IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2022 Vol.:24, Issue:1

Green Synthesis of Silver Nanoparticles Using Aqueous Extract of *Caesalpinea decapetala* Leaves and Enhance Its Microbial Activity

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Shelar Uttam B.[1]*, Pingale Shirish S.[2], Thorve Sandip S.[3], Chavan Vaibhav M.[4], Jadhav Sunil M.[5]

[1,3,4,5]Department of Chemistry, Shri Shiv Chhatrapati College Junnar, Pune, Maharashtra, India – 410 502.
[2]Department of Chemistry, ACS College Narayangaon, Junnar, Pune, Maharashtra, India – 410 504.

Submitted: 23 March 2022
Accepted: 28 March 2022
Published: 30 April 2022

Keywords: Water extract of *Caesalpinea decapetala,* Green synthesis, AgNPs, Antimicrobial activity.

ABSTRACT

The present work showed an environmental, eco-friendly, and fast process for the synthesis of silver nanoparticles. Silver nanoparticles (AgNPs) were prepared by Green synthesis method using *Caesalpinea decapetala* leaves aqueous extract. We have developed a cost-effective, rapid, and single-step method at room temperature. AgNPs start to form as soon as the reaction gets initiated and within 48 hours process gets completed. Characterization and formation of AgNPs were confirm by UV-visible spectrophotometry, FTIR spectroscopy, FE-SEM, and XRD. The average crystalline size of AgNPs was is estimated to be 12.15 nm. In IR-spectroscopy, absorption peaks were observed at 494 cm^{-1}(silver-oxygen bond), 1232 cm^{-1} (-OH bond of phenol), 1737 cm^{-1}(C=O stretching), 1375cm^{-1}(primary amine) and 1571cm^{-1}(C=N group). It showed that water extract was responsible for the reduction of silver ions into AgNPs. The plant extract acts as a stabilizing, capping, and reducing agent. By using the agar well diffusion method antibacterial activity of AgNPs was studied against Bacillus subtilis, Staphylococcus Albus, Escherichia coli, Plasmodium mirabilis, Aspergillus niger, and Candida strains. The zone of inhibition of bacteria and fungi against AgNPs was observed. The bacteria Escherichia coli shows the maximum zone of inhibition (10.25 mm) while the Staphylococcus album does not show a zone of inhibition. Both the fungal strains Candida (12mm) and Aspergillus niger(9.8 mm) showed a zone of inhibition. It provedAgNPs has significant antimicrobial activity. Hence it can be used in biomedical applications.

INTRODUCTION

A microscopic particle which is having dimensions between 1 to 100 nm is called a nanoparticle. (PackiaLekshmi et al..2012). on the earth planet, nanoparticles are formed due to combustion, food cooking, photochemical volcanic activity, and vehicle exhaust. In 1974, the scientist Prof. Norio Taniguchi first introduced the term nanotechnology during multidisciplinary research in physics, chemistry, and biology.

Nanomaterial has unique, superior, and new chemical and physical properties as compared to bulk material because of the large surface area per volume of the material/ particle. Novel compounds are produced by nanotechnology in the last two decades and are applied in different fields. Nanotechnology is a field of science that deals with manipulation, the use of materials, and production in terms of nanometers. In advanced material science, nanotechnology is the most active area for research. Nanoparticles of the required shape, size, and dispersivity are produced efficiently and by green chemistry technique with nanotechnology.[1] Property of nanoparticle changes with a decrease in dimensions. During nanomaterial synthesis with clean and green technology, these aspects play an important role.[2] The shape, size, structure, composition, and crystalline nature of nanoparticles determine their applications. The most common nanoparticles are metal nanoparticles because they are easily synthesized. The metal nanoparticles are synthesized by physical, chemical, and biological methods. The physical methods are highly expensive [3] and chemical methods produce toxic side effects to the environment.[4] Therefore it is necessary to develop a new method that does not produce toxic chemicals by obeying synthesis protocols [5] and it is the biosynthesis or green synthesis processes. Physical and chemical methods have short time stability, poor ability, safety problems, and involvement of toxic chemicals. Metal nanoparticles are prepared by using plant extract and microorganisms is called green synthesis or biosynthesis. Metal nanoparticles prepared from the plant extract method are more simple and more feasible. The rate of synthesis of the nanoparticle is faster and it is more stable by using plant extract/ products as compared to the microorganism due to the presence of metabolites. Biosynthesis of the nanoparticle is a good method because it is carried out at room temperature, neutral pH is low cost, simple, highly reproducible, environment friendly, and uses water as solvent. Mushrooms, fungi, microbial strains, enzymes, metabolites, biodegradable products, and plant extract are reported to use for the biosynthesis of metal nanoparticles.[6,7,8,9] Secondary metabolites present in medicinal plants

can convert metal ions into zero-capacity metal due to antioxidant (reducing agent) properties.

The Noble metals like platinum, palladium, zinc, copper, silver, and iron were used for nanoparticle biosynthesis. Platinum, silver, and gold nanoparticles are commonly used in human contact materials like shoes, shampoos, soaps, toothpaste, detergents, and cosmetic products. Among these metals, silver nanoparticles are preferred because silver ions act as a stabilizing agent, having antibacterial action and inhibitory effects on microbes.[10, 11] Silver has strong toxicity against microorganisms. Silver is toxic to the bacterial cell. Silver ions react with macromolecules like proteins and deoxyribonucleic acid (DNA) of the bacterial cell thereby damaging the cell wall, disrupting cell metabolism, and finally cell death. Silver nanoparticles are attractive because they are not toxic to the human body. The life span of metal in nanoparticles depends upon the stabilizing agents and processes of synthesis.

Silver nanoparticles (AgNPs) biosynthesis can be performed from the plant extract and from a microorganism. Biosynthesis of AgNPs from different plant extracts was reported. For example leaves of *Cardiospermum halicabum L.*, *Lantana camera L.*fruits and *Impatiens balsamina L.* leaves. [12,13,14] Biosynthesis of AgNPs by using fungi, yeast, and bacteria was documented.[15,16,17] Leaf extract of *Azadirachta indica* was recently found to be used for AgNPs because the silver act both as a capping and reducing agent.[18]

Caesalpinea decapetala leaves contain phenols, terpenoids, reducing sugars, flavonoids, and saponins which act as antimicrobial agents and good antioxidants. Hence leaves of plant extract act as reducing agents for the conversion of silver into AgNPs. These phytochemicals plays important role in both the reduction and stabilization of nanoparticles.

Noble metals in nanoparticles are used for the purification of drinking water.[19]AgNPs are having a wide range of applications like a catalyst, detectors, surface acting agents, optical receptors, filters, sensors.[20]AgNPs can be used in household appliances such as food storage containers, textile, medicinal devices, electronic optical fibers, agriculture, and paint. The pure metal in nanoparticle form is used for the treatment of several chronic and acute diseases like cancer, hepatitis, AIDS, and malaria.

In the present work, AgNPs are synthesized and characterized by using leaf extract of *Caesalpinea decapetala*. The biological activity of synthesized AgNPs was studied against bacterial and fungal strains in laboratory conditions.

MATERIALS AND METHODS

SAMPLE PREPARATION USING PLANT *CAESALPINIA DECAPETALA*[21]

Dried and fine powdered leaves of *Caesalpinia decapetala*, were used for extract preparation. Then plant extract was prepared by mixing 10 gm of plant extract with 100 ml deionized water in a 500 ml of (Borosil, India) round bottom flask. The mixtures were heated for about 1 hour. After heating, the supernatant solution was filtered (Whatman Filter paper 42). Then the solution was stored at 4^{o}C and further used for the reduction of silver ions (Ag+) to silver nanoparticles.

BIOSYNTHESIS OF SILVER NANOPARTICLES USING LEAVES EXTRACT OF PLANT MATERIAL

The biosynthesis of silver nanoparticles is carried out by using leaves extract of *Caesalpinia decapetala* in the following way. Exactly 3.0 mL of leaves water extract (LWE) is added to 50 mL of freshly prepared $AgNO_3$ (2 mM) aqueous solution. Then the flasks are placed on a magnetic stirrer at room temperature for the process of NPs synthesis for about 48 hrs. The appearance of yellow-brown color indicates the formation of silver nanoparticles. A control solution (2 mM $AgNO_3$) was also kept under the same condition. After completion of synthesis, the AgNPs medium was centrifuged at 10000 rpm for 30 minutes. The precipitate was frozen, dried, and used for characterization and further studies.

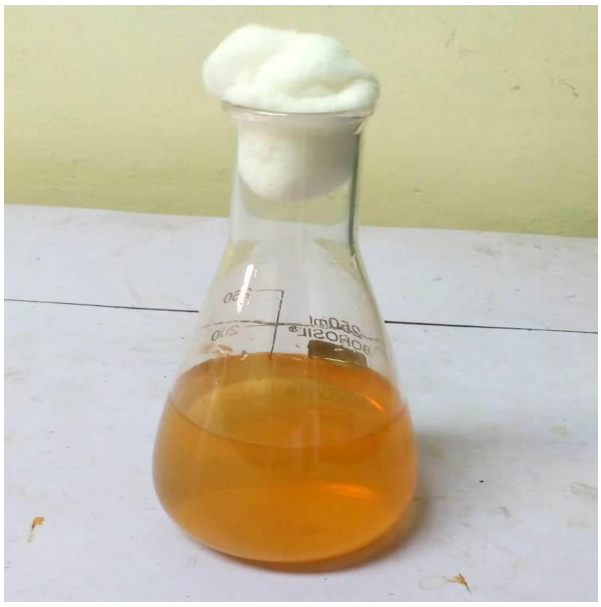
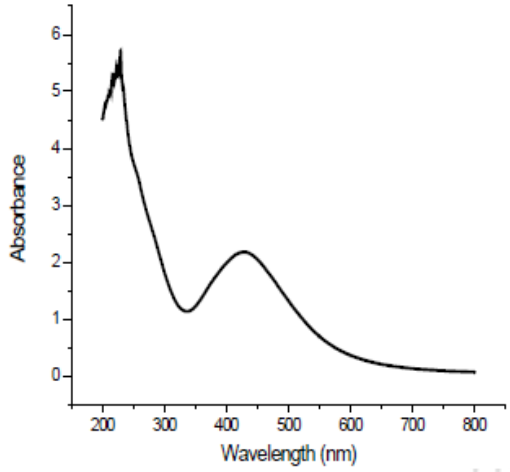


Fig.1 2mM AgNO3 + leaves extract

CHARACTERIZATION OF SILVER NANOPARTICLES

a. UV-Visible Analysis:

UV–visible spectroscopy is a preliminary and convenient tool for measuring the reduction of metal ions based on optical properties called SPR. The optical property of AgNPs was determined by a UV-Vis spectrophotometer (PerkinElmer, Lamda 35, Germany). After the addition of $AgNO_3$ solution to the plant extract, the spectra were taken. The reaction mixture after 48 hr has an absorption maximum at 430 nm suggesting the formation of Ag nanoparticles as shown in fig.2.



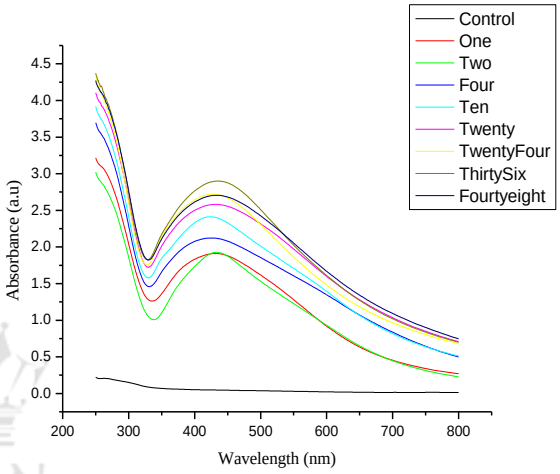


Fig.2 UV-VIS Spectra of CD- L AgNPs **Fig.3 Time dependent Biosynthesis of CD – L AgNPs at 2 mM $AgNO_3$**

The symmetrical shape of the absorption band shows the presence of spherical- polydispersed nanoparticles and which is further confirmed by FE-SEM studies. In the formation of nanoparticles at room temperature, the incubation period plays a significant role. There was no considerable change in the UV–Vis spectrum after 48 hrs. confirmed that the reaction is completed. Thus, this revealed from the experimental data that the formation of nanoparticles in solution is time-dependent as shown in fig.3.

b. IR- spectroscopy

The chemical composition of the synthesized silver nanoparticles was studied by using an FTIR spectrometer. The solutions were dried and the dried powders were characterized in the range 400 – 4000 cm^{-1}. The plant extract acts as a reducing agent, capping agent, and

stabilizing agent for biosynthesized silver NPs. The FTIR spectra for leaves water extract and corresponding CD-AgNPs are shown in Figure 4.

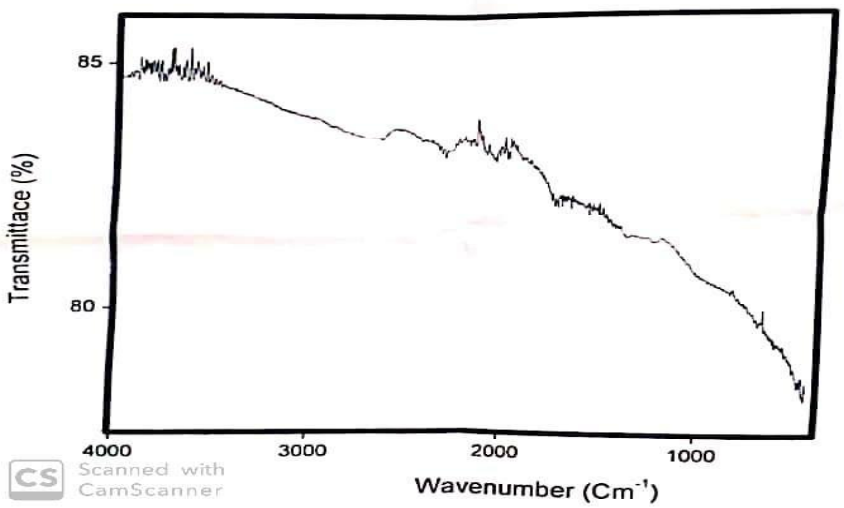


Fig. 4 IR spectra of CD-L AgNPs.

The FTIR analysis of CD-AgNPs showed sharp absorption peaks at 494,694,1232,1375, 1571, 1699, 1737, 2676, 3655, 3712 cm^{-1}. The spectral data study shows (broad, sharp) absorption peak at 494 cm^{-1} corresponding to silver-oxygen bonding, at 694, 1232, 3655 cm^{-1}corresponding to the –OH bond of phenols. The absorption peak at 1669 and 1737 cm^{-1} due to C=O stretching. The absorption peak at 1375 cm^{-1} corresponds to primary amine and 1571 cm^{-1} of the characteristic of the C=N group. This shows that –OH / >N-H groups, >C=N, >C=O groups in the water extract are responsible for the reduction of silver nitrate to AgNPs.

c. FE-SEM Analysis:

The shape and size of the silver nanoparticles synthesized by the green method were determined by scanning electron microscopy. SEM analysis of AgNPs was shown at a resolution of 3 µm in Fig.5. The biosynthesized AgNPs are spherical shaped and well distributed with aggregation. This image gives information about the organic compounds absorbed on the surface of nanoparticles which acts as a reducing as well as capping agent.

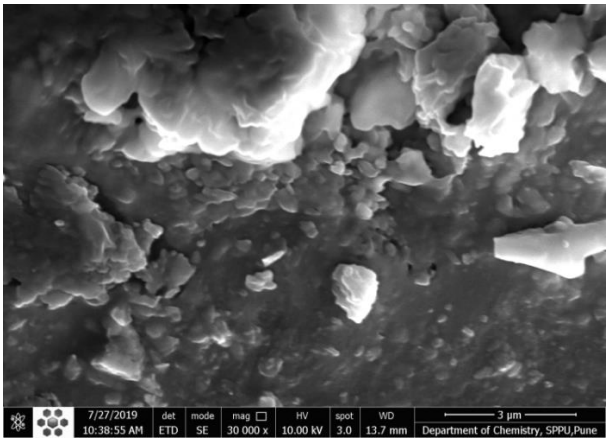


Fig. 5. SEM of CD-L AgNPs

d. XRD Analysis:

The crystal structure and average particle size of the nanoparticles were analyzed by the XRD system. The XRD patterns of vacuum-dried silver nanoparticles were synthesized by using leaves extract of *Caesalpinia decapetala.* The XRD pattern showed diffraction peaks in the range of 2θ (20–80°) which were corresponding to the (111) (200) (220) and (311) planes. The sharp diffraction peaks were observed at2θ values 27.8, 32.2, 38.2, 44.4, 64.6, and 77.4 degrees as shown in fig.6. A peak at 2θ = 38.2 shows the formation of pure silver at the start of the reaction. The average particle size of the AgNPs was determined by using Scherer's equation as follows:

$$D \approx 0.9\, \boldsymbol{\lambda\beta} \cos \theta$$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and β is the full width at half the maximum of the peak in radians.

Based on the Scherer equation, the average crystallite size of AgNPs was estimated to be 12.15nm.

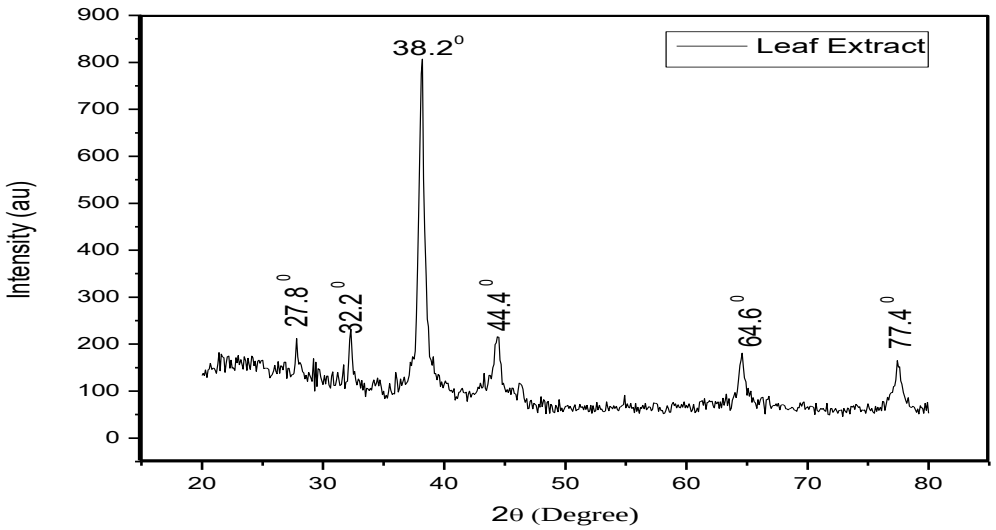


Fig. 6 XRD OF CD- L AgNPs.

ANTIMICROBIAL ACTIVITY OF AgNPs

Silver nitrate acts as a strong and natural antibiotic and antibacterial agent. Biosynthesized Silver nanoparticles are highly toxic to multidrug-resistant bacteria and fungi and hence have a great potential in biomedical applications.

Procurement of cultures- All the microbial cultures were procured from the National Collection of Industrial Microorganisms (NCIM), NCL, Pune.

Table 1: Bacterial culture

Sr. No.	**Name**	**Type**	**ATCC No.**
1	*Bacillus subtilis*	Gram positive	NCIM2063
2	*Staphylococcus Albus*	Gram positive	NCIM2178
3	*Escherichia coli*	Gram negative	NCIM 2574
4	*P.mirabilis*	Gram negative	NCIM 2388

Table 2: Fungal cultures

Sr. No.	**Name**	ATCC No.
1	*Aspergillus niger*	*NCIM545*
2	*Candida*	NCIM 3100

ANTIBACTERIAL ASSAY

To determine the antibacterial activity of AgNPs synthesized by water extract of plant leaves the agar well diffusion method was used. Muller Hinton agar and Potato Dextrose agar plates were prepared by pouring in sterile Petri plates and the overnight grew bacterial and fungal suspension was uniformly spread on the plates respectively and allowed to dry for 5 minutes. 0.5 cm diameter wells were bored using a good cutter. Pre-sterilized Whatman No. 3 filter paper discs of 0.5 cm were picked by the outer edge with sterile forceps and dipped into a prepared solution of AgNPs with a concentration of 1 mg/ml and placed on the swabbed agar plates before incubation. The incubation of plates was carried out at 37 °C for 24 hours and the zone of inhibition was measured.

RESULT:

Table 3: Zone of inhibition of AgNPs against gram-positive and gram-negative bacteria in mm.

Sr. No.	Name	Type	Zone of inhibition in mm					Mean in mm
1	*Bacillus subtilis*	Gram positive	8	9	8	9	-	8.5
2	*Staphylococcus Albus*	Gram positive	-	-	-	-	-	-
3	*Escherichia coli*	Gram negative	9	9	11	12	-	10.25
4	*P.mirabilis*	Gram negative	7	7	7	7	7	7

Table 4: Zone of inhibition of different AgNPs against fungi in mm.

Sr. No.	Name	Zone of inhibition in mm					Mean in mm
1	*Aspergillus niger*	12	9	9	11	8	9.8
2	*Candida*	12	12	12	13	11	12

Fig.7 Zone of inhibition of AgNPs against *B.subtilis*(A)and s. *Albus* (B)

Fig. 8 Zone of inhibition of AgNPs against *Escherichia coli*(C) and *S. Albus* (D)

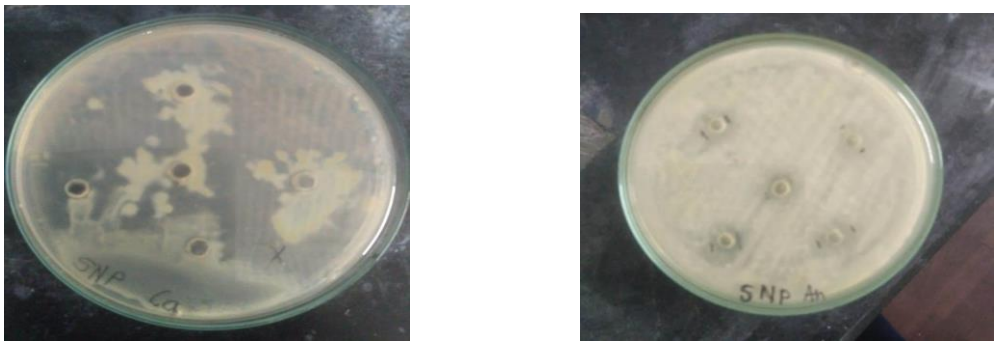


Fig. 9 Zone of inhibition of AgNPs against *Aspergillus niger*(E) and *candida*(F)

CONCLUSION

The study demonstrates that AgNPs can be green synthesized in one step by using water leaves extract of *Caesalpinia decapetala.* The UV-Vis spectroscopy, FTIR, SEM, and XRD analysis confirmed the formation of biosynthesized AgNPs, which showed the strong absorbance at 430 nm with surface Plasmon resonance. The antimicrobial activity of silver

nanoparticles has been investigated against bacterial and fungal species. The zones of inhibition of bacteria and fungi against AgNPs were observed in Table 7.2.3 and 7.2.4 respectively. The bacteria *E. Coli* show the maximum zone of inhibition of 10.25 mm and *Staphylococcus Albus does* not show any zone of inhibition. Among the fungal strains, *Candida* shows the maximum zone of inhibition of 12 mm while*Aspergillus niger* shows the minimum zone of inhibition of 9.8 mm.

The silver nanoparticles synthesized via green route using water extract of leaves of *Caesalpinia decapetala* show high toxicity to bacteria & fungi. Thus it can be utilized in biomedical applications. The present investigation end with an easy, fast and inexpensive way to synthesize silver nanoparticles.

REFERENCES

1. Nel A E, MädlerDVelegol, T Xia and EMV Hoek. Understanding bio physicochemical interactions at the nano-bio interface. Nature Materials2009; 8 :543.
2. Sharma NC, Sahi SV, Nath S, Parsons J, Gardea JL, Torresdey and Pal T.Synthesis of Plant-Mediated Gold Nanoparticles and Catalytic Role of Biomatrix-Embedded Nanomaterials. Environmental Science Technology2007; 41:5137.
3. Raffi M, Rumaiz AK, Hasan MM, Shah SI, J. Mater. Res. 2007; 22 : 3378–3384.
4. Lee KJ, JunBH, Choi J, Lee Y, Joung J, Oh YS.Nanotechnology2007; 18 : 335601.
5. Kumar A, Vemula PK, Ajayan PM, John G. Nat. Mater. 2008;7: 236–241.
6. Owaid MN and Ibraheem IJ.Mycosynthesis of nanoparticles using edible and medicinal mushrooms. European Journal of Nanomedicine 2017; 9(1):5–23.
7. Ali DM, Sasikala M, Gunasekaran M and Thajuddindig N.Biosynthesis and characterization of silver nanoparticles using marine *cyanobacterium, oscillatoriawillei.* Journal of Nanomaterial and Biostructures 2011; 6(2): 385-390.
8. Avnesh K, Sudesh KY, and Subhash CY. Biodegradable polymeric nanoparticles based drug delivery systems.*Colloids and Surfaces* B2010; 75: 1–18.
9. Bar H, BhuiDK, Sahoo GP, Sarkar P, De SP and Misra A. Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and Surfaces A- Physicochemical and Engineering Aspects.2009;339: 134–139
10. Shiraishi Y, Toshima N.Colloids Surf. A.2000;169(1): 59-66.
11. Liz-Marzan LM, Philipse A P. J. Phys. Chem.1995; 99(41):15120-15128.
12. Mahipal SS, Manokari M, Kannan N, Revathi Jand Latha R. Synthesis of silver nanoparticles using Cardiospermum halicacabumL. leaf extract and their characterization.Journal of Phytopharmacology2013; 2(5):15–20.
13. Edy Parwanto ML, Senjaya H and Jaya Edy H.Formulasisalepantibakteriekstraketanoldauntembelekan (Lantana camara L.).PharmaconJurnalIlmiahFarmasi-UNSRAT2013; 2:3.
14. Kang SN, Goo YM, Yang MR. Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of Impatiens balsaminaL. (Balsaminaceae) at different harvest times.Molecules2013;18(6): 6356–6365.
15. VarshneyR, MishraAN, BhadauriaS, GaurMS. Digest J. Nanomater. Biostruct. 2009: 349–355.
16. KowshikM, AshtaputreS, KharrazS, VogelW, UrbanJ. KulkarniSK, PaknikarKM. Nanotechnology 2003;14:95–100.
17. ShahverdiAR, MinaeianS, ShahverdiHR, JamalifarH, NohiAS. Process. Biochem. 2007; 42 :919–923.

18. Arunachalam KD,LillyBA, AnnamalaiSK and ArunachalamAM.Potential anticancer properties of bioactive compounds of Gymnemasylvestreand its biofunctionalized silver nanoparticles. International Journal of Nanomedicine. 2014;10:31-41.
19. PradeepT, Anshup. Thin Solid Films 2009;517: 6441–6478.
20. SmithaSL, NissamudeenKM, PhilipD, GopchandranKG.Spectrochim. Acta A. 2008; 71 :186–190.
21. PingaleSS, RupanarSV, ChaskarM. Plant-mediated biosynthesis of Silver nanoparticles from Gymnemasylvestreand their use in photodegradation of Methyl orange dye.J. Water Environ. Nanotechnol. Spring 2018; 3(2): 106-115.