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Green synthesis of silver nanoparticles mediated extract of various *in vitro* plants (*Bacopa monnieri*, *Coleus blumei*, *Cichorium intybus*)

Awatef M. Badrelden[1], Ahmed Ibrahim Abd Elmaksoud[2], Rana E. Abu El-Maaty[3], Amr Hassan[3], Abeer A.B. Mohamed[4] and Dalia Elebeedy[2].

[1] Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City, **Egypt.**
[2] Industrial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City, **Egypt.**
[3] College of Biotechnology, Misr University for Science & Technology (MUST), **Egypt.**
[4] Microbial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City, **Egypt.**

***Correspondence:** awatef.badrelden@gebri.usc.edu.eg **Accepted:** 08 Oct. 2017 **Published online:** 19 Jan. 2018

The present study aimed to experimentally fabricate silver nanoparticles (Ag NPs) using different *in vitro* plants extract such as *Bacopa monnieri*, *Coleus blumei*, and *Cichorium intybus* reduced solution using oxidation – reduction method. The products were characterized using UV- Visible, FTIR Zeta potential, and HR-TEM. The data reveal Ag NPs has absorption peak range from 400 to 450 nm that confirmed the Ag NPs is stable. The morphology of Ag NPs, measured by zeta potential and HR-TEM, were to have an average diameter of 40.3, 5.05, 3.16 nm for *Bacopa monnieri*, *Coleus blumei*, and *Cichorium intybus* respectively. The morphology of Ag NPs is a spherical shape. As a result, we synthesized Ag NPs using the eco-friendly, convenient and rapid method with stable for several weeks that can be utilized in various applications.

Keywords: ***Bacopa monnieri*, *Coleus blumei*, *Cichorium intybus*, Silver nanoparticles**

INTRODUCTION

Nanotechnology means the fabrication, manipulation and controls the material size to be in Nano -scale particularly between 1 and 100 nm (Villaverde, 2010).Nanobiotechnology or bio-nanotechnology is the technology which combined and overlapped the technological and application of both nanotechnology and biotechnology to keep evolving (Fakruddin et al., 2012).The manipulation of the material at Nano- scale can frequently alter their physical, electrical, optical and chemical properties (Nel et al., 2006).There is considerable attention to noble metal such as gold and silver due to their numerous applications in different fields as catalysis, photonics, photography and chemical/ biological sensing (Bu and Lee, 2012)due to their surface-enhanced Raman scattering (SERS) (Meheretu et al., 2014; Konrad et al., 2013), additionally, the antimicrobial properties of silver nanoparticles. There is great interesting with silver colloid due to their unique properties such as good conductivity, chemical stability, catalytic and antimicrobial activity(Franci et al., 2015). Silver nanoparticle (Ag NPs) have many applications in various fields such as biomarkers, broad spectrum antimicrobial agents(Stiufiuc et al., 2013; Jana and Pal, 2007), selective coating for solar energy absorption and intercalation material for electrical batteries, as biological sensors (Luo et al., 2015; Rivero et al., 2013)polarizing filters, catalysts in the chemical reaction.There are several methods of synthesis silver nanoparticles (Ag NPs) such physical method using evaporation-condensation (Kmis et al., 2000), photochemical method (Huang and Yang, 2008), the chemical method that depends on chemical reduction method (Dung Dang et al., 2012)and biological method which considered as eco-friendly method dueto thenon- toxic chemical used. It's based on the natural reducing agents such as polysaccharides, biological microorganisms such as bacteria and fungus or plants extract so itis called "Green Chemistry Technology". Although, microorganisms such fungi, algae and bacteria have become as nanofactories for the synthesis of noble metal nanoparticles. But, the researchers have drawn considerable attention to using plants extracts for biosynthesis of noble metal nanoparticles because of its rapid, eco-friendly protocol and economical method. Interestingly, it's providing a single step technique for biosynthesis process (Huang et al., 2007). In this study, we synthesize silver nanoparticle through a reduction method with green chemistry technology by using *Bacopa monnieri, Coleus blumei, and Cichorium intybus* as reducers and characterize it.

MATERIALS AND METHODS

Chemicals

Silver Nitrate (AgNO3), deionized water. All the chemicals and reagents used were of analytical grade.

Preparation of plants material

Bacopa monnieri, Coleus blumei, and Cichorium intybus were collected from the garden of the Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City, Egypt. Shoot tips were cut and washed under running tap water for 30 min, after that explants were sterilized for *Bacopa monnieri* (Abd El Maksoud et al., 2016), for *Coleus blumei*, (Rani et al., 2006) and for *Cichorium intybus* (Dolinski and Olek 2013) then, transferred on Murashige and Skoog's (1962) (MS) medium fortified with (1.0-mg /l) N_6 - benzyladenine (BA) and 7g/l agar and 30g/l sucrose for shoot multiplication for three subcultures.

Preparation of plants extract

The whole *in vitro* plants of *Bacopa monnieri, Coleus blumei*, and *Cichorium intybus* were thoroughly washed several times with deionized water to remove all the media and residuals, and shade dried in dust free condition for several days. Roots were removed from the plant that sliced into small pieces using the scalpel. Finally, plant material of small pieces was added it into 75 ml of deionized water and boiled for 3 minutes in the microwave. The extracts were filtrated to separate all impurities until the solution was clear and store in 4^0C prior using in the further experiments.

Biosynthesis of silver nanoparticles (Ag NPs)

Silver nanoparticles (Ag NPs) were synthesized using oxidation – reduction method. Silver nanoparticles were fabricated using plant extracts as reducers. In the typical synthesis, 1mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. Two ml of separate plant extracts *(Bacopa monnieri, Coleus blumei, and Cichorium intybus)* were added to 8 ml of aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and kept at room temperature until the color changed from colorless to yellow.

Characterization of silver nanoparticles (Ag NPs)

Ultraviolet–visible spectroscopy UV-Vis spectroscopy

Monitoring of bio-reduction of pure silver ions by the sampling of aliquots (0.5 ml) of the suspension then dilution of the samples with 5 ml deionized water. Subsequently, measuring UV-Visible spectroscopy analyses of silver nanoparticles were carried out as a function of the reduction of Ag+ though plant extracts at room temperature on UV –Visible spectrometer (Varian, Carey 500 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

Dynamic light spectroscopy (DLS)

The samples were sonicated in ethanol for 20 minutes, then diluted with 5 ml deionized water and subsequently measuring dynamic light spectroscopy (DLS) and Zeta Potential (ζ) using (Malvern Zeta Sizer-Nano series, Malvern, Worcestershire, UK).

Fourier-transform infrared spectroscopy (FTIR)

Centrifugation of remaining solution after reaction at 6000 rpm for 20 minutes to remove any residual or compounds that are not capping ligand of the nanoparticles. The pellet result after centrifugation was washed with ethanol for several times until obtained clear supernatant. Then, the pellet was dried to have a dried powder. Finally, the dried pellets were analyzed by FTIR (FT-IR/ FT Roman spectrometer. Circular dichroism was performed on JASCO J-805 spectrometer).

High-Resolution Transmission Electron Microscopy (HR-TEM) measurement

The sample was sonicated in ethanol for 20 minutes. A drop of this solution was loaded on carbon coating copper grid, and the solvent was allowed to evaporate for 20 minutes. HR-TEM measurement was performed on field emission transmission electron microscopy (FETEM, JSM-2100F, JEOL Inc Tokyo, Japan) at an accelerating voltage of 15Kv and 200 Kv.

RESULTS AND DISCUSSION

The formation of silver nanoparticles from extracts of *Bacopa monnieri, Coleus blumei and Cichorium intybus* (Fig. 1) as a result of the reduction of Ag+ can be showed as visual by changing the color from colorless to stable yellow as shown in (Figures 2,3,4). Silver nanoparticle characterized using UV- Visible spectroscopy. The reduction of silver metal ion using plant extract are monitoring by UV- Visible spectra as in (Fig. 2) which showed a single and strong band absorption peaks in centered at about 428 nm,411 nm and 439 nm for Bacopa monnieri, Coleus blumei and Cichorium intybus respectively. These indicating that the silver nanoparticles are isotropic in shape and uniform in size. This band called surface Plasmon resonance (SPR) (Mulvaney, 1996). Interestingly, the shift of absorption peak of silver surface plasmon resonance suggesting the formation of smaller silver nanoparticles. These observations indicate the release of proteins into filtrate that the possible mechanism for the reduction of silver ions presents in the solution (Maliszewska and Puzio, 2009). Dynamic light scattering (DLS) is applied to determine the size, size distribution profile and polydispersity index (PDI) of particles in colloidal suspension. As the (Figures 5, 6, 7) revealed the PDI of 0.277, 0.429 and 0.442 for *Bacopa monnieri, Coleus blumeiand Cichorium intybus* respectively which indicated that the silver nanoparticle is not aggregate at all. Zeta potential (ζ) measures the potential stability of the particle in the colloidal suspension. Zeta potential is used to determine the size of nanoparticle in colloidal suspension especially silver. As the (Figures 5, 6, 7) demonstrated, the zeta potential is -24.6, -24.4 and -30.2 mv with diameter 7.14 nm, 1.03 and 0.9 nm for *Bacopa monnieri, Coleus blumei, and Cichorium intybus* respectively.

The FTIR of silver nanoparticles synthesis via the extract of *Bacopa monnieri, Cichorium intybus, and Coleus blumei,* was illustrated in the following (Figures 8, 9, 10). In the (Fig. 8) of silver nanoparticle synthesis using *Bacopa monnieri*, there is a peak at 3457 cm^{-1} that corresponds to O-H stretching H- bonded alcohols and phenols. Also, there is a sharp peak at 1636 cm^{-1} that indicated as N-H primary amines. The similar peaks with slightly changed for Coleus blumei and Cichorium intybus respectively: the peaks at 3456 $cm^{-1}$and 3450 cm^{-1} (Figures, 9,10) that correspond to O-H stretching H- bonded alcohols and phenols and the peaks at 1638 cm^{-1} and 1638 cm^{-1} for both extract plants corresponds to N-H primary amines. Increasingly, their silver nanoparticles surrounded by proteins and metabolites such as terpenoids have functional groups of alcohols, ketones, aldehydes and carboxylic acids. From the analysis, It was confirmed that the carbonyl group of the amino acid residues and proteins had the greater ability to bind metal indicating that the proteins could be form capping of silver nanoparticles to inhibit agglomeration and thereby stabilize the medium. These results suggested that the biological molecules could perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Sathyavathi et al. 2010). Transmitted Electron Microscope (TEM) images of synthesized Ag nanoparticles are presented in the following (Figures 11, 12, 13). TEM images showed that the most of the Ag nanoparticles were a spherical shape with some agglomerated silver nanoparticle. This is indicating possible sedimentation at late time. The morphology of Ag NPs, measured by zeta potential and HR-TEM, were to have an average diameter of 40.3, 5.05, 3.16 nm.

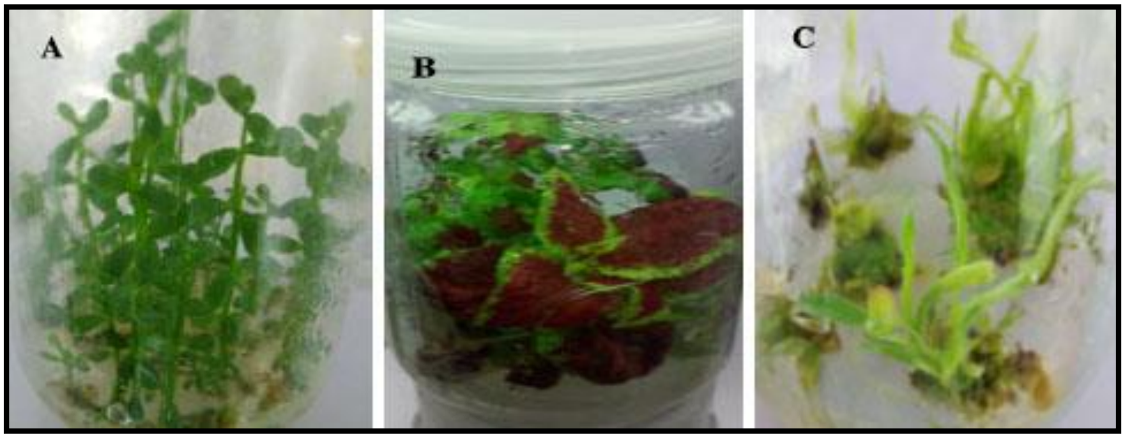


Figure 1: *In vitro* cultures A:*Bacopa monnieri. B; Coleus blumei* C: *Cichorium intybus*

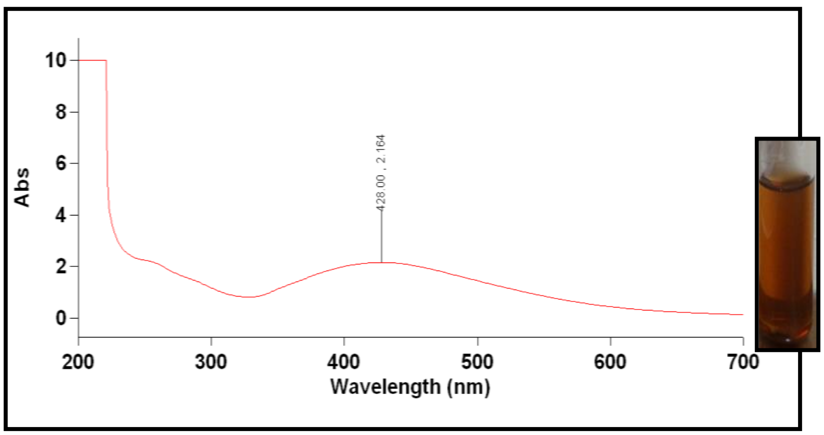


Figure 2: UV-visible absorption peak of *Bacopa monnieri* synthesized AgNPs (1mM) at 428 nm

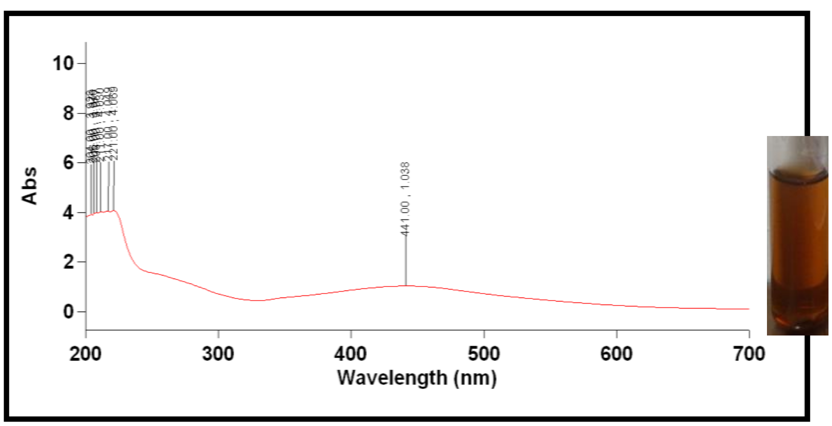


Figure 3: **UV-visible absorption peak of *Coleus blumei* synthesized AgNPs (1mM) at 441 nm**

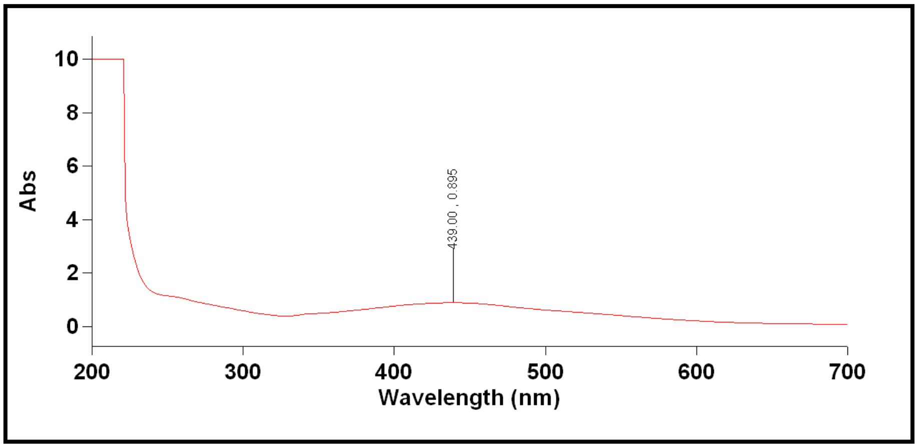
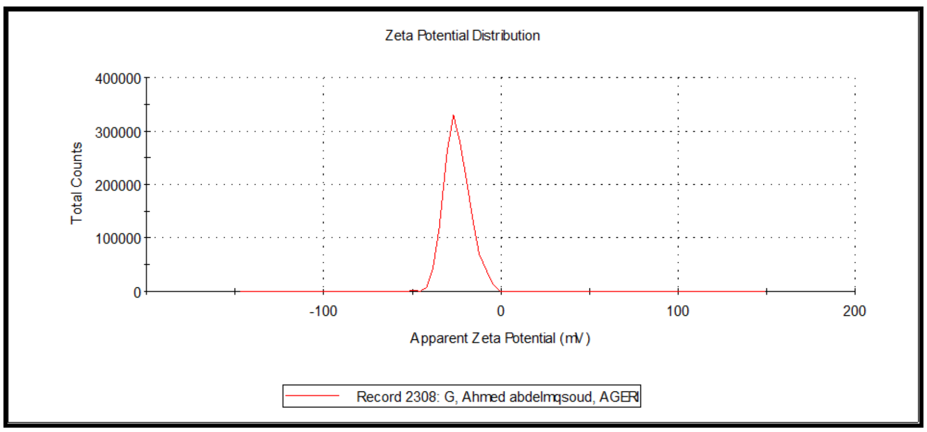


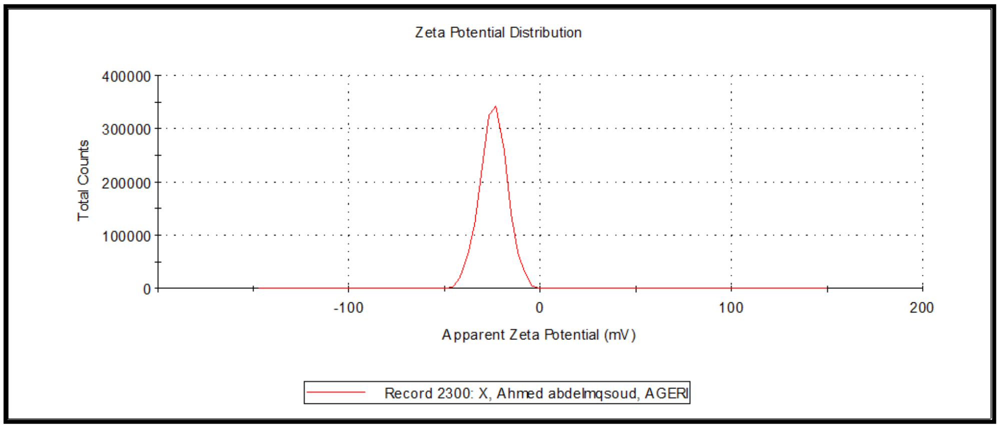
Figure 4: UV-visible absorption peak of *Cichorium intybus* synthesized AgNPs (1mM) at 439 nm



Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-24.6	Peak 1:	-24.5	99.8	7.14
Zeta Deviation (mV):	7.22	Peak 2:	-49.3	0.2	1.43
Conductivity (mS/c...	0.0495	Peak 3:	0.00	0.0	0.00

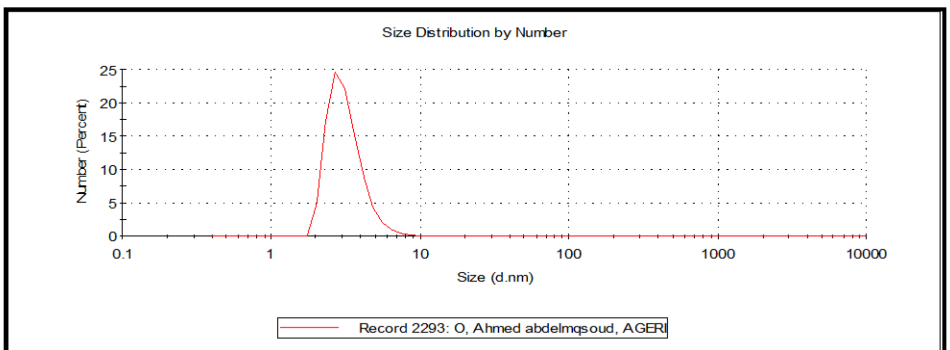
Figure 5: (a) DLS (b) Zeta potential graph of *Bacopa monnieri* mediated synthesized AgNPs (1mM)



Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-24.4	Peak 1:	-24.4	100.0	7.12
Zeta Deviation (mV):	7.12	Peak 2:	0.00	0.0	0.00
Conductivity (mS/c...	0.0646	Peak 3:	0.00	0.0	0.00

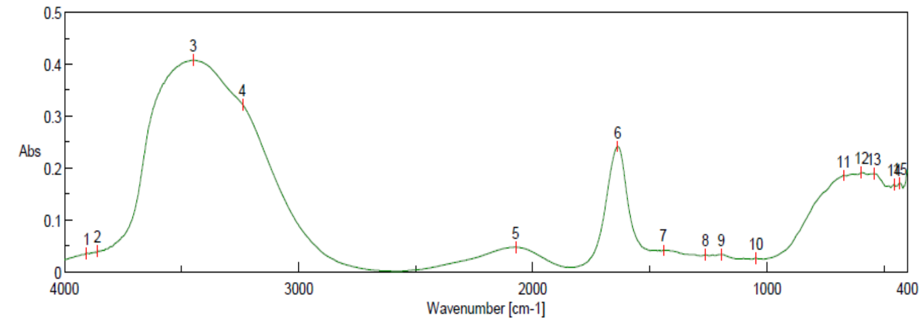
Figure 6: (a) DLS (b) Zeta potential graph of *Coleus blumei* mediated synthesized AgNPs (1mM)



Results

			Size (d.nm):	% Number	Width (d.nm):
		Peak 1:	3.168	100.0	0.9000
PdI:	0.440	Peak 2:	0.000	0.0	0.000
		Peak 3:	0.000	0.0	0.000

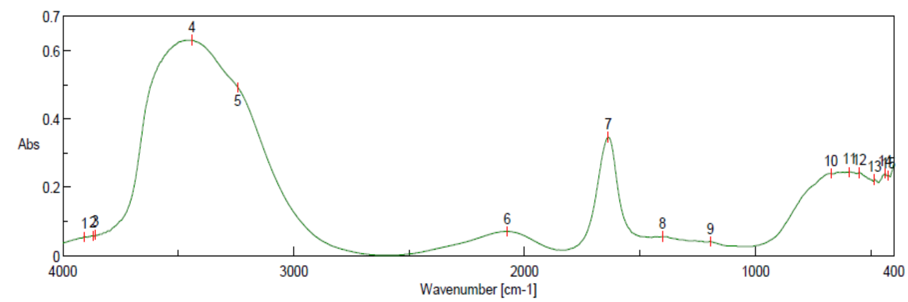
Figure 7: (a) DLS (b) Zeta potential graph of *Cichorium intybus* mediated synthesized AgNPs (1mM)



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3906.11	0.0355425	2	3858.86	0.0400278	3	3450.03	0.407476	4	3239.82	0.322167
5	2073.1	0.0471665	6	1638.23	0.241135	7	1442.49	0.0411835	8	1262.18	0.0323439
9	1194.69	0.0331825	10	1047.16	0.0257737	11	673.999	0.185375	12	595.896	0.19112
13	543.828	0.188974	14	457.047	0.168298	15	431.977	0.170178			

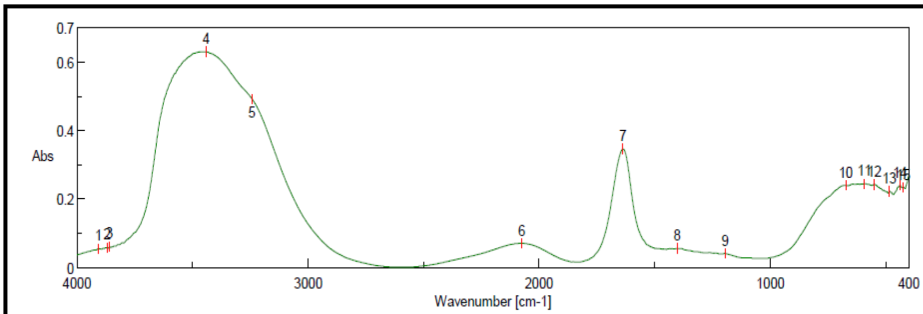
Figure 8: FTIR Spectra of AgNPs (1mM) mediated by *Bacopa monnieri*



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3907.07	0.0550824	2	3869.47	0.0577876	3	3858.86	0.0605734	4	3441.35	0.629966
5	3242.72	0.491813	6	2074.07	0.0703858	7	1638.23	0.345609	8	1402.96	0.0556791
9	1195.65	0.040623	10	673.999	0.239946	11	593.004	0.244394	12	549.613	0.242207
13	484.045	0.222862	14	438.726	0.240375	15	423.298	0.2332			

Figure 9: FTIR Spectra of AgNPs (1mM) mediated by *Coleus blumei*



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3907.07	0.0550824	2	3869.47	0.0577876	3	3858.86	0.0605734	4	3441.35	0.629966
5	3242.72	0.491813	6	2074.07	0.0703858	7	1638.23	0.345609	8	1402.96	0.0556791
9	1195.65	0.040623	10	673.999	0.239946	11	593.004	0.244394	12	549.613	0.242207
13	484.045	0.222862	14	438.726	0.240375	15	423.298	0.2332			

Figure 10: FTIR Spectra of AgNPs (1mM) mediated by *Cichorium intybu*

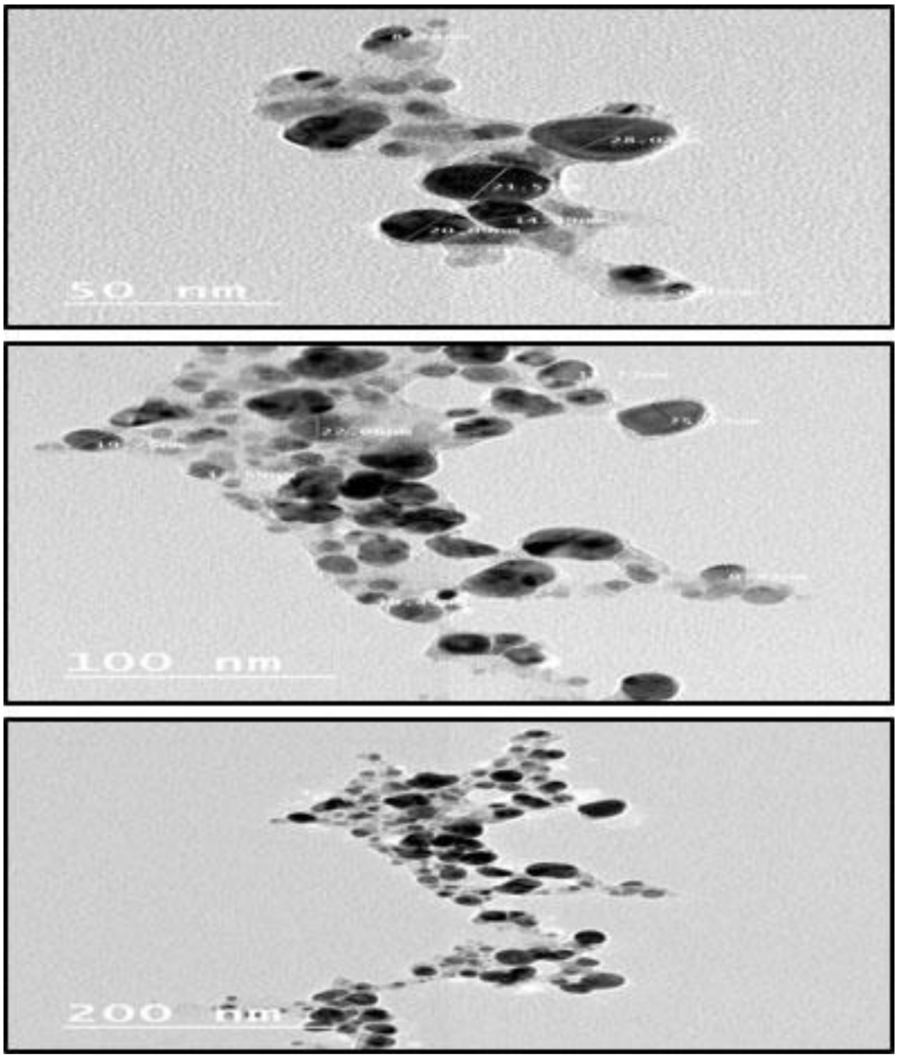


Figure 11: TEM images of AgNPs (1mM) mediated by *Bacopa monnieri* at different magnifications

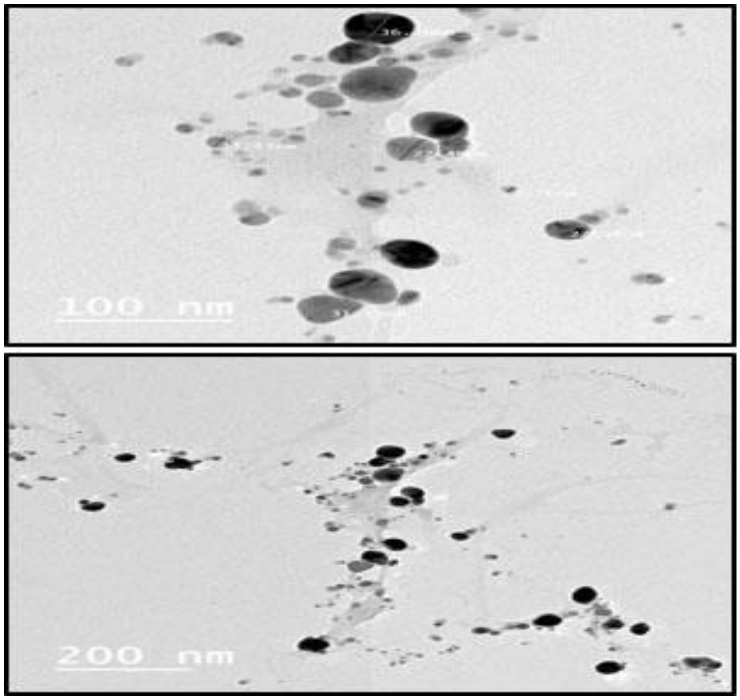


Figure 12: TEM images of AgNPs (1mM) mediated by *Coleus blumei* at different magnifications

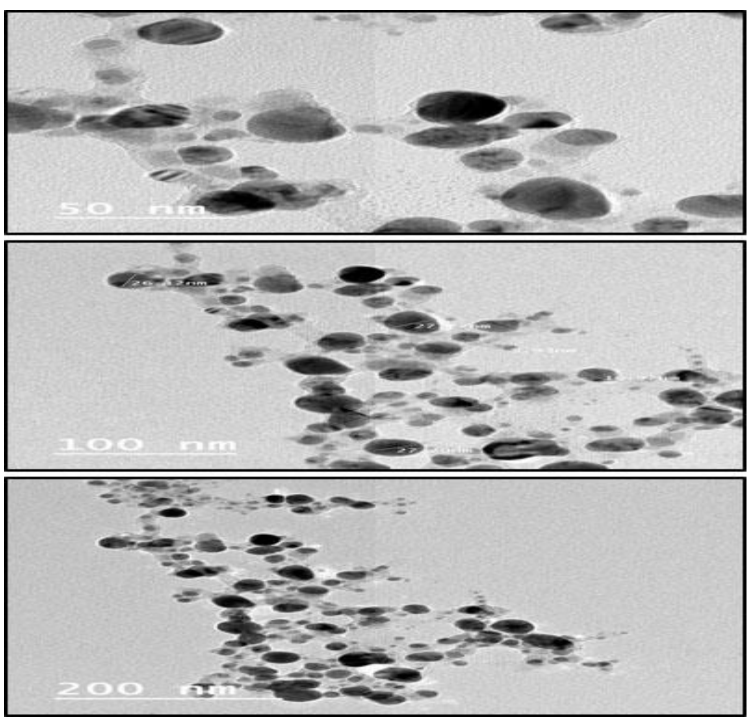


Figure 13: TEM images of AgNPs (1mM) mediated by *Cichorium intybus* at different magnifications

CONCLUSION

In this work, we have produced, by an oxidation–reduction method, a perfect round- shaped Ag-NPs of ca.40.3, 5.05, 3.16 nm diameter, using extracts of (*Bacopa monnieri, Coleus blumei, and Cichorium intybus*). The Ag-NPs were characterized by different complementary techniques UV – Visible spectroscopy, FTIR, Zeta potential and Transmission electron microscopy. This approach provides an environment-friendly, simple and efficient technique for the preparation of well-structured shaped of Ag nanoparticles

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

AB and AA designed and performled the experiments. AB and AA wrote the manuscript. AB, AA, RA, AH, AM and DE designed experiments and reviewed the manuscript. RA, AH and DE characterized the silver nanoparticle using UV- Visible spectroscopy, FTIR and TEM. AB and AA read and approved the final version.

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