IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. V (Jul – Aug 2017), PP 14-23
www.iosrjournals.org

Biosynthesis and characterization of silver nanoparticles using leaf extract of *Wedelia urticifolia*(Blume)DC and evaluation of antibacterial efficacy

[*]MohdYousuf Rather[1], K Johan Pandian[2], SM Sundarapandian[1], A Yogamoorthi[1]
[1](Department of Ecology and Environmental Science, Pondicherry University, India)
[2](Department of Pharmacology, MGMCRI, Pondicherry)
Corresponding Author: MohdYousuf Rather

Abstract: *In thepresentstudy, an easy and green method for the phytosynthesis of silver nanoparticles is reported. The leaf extract of Wedeliaurticifolia Blume DC was used for the synthesis. Overall, three ratios of leaf extract to metal salt concentrationviz 9:1, 1:1 and 1:9 were used to achieve the best ratio to be treated for getting abetter yield. The silver nanoparticles (AgNPs)synthesis was confirmed by thecolour change.The produced nanoparticles were examined by UV-Visible spectroscopy, Dynamic Light Scattering (DLS/Zeta-Sizer), XRD (X-Ray Diffraction Spectroscopy) and SEM (Scanning Electron Microscopy). All the three ratios showed a peak in UV- visible spectrum graph at 450nm, but maximum absorbance was observed in1:1 ratio followed 9:1 and 1:9. The average size of the AgNPs obtained from DLS was found to be 179.3 nm, 90.38 nm and 80.28nm for 9:1, 1:1 and 1:9 ratios respectively.SEM images indicated that the synthesized AgNPs were agglomerated, but poly-dispersed and crystalline in nature. The XRD pattern obtained for the synthesized particles matched with the ICDD standard. The results obtained from the antibacterial assay revealed that the AgNPs are more potent in inhibiting the growth of gram-negative bacterial species (Escherichia coli, 100 µg) rather than gram-positive bacterial species (Staphylococcus aureus, 400 µg).*

Keywords: *Antibacterial activity, Biosynthesis,Silver nanoparticle, Wedeliaurticifolia*

Date of Submission: 12-07-2017 Date of acceptance: 28-07-2017

I. Introduction

Metal nanoparticles, with a size between 0-100nm, have numerous applications in biology, chemistry, medical science, pharmaceutics and energy science [1] as they exhibit remarkable properties due to their specific characteristics like size, surface area. Nanoparticles are synthesized by a wide range of chemical processes. Some of them are expensive or hazardous to the environment as these processesinvolve avariety of chemicals. The green synthesis of nanoparticles is favoured over physical and chemical methods because it is cost effective, eco-friendly and does not need massive external energy or toxic chemicals [2]. Green synthesis, where a wide range of plants and microbes have been involved, is gaining a lot of attention globally because of its easy reproducibility, rapid, economic and environmental friendly techniques.Among metal nanoparticles, silver nanoparticles (AgNPs) have gained a very special focus because of their wide variety of uses downthrough human civilization. AgNPs possess a lot of unique assets like good conductivity, chemical stability, catalytic properties, and most importantly antibacterial,anti-viral, antifungal and anti-inflammatory properties [3,4].AgNPs have been produced using many plant species [5].Kasthuri et al. [6] synthesized quasi-sphericalAgNPs by using purified apiin compound, which was extracted from thehenna leaf at ambient conditions. Green tea, *Camellia sinensis* extract was used as an agent for reducing and stabilizinggold and silver nanoparticles[7]. Plant extracts from broths of lemongrass, live alfa alfaand others have aided in AgNP synthesis [8]. The reaction of aqueous leaf extract of a common ornamental geranium plant *Pelargonium graveolens* with silver nitrate ($AgNO_3$)produced AgNPs within 24 h [9].A vegetable, *Capsicum annum* was also utilized to synthesize AgNPs [10].Plants extractsfrom*Solanumtrilobatum*,*Syzygiumcumini*,*Syzygiumaromaticum*, *Centellaasiatica*, *Ocimumtenuiflorum*and*Citrus sinensis* were used for the synthesis of AgNPs from silver nitrate solution [11,12]. Besides synthesis of AgNPs using a variety of plant parts as reductants, they are also tested for their antibacterial efficacy against potential as well as common pathogenic bacterial species viz. *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*and *Streptococcus pyogens*using the extracts of *Boerhaaaviadiffusa*[13],*Tribulisterrestris*[14],*Cocusnucifera*[15],*Solanustorvum*[16],*Trianthemadecandra*[17], *Argemonemexicana*[18],*Abutilon indicum*[19],*Aloe vera*[20] and*Pistaciaatlantica*[21]. Presently, one of the

invasive plant species *Wedelia uricifolia*Blume DC has been selected and the silver nanoparticles synthesized from aqueous extract of the leaves is tested for its antibacterial potency against two pathogenic bacterial species viz.*Staphylococcus aureus*(gram positive) and *Escherichia coli*(gram negative).

II. Materials And Methods

Fresh leaves of *Wedelia urticifolia* were collected from the horticulture wing of the Pondicherry University and it has been identified with the keys in consultation with plant taxonomists in the Department of Ecology and Environmental Science. *Wedelia urticifolia* (Chinese name-Dixuegen) is an erect, weak and perennial herb having elliptic leaves, bright yellow coloured flowers, terminal heads, and a light camphor-like odour (Fig 1).

Fig. 1: Habit of Wedelia urticifolia DC plant

CLASSIFICATION: Clade: Asterid II → Order: AsteralesFamily: Asteraceae
Genus:*Wedelia*Species: *urticifolia* (Blume) DC. →

2.1 Preparation of plant extract

The aqueous plant extract from freshleaves of *W. urticifolia*was prepared based on the methods adoptedby Sigamoney et al.[22]with slight modification.The fresh and healthy plant leaves were collected, washed thoroughly first with tap water and then with distilled water to remove adhering debris and associated biotabefore use. The plant leaves were cut into fine pieces and 10 grams were ground with mortar and pestle to make a paste. To this paste, 100 ml of distilled water was added in a 250 ml beaker and the solution was boiled at 60°C for 30 minutes. The boiled solution was filtered using muslin cloth followed bycentrifugation at 5000rpm for 15 minutes and finally filtered through Whatman No. 1 filter paper. The filtrate was stored at 4°C for further experimental work.

2.2 Preparation of silver nitrate solution

Silver nitrate ($AgNO_3$) was chosen as thesource of metal for the synthesis of AgNPs. $AgNO_3$was purchased from HiMedia Pvt. Ltd. Mumbai, India. $AgNO_3$ used was of analytical reagent grade. A volume of 500 ml of 1mM $AgNO_3$ salt solutionwas prepared using double distilled waterand was stored in thedarkfor further use.

2.3 Biosynthesis of silver nanoparticles

The synthesis protocol involvedtwo phases.Phase I was to find out the suitable ratio for the synthesis of ahigher amount of particles and Phase II was to prepare more of reactants based on the suitable metal salt to extract ratio found out from Phase I to produce more nanoparticles required for further use.Phase I: Overall,three ratios were designed viz: 9:1(9ml plant extract and 1ml metal salt solution), 1:1 (5ml plant extract and 5ml metal salt

solution), and 1:9 (1ml plant extract and 9ml metal salt solution). The reacted samples after the change in colour of the mixturewere then subjected to UV spectrum analysis. The ratio which showed higher values of absorbance was considered as the suitable ratio for phase II.

Phase II: From the readings ofUV spectrum, the best ratio out of the three ratios of metal salt to plant extract was selected and thesynthesis was done ina 500ml conical flask to get more nanoparticles for further studies. The colour change and UV spectrum were obtained similar to what it was in the Phase I. For Phase II, the sample of plant extract and themetal solution was kept in an auto shaker at 250 rpm at room temperature till the reaction completion to ensure thorough mixing.The AgNPs solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 10 minutes, dried and stored until further use.

2.4 Characterization

The different techniques used to characterize the AgNPs were UV-Vis spectroscopy, DLS, XRD and SEM. UV-Visible spectroscopy is a commonly used technique to measurethe quantity of nanoparticles present in the sample interms ofthe light absorbed by the sample andis used for characterizing various metal nanoparticles.DLS is used to measure the average size of particles and their size distribution. XRD was used to determine whether the particles formed were silver metal in comparison with international standards. For XRD analysis (RigakuUltima IV), the liquid phase nanoparticle solution was dried in an oven at 60°C and then in a muffle furnace at 750°C to form a powder. The dried powder was collected for characterization by an X'pert Pro x-ray diffractometer operating at 40 kV and a current of 30mA with Cu Ka radiation in θ-2θ configuration. SEM is used for knowing the morphological characteristics viz. size and topology of particles. Dried powder of silver nanoparticles produced was placed onto carbon tape attached to aluminum stubs. The samples were viewed using scanning electron microscope, Joel India Pvt. Ltd., Model JSM-6610LV.

2.5 Antibacterial activity assay

The silver nanoparticles (AgNPs) synthesizedwere tested for their antibacterial property using broth dilution method.Two clinical cultures of pathogenic organisms,*Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram-negative) were obtained from a Private Clinical Diagnostic Centre- M/s Deveraaj Diagnostics, Pondicherry. The antibacterial susceptibility test/assay was done according to EuropeanCommittee for antibacterial susceptibility test (EUCAST, Germany) document E. Dis. 5.1, 2003. Presently, 24 hours old cultures of test organisms transferred to sterilized broth were used. The broth meets the requirements of National Committee for Clinical Laboratory Standards (NCCLS). The Minimum Inhibitory Concentration (MIC) is the lowest concentration of antimicrobial agents that inhibit 99% growth of microorganisms. The bacterial suspension is adjusted to a turbidity equivalentto that of 0.5 McFerland Standard. Each tube is inoculated with approximately 5×10^5 cfu/ml (colony forming units per milliliter). Once in four hours, the turbidity interms of OD is measured photometrically in 600nm (OD_{600}). The OD values for 24 hours with the interval of 4 hours have been plotted in the graph.

III. Results

3.1 Colourchange

The bioreduction of silver ions was visually evident from achange incolour of solution from smoky white to dark brown compared to control.Fig. 2 depicts the change in colour within 6 hours of reaction at room temperature.

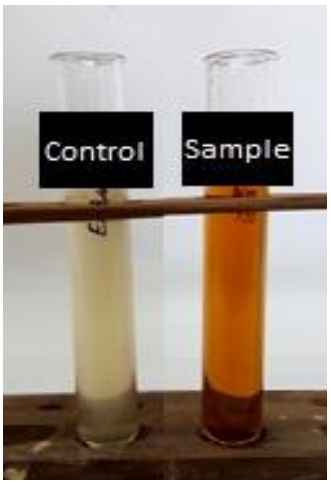


Fig. 2: Reaction tubes at the end of 6 Hours showing colour change

3.2 UV Spectrophotometer

The reduction of silver nitrate into silver ions and silver nanoparticles was monitored by analyzing the samples using the UV-Visible spectrophotometer after 6 hours of reaction time. The scanning range employed was from 200 to 800 nm. Strong surface plasmon resonance (SPR) bands were observed at 450 nm. This confirmed the formation of AgNPs. The spectra presented in Fig 3 shows the maximum absorption value of 1.9 in 1E:1M ratio at 450 nm followed by 9E:1M and 1E:9M with maximum absorption values of 1.5 and 0.5 at 450 nm and 451.5 nm respectively. The UV-Vis spectrum results concluded that among the three ratios of the extract of *W. urticifolia* evaluated, the ratio of 1:1 of leaf extract to 1 mM $AgNO_3$ solution is found advantageous for AgNPs synthesis. Hence, the same ratio was taken for Phase-II to produce more number of nanoparticles.

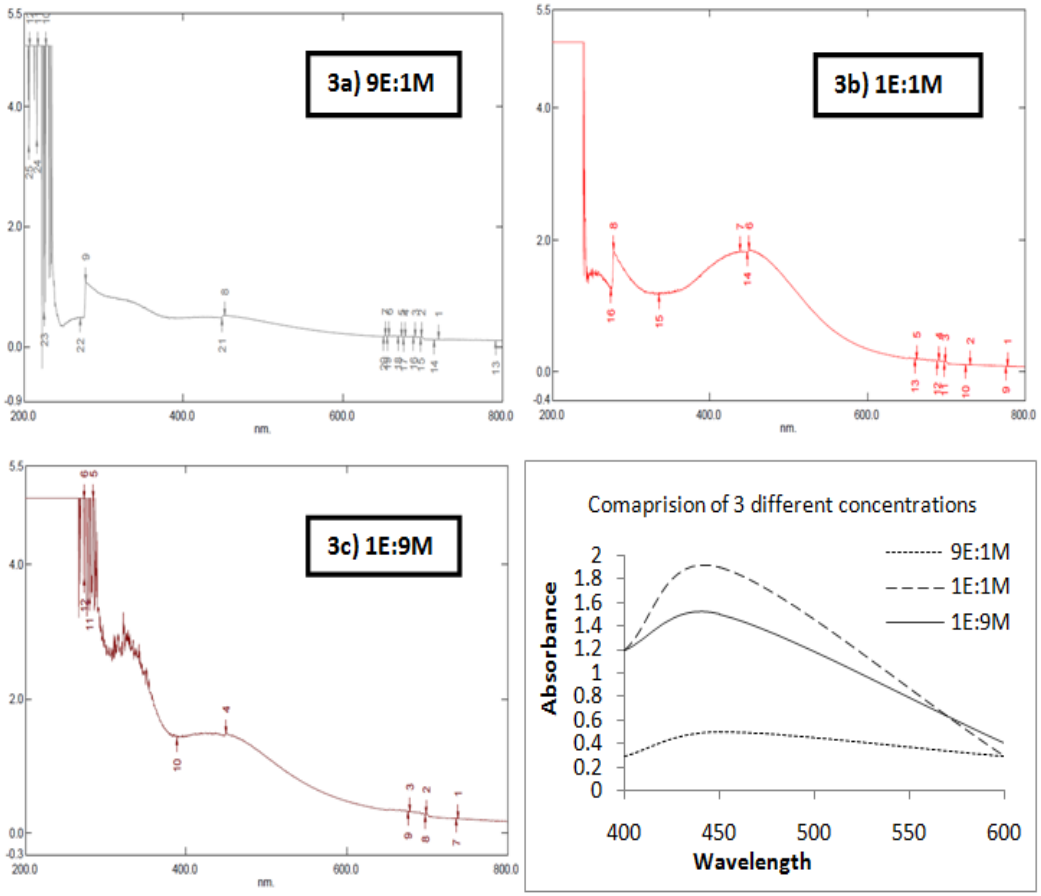
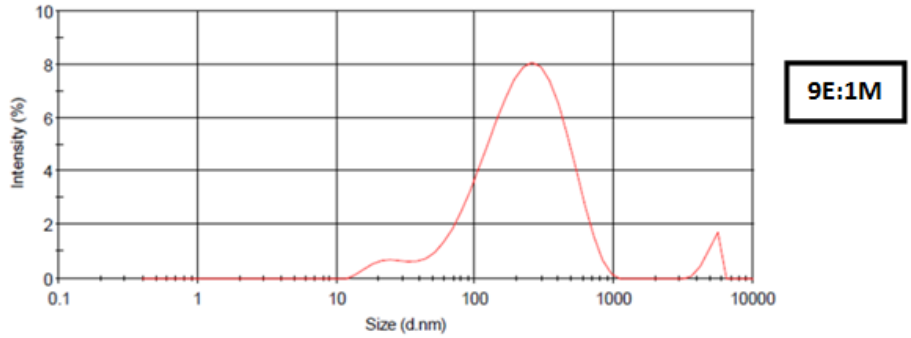


Fig 3: UV-visible spectra recorded for reaction mixture of three different ratios ofplant extract (E) to metal salt (M)

3.3 The Dynamic Light Scattering

The DLS (Dynamic Light Scattering) of the AgNPs is presented in Fig 4 and was carried out by Zeta-Sizer. The average size of the AgNPs was found to be 179.3 nm, 90.38 nm and 80.28 nm for 9E:1M, 1E:1M and 1E:9M respectively.



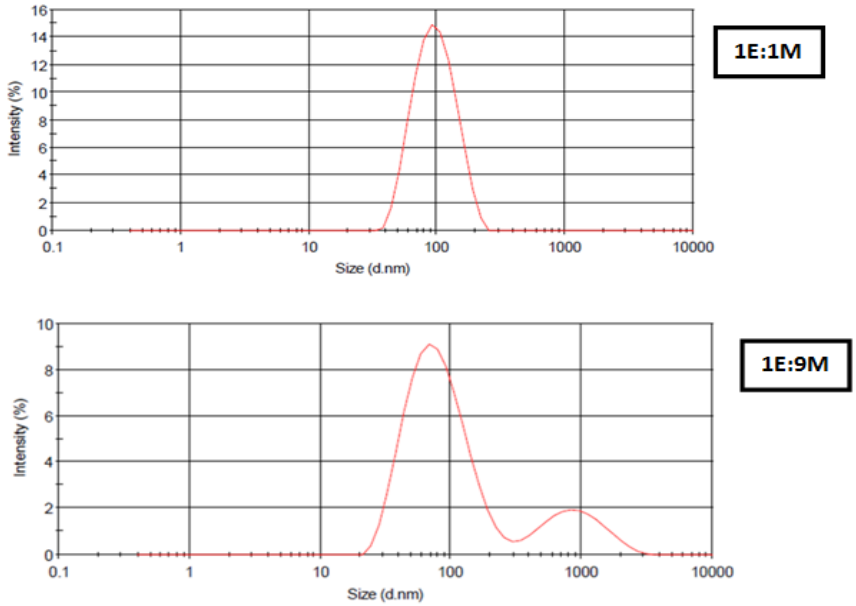


Fig 4: DLS bar graph of silver nanoparticles synthesized with different concentrations

3.4 X-Ray Diffraction (XRD)

The results of XRD are presented in spectrum i.e, Fig 5. The 2θ peak values were observed at 38.22, 44.42, 64.56 and 77.50. The results were compared with the XRD standard of AgNPs (PDF Card - 01-087-0720_Ag) which has values of 38.20, 44.40, 64.60 and 77.60 and corresponds to Bragg's diffraction at 111, 200, 220 and 311 planes of the lattice structure. Maximum peak was obtained at 38.22. These results confirmed that the synthesized nanoparticles are silver metal with the crystalline structure.

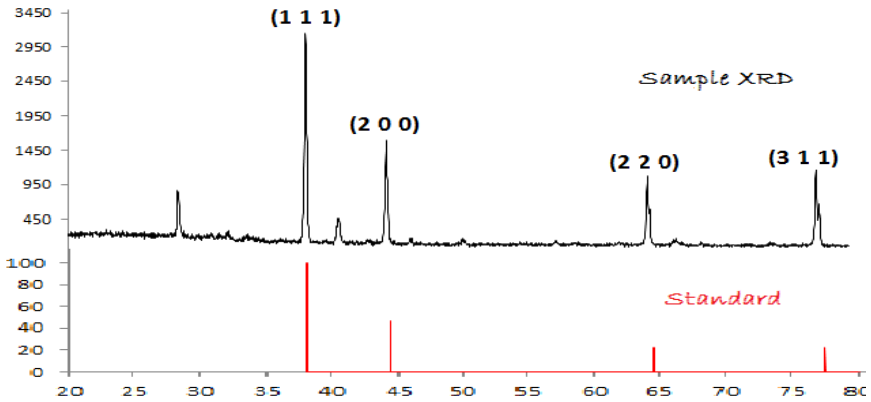


Fig 5: XRD spectrum of nanoparticles obtained withstandard

3.5 SEM

The SEM image of AgNPs is presented in Fig 6. The synthesized AgNPs were found to be agglomerated, but poly-dispersed and crystalline in nature.

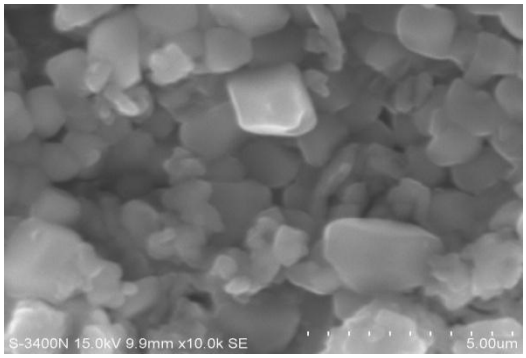


Fig 6: SEM image of silver nanoparticles

3.6 Antibacterial activity

The antibacterial activity test revealed that the overall inhibitory effect is found to be dosedependantin boththe bacterial species. The MIC of AgNPs is found to be400µg for gram-positive bacteria (*S.aureus*) and 100µg for gram-negative bacteria(*E.coli*). AgNPs exhibited a high level of inhibitory action against *E.coli*.The growth profile of the two species with variation in concentration of AgNPs is depicted in Fig. 7.

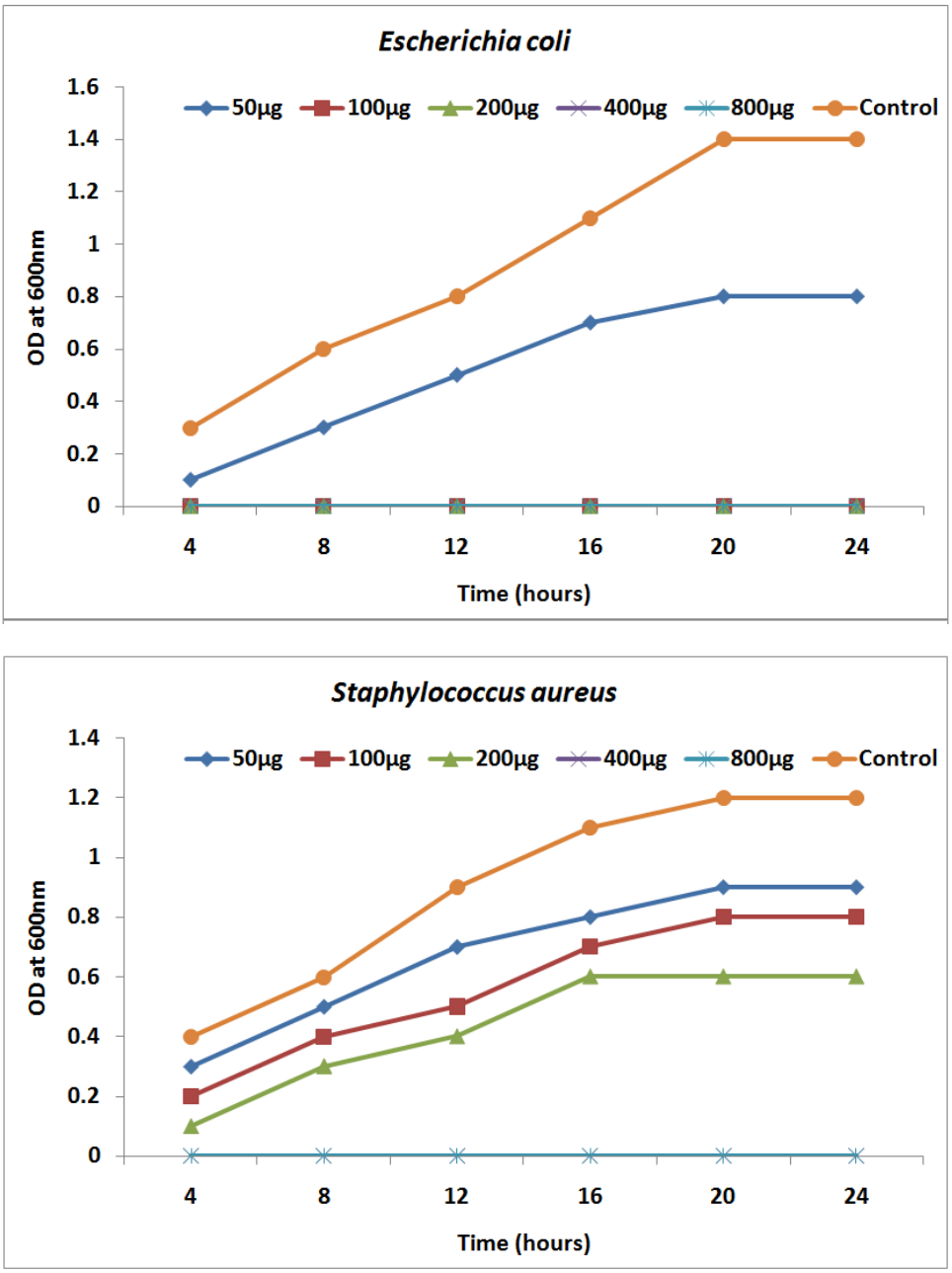


Fig. 7: Assessment of MIC using broth dilution method

IV. Discussion

In the present study, taking the leaf extract of *Wedeliaurticifolia* based on its phytochemical constituents, an attempt was made to synthesiseAgNPs. During the reaction, in 6 hours the brown colourformation took place indicating the formation of AgNPs. This kind of colour change in AgNPs synthesis was also reported by [1,23,24,25,26,27,28] using anaqueous extract ofdifferent plant parts.Typical peak for AgNPsobserved generally ranges from 418 to 460nm [1].UV spectra obtained in the present study displayed absorption peak at 450nm which is specific to AgNPs.The results were similar to the previous study done by Jain etal. [29], who observed the absorbance peak at 450 nm. Peaks between 400 and 450nm were observed in UV-Vis spectrum of AgNPs by Sandeep et al. [27].Nestor et al. [7] reported that thepeak in absorbance around

430 nm in UV-Vis spectra is a characteristic of AgNPs. AgNPs synthesis using *Cinnamomumcamphora*showeda sharp absorbance at around 440 nm[30].Subramani et al. [31]reported that the AgNPsabsorption spectra have absorbance maxima at 421 nm. The absorbance peak of AgNPs occurs at 451nm which is a narrow peak with an increase in absorbance due to increase in the formation of a number of nanoparticles as a result of the silver ions reduction present in the aqueous solution. AgNPs synthesized using extracts of *Ananascomosus* displayedthe characteristic UV-Vis absorption peak at 430 nm whichconfirmed their formation[32]. Absorption spectra of AgNPs formed in the reaction mixturehave anabsorption peak at 420-510 nm and the broadening of thepeakindicates the polydispersed nature of particles [33]. Daniel et al. [34] stated SPR peaks were observed from 410 to 440nm for AgNPs synthesized using plants.Balazet al. [35] also synthesizedAgNPs using the plant extract of *Origanumvulgare* and obtained peak at 445nm.Manikandan et al. [28] also observed the peak at 445 nm in AgNPs solution obtained from anaqueous extract of *Phyllanthusacidus* fruit.

The XRD showed 2θ values ranging from 20˚ to 80˚. A comparison of XRD spectrum obtained for the NPs synthesized in the present study with the international standard spectrum confirmed that the nanoparticles formed in the present experiment were of the silver metal particles, as demonstrated by the four peaks at 2θ values of 38.45˚, 44.48˚, 64.69˚ and 77.62˚, which correspond to 111, 200, 220 and 311 planes for silver respectively and which showed similarity with the database of Joint Committee on Powder Diffraction Standards (JCPDS file No. 04-0783).The XRD peaks exhibited by the synthesizedAgNPs of *Ananascomosus*have also shown peaks at 38.45, 44.48, 64.69 and 77.62[32].A similar pattern was also obtained by Kalidasan and Yogamoorthi [25], Manikandan, et al. [28], Krishnaraj et al. [36],Santhoshkumar et al. [37],and Kudle et al. [38]. The results, thus illustrate that the synthesizedAgNPs are crystalline in nature. Characterization by Scanning Electron Microscopy was carried out to know the structure of the reaction product that was formed. SEM was conducted to know the size and topology of silver nanoparticles obtained. The SEM image of the AgNPs synthesized in the present study indicatesthe individual silver particles as well as agglomerated particles with arough surface. Manikandan et al. [28] observed AgNPs under SEM and found that most of the nanoparticles were aggregated and just a few of them were scattered. Ali et al. [39] attributed that the agglomeration of nanoparticles is due to induced-dehydration. Similar observations were also made by Kalidasan and Yogamoorthi [25], Daniel et al. [34], Song and Kim [40] and Prashanth et al. [41]. So, further particle processing is needed before it can be used for any other purpose.Formation of nanoparticles by reducing the silver salt might be due to the type of phytochemical constituents present in the study plant, *Wedelia urticifolia*. It has been reported that members of the genus *Wedelia*contain terpenes, steroids, flavonoids, coumarins, cyclitols, and organic acids [42] and eleven monoterpene hydrocarbons (53.09%) were found in leaves, with a-pinene (15.57%), d-limonene (11.19%), a-phellandrene (9.69%) and g-terpinene (9.01%) as the main ones and also some relatively similar compounds [43]. Probably, these phytochemical constituents might have actedasa reducing agent reacting with silver nitrate to form AgNPs. The concentration of the extract might have also influenced the efficiency of nucleation of nanoparticles.

Secondly, the antibacterial efficacy of the presentlysynthesized AgNPs was tested.The antibacterial activity test revealed that the overall inhibitory effect is found to be dose-dependent in both the tested bacterial species. The MIC of AgNPs is arrived at as 400 µg for gram-positive bacteria (*S. aureus*) and 100 µg forgram-negative bacteria (*E.coli*). Silver nanoparticles exhibited a higher level of antibiotic potential on *E.coli*. Similar results were also reported by Kaviya et al.[44] who synthesized AgNPs using anextract of orange peelings (*Citrus sinensis*) and found their highest antibacterial activity against *E. coli*than*S. aureus*.Benakashani et al.[45] reported that the gram negative bacteria were more subtle than gram-positive bacteria to biologically synthesize AgNPs using *Capparisspinosa*leaf extract.In the present study, *S. aureus*, the gram-positivebacteria was less sensitive to synthesized AgNPs thangram-negative bacteria*E.coli*. The probable factor for the less susceptibility of*S.aureus* observed in the present study could beattributed to the thickness of their cell wall; the gram-positive bacteria have thicker cell wall than gram negative bacteria, as the cell wall is made up of peptidoglycan molecules. Ankanna et al. [46] explained that the peptidoglycan is negatively charged and silver ionshave positive charge, due to which more silver ions may get fixedto peptidoglycan. Kim et al, [47] also attributed the less susceptibility of gram-positive bacteria to their thicker cell walls compared to that of gram-negative bacteria.With the increase in the AgNPs concentration, the greater lag phase in growth was seen and lesser OD values were found at higher concentrations indicating a dose-dependent activity. Similar kind of lag phase in growth profile was observed in previous studies [48,49]. With the increase in the AgNPs concentration, the growth of the bacteria got reduced and then stopped. The differences in the MIC could be credited to the differences in surface charge and size of AgNPs [50,51]. AgNPs with 1-10nm size range has been reported to be most effective against bacterial species [52]. Zarei [53] reported MIC in the range of 3-25 µg/mL of 2-25nm colloidal AgNPs for *E. coli* at initial concentration of 105-108 cfu/mL. Moreover, Pal et al. [54] found that the nanoparticle and *E. coli* bacteria surface interaction is shape-dependent. Thus, it is understood that as the size of nanoparticles used (90.38nm) in the current bioassay tests is more than the previous studies, the MIC values for both the species *E. coli* and *S. aureus* are found to be higher.

The exact mechanisms of antimicrobial or toxicity activities by AgNPsare still a debatable subject matter among microbiologists. However, referring to the biophysical properties of nanoparticles in general and characteristics of silver ions/NPs, in particular,the mechanism might be involving more than one cellular biochemical kinetics. Ag^{2+} ions/nanoparticles are able to form ligandswith cell organelles which in turn form ligands with nucleic acids and they specifically interact with the nucleosides instead of phosphate groups of nucleic acids[48,52,55]. Besides, there is anelectrostatic attraction betweenthe positive charge of nanoparticles and negative charge of bacterial cells[56] which facilitate ligand formation.Other mechanismsinvolve theinteraction of silver molecules with the biological macromolecules which include DNA and enzymesby an electron-release mechanism[57] or free radical production[48]. The three-dimensional structure of proteins is changed by both AgNPsand silver ions as they interfere with the disulphide bonds and chunk the functional processes of the microorganism[58,59,60].It has been proposed that the AgNPs induce the inhibition of protein and cell wall synthesis [61]. Nanoparticles curb thephosphotyrosine profile of bacterial peptide which in turn disturbssignal transduction and inhibits thegrowth of micro-organisms[5].

V. Conclusion

In the present study, the aqueous leaf extract of *Wedelia urticifolia* at 1:1 ratio (metal salt:extract) is found to be more suitable for harvesting higher amount of AgNPs within 6 hours. Further, it is also reported from the present study that the leaf of *W. urticifolia*is a potential candidate for the synthesis of AgNPs as the UV-Vis absorbance is as high as 1.5. The results obtained from the antibacterial assay reveal that AgNPs are more potent in inhibiting thegrowth ofgram-negative bacterial species rather than gram-positive bacterial species. It would be more effective if further studies are done on the isolation of individual organic reductants present in the leaf extract for the synthesis of AgNPs and it is hopefully envisaged that AgNPs would serve as magic nanorobots that could search and kill the target cell and/or pick and place the drug in the specified site precisely in the near future.

Acknowledgements

The authors are grateful to the UGC for providing scholarship during the study period to MYR. Special thanks are to CIF, Pondicherry University for DLS reports. The authors are also sincerely grateful to the Head, Department of Nanosciences for XRD characterization and Annamalai University for SEM analysis. We also express our thanks toa Private Clinical Diagnostic Centre- M/s Deveraaj Diagnostics, Pondicherry.

References

[1] M. Vanaja andG. Annadurai.*Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. Applied Nanoscience. 3, 2013,211–223.DOI 10.1007/s13204-012-0121-9

[2] S. Dhuper, D. Panda and P. L. Nayak. Green synthesis and characterization of zero-valent iron nanoparticles from the leaf extract of *Mangiferaindica*. Nano Trends: Journal of Nanotechnology. 13(2), 2012, 16–22.

[3] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar and M. Sastry**.**Extracellular biosynthesis of silver nanoparticles using the fungus *Fusariumoxysporum*. Colloids and Surfaces B: Biointerfaces. 28(4), 2013, 313–318.https://doi.org/10.1016/S0927-7765(02)00174-1.

[4] T. Klaus-Joerger,R. Joerger,E. Olsson and C. Granqvist.Bacteria as workers in the living factory: metal accumulating bacteria and their potential for materialsscience. Trends in Biotechnology. 19(1), 2001, 15–20.

[5] S. Ahmed, M. Ahmad, B. L. Swami and S. Ikram. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise.Journal of Advanced Research. 7(1), 2016, 17–28.https://doi.org/10.1016/j.jare.2015.02.007.

[6] J. Kasthuri, S. Veerapandian, and N. Rajendiran.Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids and Surfaces. B: Biointerfaces. 68(1), 2009, 55–60.DOI: 10.1016/j.colsurfb.2008.09.021

[7] A. R. V. Nestor, V. S. Mendieta, M. A. C. Lopez, R. M. G. Espinosa, M. A. C. Lopez and J. A. Alatorre**.** Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camielliasinensis* extract.Materials Letters. 62(17-18), 2008, 3103–3105.https://doi.org/10.1016/j.matlet.2008.01.138.

[8] J. L. Gardea-Torresdey, E. Gomez, J. R. Peralta-Videa, J. G. Parsons, H. Troiani and M. Jose-Yacaman. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir. 19(4), 2003, 1357– 1361.DOI: 10.1021/la020835i

[9] S. S. Shankar, A. Ahmad and M. Sastry. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnology Progress. 19(6), 2003, 1627– 1631.DOI: 10.1021/bp034070w.

[10] S. Li, L. Qui, Y. Shen, A. Xie, X. Yu, L. Zhang and Q. Zhang. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract. Green Chemistry. 9, 2007, 852–858.DOI:10.1039/B615357G.

[11] P.Logeswari, S. Silambarasan and J. Abraham. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society. 19(3), 2015, 311–317.https://doi.org/10.1016/j.jscs.2012.04.007.

[12] K.Venugopal, H. A. Rather, K.Rajagopal, M. P.Shanthi, K. Sheriff, M.Illiyas, R. A. Rather, E.Manikandan, S.Uvarajan, M.Bhaskar and M. Maaza. Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of *Syzygiumaromaticum*. Journal of Photochemistry & Photobiology, B: Biology 167, 2017, 282–289.https://doi.org/10.1016/j.jphotobiol.2016.12.013.

[13] A. Nabikhan, K. Kandasamy, A. Rajand N. M. Alikunhi. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuviumportulacastrum* L. Colloids and Surfaces B: Biointerfaces 79(2), 2010, 488–93.doi: 10.1016/j.colsurfb.2010.05.018.

[14] V. Gopinatha, M. D. Ali, S. Priyadarshini, N. M. Priyadharsshini, N. Thajuddinb andP. Velusamy. Biosynthesis of silver nanoparticles from *Tribulusterrestris* and its antimicrobial activity: a novel biological approach. Colloids andSurfaces B: Biointerface, 96, 2012, 69–74.DOI: 10.1016/j.colsurfb.2012.03.023.

[15] R. Mariselvam, A. J. Ranjitsingh, A. Usha Raja Nanthini, K. Kalirajan, C. Padmalatha, and P. M.Selvakumar. Green synthesis of silver nanoparticles from the extract of the inflorescence of *Cocosnucifera* (Family: Arecaceae) for enhanced antibacterial activity. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy.129, 2014, 537–41.https://doi.org/10.1016/j.saa.2014.03.066

[16] K. Govindaraju, S. Tamilselvan, V. Kiruthiga andG. Singaravelu. Biogenic silver nanoparticles by *Solanumtorvum* and their promising antimicrobial activity. Journal ofBiopesticides. 3(1), 2010, 394–399.

[17] R. GeethalakshmiandD. V. L. Sarada. Synthesis of plant-mediated silver nanoparticles using *Trianthemadecandra* extract and evaluation of their anti-microbial activities.International Journal of Engineering Sciences and Technology. 2(5), 2010, 970–975.

[18] N. Khandelwal, A. Singh, D. Jain, M. K. Upadhyaand H. N. Verma. Green synthesis of silver nanoparticles using *Argimonemexicana*leaf extract and evaluation of their antimicrobial activities. Digest Journal of Nanomaterials andBiostructures. 5(2), 2010, 483–489.

[19] S. A. Kumar,S. Ravi, V. Kathiravan and S. Velmurugan. Synthesis of silver nanoparticles using *A. indicum* leaf extract and their antibacterial activity. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy. 134 (3), 2015,34–39.https://doi.org/10.1016/j.saa.2014.05.076

[20] Y. Zhang, D. Yang, Y. Kong, X. Wang, O. Pandoli and G. Gao. Synergetic antibacterial effects of silver nanoparticles *Aloe Vera* prepared via a green method. Nano Biomedicine and Engineering.2(4), 2010, 252–7.

[21] B. Sadeghi, A. Rostami andS. S. Momeni. Facile green synthesis of silver nanoparticles using seed aqueous extract of *Pistaciaatlantica* and its antibacterial activity. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy.134, 2015, 326–32.doi: 10.1016/j.saa.2014.05.078

[22] M. Sigamoney, S. Shaik, P. Govender, S. B. N. Krishna andSershen. African leafy vegetables as bio-factories for silver nanoparticles: A case study on *Amaranthusdubius* C. Mart. Ex Thell. South African Journal of Botany. 103,2016, 230–240. https://doi.org/10.1016/j.sajb.2015.08.022.

[23] R. R. R. Kannan, W. A. Stirk andJ. V. Staden. Synthesis of silver nanoparticles using the seaweed *Codiumcapitatum* P.C Silva (Chlorophyceae). South African Journal of Botany. 86,2013, 1–4.https://doi.org/10.1016/j.sajb.2013.01.003

[24] M. Vijayakumar, K. Priya, F.T. Nancy, A. Noorlidah and A.B.A. Ahmed. Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. IndustrialCrops and Products. 41, 2013, 235–240.https://doi.org/10.1016/j.indcrop.2012.04.017.

[25] M. Kalidasan andA. Yogamoorthi. Biosynthesis of silver nanoparticles using *Achyranthusaspera* and its characterization. International Journal of Nanomaterials and Biostructures. 4(1), 2104, 5-11.

[26] D. Bose andS. Chatterjee. Biogenic synthesis of silver nanoparticles using guava (*Psidiumguajava*) leaf extract and its antibacterial activity against *Pseudomonas aeruginosa*. Applied Nanoscience. 6, 2016, 895–901. DOI 10.1007/s13204-015-0496-5.

[27] S. Patil, G. Chaudhari, J. Paradeshi, R. Mahajan and B. L. Chaudhari. Instant green synthesis of silver-based herbo-metallic colloidal nanosuspension in *Terminaliabellirica* fruit aqueous extract for catalytic and antibacterial applications. 3 Biotech. 7(1), 2017 7:36. DOI 10.1007/s13205-016-0589-1.

[28] R. Manikandan, M. Beulaja, R. Thiagarajan, S. Palanisamy, G. Goutham, A. Koodalingam, N.M. Prabhu, E. Kannapiran, M. JothiBasu, C. Arulvasu and M. Arumugam. Biosynthesis of silver nanoparticles using aqueous extract of *Phyllanthusacidus* L. fruits and characterization of its anti-inflammatory effect against H_2O_2 exposed rat peritoneal macrophages. Process Biochemistry. 55, 2017, 172–181.https://doi.org/10.1016/j.procbio.2017.01.023.

[29] D. Jain, S. Kachhwahaa, R. Jain, G. Srivastavaa and S. L. Kotharia. Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. Indian Journal of Experimental Biology. 48, 2010, 1152-1156.

[30] J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He, J. Hong and C. Chen. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomumcamphora* leaf.Nanotechnology. 18(10), 2007, 105104.doi:10.1088/0957-4484/18/10/105104

[31] V. Subramani, J. J.Jeyakumar, M. Kamaraj and B. Ramachandran. Plant Extracts Derived Silver Nanoparticles. International Journal of Pharma Research & Review. 3(3), 2104, 16-19.

[32] N. Ahmad and S. Sharma. Green Synthesis of Silver Nanoparticles Using Extracts of *Ananascomosus*. Green and Sustainable Chemistry. 2, 2012, 141-147.

[33] S. P. Chandran, M.Chaudhary, R. Pasricha, A. Ahmad and M.Sastry. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnology progress. 22(2),2006, 577-583.

[34] S.C.G. K. Daniel, B. N.Banu , M. Harshiny, K. Nehru, P. S. Ganesh, S. Kumaran and M. Sivakumar. *Ipomeacarnea*-based silver nanoparticle synthesis for antibacterial activity against selected human pathogens. Journal of Experimental Nanoscience. 9(2), 2014, 197–209. http://dx.doi.org/10.1080/17458080.2011.654274

[35] M. Balaz, L. Balazova,N. Daneu,E. Dutkova, M. Balazova, Z. Bujnakova and Y. Shpotyuk. Plant-Mediated Synthesis of Silver Nanoparticles and Their Stabilization by Wet Stirred Media Milling. Nanoscale Research Letters. 12:83, 2017. DOI:10.1186/s11671-017-1860-z.

[36] C. Krishnaraj, E. G. Jagan,S. Rajasekar, P. Selvakumar, P. T. Kalaichelvan and N. Moha. Synthesis of silver nanoparticles using *Acalyphaindica* leaf extracts and its antibacterial activity against water borne pathogens.Colloids and Surfaces B: Biointerfaces 76(1), 2010, 50–56.https://doi.org/10.1016/j.colsurfb.2009.10.008.

[37] T. Santhoshkumar, A. A. Rahuman, G. Rajakumar, S. Marimuthu, A. Bagavan, C. Jayaseelan, A. A. Zahir, G. Elango and C. Kamaraj. Synthesis of silver nanoparticles using *Nelumbonucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res. 108, 2011, 693–702. DOI:10.1007/s00436-010-2115-4

[38] K. R. Kudle,M. R.Donda, J. Alwala, R. Koyyati, V. Nagati, R. Merugu andM. P. Rudra. Biofabrication of silver nanoparticles using *Cuminumcyminum* through microwave irradiation. International journal of Nanomaterials and Biostructures,2(4), 2012, 65-69

[39] D. M. Ali, N.Thajuddin, K. Jeganathan and M.Gunasekaran. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces 85(2),2011, 360–365.https://doi.org/10.1016/j.colsurfb.2011.03.009.

[40] J. Y. Song and B. S. Kim. Biological synthesis of bimetallic Au/Ag nanoparticles using Persimmon (*Diopyros kaki*) leaf extract. Korean Journal of Chemical Engineering, 25(4), 2008, 808-811.

[41] S. Prashanth, I. Menaka, R. Muthezhilan andN. K. Sharma. Synthesis of plant-mediated silver nanoparticles using medicinal plant extract and evaluation of its antimicrobial activities. International Journal of Engineering Science and Technology. ISSN, 0975-5462, 3(8), 2011, 6236-6250.

[42] X. Li, M. Dong, Y. Liu, Q.W. Shi andH.Kiyota. Structures and biological properties of the chemical constituents from the genus *Wedelia*. Chemistry and Biodiversity. 4,2007, 8-23.DOI: 10.1002/cbdv.200790070.
[43] L. Zhu, Y.J Tian, Y.C Yin and S.M. Zhu. Chemical composition and antimicrobial activities of the essential oils from flowers, leaves, and stems of *Wedelia urticifolia*. Italian Journal of Food Science. 24(1),2012, 19-25.
[44] S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary and K. Srinivasan. Biosynthesis of silver nanoparticles using *Citrus sinensis*peel extract and its antibacterial activity. SpectrochimiaActa Part A: Molecular and Biomolecular Spectroscopy. 79(3), 2011, 594-598.
[45] F. Benakashani, A. R. Allafchian and A.H. Jalali. Biosynthesis of silver nanoparticles using *Capparisspinosa* L. leaf extract and their antibacterial activity. Karbala International Journal of Modern Science. 2(4), 2016, 251-258.
[46] S. Ankanna S, T. N. V. K. V. Prasad, E. K. Elumalai, and N. Savithramma. Production of biogenic silver nanoparticles using *Boswelliaovalifoliolata* stem bark. Digest Journal of Nanomaterials andBiostructures. 5(2), 2010, 369–72.
[47] J. S. Kim, E. Kuk, K. N. Yu, J. H. Kim, S. J. Park, H. J. Lee, S. H. Kim, Y. K. Park, Y. H. Park, C. Y. Hwang, Y. K. Kim, Y. S. Lee, D. H. Jeong and M. H. Cho. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 3, 2007, 95–101.
[48] I. Sondi and B. Salopek-Sondi. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. Journal of Colloid and Interface Science. 275(1), 2004, 177–82.https://doi.org/10.1016/j.jcis.2004.02.012.
[49] J. P. Ruparelia, A. K. Chatterjee, S. P. Duttagupta and S.Mukherji. Strain specificity in antimicrobial activity of silver and copper nanoparticles. ActaBiomaterialia 4(3), 2008, 707–716.https://doi.org/10.1016/j.actbio.2007.11.006.
[50] Z. Khan, S. A. Al-Tnabaiti,E. H. El-Mossalamy and A. Y. Obaid. Effect of macromolecule poly(vinyl alcohol) on the growth of cetyltrimethylammonium bromide stabilized Ag-nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 352(1-3), 2009, 31–37. https://doi.org/10.1016/j.colsurfa.2009.09.045.
[51] H. Y. Song, K. K. Ko, I. H. Oh and B. T. Lee. Fabrication of silver nanoparticles and their antimicrobial mechanisms. European Cells and Materials. 11(1), 2006,58–59.
[52] J. R. Morones, J. L.Elechiguerra, A. Camacho, K. Holt, J. B.Kouri, J. T. Ramfrez and M. J. Yacaman. The bactericidal effect of silver nanoparticles. Nanotechnology 16, 2005, 2346–2353.doi:10.1088/0957-4484/16/10/059
[53] M. Zarei, A. Jamnejad andE. Khajehali. Antibacterial effect of silver nanoparticles against four foodborne pathogens. Jundishapur Journal of Microbiology. 7(1), 2014, e8720. doi: 10.5812/jjm.8720. [PubMed: 25147658].
[54] S. Pal, Y. K. Tak, and J. M. Song. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. Applied and Environmental Microbiology. 73(6), 2007, 1712–1720.doi:10.1128/AEM.02218-06.
[55] Y. Yakabe, T. Sano, H. Ushio and T. Yasunaga. Kinetic studies of the interaction between silver ion and deoxyribonucleic acid. Chemistry Letters. 9(4), 1980, 373–376.https://doi.org/10.1246/cl.1980.373.
[56] Y. W. Cao,R. Jin and C. A. Mirkin. DNA-modified core–shell Ag/Au nanoparticles. Journal of American Chemical Society.123, 2001, 7961–7962.
[57] V. K. Sharma, R. A. Yngard and Y. Lin. Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science.145(1-2), 2009, 83–96.https://doi.org/10.1016/j.cis.2008.09.002.
[58] B. Sadeghi and F. Gholamhoseinpoor. A study on the stability and green synthesis of silver nanoparticles using *Ziziphoratenuior* (Zt) extract at room temperature. SpectrochimicaActa Part A: Molecular andBiomolecuular Spectroscopy.134, 2015, 310–315.http://dx.doi.org/10.1016/j.saa.2014.06.046.
[59] X. Jia, X. Ma, D. Wei, J. Dong, and W. Qian. Direct formation of silver nanoparticles in cuttlebone derived organic matrix for catalytic applications. Colloids and Surfaces A: Physicochemical and Engineering Aspects.330(2-3), 2008, 234–240.https://doi.org/10.1016/j.colsurfa.2008.08.016.
[60] M. Rai, A. Yadav and A. Gade, Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 27(1), 2009, 76–83.https://doi.org/10.1016/j.biotechadv.2008.09.002.
[61] J. Park, D. H. Lim, H. J. Lim, T. Kwon, J. S. Choi, S. Jeong, I. H. Choi and J. Cheon. Size dependent macrophage responses and toxicological effects of Ag nanoparticles. Chemical Communications. 47, 2011, 4382–4384.DOI: 10.1039/c1cc10357a

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

MohdYousuf Rather. "Biosynthesis and characterization of silver nanoparticles using leaf extract of *Wedelia urticifolia*(Blume) DC and evaluation of antibacterial efficacy." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 12.4 (2017): 14-23.