Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 4, 2015

Original Article

GREEN SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND CYTOTOXIC EFFECTS OF SILVER NANOPARTICLES USING *ORIGANUM HERACLEOTICUM* L. LEAF EXTRACT

RAVIKUMAR RAJENDRAN[1], NITHYA GANESAN[2], SATHEESH KUMAR BALU[3], SRINIVASAN ALAGAR[4], PREMKUMAR THANDAVAMOORTHY[1], DEVAKI THIRUVENGADAM[1]*

[1]Department of Biochemistry, Guindy Campus, University of Madras, Chennai 600025, TN, India, [2]Bharathidasan University, Trichy, TN, India, [3]Department of Bioelectronics and Biosensors, Alagappa University, Karaikudi, TN, India, [4]Department of Nanoscience and Technology, Alagappa University, Karaikudi, TN, India
Email: devakit@yahoo.co.uk

Received: 28 Jan 2015 Revised and Accepted: 25 Feb 2015

ABSTRACT

Objectives: Silver nanoparticles (AgNPs) possess unique features among noble metal nanoparticles due to their wide range of applications in various field including medicine, environmental safety, etc. The present study relies on eco-friendly, cost effective method for the synthesis of AgNPs.

Methods: AgNPs were rapidly synthesized from $AgNO_3$ using aqueous leaf extract of *O. heracleoticum* L. as reducing as well as capping agent. Synthesized AgNPs were characterized with the help of FE-SEM, XRD, EDS and UV–vis absorption spectroscopy. FE-SEM showed the spherical nature of nanoparticles with the size ranging from 30-40 nm. In addition, their antibacterial activities against standard bacterial pathogens were evaluated as per the standard disk diffusion method. Further, cytotoxicity potential of AgNPs were also determined using the MTT assay.

Results: X-ray diffraction analysis showed that the nanoparticles formed in the present synthesis were crystalline in nature. FT-IR analysis showed the presence of possible biomolecules needed for reducing silver ions. Further, antibacterial activity of synthesized AgNPs showed effective inhibition against pathogenic microbes. In addition, these green synthesized nanoparticles were shown to exhibit potential cytotoxic effect on MCF-7 cell line in dose dependent manner.

Conclusion: It has been demonstrated that *O. heracleoticum* L. plant extract could be used as a proficient green reducing agent for the synthesis of AgNPs. Further, these synthesized AgNPs were shown to exhibit effective antibacterial effect against pathogens and potential cytotoxic effect on MCF-7 cells.

Keywords: AgNPs, *O. heracleoticum* L., Aqueous extract, Pathogenic microbes, Cytotoxicity.

INTRODUCTION

For the past 40 years, the foundations were set down for nanotechnologies to deliver diagnostic and therapeutic agents in a secure and more systematic manner [1]. Attaining this prescience became more pragmatic in recent years, with increasing numbers of nano based therapeutics and diagnostics being profit-oriented or having reached clinics [2]. Advances in Nanoscience are promptly enabling the evolution of nanoparticles with distinct properties which address the limitations of traditional disease diagnostic and therapeutic agents [3]. In 1959, Richard P. Feynman, Physicist at California Institute of Technology stated that "There is plenty of room at the bottom" starting from atomic level and scaling down to the nanolevel which provides a route to forthcoming technology and advancement. From there, Nanotechnology has now educed into much innovation in all areas of Science including Medicine, new therapeutic, tissue engineering, diagnostic concepts, drug delivery, gene silencing and vehicles for targeted drug delivery system [4, 5]. Nanomedicines were declared to augment adequacy, specificity, durability and therapeutic index of analogous drugs [6, 7]. Nanoparticles have immense application including their use in highly sensitive diagnostic assays, drug and gene delivery, thermal ablation and radiotherapy enhancement, antimicrobial agents, sensors, optical, electronic, and catalytic application [8]. Green synthesis and characterization of nanoparticles have attained a notable segment of Nanotechnology in the past decade, peculiarly for noble metals such as Au, Ag, Pt and Pd. Moreover, the physical or chemical approaches are normally engaged to synthesize metal nanoparticles because of their indelible benefit in assembling well-defined NPs with quite tractable sizes and shapes. These methods involve more expensive and tedious treatments such as laser ablation, hydrothermal synthesis, solvothermal synthesis, pyrolysis and inert gas condensation [9]. In contrast to this, several research groups have brought about success in the synthesis of NPs profit by biological raw materials, an alternative to lethal chemicals and the expensive physical methods. At present, feasible initiatives that use green Chemistry to enhance and/or protect our surroundings are becoming important affair in various fields of research. Natural products exhibit one of the most abiding approaches to this goal. Plants have been confirmed to be one of affluent sources of natural products. Moreover, using this kind of approaches represent a development of nature-friendly and cost-effective approach [10].

Metal nanoparticles have received great attention due to their eccentric and unusual physico-chemical properties. At present, synthesis of nanoparticles is a crucial area of research, probing for a nature-friendly manner for current science. Countless methodologies are emerged to synthesize noble metal nanoparticles of specific shape and size depending upon requirement. Green synthesis of nanoparticles has an emerging acme of junction between Biotechnology and Nanotechnology which has earned notable attention to a growing need to establish environmentally amiable technologies in nanoparticle synthesis [11, 12]. In particular, extracts obtained are more beneficial because using them eliminates the tedious process of maintaining cell cultures and can be suitably restrained for extensive production under non aseptic environments. Amongst various metal nanoparticles, AgNPs are globally recognized owing to its immense range of applications in biosensing, photonics, photocatalysis, pharmaceuticals, micro-electronics, etc [13]. In addition, AgNPs have shown to exhibit antifungal, antiviral, antibacterial, antiplatelet, anti-inflammatory and anticancerous activity [14]. The boundless antimicrobial properties of AgNPs encourage its potential in a large number of environmental and biomedical applications as well as in clothing, cosmetics and different consumer products [15]. Several research papers reported the synthesis of silver nanoparticles using plant

extracts such as *Oryza sativa*, *Zea mays* [16]; *Jatropha curcas* seeds [17]; *Acalypha indica* leaf [18]; banana peel [19]; *Ocimum sanctum* leaf, stems and roots [20, 21]; *Murraya koenigii* (curry) leaf [22]; *Ocimum tenuiflorum*, *Solanum trilobatum*, and *Citrus sinensis* leaves [23] and mulberry leaves [24].

The present work is targeted to accomplish the synthesis of AgNPs through green-mediated synthesis strategy. The present study reveals the rapid synthesis of AgNPs by reducing the silver nitrate solution using aqueous leaf extract of *O. heracleoticum* L. *O. heracleoticum* L. (syn. *O. hirtum* L.; *O. creticum* Sieber & Bentham; *O. vulgare* L. subsp. *hirtum* (Link) Ietswaart) (Lamiaceae) that is native to Mediterranean Europe from Spain to Northern Balkan and Asia [25]. According to the traditional medicine, *O. heracleoticum* L. is diuretic and antidote for epilepsy and psoriasis [26]. Previous studies showed that, the plant acquire antioxidant [26, 27], anti-fungal [25] and potent anti-bacterial properties [28-30]. The green synthesized nanoparticles were characterized by different technique and it showed improved antimicrobial activity against pathogenic strains. Cytotoxicity of the nanoparticles was also assessed against human breast cancer MCF-7 cell line and it gave conclusive evidence.

MATERIALS AND METHODS

Green synthesis of silver nanoparticles

The collected *O. heracleoticum* L. leaves were washed thrice in distilled water and shade dried for two weeks. The dried leaves were grinded into fine particles using a pulverizer. For the preparation of aqueous leaf extract, 10 g of leaf powder was dissolved in 100 ml of deionized water followed by boiling at 60 °C for 10 min. The aqueous leaf extract was allowed to cool, filtered through nylon mesh, followed by Millipore filter (0.45 µm) and refrigerated until further use. For the green synthesis of AgNPs to occur, 10 ml of *O. heracleoticum* L. leaf extract was mixed with 90 ml of silver nitrate ($AgNO_3$, 1 mM) solution. The obtained product was heated gradually at 60–90 °C for 10 min using a water bath. The conversion of pale brown to reddish brown color evidenced the formation of AgNPs.

Characterization of silver nanoparticles

Synthesized AgNPs were confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV–vis spectra, at the wavelength of 200-700 (nm) Beckman-DU 20 spectrophotometer. An aliquot of this filtrate containing silver nanoparticles was used for FE-SEM, XRD and EDS studies. FE-SEM images were acquired by Carl Zeiss, SIGMA instrument (UK). For XRD studies, dried nanoparticles were coated on XRD grid and the spectra was obtained by using Philips PW1830 X-ray generator operated at a voltage of 40 kV and a current of 30mA with Cu Kα1 radiation. In addition the presence of metals in the sample was also analyzed by energy dispersive spectroscopy (EDS).

Antimicrobial activity assay

The antimicrobial activity of green synthesized AgNPs was performed by agar well diffusion method [31]. A total of six microbial strains namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae and Candida albicans* were procured from Microbial type culture collection, Chandigarh, India. Stock cultures were maintained at 4 °C on agar slants of nutrient media. Prior to the experiment, microbial pure cultures were sub cultured onto Muller Hinton broth (Bacteria) and Sabouraud Dextrose broth (Fungi) and incubated overnight at 37 °C. Later Muller Hinton agar plates and Sabouraud Dextrose agar plates were prepared and punctured for wells and loaded with 50 µl AgNPs. After incubation at 37°C for 24 h, the diameter of Zone of Inhibition (ZoI) was measured in millimeter (mm) and was recorded as mean±SD of triplicate experiments.

Cell culture

Human breast cancer MCF-7 cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India. It was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Hi Media Laboratories, India).

Effect of AgNPs on cytotoxicity

Effect of AgNPs on cell proliferation was determined using MTT (dimethyl thiozolyl diphenyl-tetrazolium bromide) assay. Briefly, the cells were suspended at 3×10^5 Cell mL^{-1}. The cells were placed in 96-well microtiter plates (200 µl $well^{-1}$) and incubated for 24 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. MCF-7 cancer cells were treated with a series of concentration (10–500 µg/ml) of green synthesized AgNPs. Control cultures were treated with Dimethyl sulfoxide (DMSO). After 5 days, cell viability was measured by MTT assay, from which cytotoxic concentration (CC_{50}) was calculated [32].

Statistical analysis

The statistical analysis was done using SPSS software Version 16.0 (SPSS Inc., Chicago, IL, USA). The one-way ANOVA was done for expressing the significance of the present study. Statistical significance was accepted at a level of $p<0.05$. Values were presented as the mean±SD of the three replicates of each experiment.

RESULTS AND DISCUSSION

AgNPs are promising agents in the Nanotechnology, because of their novel activity against many of the inimical processes in Bioscience. Many approaches have been utilized to get a better option for the synthesis of AgNPs such as physical, chemical and biological methods. In recent times, synthesis of AgNPs using aqueous extracts of green leaves has become crowd-pleasing, since it avoids tedious and expensive process [33]. Extracts from bio-organisms may serve as reducing, as well as capping agents in AgNPs synthesis. Examining the synthesis of AgNPs with purified bio-organics may pledge fitter acumen into the system mechanism. Many scientists have revealed the green synthesis of metal nanoparticles from aqueous extracts of plants for wide range of global applications (mentioned earlier). In the present investigation, synthesis of silver nanoparticles using aqueous leaf extracts of *O. heracleoticum* L. was performed. To fabricate AgNPs, temperature dependent approach was carried out by reducing silver nitrate ($AgNO_3$) solution. It has been proved that, the elevation in temperature results in the rapid biosynthesis of nanoparticles [34]. Formation of AgNPs was indicated by the conversion of pale brown to dark brown color (fig.1) in the colloidal solution due to excitation of SPR effect and reduction of $AgNO_3$ [18]. No color change was observed in $AgNO_3$ (1 mM) solution.

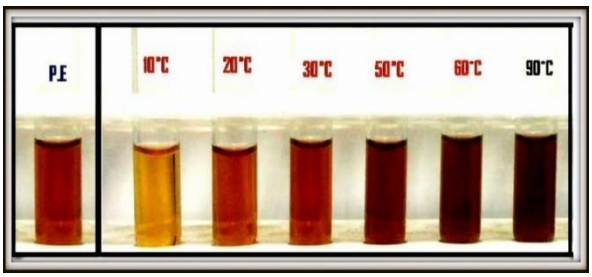

Fig. 1: Photograph showing colour change

The reduction of metal ions was progressively observed as well as measured with UV–visible spectrometer in the wavelength range 200–700 nm. The characteristic absorption peak at 430 nm in UV–vis spectrum (fig.2) which steadily increases in intensity as a function of time of reaction (ranging from 5 min to 30 min), confirmed the formation of silver nanoparticles. The size and shape of the nanoparticles are decided by the existence of the absorption peak. It is stated that, appearance of single SPR in the early stages of synthesis may correspond to the spherical nature of the AgNPs [18] and increase in temperature may lead to the hoisted concentration of AgNPs [32].

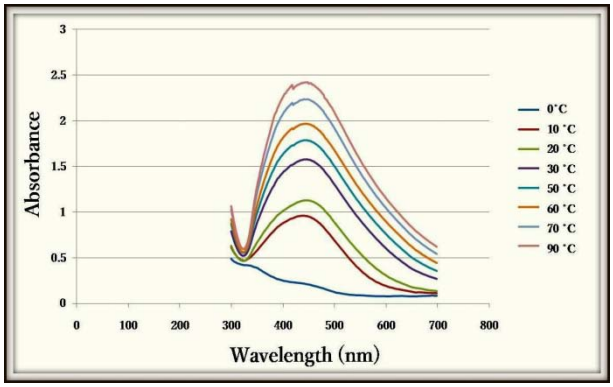

Fig. 2: UV spectral peak of temperature dependent silver Nanoparticles synthesized at different temperature

Electron Microscope study

FE-SEM analysis of the $AgNO_3$ and synthesized silver nanoparticles was performed to understand the morphology and it was clearly distinguishable owing to their size difference. From the FE-SEM image the size of the $AgNO_3$ obtained was greater than 1000 nm size, where as synthesized AgNPs measured 30–40 nm in size (fig.3). Synthesized AgNPs were found to be spherical in shape with clusters (fig.3). Formation of clusters is due to the presence of elevated concentration of bioactive compounds in the colloids [32].

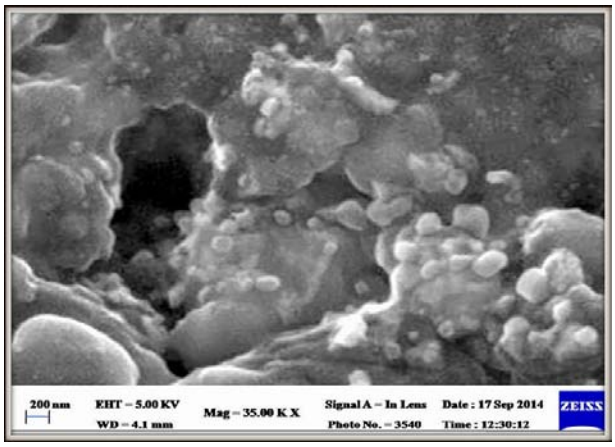

Fig. 3: Field emission-scanning electron microscopic image of AgNPs

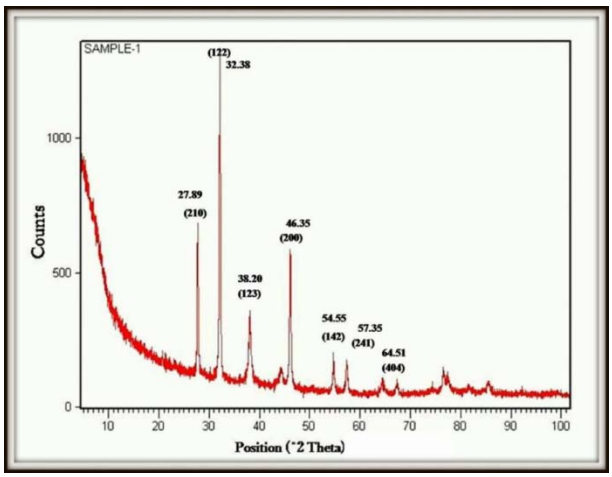

Fig. 4: X-ray diffraction analysis

X-ray diffraction (XRD) analysis

The XRD patterns of vacuum-dried AgNPs synthesized using the leaf extract of *O. heracleoticum* L. showed distinct diffraction peaks at 27.89°, 32.38º, 38.20°, 46.35°, 54.55° and 57.35° which indexed the planes of 210, 122, 123, 200, 142, 241 of the cubic face-centered silver (fig.4).

It confirms that the prepared AgNPs were biphasic in nature and it clearly illustrates that the biosynthesized AgNPs are crystalline in nature. Similar reports for XRD were shown in *Ocimum canum* leaf extract and *Morinda citrifolia* root extract for synthesized silver nanoparticles [35, 36]. The slight shift in the peak positions indicates the occurrence of strain in the silver crystal, which is a characteristic feature of nanocrystallites [36]. Thus, the XRD pattern proves to be strong evidence in favour of the UV–vis spectra for the presence of silver nanocrystals. The average distribution of silver nanoparticles formed in the bioreduction process was found to be around 35 nm (fig.5).

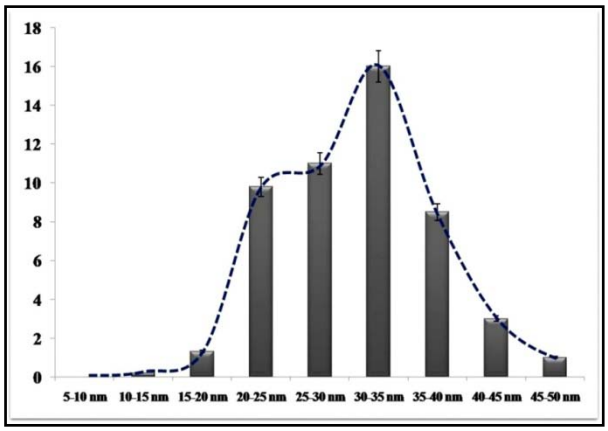

Fig. 5: Particle size distribution

Fourier transform infra-red spectroscopy (FT-IR) analysis

FTIR spectroscopy is a unique tool in analyzing, understanding and to identify the biomolecules accountable for the reduction of Ag^+ions and capping of the bioreduced AgNPs synthesized using plant extract. Fig. 6 shows spectral peaks, indicating the presence of bands relevant to amide N-H stretching (3294 cm^{-1}), nitrile C=N stretching (2302 cm^{-1}) and aromatic C=C bending (1636 cm^{-1}). The existence of the band at 1400 cm^{-1} and 1040 cm^{-1} suggested a strong contact between C–O [37]. These functional groups might have a potent role in the green synthesis of AgNPs [32].

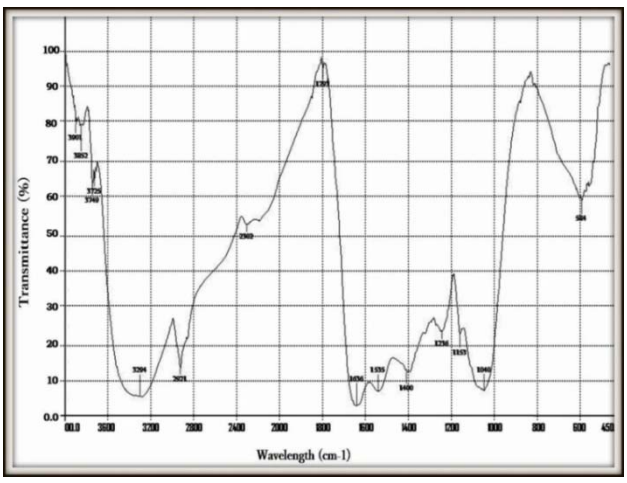

Fig. 6: FT-IR Spectrum analysis

Energy dispersive X-ray spectroscopy (EDS) analysis

The energy dispersive spectrum (fig.7) illustrates strong signal in the silver region and confirms the formation of AgNPs, which suggests the presence of Ag as the predominant element. Metallic silver nanoparticles typically showed an optical absorption peak at 3 keV due to the SPR effect [38]. Several elemental signals along with silver nanoparticles were also recorded, which were not perceived for the biosynthesis of many other nanoparticles [39].

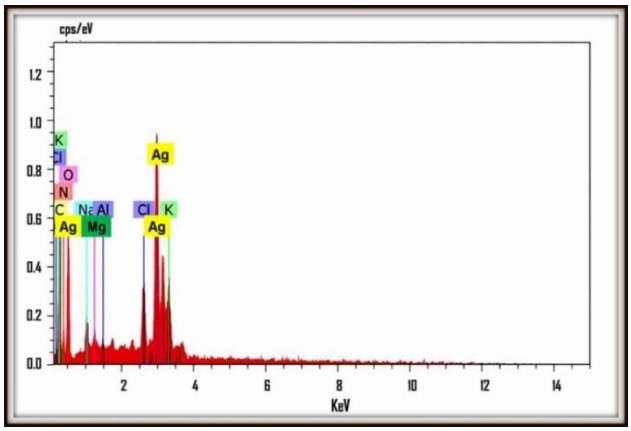

Fig. 7: Energy dispersive X-ray spectrum of Nanoparticles

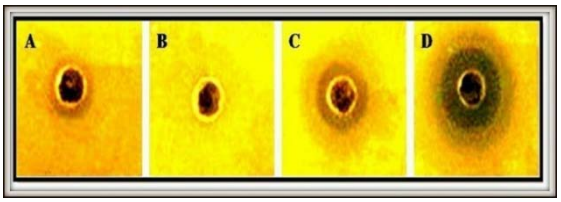

Fig. 8: Antibacterial activity assay against *S. aureus. A. Origanum heracleoticum* L. leaf extract, B. $AgNO_3$ without leaf extract, C. 0.01 mmol/ml synthesized AgNPs and D. 0.02 mmol/ml synthesized AgNPs

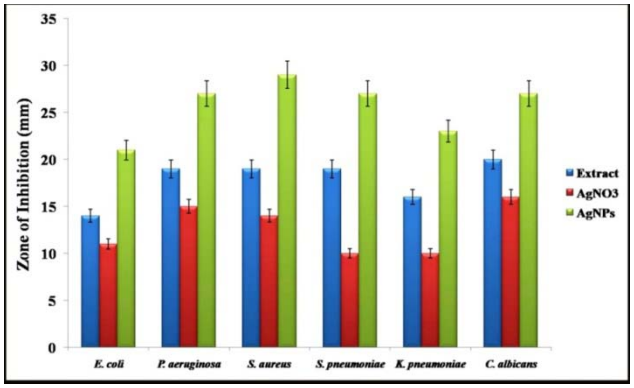

Fig. 9: Antibacterial activity against pathogenic microbes

Antimicrobial activity of AgNPs

It has been demonstrated that the highly reactive metal oxide nanoparticles possesses peerless bactericidal action against Gram-positive and Gram-negative bacteria. The toxicity of Ag against wide range of microorganisms is well understood and AgNPs have been recently shown to be a potent antimicrobial material [40]. In the present study, the antimicrobial activity of Ag against pathogenic organism including bacteria and fungi was investigated. It is stated that, the antimicrobial effect was dose dependent. AgNPs (0.02 mmol/ml) showed clear inhibition after incubation of 24h against *E. coli* (21 mm), *P. aeruginosa* (27 mm), *K. pneumoniae* (23 mm), *S. aureus* (29 mm), *S. pneumoniae* (27 mm) and *C. albicans* (27 mm) (fig.8 & 9).

This may comment the antimicrobial nature of synthesized AgNPs. It is proved, AgNPs by green synthesis can compete commercial antimicrobial agents (antibiotics) used for the treatment of bacterial infections [32]. Silver is well known for its antimicrobial activity and has been used for years in the medicine and even has shown to prevent binding of HIV to host cells. Additionally, Ag has been used in water and air filtration to eliminate microorganisms [41].

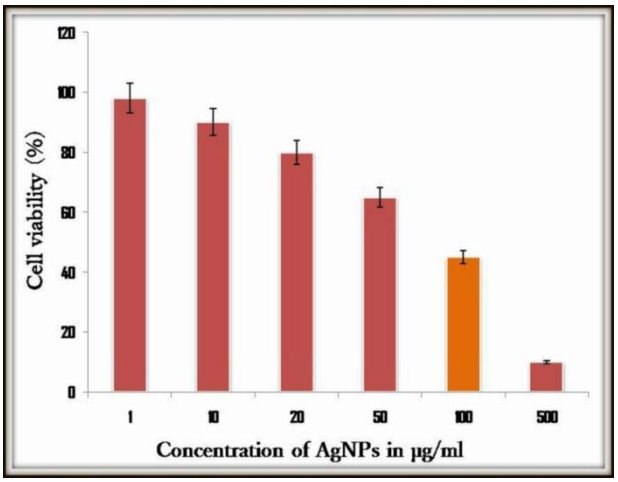

Fig. 11: Cytotoxic effect of AgNPs on MCF-7 cells (concentration vs. inhibition)

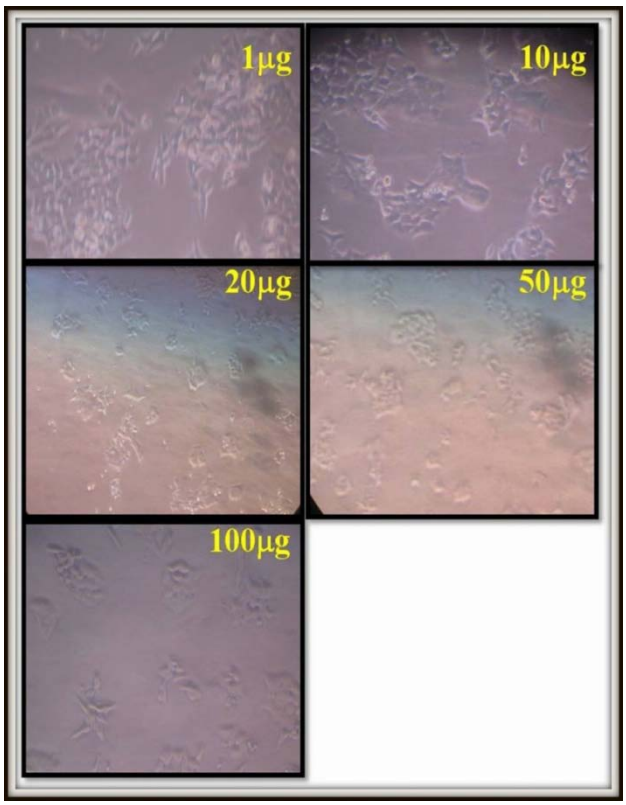

Fig. 10: Images of MCF-7 cell line at varied concentration of AgNPs treated Cells

Cytotoxicity studies

In vitro cytotoxicity of the AgNPs was screened against human breast cancer MCF-7 cells at different concentrations (10–500 µg/ml) and the viability of tumor cells was confirmed by using MTT assay. The synthesized AgNPs illustrates a substantial cytotoxicity against MCF-7 cell lines (fig.10 & 11). The significant death in the MCF-7 cancer cells was observed in 100µg/ml of AgNPs in a dose dependent manner. Higher concentration (500µg/ml) of AgNPs may result in additional toxicity to the cells that may lead to 85% dead cells after incubation. The result suggested that the leaf mediated synthesized AgNPs reveals great selectivity to tumor cells and can deliver quiescent application in cancer prevention. Earlier studies showed that the presence of AgNPs may induce reactive ROS and cause damage to cellular components leading to intracellular oxidative stress [42] and may end up in apoptosis and necrosis [43].

The cytotoxic effect of *O. heracleoticum* L. extract along with lipopolysaccharide (LPS) (1µg/ml) was already reported but found to be negligible [44]. In that sense, this is pilot study which gives a strong evidence for cytotoxicity of green synthesized AgNPs from *O. heracleoticum* L. The cytotoxic effects of Ag are due to active physicochemical interaction of Ag atoms with the functional groups of intracellular proteins, nitrogen bases and phosphate groups in DNA [45].

CONCLUSION

In this paper, we report simple, rapid and systematic approach for the green synthesis of AgNPs using *O. heracleoticum* L. without using harmful substance and it completely eliminates the tedious process (fig.12).

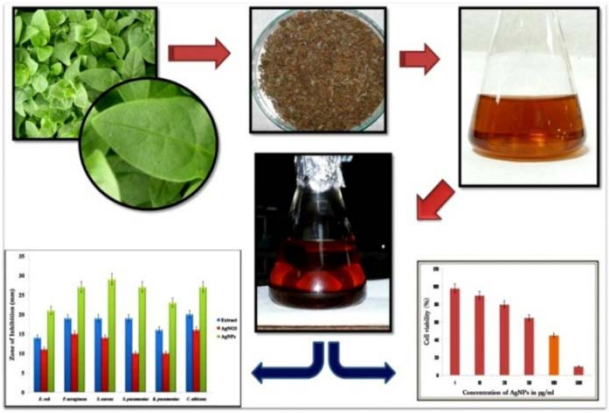

Fig. 12: Schematic diagram of synthesis of AgNPs from *O. heracleoticum* L

The characterization with UV–vis spectroscopy, Fourier transmission infrared (FT-IR), field emission-scanning electron microscopic (FE-SEM), X-ray diffraction (XRD) analysis and Energy dispersive X-ray spectroscopy (EDS) analysis confirms the formation of AgNPs. In addition, the synthesized AgNPs showed potent antimicrobial activity against pathogenic microbes. Interestingly, this is the pilot study evidencing the cytotoxicity of AgNPs from the extracts of *O. heracleoticum* L. Further investigations are needed to explain the possible mechanism underlying the anticancer activity of *O. heracleoticum* L.

ACKNOWLEDGEMENT

The authors are thankful to University of Madras for providing facilities to carry out this work. The authors are also thankful to Alagappa University and Indian Institute of Sciences for providing facilities to analyze the samples by XRD, FT-IR, EDS and FE-SEM.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer 2008;8(6):473-80.
2. Davis M, Zuckerman J, Choi C. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nat 2010;464(7291):1067-70.
3. Veiseh O, Gunn J, Zhang M. Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. Adv Drug Delv Rev 2010;62(3):284-304.
4. Rajan B, Sathish S, Jayakumar S. Synthesis and *in vitro* anticancer evaluation of 2-isopropyl-5-methylphenol Loaded PLGA based iron oxide nanoparticles. Biomed Prev Nut 2014;4(2):137-41.
5. Appenzeller T. The man who dared to think small. Sci 1991;254(5036):1300.
6. Lai S, Wang Y, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Delv Rev 2009;61(2):158-71.
7. Mishra B, Patel B, Tiwari S. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomed: Nanotechnol, Biol Med 2010;6(1):9-24.
8. Awwad A, Salem N, Abdeen A. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. Int J Industrial Chem 2013;4(1):29.
9. Moreno-Ãlvarez S, Martinez-Castanon G, Nino-Martinez N. Preparation and bactericide activity of gallic acid stabilized gold nanoparticles. J Nanopart Res 2010;12(8):2741-6.
10. Karthiga P, Soranam R, Annadurai G. Alpha-mangostin. The major compound from *Garcinia mangostana* Linn. responsible for synthesis of Ag nanoparticles: Its Characterization and evaluation Studies. Res J Nanosci Nanotechnol 2012;2(2):46-57.
11. Mandal D, Bolander M, Mukhopadhyay D, Sarkar G, Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. Appl Microbiol Biotechnol 2005;69(5):485-92.
12. Basavaraja S, Balaji S, Lagashetty A, Rajasab A, Venkataraman A. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. Mater Res Bull 2008;43(5):1164-70.
13. Li D, Li C, Wang A, He Q, Li J. Hierarchical gold/copolymer nanostructures as hydrophobic nanotanks for drug encapsulation. J Math Chem 2010;20(36):7782.
14. Vaidyanathan R, Kalishwaralal K, Gopalram S, Gurunathan S. Retracted nanosilver-The burgeoning therapeutic molecule and its green synthesis. Biotechnol Adv 2009;27(6):924-37.
15. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009;27(1):76-83.
16. Leela A, Vivekanandan M. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. Afr J Biotechnol 2008;7:3162-65.
17. Bar H, Bhui D, Sahoo G, Sarkar P, Pyne S, Misra A. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf A 2009;348(1-3):212-16.
18. Krishnaraj C, Jagan E, Rajasekar S, Selvakumar P, Kalaichelvan P, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B 2010;76(1):50-6.
19. Bankar A, Joshi B, Kumar A, Zinjarde S. Banana peel extract mediated novel route for the synthesis of palladium nanoparticles. Mat Lett 2010;64(18):1951-3.
20. Ahmad N, Sharma S, Alam M. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B 2010;81(1):81-6.
21. Singhal G, Bhavesh R, Kasariya K, Sharma A, Singh R. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanopart Res 2011;13(7):2981-8.
22. Christensen L. Biosynthesis of silver nanoparticles using *Murraya Koenigii* (Curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. Adv Matter Lett 2011;2(6):429-34.

23. Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. J Saudi Chem Soc 2012. [Article In Press].
24. Awwad A, Salem N. Green synthesis of silver nanoparticles by mulberry leaves extract. Nanosci Nanotechnol 2012;2:125-8.
25. Dzamic A, Sokovic M, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin P. Chemical composition and antifungal activity of *Origanum heracleoticum* essential oil. Chem Nat Compd 2008;44(5):659-60.
26. Tsimogiannis D, Stavrakaki M, Oreopoulou V. Isolation and characterisation of antioxidant components from oregano (*Origanum heracleoticum*). Int J Food Sci Technol 2006;41(s1):39-48.
27. Zheng Z, Tan J, Liu H, Zhou X, Xiang X, Wang K. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). Aquaculture 2009;292(3-4):214-8.
28. Skandamis P, Nychas G. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J Appl Microbiol 2001;91(6):1011-22.
29. Hammer K, Carson C, Riley T. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999;86(6):985-90.
30. Dorman H, Deans S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88(2):308-16.
31. Perez C, Paul M, Bazerque P. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp 1990;15:13.
32. Sankar R, Karthik A, Prabu A, Karthik S, Shivashangari K, Ravikumar V. *Origanum vulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. Colloids Surf B 2013;108:80-4.
33. Song J, Kim B. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 2008;32(1):79-84.
34. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 1996;12(3):788-800.
35. Suman T, Elumalai D, Kaleena P, Rajasree S. GC-MS analysis of bioactive components and synthesis of silver nanoparticle using *Ammannia baccifera* aerial extract and its larvicidal activity against malaria and filariasis vectors. Ind Crops Prod 2013;47:239-45.
36. Jayaseelan C, Rahuman A. Acaricidal efficacy of synthesized silver nanoparticles using aqueous leaf extract of Ocimum canum against *Hyalomma anatolicum* and *Hyalomma marginatum* (Acari: Ixodidae). Parasitol Res 2011;111(3):1369-78.
37. Kumar P, Senthamil Selvi S, Lakshmi Prabha A, Prem Kumar K, Ganesh Kumar S, Govindaraju M. Synthesis of silver nanoparticles from *Sargassum tenerrimum* and screening phytochemicals for its anti-bacterial activity. Nano Biomed Engg 2012;4(1):12-6.
38. Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of silver nanoparticles using citrus sinensis peel extract and its antibacterial activity. Spectrochim Acta Part A 2011;79(3):594-8.
39. Mallikarjuna K, John Sushma N, Narasimha G, Manoj L, Deva Prasad Raju B. Phytochemical fabrication and characterization of silver nanoparticles by using Pepper leaf broth. Arabian J Chem 2014;7(6):1099-103.
40. Kim B, Salunke B, Sawant S, Alkotaini B. Biological synthesis of silver nanoparticles using plant leaf extracts and their specific antimicrobial activity. New Biotechnol 2014;31:173-8.
41. Sharma V, Yngard R, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 2009;145(1-2):83-96.
42. Nel A. Toxic potential of materials at the nanolevel. Sci 2006;311(5761):622-7.
43. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis 2007;12(5):913-22.
44. Conforti F, Marrelli M, Menichini F. Chemical composition and protective effect of oregano (*Origanum heracleoticum* L.) ethanolic extract on oxidative damage and on inhibition of NO in LPS-stimulated RAW 264.7 macrophages. J Enzyme Inhib Med Chem 2011;26(3):404-11.
45. Satyavani K, Ramanathan T, Gurudeeban S. Plant mediated synthesis of biomedical silver nanoparticles by using leaf extract of *Citrullus colocynthis*. Res J Nanosci Nanotechnol 2011;1(2):95-101.