World Applied Sciences Journal 22 (6): 836-843, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.22.06.7394

Optimization and Effects of Different Reaction Conditions for the Bioinspired Synthesis of Silver Nanoparticles Using *Hippophae rhamnoides* Linn. Leaves Aqueous Extract

[1]Bashir Ahmad, [1,2]Javid Ali and [3]Shumaila Bashir

[1]Center of Biotechnology and Microbiology, University of Peshawar, KPK Pakistan
[2]PCSIR Laboratories Complex Jamrud Road Peshawar, KPK Pakistan
[3]Department of Pharmacy, University of Peshawar, KPK Pakistan

Abstract: The use of plant extracts for the synthesis of nanoparticles plays a significant role in nanotechnology field as it is simple, fast, environment friendly and no use of toxic chemicals. The current research work investigate the findings of optimization of different experimental variables conditions like reaction pH, silver nitrate concentrations, time, temperature and mixing ratio of the reactants on silver nanoparticles synthesized using aqueous extract of *Hippophae rhamnoides* L. (Seabuckthorn) leaves. The formation of yellowish brown color was confirmed the synthesis of silver nanoparticles. The optimized condition for the bioinspired synthesis of silver nanoparticles revealed that silver nitrate concentration was 1mM, temperature was 75°C, pH was 7, incubation time was 1 h and aqueous extract and silver nitrate ratio was 5:95. It can be observed from the finding that good modification of the bioprocess parameters will improve potential of desired nano-product for particular applications.

Key words: Silver Nanoparticles Preparation · Temperature · pH · Time · Concentration of $AgNO_3$ · Reactant Ratio

INTRODUCTION

The word "nano," is derive as of the Greek "nanos" which means of "dwarf" and nearly all of the thrilling properties of 'nano' commence to be obvious in systems lesser than 1000 nm, or 1 micrometer, 1 µm. Nanotechnology can be defined as the design, production and application of materials and devices whose size and shape have been drawing at the nanoscale [1].

The biogenic synthesis for nanoparticle production is easy, trouble-free, environment friendly, low cost and employed to get optimized parameters for controlled synthesis of nanoparticles which can be applied with particular ingredients as catalysts, while traditional methods cannot exhibited these properties [2]. Biological fabrication approach gives superiority over physical and chemical process because it is environment friendly, easily optimized for large-scale production, no need of hazardous chemicals, heat, power, high pressure and further it is cost effective. Utilization of plants for the synthesis of nanoparticle can be preferred over further green (biological) techniques due to easily optimized for the pilot scale production, no involvement of cell cultures maintenance and no requirement of aseptic environment [3].

Significant interest has arisen in the research of NPs during the last decade, in particular for biomedical applications. The integration of nanotechnology into the field of medical science has opened new possibilities. Working with nanomaterials has allowed a better understanding of molecular biology. As a consequence, there is the potential of providing novel methods for the treatment of diseases which were previously difficult to target due to size restrictions. For biomedical applications, the synthesis of biofunctional NPs is very important and it has recently drawn attention of numerous research groups, making this area constantly evolve [4].

Due to attractive physiochemical properties of silver nanoparticles, it has a great potential in the field of medicine and biology. Silver based products have been recognized as potent antibacterial and antifungal activities, which has been used from prehistory times

Corresponding Author: Javid Ali, Center of Biotechnology and Microbiology, University of Peshawar, KPK Pakistan, PCSIR Laboratories Complex Jamrud Road Peshawar, KPK Pakistan.

against for the treatment and prevention of different disease especially infections [5].

In this study, we account for the bioinspired synthesis of silver nanoparticles by aqueous leaves extract of *Hippophae rhamnoides* Linn. (Seabuckthorn). Furthermore these bioinspired fabricated nanoparticles were optimized by using different factors like temperature, pH, time, $AgNO_3$ concentration, leaf extract and silver nitrate concentration ratio.

MATERIALS AND METHODS

Plants Leaves Procurement: The fully matured healthy leaves of seabuckthorn were collected from PCSIR Skardu Gilgit Baltistan, Pakistan. The leaves were slightly washed, dried and powdered with a laboratory mill.

Plants Leaves Extract: Twenty five (25 g) of the leaves powdered of seabuckthorn were weighed and kept into 1000-ml conical flask containing 500 ml double distilled water, well mixed and then boiled for 25 min. The extract obtained was filtered through muslin clothe and then filtered through filter paper (Whatman No.1) and the filtrates were received in Erlenmeyer flask (500 ml) and kept at low temperature to use further.

Bioinspired Fabrication of Silver Nanoparticles: Silver nitrate aqueous solution (1 mM) was prepared and used for the bioinspired synthesis of silver nanoparticles. Seabuckthorn leaves extract 5 ml was added into 95 ml of aqueous solution of 1 mM $AgNO_3$ and heated on horizontal shaking water bath at 75°C for 60 min in a dark room. Reduction of $AgNO_3$ to silver ions was confirmed by change the color from colorless to brown. Furthermore confirmation of AgNPs synthesis was carried out by spectrophotometric determination. The fully reduced solution was concentrated on rotary evaporator (R-200, Buchi Rotavapor, Switzerland) on 50°C. The concentrated silver nanoparticles (AgNPs) were dried in an oven overnight at 50°C and grinded in mortar pestels.

UV–Vis Spectra Analysis: The silver ions reduction confirmation was carried out by UV-Visible Spectrophotometer UV-1700 (Shimadzu, Japan) spectrum of the reaction solution after cooling at room temperature were measured by mixing 20 ml distilled water with 1ml sample reduced solution (silver nanoparticles).

Optimization of Different Parameters: To improve the optimization and reproducibility of the trial results of the bioinspired synthesis method as a whole, the factorial Design of Experiments, the ‘‘one factor- at-a time’’ method, was used in this research work. Here, the investigational factors are different one at a time with the left over factors detained constant.

Temperature: The described protocol was repeated for temperature optimization, where the temperature of the reaction was set at 25, 35, 45, 55, 65, 75 and 85°C. The resulting solutions (AgNPs) absorbance was monitored UV-Vis spectrophotometrically.

pH: The above described procedure was repeated for optimization of pH where the interaction pH was adjusted at 4, 5, 6, 7 and 8, respectively. For pH adjustment, solution of NaOH (0.1N) and 0.1 N HCl was used. At the end the absorbance of the resultant mixture solutions (AgNPs) was noted by UV-Vis spectrophotometrically.

Time: The above described protocol was repeated to optimize the time required for the reaction completion, for this purposes different reactions periods (10 min, 20 min, 30 min, 40 min, 50 min, 60 min and 70 min) were choose. At the end the absorbance was observed UV-Vis spectrophotometrically.

Concentration of Silver Nitrate Solution: The above described approach was repeated for optimization of silver nitrate concentration, where the reaction was monitored using different concentration of silver nitrate (0.25, 0.5, 0.75, 1 and 2 mM). At the end the absorbance of the resultant mixture solutions was calculated by UV-Vis spectrophotometrically.

Leaf Extract Concentration and Silver Nitrate Ratio: The above described method was also repeated for optimization of leaf extract concentration and silver nitrate required for the maximum production of AgNPs, where the reaction was monitored by using different ratio of leaf extract and silver nitrate solution (0.5:99.5, 1:99, 2.5:97.5, 5:95 and 10:90). At the end the absorbance of the resultant mixture solutions was calculated by UV-Vis spectrophotometrically.

RESULTS AND DISCUSSION

Temperature: On addition the aqueous extract of seabuckthorn leaves (Fig. 1) to silver nitrate solution, the reaction medium rapidly changed colour from colorless to brown (Fig. 2). Reaction temperatures play an important

Fig. 1: *Hippophae rhamnoides* L. leaves used in the biosynthesis of AgNPs.

Fig. 2: $AgNO_3$ = 1 mM silver nitrate solution, SBL Extract (aq.) = Seabuckthorn leaves aqueous extract, AgNPs = Silver nanoparticles.

role to control the nucleation process of nanoparticle configuration. Absorbance increased with raise in the temperature from 25 to 75°C and thereafter decreased at higher temperatures. The changeable temperatures effect on AgNPs synthesis by seabuckthorn leaves is presented in Figure 3. Experimental temperature range is from 25°C to 85°C, maximum synthesis was get at 75°C. Temperature is an important aspect affect AgNPs synthesis. Our current record indicates that the most favorable temperature of 75°C is quite precise for AgNPs synthesis by seabuckthorn leaves which observe maximum absorbance at 435 nm.

Increase in absorbance of reaction mixture with the increase in incubation temperature obviously depicts the higher synthesis of AgNPs at high temperatures [6]. Moreover, nanoparticles synthesized at higher temperature exhibit surface plasmon resonance at narrow absorption range indicating monodispersity. Increase in temperature increased the rate of formation of silver nanoparticles from silver ions, retarding the secondary reduction process. The study of Amit, Abhishek and Uttam [7] reported that absorbance increased with increase in the temperature from 25 to 45°C and thereafter decreased at higher temperatures. These observations follow the same pattern as follow in our study. Again our study finding was close agreement of the previous study [3] in which the temperature increased, the rate of silver nanoparticles formation also increased. Because of the reduction in aggregation of the growing nanoparticles the size is reduced initially. Rising the temperature further than 75°C help the crystal formation around the nucleus which cause the absorption reduce. It was observed [8] that maximum formation of AgNPs was observed at 50°C. At low temperature the reaction was sluggish and at 4°C, 20°C and 30°C no major dissimilarity was found. But an increase in temperature was observed with an increase in the reaction rate. The UV-Visible absorption spectra showed [9] that maximum absorption peak was observed at 40°C during the study temperature range (25-45°C). The absorption was decreases at 45°C, our study reported that maximum absorption was showed at 75°C, increase in temperature (85°C) the absorption was decreases (Fig. 3).

Optimization of pH: Effect of pH on the bioinspired synthesis of silver nanoparticles by seabuckthorn leaves was tested over a wider pH range (pH 4 - 8) as shown in Fig. 4. The UV-Visible absorption spectra can be clearly seen that the maximum absorption shows at pH 7.0. Increase in pH observes decrease in absorption. So in the current study the optimize pH observe was 7. It was observed [7] that pH play a significant role during silver nanoparticles synthesis to control shape and size. Larger size nanoparticles were synthesized at low pH (acidic), whereas, at higher pH (alkaline), smaller size NPs synthesis was observed. The investigation carried out by Sathishkumar, Sneha and Yun [10] to study the effects of pH on the shape and size of AgNPs and found that nanoparticle aggregation seems to outdo the nucleation process in acidic conditions. At pH (alkaline), though, the great numbers of nuclei formation, instead of aggregation, led to the synthesis of more of NPs with smaller diameter. Blue shift in absorption pattern confirmed formation of relatively smaller NPs. The study of Ravichandran et *al*. [3] described that low pH (acidic) state decreases the synthesis of AgNPs, while high pH (basic) state increases the synthesis AgNPs. At lower pH (pH4) large nanoparticles were synthesized, where as at high pH (pH 8) highly dispersed and small nanoparticles were synthesized. At acidic pH (low), the aggregation of AgNPs to prepared bigger nanoparticles was supposed to be preferred over the nucleation. At alkaline pH (higher), though, the great number of functional groups accessible for silver binding to create a higher number of AgNPs to attach and afterward synthesize an enormous number of smaller diameters of nanoparticles. But agglomeration of nanoparticles took place at alkaline pH. The optimum conditions (pH and temperature) [11] were found to be 7.0 and 80°C respectively. In our study the same pH effect is found.

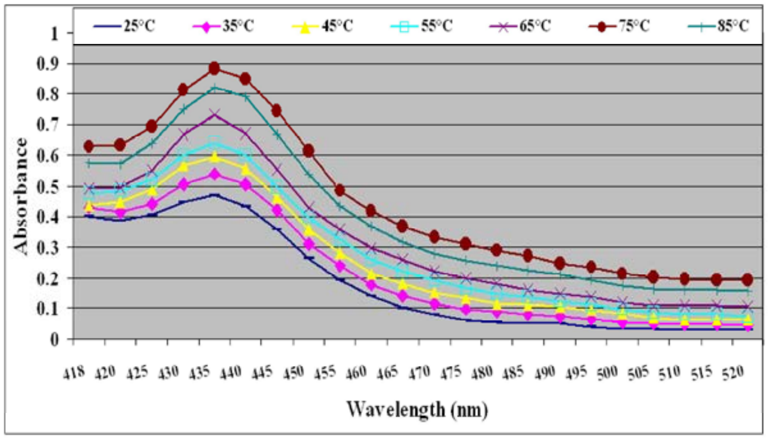

Fig. 3: UV-Visible spectra of AgNPs showing effect of different reaction temperature

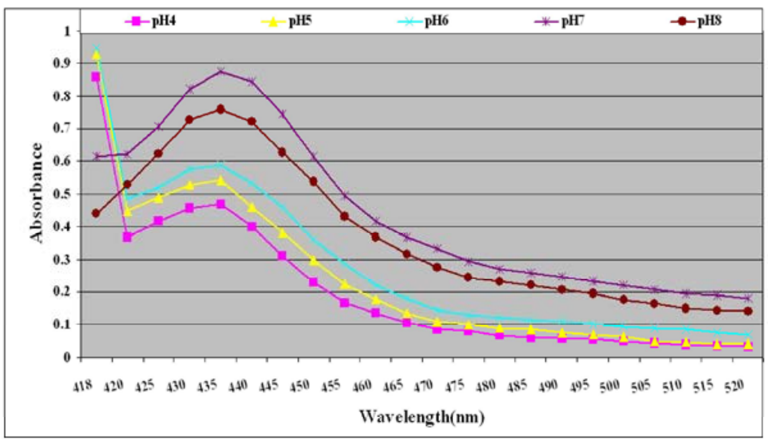

Fig. 4: UV-Visible spectra of AgNPs showing effect of different reaction pH

It was pointed out [12] that at low pH (2 and 5) nanoparticles synthesis doesn't take place. But at high pH (9 and 11) rapid colour appearance was observed but shifted peak to 500 nm. At pH 13 agglomerations was seen instantly following addition of $AgNO_3$ into the leaf extract. The reaction was happening as soon as the $AgNO_3$ was added into the leaf extract at pH 7 (neutral) and the synthesis was found in 30 min duration. It was observed [13] that at 0.5 h of interaction time of fruit extract with salt solution, red shift in the SPR observed in acidic pH in the colloidal solution of AgNPs. This means that the large size particles were formed in acidic pH. In alkaline medium blue shift in SPR clearly indicated the formation of small particles in alkaline pH. At pH 2, there was no indication of AgNPs formation in UV–Vis spectra while flocculation observed in reaction medium. This was due to the fact that higher positive charges at the surface of nanoparticles attracted negative charge biomass, which lead to flocculation. A great fall in the flocculation factor in high pH (alkaline) was also observed. Thus, change in SPR was a function of solution pH. In alkaline pH, due to the presence of high concentration of hydroxyl ion on the surface of nanoparticles, repulsive force dominated in colloidal solution and thus, particles aggregation reduced. This leads to the size reduction and blue shift in SPR spectra. The finding of Afreen and Vandana [9] shows that a maximum absorption sharp peak was show at pH 7.0, these findings are a close agreement to the present study.

Time: Absorbance of AgNPs was amplified with the incubation duration raises and maximum absorbance was investigated at 1 h of reaction time (Fig. 5). Increases incubation time observe no significant increase in absorption. The study of Amit, Abhishek and Uttam [7] found that absorbance of silver colloidal solution increased with span of time and maximum absorption was observed after 12 hrs of reaction. Aggregation of NPs after 36 hrs of reaction was observed, a sign of instability.

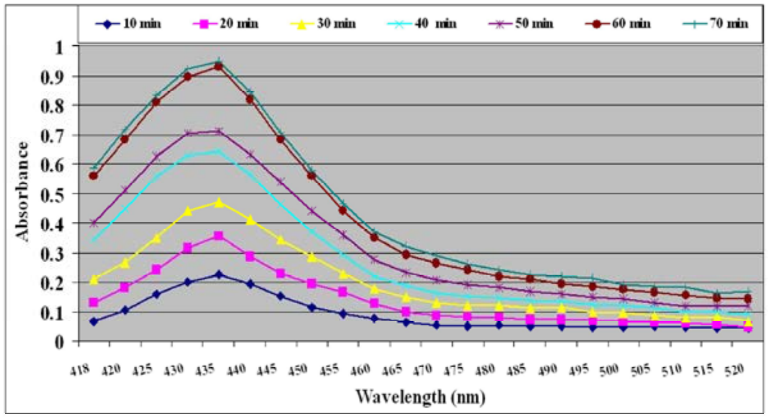

Fig. 5: UV-Visible spectra of AgNPs showing effect of reaction time

Reaction time of more than 10 hrs was due to less redox potential of silver ions. These findings are in agreement with observations made by Singh *et al*. [14]. The finding of Ravichandran *et al*. [3] observed that more silver nanoparticles are formed as the period of reaction increases. An optimum period is required due to the unsteadiness of the AgNPs synthesis, as AgNPs agglomeration after the optimum incubation resulting in larger sizes of particles. The optimum duration necessary for the end of reaction was 60 min. In the present study the same duration condition is observe (Fig. 5). It was found that at 0 and 30 minutes no formation of NPs [8] but formation rate start quickly at 60 minutes and was ended in 5 hours. It was observed [12] that the reaction was happening as rapidly as the $AgNO_3$ was mixed with leaves extract and the synthesis was achieved within 30 min of duration. After 30 min increasing duration of the reaction absorption also increase but silver nanoparticles take place at 30 min. The formation of AgNPs started [13] within 0.5 h incubation and reaction mixture color was changed from translucent to orange-yellow because of the SPR vibration excitation. The intensity of SPR and absorbance increases as the reaction time increased up to 8 hrs. There was no change in SPR means that the stability of the AgNPs colloidal solution within the reaction time period. SPR peak centered at 435 nm (λmax), which is a close agreement to our finding. Silver nanoparticles were fabricated quickly within 30 min of incubation time [15], with the addition of leaf extract the $AgNO_3$ aqueous solution was changed to brown color in 30 min. The incubation time increases the intensity of brown color was also increases, it may be due to the reduction of $AgNO_3$ and surface plasmon resonance excitation effect. The study of Afreen and Vandana [9] was found that the absorption peaks were increase at the incubation time increases (24 hrs - 120 hrs). Silver nanoparticles were prepared from 0.5% gum at 1 mM $AgNO_3$ [16] with varying the reaction time (10–60 min). It was noticed from the UV peaks that absorbance increases when the time increase. Perhaps more and more OH groups are being transformed to carbonyl groups through air oxidation, as a results silver ions reduction take place. These observations were a close agreement to our findings.

The SPR band increases [17] as the time increases (0, 3, 6, 9 and 12 hrs) obviously confirmed that the synthesis of silver nanoparticles was accomplished in 9 hrs. The UV absorbance was not vary considerably with additional reaction time for 12 hrs; so, optimize time condition was 9 hrs for silver nanoparticles synthesis using water extract 5% (v/v). These findings support our results, as in our investigation the optimize time was 60 min, but increase in absorption and then no significant increase in absorption phenomena were the same. The UV absorbance spectra [18] from the AgNPs observed from small exposure incubation time experiment. With rising incubation duration from 30 min to 4 h 15 min, a red move in λmax from 420 to 444 nm was indicated. Further interaction time increasing up to 24 h, the value of λmax did not change drastically, although values of absorbance were increased. The maximum absorbance 0.77 was noted at 24 hrs.

Concentration of Silver Nitrate Solution: Effect of initial $AgNO_3$ concentration was studied by varying the concentration of $AgNO_3$ from 0.25 - 2 mM. Increased in yield of concentration was observed when metal salts concentration was increased from 0.25-1 mM. Beyond this there was again fall in absorbance (Fig. 6). The study of Amit, Abhishek and Uttam [7] found that increase in yield of AgNPs was observed when metal salt concentration was increased from 0.5 - 4 mM. Beyond this there was

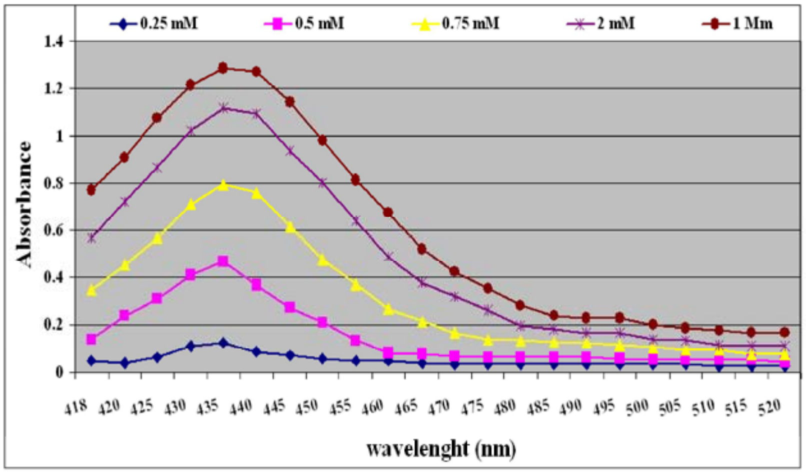

Fig. 6: UV-Visible spectra of AgNPs showing effect of concentration of $AgNO_3$

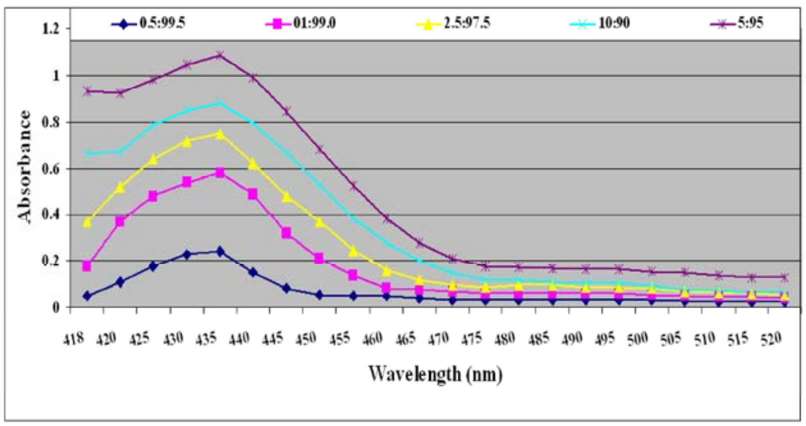

Fig. 7: UV-Visible spectra of AgNPs showing effect of leaves extract and $AgNO_3$

again fall in absorbance; hence 4 mM concentration of AgNO3 was selected for further experiments. Similar effect of varying concentration of plant extract and silver salt on yield, size and disparity of silver nanoparticles was investigated [19]. But in the present study 1 mM concentration was the optimum condition for AgNPs as shown in Figure 6. The study of Srivastava *et al*. [20] has been found that the maximum peak was observed in 0.5 mM silver nitrate. In another study [3] a maximum yield of AgNPs was found with silver nitrate (1 mM) solution, support our results data (Fig.6). The rate of plant extracts based synthesis of AgNPs was reported [8] that the slowest rate of synthesis were found with $AgNO_3$ (0.3 mM), but low rate of bioreduction was observed with the higher concentrations and the 0.7 mM was the optimum concentration for the maximum synthesis (very close to our findings). Study showed [12] that 1 mM $AgNO_3$ observed fast synthesis while at 2 mM and 3 mM concentrations of $AgNO_3$ shifted of peak take place.

Concentration Ratio of Silver Nitrate and Leaves Extract: Optimization of concentration ratio of silver nitrate and leaves extract results are shown in Fig. 7. It was observed that ratio of 5:95 found the maximum absorption and at high leaf extract (10 ml) with 90 ml of 1 mM $AgNO_3$, the absorption was decreases. It was reported in literature [21] that optimal extract and metal salt ratio was necessary for the symmetrical nanoparticles preparation. Diluted extract of flower [7] containing 2 ml extract in water (100 ml) i.e. 0.02 dilution was capable to fabricate the highest concentration of silver nanoparticles as showed by maximum absorbance at 420 nm. Sharp peak was observed at 0.02 dilution of flower extract as matched with other peaks. The study of Ravichandran *et al.* [3] was found that the optimum ratio for the reaction is 1:19 based on the number of trials and the optimum yield, these observations was a close agreement to our results. It was concluded Krishnaraj *et al*.[12] that 50 ml reaction mixture containing leaves extract (6 ml) and $AgNO_3$(1 mM) was changed to brown colour in incubation time (30 min), signifying fast synthesis of AgNPs. Increasing the leaves extract shifted the peaks towards 500 nm. It was reported that [19] the resultant color changes were noticed from reddish-yellow to deep red with rising ratio of leaves extract. It was observed that the 1:30 ratio of leaves extract in the mixture of reaction were efficient for the synthesis of silver and gold nanoparticles Synthesis.

CONCLUSION

A significant requirement in the nanotechnology field was the progress of an eco-friendly and reliable approach for the fabrication of metals base nanoparticles. We have reported that use of an economic feasible biological reducing agent and natural seabuckthorn leaves aqueous extract can fabricate AgNPs. This plant base nanochemistry method, free from poisonous and dangerous solvents and waste. The overall optimized reaction condition was: temperature = 75 °C, time= 60 min, concentration of silver nitrate= 1 mM, pH – neutral and the concentration ratio of silver nitrate and seabuckthorn leaves extract = (95:5).

REFERENCES

1. Cristina, B., I.P.B. Ivan and K. Robbie, 2007. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases, 2(4): MR17 - MR172.
2. Popescu, M., A. Velea and A. Lorinczi, 2010. Biogenic Production of Nanoparticles. Digest Journal of Nanomaterials and Biostructures, 5(4): 1035-1040.
3. Ravichandran, V., Z.X. Tiah, G. Subashini, F.W.X. Terence, F.C.Y. Eddy, J. Nelson and A.D. Sokkalingam, 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society, 15: 113-120.
4. Cristina, B.A., L.D. Tung and N.T.K. Thanh, 2010. Synthesis of nanoparticles for biomedical applications. DOI: 10.1039/b920666n. Annual Reports on the Progress of Chemistry Section. A, 106: 553-568.
5. Shankar, S.S., A. Rai, A. Ahmad and M.J. Sastry, 2004. Rapid synthesis of Au, Ag and bimetallic Au shell nanoparticles using Neem. Journal of Colloid and Interface Science, 275: 496-502.
6. Van, H.D.L., W.G. Klemperer and C.F. Zukoski, 2001. Silver nanoparticle formation: Predictions and verification of the aggregative growth model. Langmuir, 17: 3128-3135.
7. Amit, K.M., K. Abhishek and C.B. Uttam, 2012. Free Radical Scavenging and Antioxidant Activity of Silver Nanoparticles Synthesized from Flower Extract of Rhododendron dauricum. Nano Biomedicine Engineering, 4(3): 118-124.
8. Sougata, G., P. Sumersing, A. Mehul, K. Rohini K. Sangeeta, P. Karishma, S.C. Swaranjit, B. Jayesh, D.D. Dilip, J. Amit and A.C. Balu, 2012. Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. International Journal of Nanomedicine, 7: 483-496.
9. Afreen, B. and A. Vandana, 2011. Synthesis and Characterization of silver nanoparticles by Rhizopus stolonier. International Journal of Biomedical and Advance Research, 02(05): 148-158.
10. Sathishkumar, M., K. Sneha and Y.S. Yun, 2010. Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bactericidal activity. Bioresource Technology, 101: 7958-65.
11. Karthik C. and K.V. Radha, 2012. Biosynthesis and characterization of silver nanoparticles using Enterobacter aerogenes: A kinetic approach. Digest Journal of Nanoparticles and Biostructures, 7(3): 1007-1014.
12. Krishnaraj, C., R. Ramachandran, K. Mohan and P.T. Kalaichelvan, 2012. Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 93: 95-99.
13. Preeti, D. and M. Mukhopadhyay. 2013. *In-vitro* free radical scavenging activity of biosynthesized gold and silver nanoparticles using Prunus armeniaca (apricot) fruit extract. Journal of Nanoparticles Research, 15: 1366-1376.
14. Singh, C., V. Sharma, P.K. Naik, V. Khandelwal and H. Singh, 2011. A green biogenic approach for synthesis of gold and silver nanoparticles using Zingiber officinale. Digest journal of Nanoparticles and Biostructures, 6: 535-42.

15. Karthikeyan, A.P., M. Kadarkarai, P. Chellasamy, P. Sekar, H. Jiang-Shiou and N. Marcello, 2012. Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against Anopheles stephensi Liston (Diptera: Culicidae). Parasitology Research, 111: 997-1006.
16. Aruna, J.K., R.B. Sashidhar and J. Arunachalam, 2012. Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (*Anogeissus latifolia*) and its biological activity. Organic and Medicinal Chemistry Letters, 2: 17-26.
17. Im, A.R., H. Lina, K.E. Ray, K. Jinwoong, S.K. Yeong and P. Youmie, 2012. Enhanced Antibacterial Activities of Leonuri Herba Extracts Containing Silver Nanoparticles. Phototherapy Research, 26: 1249-1255.
18. Tripathy, A., M.R. Ashok, N. Chandrasekaran, T.C. Prathna and M. Amitava, 2010. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. Journal of Nanoparticles Research, 12: 237-246.
19. Dwivedi, A.D. and K. Gopal, 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 369: 27-33.
20. Srivastava, A.A., A.P. Kulkarni, P.M. Harpale and R.S. Zunjarrao, 2011. Plant mediated synthesis of silver nanoparticles using a bryophyte: Fissidens minutus and its anti-microbial activity. International Journal of Engineering Science and Technology, 3(12): 8342-8347.
21. Iravani, S., 2011. Green synthesis of metal nanoparticles using plants. Green Chemistry, 13: 2638-2650.