kamili, et al, 2019).

UV-Vis spectrophotometer analysis

The analysis carried out using a quartz cuvette with control as reference. The reduction of silver ions was monitored spectrophotometrically. It is reported that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420 nm and 450 nm (Date, et al, 2007).

Droplet size

The droplet size of silver nanoparticles formulations evaluated by Photon correlation spectroscopy (Malvern Instrument UK) measures the size range of 10 to 5000 nm. Measurements are done in triplicate (Gang, et al, 2003).

Zeta potential

The zeta potential of the dilute silver nanoparticles formulation (1:2500 v/v) homogenized evaluated with zeta meter system measured with zetasizer. (Malvern, Nano Series ZS90, Malvern Instruments, Ltd., UK). The studies were performed in triplicate at 25 °C (Shaikh, et al, 2019).

TEM analysis

The transmission electron microscopic (TEM) study a few drops of silver nanoparticle solution were dropped onto a TEM grid, and the residue was removed by a filter paper beneath the TEM grid.

Antimicrobial activity

Synthesized silver nanoparticles in this research were tested for antimicrobial activity using agar gel diffusion method against pathogenic bacteria, *E. coli*, and *S. aureus*. 20 mL of molten and nutrient agar media placed on sterilised petri dishes and left overnight at room temperature to allow any contamination to appear. A 100 mL nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Silver colloidal solution (40 µL) was applied on a thick paper disk of 0.5 cm diameter, dried and then applied on the surface of the media plate. The plates containing the bacterial and extract AgNPs were incubated at 37 °C for 24 h, and then examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a metre ruler, and the mean value for each organism was recorded and expressed in millimetres (Kamili, et al, 2020; Nasseri, et al, 2019; Mandal Pinaki, 2017).

Chick Chorioallantoic Membrane (CAM) Assay

It is one of the best *in ovo* assay designed intended for recognition and quantification of antiangiogenic drugs. Eggs were obtained from hatchery on 0 day. They were examined for damages. Eggs were then randomly labelled as control, Bevacizumab and three test concentrations of extracts, each containing six eggs. They were sanitized using ethanol, incubated at constant humidity and temperature. On third day, a window was made on the slim end and 2 ml of albumin was drawn out. Opening was sealed and incubated. It was opened again on seventh day and a sponge of 3mm length, 3mm width and 1mm thickness piece was kept on CAM. Saline was put in the control group; drug doses were respectively given to test and standard groups. Then the eggs were placed back for incubation till day fourteen. Same day, CAM under the sponge were dissected from all the groups. These membranes were kept in 10% formalin, hematoxylin-eosin was added, and observed in microscope. The new branches from the vessels were counted under the square area. The angiogenesis index was represented as mean ± SEM. 'Angiogenesis score 1-4 was given. For branching points ≥ thirty-five, score is four. For branches between twenty-five-thirty-four, score is three and if it is fifteen-twenty four, score is two. For branches <fifteen, score is one. The test doses of nano extracts (10^{-6}M, 10^{-5}M, 10^{-4} M) were chosen based on the pilot studies. Pilot study showed the concentration of 10^{-5} M as sub maximal efficacy of the nano extracts. The molarity formula M=m/V was used to calculate the drug amount to provide 10^{-4}M concentration. Firstly, concentration of 10^{-4}M was prepared, and then the other concentrations were prepared from the earlier by serial dilutions (Aminu, et al, 2014).

IV. RESULTS

Silver nanoparticles were synthesized using leaves of the two plants. Surprisingly, silver nanoparticles were synthesized simply within various incubation periods. In our research, the aqueous silver nitrate solution was turned to yellowish brown colour within appropriate time, with the addition of plant extract (for example colour changes *Aerva lanata* AgNps (AL- AgNps) and *Momordica charantia* AgNps (MC-AgNps) at various times.

UV-Vis analysis

Silver nanoparticle formations from silver ions show an absorption peak around 420 nm.(Table 1)

Table 1. Absorbance max of AL-AgNps and MC-AgNps at different time intervals

Plant extract	Absorbance at λ_{max}					
	After 2 h	After 6 h	After 12 h	After 24 h	After 48 h	After 60 h
AL-AgNps	0.177	0.356	0.466	0.666	1.234	1.156
MC-AgNps	0.018	0.133	0.234	0.277	0.355	0.567

Particle size analysis of silver nanoparticles

The particle size of the silver nanoparticles was found to be 36.9 nm & Z-Average of 26.8 nm indicating all the particles were in the nanometer range. (Figure 1).

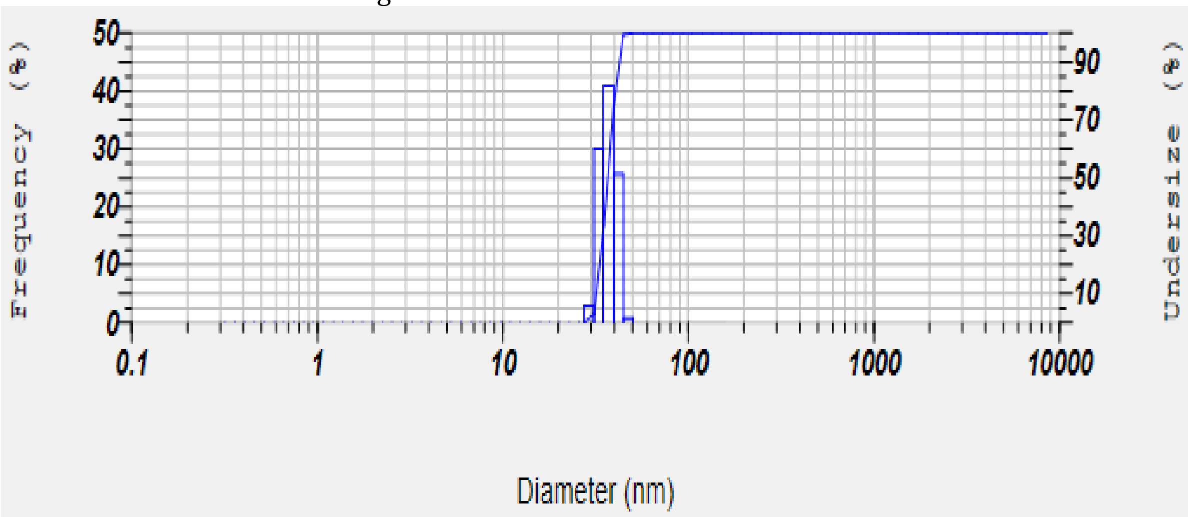


Fig-1: Particle size analysis of silver nanoparticles

Zeta potential is responsible for the degree of repulsion between adjacent, similarly charged, dispersed droplets. A zeta potential value of ±30 mV is sufficient for the stability of the silver nanoparticles. The zeta potential of the silver nanoparticles was found to be −26.8 mV which comply with the requirement of the zeta potential for stability. (Figure 2)

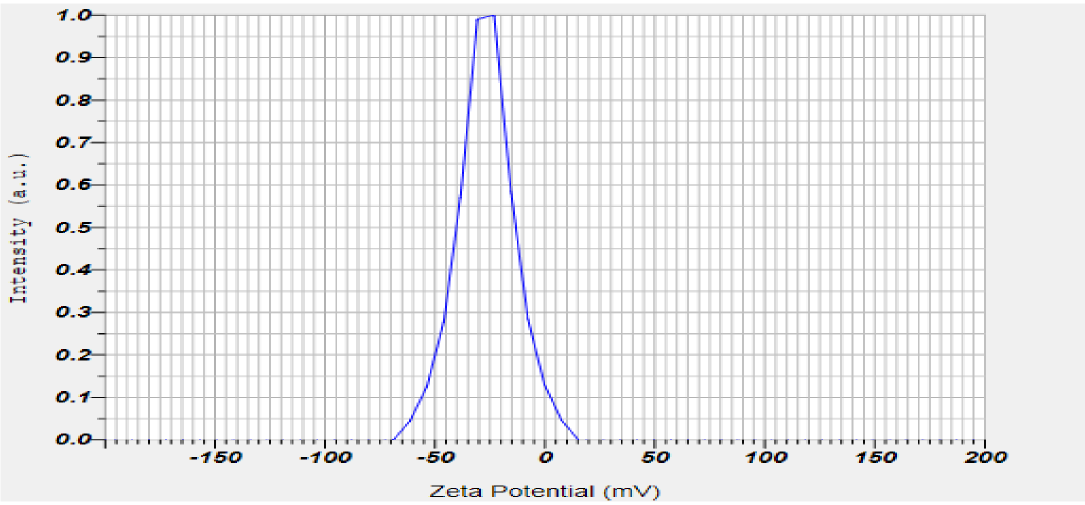


Fig-2: Zeta potential of the silver nanoparticles

TEM studies

Nanoparticles observed from the micrograph majority are spherical with a small percentage of elongated particles and ranged in size of 5–30 nm with an average size of 20 nm in Figure 3.

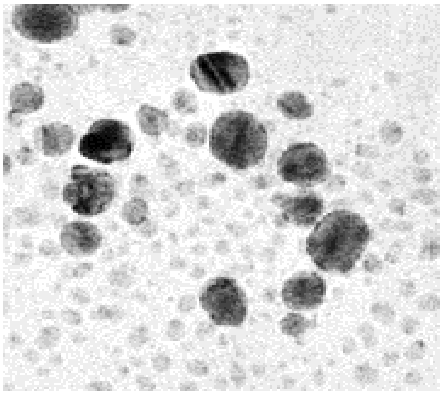


Fig-3: TEM image of biosynthesised AgNps

Antimicrobial activity of aqueous extracts

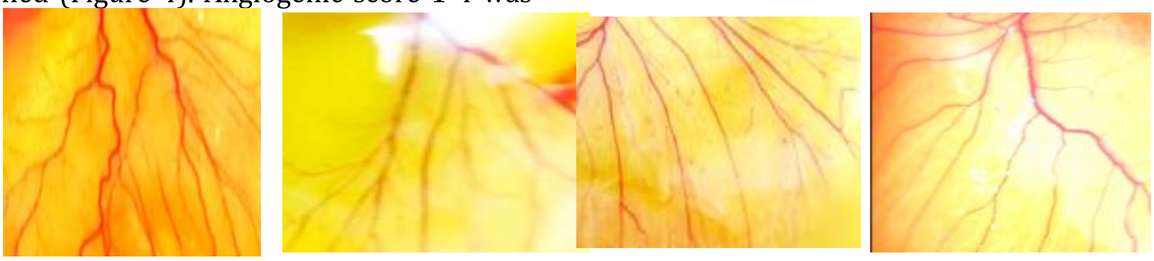
The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria. The aqueous extracts exhibited better activity compared to ethyl acetate extracts. The maximum ZI was shown by aqueous extract against E.coli. Increasing dose response was observed with the aqueous extract of both *Momordicacharantia* and *Aervalanata*. Both extracts showed similar activity with aqueous extract. The higher dose showed greater ZI against both *E.coli and Bacillus subtilis.* The effective antimicrobial doses for aqueous extracts of *Momordica charantia* and *Aerva lanata* are 100 &200µg/ml. (Table.2)

Table 2. ZI of test aqueous extracts and standard drug

S.no	Micro organism	ZI of chloramphenicol (10µg/ml)	ZI of AL-AgNps		ZI of MC-AgNps	
			100µg/ml	200µg/ml	100µg/ml	200µg/ml
1.	*E.coli*	9.0 mm	8.90 mm	9.0 mm	8.60 mm	9.50 mm
2.	*Bacillus subtilis*	8.50 mm	8.6 mm	9.50 mm	8.2 mm	8.50 mm

Chick Chorioallantoic Membrane (CAM) Assay

On the 14th day of the CAM method, membrane under the sponge was dissected. The new vessels formed in the unit square area were quantified (Figure 4). Angiogenic score 1-4 was specified. The results of three doses of SP, the standard antiangiogenic drug Bevacizumab and VEGF were statistically compared with the control results. Significant results were observed with the highest test dose selected at 10^{-4}M.



CONTROL

BEVACIZUMAB (1µM)

AL-AgNps (10-4M)

MC-AgNps (10-4M)

Fig-4: Photographic images showing the antiangiogenic action of various groups in the CAM assay

V. DISCUSSION

Plants extracts can be efficiently used in the synthesis of silver nanoparticles as a green method. In UV-Vis spectroscopy colour change was due to the excitation of surface plasmon resonance (SPR) effect and reduction of AgNO3[25-28]. The silver surface plasmon resonance was recorded at 420 nm whose frequency eventually increased with time of reaction (ranging from 1 h to 60 h) without showing any shift of the wavelength maximum. Initially , 0.5 mL of each plant extracts was mixed with 20 mL silver nitrate and incubated for several time intervals at 30 °C for testing silver intervals by UV–Vis spectroscopy[29]. The shape and size of the obtained silver nanoparticles were elucidated with the help of TEM images. In the CAM assay, number of branching points and angiogenic score were evaluated and significant results were observed with both the extracts.

The anti-microbial and antiangiogenic activity of *Aerva lanata* and *Momordica charantia* are well studied in since ancient times. This study scientifically proves the better antiangiogenic and antimicrobial effect of silver nanoparticles over the extracts alone. Green synthesis of silver nanoparticles using the aqueous leaf extract of *Aerva lanata* and *Momordica charantia* were screened for antimicrobial activity using the cup plate method against gram positive and gram negative reference organisms (*Bacillus Substilis* and *Escherichia coli)*, and showed good antibacterial activity against these two clinically active strains. The nano extracts also showed significant antiangiogenic property in the CAM assay[30-33].

VI. CONCLUSION

In the present investigation an effort has been made for green synthesis of silver nanoparticles using the aqueous leaf extract of *Aerva lanata* and *Momordica charantia* were screened for antimicrobial activity by chick chorioallantoic membrane assay (CAM) (*in-ovo* method). The silver nanoparticles prepared were found to be spherical in shape with nano size and stable. The biosynthesized silver nanoparticles (AgNPs) showed good antibacterial activity against clinical strains of two bacteria. The antiangiogenic activity of extract was screened by chick chorioallantoic membrane assay (CAM) (*in-ovo* method). In CAM assay, number of branches and angiogenic-score were evaluated and significant results were observed.

ACKNOWLWDGEMENT

Authors are thankful to CMR College of Pharmacy management for providing facilities to carry out the research work.

CONFLICT OF INTREST

No conflict of interest

AUTHORS COUNTRIBUTION

All the authors contributed equally towards the article

REFERENCES

1. Velusamy P, Kumar GV, Jeyanthi V, Das J, Pachaiappan R. Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application. Toxicol Res 2016;32 (2): 95-102.
2. Li X, Xu H, Chen ZS, Chen G. Biosynthesis of nanoparticles by microorganisms and their applications. J Nano 2011;21(1):1-12.
3. Wang S, Chen T, Chen R, Hu Y, Chen M, Wang Y. Emodin loaded solid lipid nanoparticles: preparation, characterization and antitumor activity studies. Int J pharm 2012;430 (1-2): 238-46.
4. Yamasaki S, Yamada T, Kobayashi H, Kitagawa H. Preparation of Sub-10 nm AgI Nanoparticles and a Study on their Phase Transition Temperature. Chemistry–An Asi J 2013;8(1): 73-5.
5. Zare B, Babaie S, Setayesh N, Shahverdi AR. Isolation and characterization of a fungus for extracellular synthesis of small selenium nanoparticles. Nanomed J 2013;1(1): 13-9.
6. Mohammadi F, Yousefi M, Ghahremanzadeh R. Green synthesis, characterization and antimicrobial activity of silver nanoparticles (AgNPs) using leaves and stems extract of some plants. Adv J of Chem-Section A

(Theoretical, Engineering and Applied Chemistry) 2019;2(4):266-385:266-75.

7. Hu J, Cai W, Li Y, Zeng H. Oxygen-induced enhancement of surface plasmon resonance of silver nanoparticles for silver-coated soda-lime glass. J Phys Condens Matter 2005;17(35): 5349.

8. Kim MJ, McNally B, Murata K, Meller A. Characteristics of solid-state nanometre pores fabricated using a transmission electron microscope. Nanotech 2007;18(20): 205302.

9. Lu Y, Spyra P, Mei Y, Ballauff M, Pich A. Composite hydrogels: robust carriers for catalytic nanoparticles. Macromol Chem Phys 2007;208(3): 254-61.

10. Qasim Nasar M, Zohra T, Khalil AT, Saqib S, Ayaz M, Ahmad A, Shinwari ZK. Seripheidium quettense mediated green synthesis of biogenic silver nanoparticles and their theranostic applications. Green Chem Lett Rev 2019;12(3): 310-22.

11. Soleimani M, Habibi-Pirkoohi M. Biosynthesis of silver nanoparticles using Chlorella vulgaris and evaluation of the antibacterial efficacy against Staphylococcus aureus. Avicenna J Med Biotechnol 2017;9(3): 120.

12. Vivek M, Kumar PS, Steffi S, Sudha S. Biogenic silver nanoparticles by Gelidiella acerosa extract and their antifungal effects. Avicenna J Med Biotechnol 2011;3(3): 143.

13. Vanaja M, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Malarkodi C, Annadurai G. Kinetic study on green synthesis of silver nanoparticles using Coleus aromaticus leaf extract. Adv Appl Sci Res 2013;4(3): 50-5.

14. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK. Antimicrobial effects of silver nanoparticles. Nanomed: Nanotechnology. Bio and Med 2007;3(1): 95-101.

15. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotech 2007;18(22): 225103.

16. Bajpai SK, Mohan YM, Bajpai M, Tankhiwale R, Thomas V. Synthesis of polymer stabilized silver and gold nanostructures. J Nanosci 2007;7(9): 2994-3010.

17. Guari Y, Thieuleux C, Mehdi A, Reyé C, Corriu RJ, Gomez-Gallardo S, Philippot K, Chaudret B. In situ formation of gold nanoparticles within thiol functionalized HMS-C16 and SBA-15 type materials via an organometallic two-step approach. Chem of materials 2003;15(10): 2017-24.

18. Mayya KS, Schoeler B, Caruso F. Preparation and organization of nanoscale polyelectrolyte-coated gold nanoparticles. Adv Funct Mater 2003;13(3): 183-8.

19. Ohno K, Koh K, Tsujii Y, Fukuda T. Fabrication of Ordered Arrays of Gold Nanoparticles Coated with High-Density Polymer Brushes. Angewandte Chemie Int Edn 2003;42(24): 2751-4.

20. Zhao XG, Shi JL, Hu B, Zhang LX, Hua ZL. In situ formation of silver nanoparticles inside pore channels of ordered mesoporous silica. Materials Letters. 2004;58(16): 2152-6.

21. Tan WB, Zhang Y. Surface modification of gold and quantum dot nanoparticles with chitosan for bioapplications. J Biomater Res 2005;75(1): 56-62.

22. Tanori J, Pileni MP. Control of the shape of copper metallic particles by using a colloidal system as template. Langmuir 1997;13(4): 639-46.

23. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat Res Appl SC 2016;9(1): 1-7.

24. Nasrollahzadeh M, Sajadi SM, Issaabadi Z, Sajjadi M. Biological sources used in green nanotechnology. In Interface Sci Technol 2019;28: 81-111.

25. Priyadarshini JF, Sivakumari K, Selvaraj R, Ashok K, Jayaprakash P, Rajesh S. Green synthesis of silver nanoparticles from propolis. Res J Life Sci Bioinform Pharm Chem Sci 2018;4: 23-36.

26. Kamili C, Kakaraparthy RS, Vattikuti UM. Anti-Angiogenic Activity of Flunarizine by In Ovo, In Vitro, and In Vivo Assays. Turk J Pharm Sci 2019;16(3): 303.

27. Date AA, Nagarsenker MS. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. Int J Pharm 2007;329(1-2): 166-72.
28. Gang R, Si-Shen F. Preparation and characterization of poly (lactic acid)-poly (ethylene glycol) microspheres for controlled release of microspheres. Biomate 2003;24: 5037-44.
29. Shaikh R, Zainuddin Syed I, Bhende P. Green synthesis of silver nanoparticles using root extracts of Cassia toral L. and its antimicrobial activities. Asian J Green Chem 2019;3(1): 70-81.
30. Kamili C, Kandoti HS, Radhakrishnan S, Konde A, Vattikutti UM. Anti-angiogenic activity of chloride and potassium channel modulators: repurposing ion channel modulators. FJPS 2020;6(1): 1-8.
31. Nasseri MA, Shahabi M, Allahresani A, Kazemnejadi M. Eco-friendly biosynthesis of silver nanoparticles using aqueous solution of Spartium junceum flower extract. Asian J Green Chem 2019;3(3): 382-90.
32. Pinaki Mandal. Green Synthesis and Characterization of Polyvinyl Alcohol Embedded Silver Nanoparticles Using Simul (Bombax ceiba) Flower Extract. Int J Cur Res Rev 2017; 9(14): 1-6.
33. Aminu A , Ibrahim B , Shiwani C , Geetika S , Ramesh N. Biosynthesis and characterization of silver nano particles using black carrot root extract. Int J Cur Res Rev 2014;6(17):5-8.