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BIOGENIC SILVER NANOPARTICLES FROM *SPINACIA OLERACEA* AND *LACTUCA SATIVA* AND THEIR POTENTIAL ANTIMICROBIAL ACTIVITY

AMARNATH KANCHANA[a*], ISHA AGARWAL[b], SWETHA SUNKAR[c], JAYSHREE NELLORE[d], KARTHICK NAMASIVAYAM[d]
[a]*Associate Professor, Department of Biochemistry, Sathyabama University Dental College and General Hospitals.*
[b]*Research Scholar, Department of Biotechnology, Sathyabama University*
[c]*Associate Professor, Department of Biotechnology, Sathyabama University*
[d]*Senior Lecturers, Department of Biotechnology, Sathyabama University.*

Development of biologically inspired experimental processes for the synthesis of nanoparticles is an important branch of nanotechnology and is expected to open new avenue to fight and prevent disease. Many researches and studies are evolving regarding the green synthesis of nanoparticles due to the challenges faced by chemical synthesis protocol of nanoparticles in being toxic, flammable and unstable. In this study we report the synthesis of silver nanoparticles from *Spinacia oleracea* and *Lactuca sativa* leaves by 'exploiting' the reduction capabilities of varied phytochemicals present in it, as confirmed by the FTIR characterization analysis technique. UV-Vis Spectroscopy interpreted the formation of silver nanoparticles by the reduction of silver salts, respectively. The biogenic nanoparticles synthesized using lettuce and spinach leaves exhibited variety of shapes, exposed by SEM and TEM. Due to increasing development of the resistance to the existing antibiotic and various drugs, we extended our research to utilize the efficacy of these synthesized nanoparticles as antimicrobial agents, against *B.subtilis, S.aureus, K.pneumonia* and *E. faecalis* strains. Our results specify that the bactericidal properties of the nanoparticles present a direct interaction with the bacterias.

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Keywords: Silver nanoparticle; Spinacia oleracea and Lactuca sativa; Antibacterial activity; Phytonano synthesis

1. Introduction

Research on nanoparticles is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields [1]. Synthesis of nanoparticles can be carried out by using various chemical and physical methods. But use of such methods is harmful in one or the other way as the chemicals often used are toxic, flammable, not easily disposable due to environmental issues, have low production rate, etc [2]. As a result, a great deal of effort has been put into the search for methods utilizing biological systems, such as the micro-organisms and plants, for the synthesis of metal nanoparticles. Various micro-organisms such as bacteria, fungi, and yeasts have been suggested as nanofactories for synthesizing metal nanoparticles of silver and gold. But, the use of plants for the fabrication of nanoparticles has drawn the attention of researchers as a rapid, low cost, eco-friendly and a single step method for the biosynthesis process [3]. It has been reported that the rate of reduction of metal ions using plants has been found to be much faster as compared to micro-organisms and stable formation of metal nanoparticles.

* Corresponding author: kanchibms@yahoo.co.in

The synthesis of nanoparticles can be carried out by both intracellular and extracellular methods such as leaf broth, sun dried leaves, fruits, growing plants on metal-rich soil, gold-rich agar media, etc. and the results obtained were positive in all cases. Interestingly silver [4] and gold [5] nanoparticles were synthesized from alfalfa sprouts. More specifically, Shiv Shankar et al. (2004) [6] and Richardson et al. (2006) [7] provided a cost-effective and easy method for the synthesis of silver nanoparticles, using *Geranium* leaves. Harris and Bali (2007) [8] have focused their research on the uptake of silver by *Brassica juncea* and *Medicago sativa* and reported that these plants can be used as hyper accumulators. Huang and his coworkers (2007) [2] made an effort to synthesis of silver and gold nanoparticles using a sun-dried leaf extract of *Cinnamomum camphora* and the reductive biomolecules attributed to the control of shape and size of nanoparticles. The shape and size of the nanoparticles synthesized using plants can be controlled and modulated by changing the pH. The other factors responsible for the control of shape and size of metallic nanoparticles are the presence of protective and reductive biomolecules [9].

Production of nanoparticles under nontoxic green conditions is of vital importance to address growing concerns on the overall toxicity of nanoparticles for medical and technological applications [10, 11, and 12]. Deciphering the endless antioxidant power of lettuce and spinach leaves, we investigated the synergistic potentials of various phytochemicals present in *Spinacia oleracea* and *Lactuca sativa* leaves for the reduction reactions of silver salts to produce silver nanoparticles respectively, which have potential applications in the diagnosis and therapy of various deadly diseases including cancer. Further the synthesized nanoparticles were characterized using UV-Vis Spectroscopy, FTIR, SEM and TEM that confirmed the formation of silver nanoparticles via reduction of silver salts, role of phytochemicals in their synthesis and revealed its various sizes, respectively. Current research in bactericidal nanomaterial has opened a new era in pharmaceutical industries. However, the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium–nanoparticle interaction. Thus in the present investigation we also report the synthesis of highly stable nanoparticles of silver from lettuce and spinach leaf extracts endowed with significant antibacterial properties against *B.subtilis, S.aureus, K.pneumonia* and *E. faecalis.*

2. Material and methods

Silver nitrate obtained from Sigma–Aldrich Chemicals. All glasswares have been washed with lavolene and distilled water and dried in oven before use. Fresh leaves of spinach and lettuce have been collected from local markets, Chennai, India.

2.1. Preparation of leaves extract

The fresh leaves of *Spinacia oleracea* and *Lactuca sativa* were washed several times with ultra pure water to remove the dust. Leaf extracts used for the synthesis was prepared from 20 g of thoroughly washed leaves in a 500mL Erlenmayer flask and boiled in 250mL ultrapure water for 20 min. Filtered leaf extracts were stored at −15 ◦C for further use, being usable for 1 week.

2.2. Development of silver nanoparticles

60mL aqueous solution of 1mM of silver nitrate was reduced using 2.5mL of leaves extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of silver (AgNPs) nanoparticles.

2.3. Characterization of the synthesized nanoparticles

2.3.1. UV–vis spectra analysis

Synthesis of AgNPs by reducing respective metal ion solution with leaves extract may be easily observed by UV–vis spectroscopy. The absorption spectra at different leaves extract quantities and metal concentrations were measured using a Perkin-Elmer Lamda-45 spectrophotometer in 300–1000nm range. Naturally synthesized AgNPs of diameters (10–35 nm) gave sharp peaks in the visible region of the electromagnetic spectrum.

2.3.2. TEM analysis of silver nanoparticles

Transmission Electron Microscopic (TEM) analysis was done by using a TEM, JEM-1200EX, JEOL Ltd., Japan. 3_L of the sample was placed on the carbon coated copper grid, making a thin film of sample on the grid and extra sample was removed using the cone of a blotting paper and kept in grid box sequentially.

2.3.3 SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

2.3.4 FTIR analysis of dried biomass after bioreduction

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

2.3.5 Antimicrobial Studies

The antimicrobial activity of silver nanoparticles was evaluated against *Bacillus subtilus, Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 9027 and *Klebsiella pneumoniae* (clinical isolate) by agar disc diffusion method. The 18 hrs-revived cultures were prepared in nutrient broth (Composition (gm/litre) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose 1.0). Two replicas of respective microorganism were prepared by spreading 100 µl of revived culture on the nutrient agar plate (Composition (gm/litre) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose 1.0; agar-agar 12.0) with the help of spreader. Well was made having a diameter of about 7mm. 50 µl, 75 µl, and 125 µl samples of synthesized silver nanoparticles was placed in the second well. The Petri plates were incubated in dark at 37°C.

3. Results and discussion

Since the development of the concept of green nanoparticle preparation by Raveendran et al. 2003, [13] there has been growing need for environmentally benign metal-nanoparticle synthesis process that do not use toxic chemicals in the synthesis protocols to avoid adverse effects in medical applications. Thus the inspiration for green chemistry and bioprocesses comes from nature through yeast, fungi, bacteria and plant extracts in the synthesis of biocompatible metal and semiconductor nanoparticles [14, 15]. Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications in catalysis, electronics, photonics, optoelectronics, sensing, and pharmaceuticals. Specifically, these nanoparticles are strong candidates for Surface Enhanced Raman Spectroscopic (SERS) studies that yet again prompt the interest of the scientific community to develop newer green synthetic methods for obtaining these nanoparticles. Owing to the therapeutic benefits of the phytochemicals, vitamins, minerals and fibers present in *Spinacia olerace* [16] and *Lactuca sativa* [17] leaves, the present study highlights its utility in the green synthesis of silver nanoparticles and its stability without the intervention of any external man-made chemicals.

The use of phytochemicals in the synthesis of nanoparticles has become an important symbiosis between nanotechnology and green Chemistry [7]. Thus, **o**n challenging the 'fight-o-chemicals' of the broths (20 g of leaf biomass) with Ag^{+} ions, the present results confirm that the solution changed from yellowish green to golden brown (Fig. 1a & 1b) the final brownish color appeared gradually with time. The entire reaction mixture turned to yellowish brown color after 8 hrs of reaction, and exhibited an absorbance peak around 440 nm characteristic of silver nanoparticle (AgNps), due to its surface plasmon resonance absorption band (SPR) (Fig. 1a & 1b)**.** It is observed that the silver SPR band occurs initially at 450 nm and after completion of the reaction, the wavelength of the SPR band stabilizes at 480 nm [18].

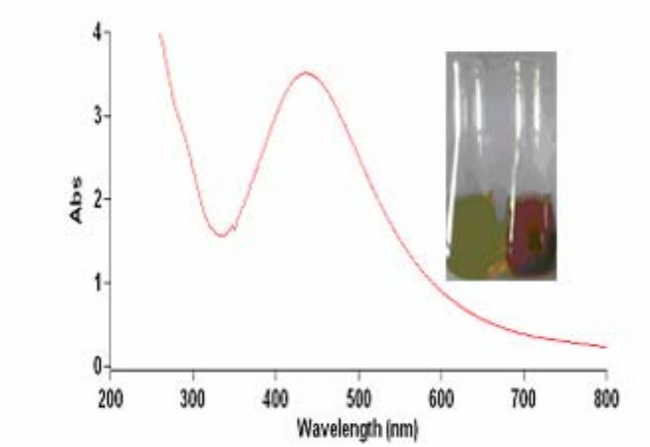

Fig. 1(a) UV-vis absorption spectra of colloidal Ag nanoparticles synthesized using Lactuca Sativa leaf extracts. The inset of the figure shows glass beakers of the silver nanoparticle solution formed at the end of the reaction.

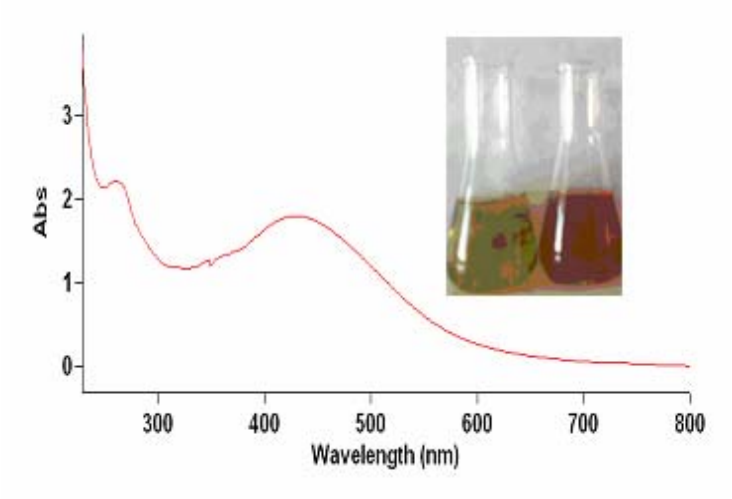

Fig. 1(a) UV-vis absorption spectra of colloidal Ag nanoparticles synthesized using Spinacia oleracea leaf extracts. The inset of the figure shows glass beakers of the silver nanoparticle solution formed at the end of the reaction.

Rapid synthesis of stable AgNps using *Geranium* leaf broth (20 g of leaf biomass) and 1mM aqueous $AgNO_3$ have been reported by Sastry et al. 2003 [19], where bioreduction was found to be completed within 24 hrs. Similarly, Govindaraju et al. 2010 [20], reported rapid synthesis of stable silver, nanoparticles using 20 g of leaf biomass of *solanum toruvum* and 1mM aqueous $AgNO_3$, within 60 minutes. The position and shape of plasmon absorption of silver nanoclusters are strongly dependent on the particle size, dielectric constant of the medium and surface adsorbed species. According to Mie's theory, only a single SPR band is expected in the absorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles. The number of SPR peaks increases as the symmetry of the nanoparticle decreases [21]. Thus, spherical nanoparticles, disks, and triangular nanoplates of silver show one, two, and more peaks, respectively. In the present investigation, the reaction mixtures showed a single SPR band revealing spherical shape of silver nanoparticles, which was further confirmed by SEM and TEM images**.**

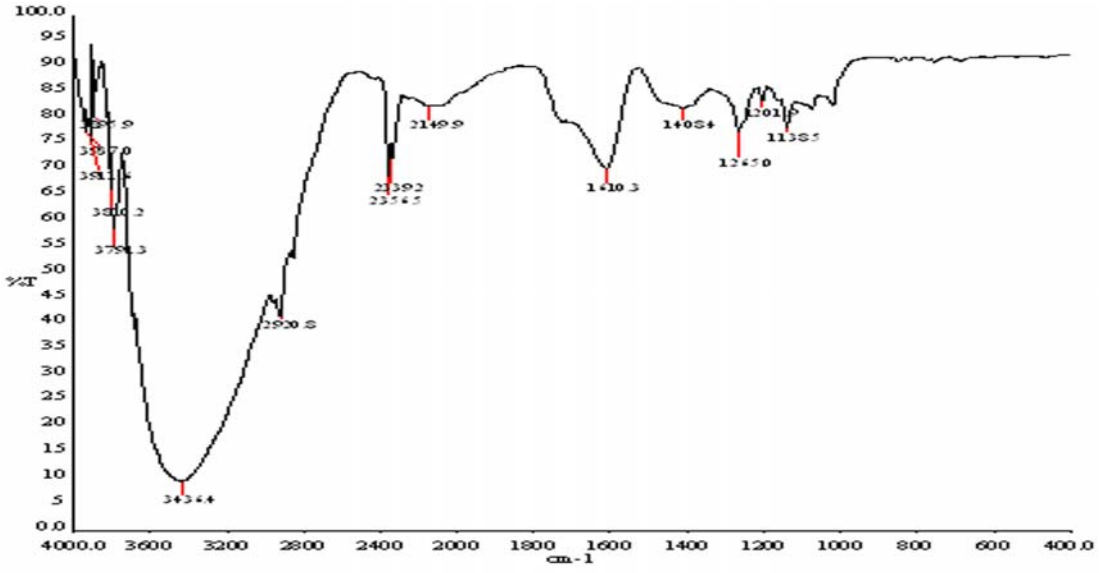

Fig. 2. aFTIR spectra of Ag nanoparticles synthesized using Lactuca sativa leaf extracts.

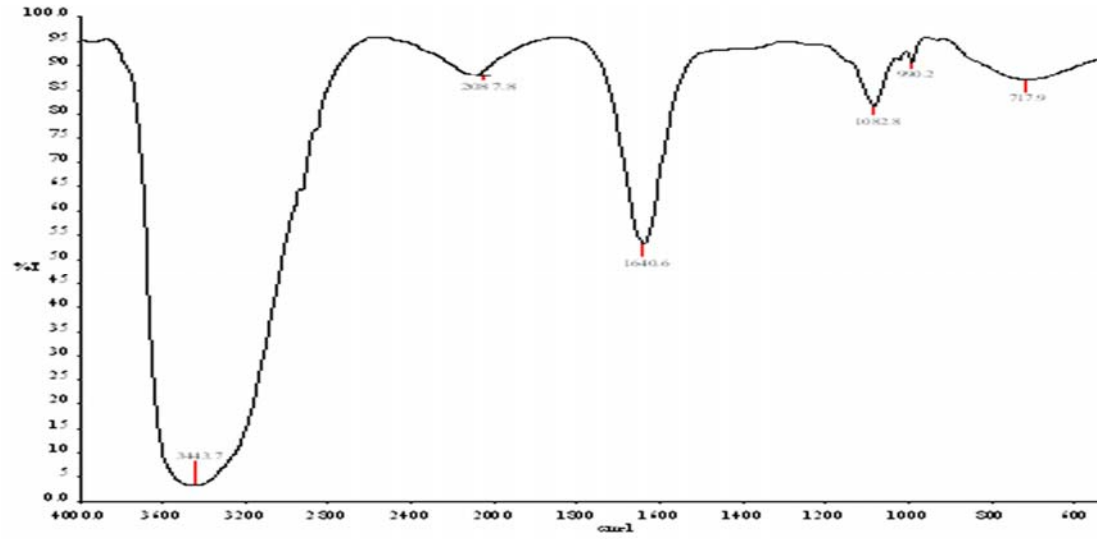

Fig. 2. bFTIR spectra of Ag nanoparticles synthesized using Spinacia oleracea leaf extracts.

The overall morphology of the AgNps produced by reduction of Ag+ ions with 20 g of biomass and 1mM AgNO3 is composed of a large quantity of uniform nanoparticles. SEM (Fig. 3a & 3b) and TEM (Fig. 3c & 3d) analysis expose predominantly spherical shaped Ag nanoparticles are. At a magnification range of 50 the morphology of silver nanoparticles was more clearly seen and the particles are being predominantly spherical, polydispersed and ranged in size from 40-70 nm. These nanoparticles appear to have assembled into very open, quasi-linear superstructures rather than a dense closely packed assembly [24]. The TEM images also reveal that nanoparticles are not in physical contact but are separated by uniform interparticle distance. The reason for these large-sized particles is due to the aggregation of two or more nanoparticles together which in turn result due to the presence of excess amounts of reducing moieties and the interactions between stabilizing molecules bound to the surface of particles and secondary reduction process on the surface of the preformed nuclei [25]. It was noticeable that the edges of the particles were lighter than the centers, suggesting that some bioorganic compounds such as proteins in *Spinacia oleracea* and *Lactuca sativa*, capped the silver NPs [26] contributing to reduction of Ag^{+} ions to

Ag^0. However, it is not yet clear which protein or compound is responsible for bioreduction of silver.

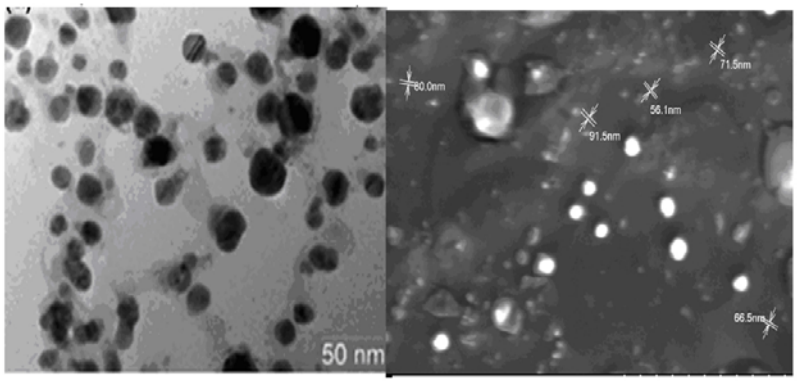

Fig. 3a TEM images of Ag nanoparticles synthesized from *Lactuca sativa* leaf Extracts

Fig. 3b SEM images of Ag nanoparticles synthesized from *Lactuca sativa* leaf Extracts

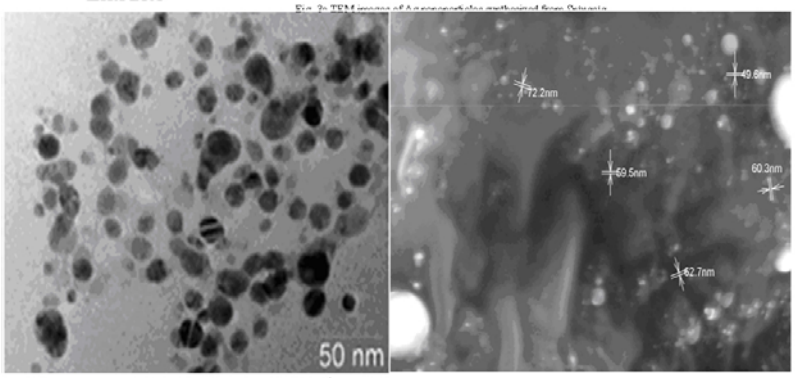

Fig. 3c TEM images of Ag nanoparticles synthesized from *Spinacia oleracea* leaf extracts

Fig. 3d SEM images of Ag nanoparticles synthesized from *Spinacia oleracea* leaf extracts

FTIR measurements were carried out to identify the possible biomolecules accountable for the stabilization and for the reduction of the Ag+ ions and the capping of the bioreduced silver nanoparticles synthesized by the broth. The leaf broth after complete reduction of Ag+ was centrifuged at 15000 rpm for 15 mins to isolate the silver nanoparticles free from proteins or other compounds present in the solution. The representative spectra of stabilized silver nanoparticles obtained from *Spinacia oleracea* (Fig. 2a) and *Lactuca sativa* (Fig. 2b) leaf broths manifests absorption peaks located at about at 3443, 2087, 1640, 1082, 990, 717 cm-1 and 3436, 2920, 2356, 1610, 1408, 1265, 1138 cm-1 respectively. The peaks at 3443 cm-1 of fig 2a and 3436 cm-1 of fig 2b corresponds to the O–H stretching and 2087 cm-1 of fig 2a and 2364.00 cm-1of fig 2b is assigned to and aldehydic C–H stretching.. Absorbance bands which are observed in the region of 2087, 1640, 1082, 990, 717 cm-1 of *Spinacia oleracea* (fig 2a) and 2920, 2356, 1610, 1408, 1265, 1138 cm-1 of *Lactuca sativa* (fig 2b) are known to be associated with the stretching vibrations for –C C–C O, –C C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols), respectively. Moreover, the peak at 1650 cm−1 of *Spinacia oleracea* and 1610 of *Lactuca sativa* are assigned to the amide I bonds of proteins that may arise due to carboxyl stretch and N–H deformation vibrations [22, 23]. Studies have confirmed the fact that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium [2]. Furthermore experimental evidences have been reported that *Lactuca sativa* [17] and *Spinacia oleracea* [27]

contain chemically different groups of compounds (polyphenols, flavonoids, sterols, triterpenes, triterpenoid saponins, beta-phenylethylamines, tetrahydroisoquinolines, reducing sugars like glucose and fructose, amino acids and proteins) that possess free radical scavenging activities [28] which could be responsible for the reduction of silver and synthesis of nanoparticles through biogenic routes [29].

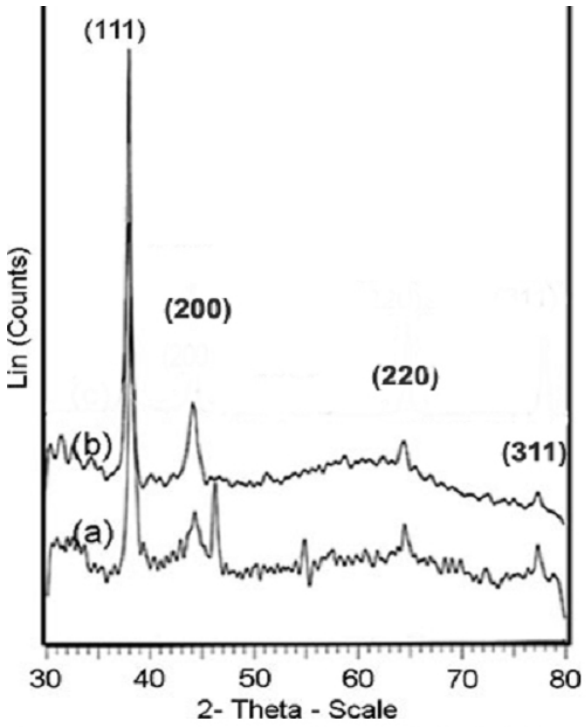

Fig. 4. XRD Patterns of Ag nanoparticles synthesized using (a) lactuca sativa (b) Spinacia oleracea The Bragg reflections are indexed on the basis of the fcc silver structure.

The probability of reduction of $AgNO_3$ to silver may also be illustrated due to the absorption of light by the photosynthesizing organism that produces carbohydrates which are utilized by the cell as glucose by glycolysis [7]. However, the presence of water-soluble antioxidant vitamin and a reducing agent like ascorbate at high levels in all parts of plants may also be the reason for the reduction, and thereby neutralizing reactive oxygen species leading to the formation of ascorbate radical and an electron that reduces the Ag^+ ion to Ag^0 [26]. Using XRD, further structural analysis was carried out for lettuce and spinach plants for Ag nanoparticles. A characteristic XRD pattern of the Ag synthesized by bio based process was found to possess a foci structure as shown in Fig. 4a and 4b. The Bragg reflections at 2h = 38.18, 44.37, 64.48 and 77.63 can be indexed to the (111), (200), (220) and (311) orientations, respectively, confirmed the presence of silver nanoparticles. These results evidently point out that the nanoparticles were poised of highly crystalline Ag [30].

Promising nanomaterials with antibacterial properties are metallic nanoparticles, which exhibit increased chemical activity due to their large surface volume ratios and crystallographic surface structure. The study of bactericidal nanomaterials is particularly timely considering the recent increase of new resistant strains of bacteria to the most potent antibiotics. Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells [31, 32, 33 & 34], catheters coated with AgNPs prevent biofilm formation from gram positive and gram negative bacterias thereby showing significant *in vitro* antimicrobial activity [35]. Reactive oxygen species (ROS), a

scavenger, and Ag^+ ion, a neutralizing agent, suggested a role of ROS in the strong bactericidal activity of carbon filter supporting silver [36]. This has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles.

Nanoparticles with a larger surface area possess higher antibacterial effects compared to the macrosized particles. In spite of the studies conducted on the antibacterial activity and efficacy of many plants, little is known about the antibacterial activity of the nanoparticles synthesized using these species. The present study demonstrated the effect of silver nanoparticles in the range of 1-100 nm on Gram-negative bacteria and gram positive bacteria. The number of bacterial colonies grown on agar plates corresponds to different concentration of silver nanoparticles and declines when the concentration of nanoparticles increased. As such *Spinacia oleracea* and *Lactuca sativa* silver nanoparticles were found highly toxic against four bacterial human pathogens, viz. *E.faecalis, Klebsilla pneumonia* (gram positive) *Staphylococcus aureus Bacillus subtilis* (gram negative) at different concentrations by using standard zone of inhibition (ZOI) microbiology assay, with a well size of 7 mm diameter. Ofloxacin was used as a control antimicrobial agent **(Table 1).** The results indicated that silver nanoparticles synthesized from *Spinacia oleracea* and *Lactuca sativa* has stronger activity than silver nitrate and standard antibiotic ofloxacin. The percentage fold increase of spinach AgNps was higher by 23% to lettuce AgNps and 38.1% to the plant extract as indicated by the ZoI. The increase in bacterial impediment between the AgNps of spinach and lettuce leaf extracts is highly probable due to the polyphenols and other phytochemical compounds of the plant determining that they are capped on the surface of nanoparticles [27].

The bactericidal effect of silver and AgNPps can be attributed to the attachment of AgNPs to the surface of the cell membrane disturbing permeability and respiration functions of the cell **[37].** It is also possible that Ag Nps not only interact with the surface of membrane, but can also penetrate inside the bacteria [38]. Smaller AgNps having the large surface area available for interaction would give more bactericidal effect than the larger AgNps **[37].** Additionally reports suggest that ionic silver strongly interacts with thiol group of vital enzymes and inactivates them [33, 34 & 39]. Experimental evidence also proposes that DNA may lose its replication ability once the bacteria have been treated with silver ions [40]. The differences between gram-positive and gram-negative bacteria essentially rest in the structure of their respective cell walls. The gram-negative bacteria have a layer of lipopolysaccharide at the exterior, followed underneath by a thin (about 7–8 nm) layer of peptidoglycan consisting of linear polysaccharide chains cross-linked by short peptides to form a three dimensional rigid structure [41]. Although the lipopolysaccharides are composed of covalently linked lipids and polysaccharides, they lack strength and rigidity. Negative charges on the lipopolysaccharides are attracted towards weak positive charges available on silver nanoparticle [42] thereby contributing to the sequestration of free Ag^+ ions. Thus the relations between nanoparticles and the cell wall of bacteria would be facilitated by the relative wealth of negative charges on the gram-negative bacteria, which was amiable to the fact that growth of gram-negative bacteria was supplementary. Once inside the cell, nanoparticles would impede with the bacterial growth signalling pathway by amending tyrosine phosphorylation of putative peptide substrates decisive for cell viability and division. Thus, gram-positive bacteria may allow less Ag^+ to reach the cytoplasmic membrane than the gram-negative bacteria.

4. Conclusion

The green synthesis of stable and spherically shaped AgNPs using *Spinacia oleracea* and *Lactuca sativa* leaf extracts depends on the selection of solvent medium, environmentally benign reducing agent, and nontoxic substances. The silver nanoparticles synthesized and investigated in this study establishes a stronger antibacterial potency which was dose dependent and was more pronounced against gram negative than gram-positive organisms. The silver nanoparticles manifested antibacterial properties by anchoring to and stabbing the bacterial cell wall, and altering cellular signalling by dephosphorylating key peptide substrates on tyrosine residues. However, further studies must be conducted to examine cytotoxicity of nanoparticles towards human cancer cells before proposing their therapeutic use. Outstandingly, the reaction was

graceful and suitable to handle, and it is understood that it has advantages over other biological synthesis.

Table 1 Mean zone of inhibition (mm) of silver nanoparticles synthesized using aqueous extract of Spinacia oleracea and Lactuca sativa and Ofloxacin against 5 different bacterial species. Disk diameter was 6 mm.

Micro organism	Spinach-AgNps	Lettuce-AgNps	Ofloxacin	Percentage increase (%) A–B	B–C
Bacillus subtilis	12	10	10	10	40
Escherichia coli	13	12	12	16.7	16.7
Klebsiella pneumoniae	14	12	10	16.7	40
Pseudomonas aeruginosa	11	12	13	16.7	7.69

Percentage increase (%) of bacterial inhibition between spinach-AgNPs and Lettuce-AgNPs was calculated using the formula (A − B)/B × 100, which indicated an overall 21.12%.

References

[1] M.A. Albrecht, C.W Evan, C.L. Raston, Green Chemistry. **8**, 417–432 (2006).
[2] J. Huang, C. Chen, N. He, Lu. Y. Hong, L. Qingbia, W. Shao, D. Sun, X. Wang, Y.Wang, X. Yiang, Nanotechnology. **18**, 105 (2007).
[3] M. Kowshik, S. Ashataputre, S. Kharrazi, S.K. Kulkarni, K.M. Paknikari, W.Vogel, J. Urban, Nanotechnology. **14**, 95 (2003).
[4] J.L. Gardea-Torresedey, V. Armendariz, I. Herreira, J.G. Parsons, Peralta-Videa, J.R. Teimann, K.J. Torresday, Journal of Hazardous Substance Research. **4**, 1 (2003).
[5] J.L. Gardea-Torresdey, K.J. Tiemann, J.G. Parsons, G.Gamez, I. Herrera, Jose M. Yacaman, Microchemical Journal. **71**,193 (2002).
[6] S.Shivshankar, A.Rai, A.Ahmad, M. Sastry, J. Colloid Interface Sci. **275**, 496 (2004).
[7] A. Richardson, B.C. Chan, R.D. Crouch, A. Janiec, B.C. Chan, R.D. Crouch, Chemical educator. **11**, 331 (2006).
[8] A.T. Harris, R. Bali, Journal of Nanoparticle Research. **11051**, 9288 (2007).
[9] L. Jorge, G. Torresdey, E. Gomez, J.R. Peralta-Videa, J.G. Parsons, H. Troiani, M.J. Yacaman, Langmuir. **19**, 1357, (2003).
[10] R. Hardman, Toxicologic Review of Quantum Dots: Toxicity Depends on Physicochemical and Environmental Factors. Environ. Health. Perspect. **114**, 165 (2006).
[11] J. Curtis, M. Greenberg, J. Kester, S. Phillips, G. Krieger, Toxicol. Rev. **25**, 245 (2006).
[12] N. Lewinski, V. Colvin, R.Drezek, Cytotoxicity of nanoparticles. Small. **4**, 26 (2008).
[13] P.Raveendran, J. Fu, S.L. Wallen, Completely "green" synthesis and stabilization of metal nanoparticles. J. Am. Chem. Soc. **125**, 13940 (2003).
[14] S.S. Shankar, A.Ahmad, R. Pasricha, M. Sastry, Journal of Material chemistry. **13**, 1822 (2003).
[15] M.F. Lengke, M.E. Fleet, G. Southam. Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold (I)-thiosulfate and gold (III)-chloride complexes.

Langmuir. **22**, 2780 (2006).
[16] L.R. Howard, N. Pandjaitan, T. Morelock, M.I. Gil, J Agric Food Chem. **50**, 5891 (2002).
[17] L. Rafael, M.S. Ascensión, A. Francisco, B, I. Tomás- María Gil Federico, Food Chemistry. **108**, 1028 (2008).
[18] P. Mulvaney, Surface Plasmon Spectroscopy of Nanosized Metal Particles Langmuir. **12**, 788 (1996).
[19] M. Sastry, A. Ahmad, M.I. Khan, R. Kumar, Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr. Sci. **85**, 162 (2003).
[21] I. O. Sosa, C. Noguez, R.G. Barrera, Optical properties of metal nanoparticles with arbitrary shapes. J. Phys. Chem. B. **107**, 6269 (2003).
[22] D. Philip, Mangifera Indica leaf-assisted biosynthesis of well-dispersed silver nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy Spectroscopy. **78**, 1327 (2011).
[23] S. Basavaraja, S.D. Balaji, A.Lagashetty, A.H. Rajasab, A. Venkataraman, *Mater.* Res. Bull. **43**, 1164 (2008).
[24] S. Shivshankar, A. Ahmad, M. Sastry, Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. **191**, 627 (2003).
[25] J.Y. Song, B.S. Kim, Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng. **32**, 79 (2008).
[26] Y.Li, J.A.Yates, J.J. Chen, Identification and characterization of sea squirt telomerase reverse transcriptase. Gene. **400**, 16 (2007).
[27] M.I. Gil, F. Ferreres, F.A. Tomas-Barberan, J Agric Food Chem. **47**, 2213 (1999).
[28] M. Bergman, A. Perelman, Z. Dubinsky, S. Grossman. Phytochemistry. **62**, 753 (2003).
[30] N. Vigneshwaran, N.M. Ashtaputre, P.V. Varadarajan, R.P. Nachane, K.M. Paralikar, R.H. Balasubramanya, Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavu.s* Mater. Lett. **61**, 1413(2007).
[31] N. Nino-Martinez, G.A. Martinez-Castanon, A. Aragon-Pina, F. Martinez-Gutierrez, J.R. Martinez-Mendoza, F.Ruiz, Nanotechnology. 19, 065711.1-065711.8 (2008).
[32] T. Bechert, V. Alt, P. Steinrücke, M. Wagener, P. Seidel, E. Dingeldein, Biomaterials. **25**, 4383 (2004).
[33] H.Y. Lee, H.K. Park, Y.M. Lee, K. Kim, S.B. Park, Chem.Commun. **2007**, 2959 (2007).
[34] S. Jeong, S. Yeo, S. Yi, J Mater Sci. **40**, 5407 (2005).
[35] D. Roe, B. Karandikar, N. Bonn-Savage, B. Gibbins, J.B. Roullet, J Antimicrob Chemother. **61**, 869 (2008).
[36] H. Lepape, F.P. Solano-Serena, C. Contini, Devillers, A. Maftah, P. Leprat, J Inorg Biochem. **98**, 1054 (2004).
[37] L. Kvitek, A.Panacek, J. Soukupova, M. Kolar, R. Vecerova, R. Prucek, J Phys Chem C. **112**, 5825 (2008).
[38] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J. Kouri, J.T. Ramirez M. Yacaman, Nanotechnology. **16**, 2346-2353 (2005).
[39] T.H. Wu, F.L. Yen, L.T. Lin, T.R. Tsai, C.C. Lin, International Journal of Pharmaceutics. **346**, 160–168 (2008).
[40] S. Pal, Y.K. Tak, J.M. Song, Applied and Environmental Microbiology. **73**, 1712–1720 (2007).
[41] M. Madigan, J. Martinko, Brock Biology of Microorganisms 11th edn (Englewood Cliffs, NJ: Prentice Hall). (2005).
[42] Z.M. Sui, X. Chen, L.Y. Wang, L.M. Xu, W.C. Zhuang, Y.C. Chai, C.J. Yang, Capping effect of CTAB on positively charged Ag nanoparticles. *Physica E.* **33**, 308 (2006).