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Review Article

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Microbial Silver Nanoparticles –Review

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Abstract

Nanotechnology is one of the most interesting areas which are used to describe the creation and utilization of materials with structural features between those of atoms and bulk materials with at least one dimension in the nano range (Song and Kiml 2009, Nalwa 2000). It has been defined as a technology that mainly consists of the process of separation, consolidation and deformation of materials by one atom or molecule (Taniguchi, 1974). Nanotechnology is currently fast growing niche in the field of nano science (Mandal *et al.,* 2006). It has blossomed over the last twenty years and the need for nanotechnology will only increase as miniaturization becomes more important in the areas such as computing, sensors and biomedical application.

Keywords: Nanotechnology, Microbes, Silver Nanoparticles.

Introduction

Rapid synthesis of silver nanoparticles can be done by the combination of culture supernatant of bacteria. Saifuddin *et al.,* (2009) revealed that the culture supernatant of *Bacillus subtilis* with the help of microwave irradiation to silver ions lead to the production of silver nanoparticles. The examinations of the nanoparticles were done by UV visible spectroscopy and Transmission Electron Microscopy (TEM) and the range of the nanoparticles were found to be 5-60nm.

Sathish kumar *et al.,* (2009) reported in their study, green synthesis of Nano - crystalline silver nanoparticles from *Cinnamon Zeycanicum* bark extract and powder for its enhanced bactericidal activity. Silver nitrate and *C. Zeylanicum* powders (CBP) and extracts (CBPE) were used to synthesize silver nanoparticles separately. CBPE synthesized more silver nanoparticles when compared with CBP due to the presence of large reducing agent in CPBE. It was characterized by UV-Vis spectra analysis, TEM observation and XRD. The UV- Visible spectra of the sample were monitored as a function of reaction time and biomaterial dosage at a resolution of 1nm. The cubic and hexagonal phases of nanocrystals were confirmed by XRD pattern. The bactericidal activity of silver nanoparticles was checked against the *E.coli* strain in Luria Bertani (LB) broth media. The growth was monitored for every 24 hours by using UV Spectrophotometer at 600nm. EC_{50} value of 11+ 1.72mg/L was obtained against *E.coli* for the synthesized silver nanoparticles.

In the study reported by Arima nanda and Saravanan (2009) *Staphylococcus aureus* was maintained in muller hinton broth. The broth culture was centrifuged

at 12, 000 rpm for 3 mins and the supernatant was used as a solvent in the synthesis of AgNPs. Then the supernatant was added to the vessel containing silver nitrate solution. The reaction of this solution was monitored and measured in the UV visible spectrophotometer at a resolution of 1nm. The AgNPs were characterized by AFM. The AgNP synthesized from *S.aureus* was tested for antimicrobial activity by well diffusion method, against pathogenic organisms such as MRSA, MRSE, *Streptococcus pyogenes, Salmonella typhi, Klebsiella pneumoniae* and *Vibrio cholorae.* The cultures mentioned was swabbed as a broth on agar plates and the samples of nanoparticles was poured in to wells of the plates and incubated. After 18 hrs the different levels of zone of inhibition was measured.

Nitya and Ragunathan, (2009) synthesized silver nanoparticles using *Pleurotus sajor*. The fungal filtrate of white rot fungi was reacted with silver nitrate solution and observed for the color change from pale yellow to light brown. The intensity of the colour increased as the time increased. It was then characterized by using UV-Vis studies, SEM analysis and conformed as silver nanoparticles. The spherical shaped, nanoparticle with 5-50nm was obtained. The susceptibility of silver nanoparticles against positive and negative organism was carried out by agar well diffusion method. The antibacterial activity was observed more in the gram negative organisms such as *Escherichia coli, Pseudomonas aeruginosa* when compared with gram positive organism such as *Staphylococcus aureus*.

There was an interesting article on green synthesis of small silver nanoparticles using *Geroniol* and its cytotoxicity against fibrosarcoma- wehi 164. The researchers successfully produced the silver nanoparticles from *Geroniol.* The synthesized nanoparticles were characterized by UV- Visible spectrum and Transmission Electron Microscope. The biologically synthesized silver showed significant cytotoxicity against fibrosarcoma- wehi 164. (Mona Safaepour *et al*., 2009).

The green synthesis of nanosilver particles from extract of *Eucalyptus hybrid* leaf was investigated. The plant extract was prepared by soxhlet extraction method. The identification of active constituents was done with the help of phytochemical analysis tests. The biologically synthesized silver nanoparticles were characterized by the means of UV-Visible spectra analysis, XRD analysis, SEM, and EDX analysis. And the presence of silver nanoparticles was confirmed. (Manish *et al.,* 2009).

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One study was reported on the extracellular synthesis of silver nanoparticles using *Euphorbia hitra* and their antibacterial activities. The *Euphorbia hitra* leaves were collected from Regional Agriculture Research Station Tirupathi, Andra Pradesh India. 1mM silver nitrate was added to the plant extract to produce the silver nanoparticles. The colour change of the solution indicated the presence of the silver nanoparticles. The UV-Vis spectrum was used to monitor the reduction of pure Ag^{+} ions in the reaction medium. Scanning Electron microscopic analysis was done using Hitachi-S-4500. The antibacterial activity was checked against

5 pathogenic organisms, namely *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa,* and the synthesized silver nanoparticles showed high activity against *Staphylococcus aureus* (Elumalai *et al*., 2010). Prabhu *et al*., (2010) reported a study on synthesis of silver phyto nanaoparticles and their antibacterial efficacy. *Ocimum sancta* and *Vitex negundo* were used for the studies, both plants were able to produce the silver nanoparticles. The biologically synthesized silver nanoparticles were used against five pathogenic organisms namely, *Staphylococcus aureus, Proteus mirabilis, Vibrio cholera,* and *Pseudomonas aeruginosa*. The plant *Ocimum sancta* showed high antibacterial activity against *Pseudomonas aeruginosa,* where as the *Vitex negundo* showed comparatively high activity against *Proteus mirabilis*. But compared to *Vitex negundo*, the *Ocimum sancta* showed more antibacterial activity.

One study was reported on synthesis of plant mediated silver nanoparticles using papaya fruit extract and evolution of their anti microbial activities. 1mM aqueous solution of silver nitrate was used to synthesize the silver nanoparticles. The colour change of the solution gave first evidence of synthesis of silver nanoparticles. And the confirmation was done by UV- Vis spectra analysis, XRD measurement, SEM analysis and FTIR analysis. The antibacterial assays were done against *Escherichia coli* and *Pseudomonas aeruginosa*. The nanoparticles showed better activity against *E.coli*. (Jain *et al*., 2010). Mohammad Gilaki, (2010) reported the biosynthesis of silver nanoparticles using plant extracts. The plants belong to Asteraceae, Basellaceae, and Poaceae families were selected for the study. The author was successfully produced silver nanoparticles from all plants. And the characterization of biologically synthesized silver nanoparticles was done by the means of X-ray diffraction and electron microscopy.The spore crystal of *Bacillus thuringiensis* was used for the synthesis of silver nanoparticles. Aqueous solution of silver nitrate was mixed with the spore crystal mixture for the reduction of silver ions and incubated for 5 hours at room temperature. The synthesized silver nanoparticles were characterized using UV-Vis spectra analysis, XRD measurements, TEM analysis. The antibacterial activities of synthesized nanoparticles were carried out against multi- drug resistant human pathogens and antibiotics. The clear zone inhibition was observed against *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* but the standard antibiotics were resistant against the pathogen. (Devendra Jain *et al*., 2010).

Silver nanoparticles were synthesized from *Streptomyces species* by reacting the extract with silver nitrate solution. The synthesis of silver nanoparticles was confirmed by the colour change from pale yellow to brownish due to the Surface Plasmon Resonance. The antibacterial activity was examined by agar well diffusion method against pathogenic Gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*) and Gram negative bacteria (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris*). The silver nanoparticles showed high activity against *Salmonella typhi* followed by *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, E.coli* and least activity was against *Klebsiella pneumoniae* (Shirley *et al*., 2010). Vijay *et al*., (2010) synthesized silver nanoparticles from *Aspergillus clavatus* isolated from *Azadirachta indica*. It was then reacted with silver nitrate solution and incubated for the colour change from colourless to brown. It was then characterized using X ray diffraction, spectrometry, Atomic Force Microscopy and Transmission electron microscopy. The size of the nanoparticles ranges from 10-25 nm. Antimicrobial activity was checked against *Candida albicans*, *Pseudomonas fluorescens* and *Escherichia coli* and the average minimum inhibition concentration was showed against *Candida albicans*.

The synthesis of silver nanoparticles can be done by physical, chemical, and biological methods. Compare to biological methods, chemical and physical methods are highly expensive. Therefore biologically or biosynthesized silver nanoparticles has high market values. In the year of 2010, a couple of researchers successfully produced silver nanoparticles from *E.coli* and these silver nanoparticles were characterized by UV- Vis spectroscopy and particle size analyser. The nanoparticles exhibited high absorbance at 400nm in UV-Visible spectroscopy and the particle size ranges from 35 to 45nm (Kannan Natarajan *et al.*, 2010).
Biosynthesis of silver nanoparticles by a fungus *Bipolaris nodulosa* and its antimicrobial activity was checked. In this study the infected leaves of *Eleusine indica* was used to isolate the pathogens. Silver nitrate solution was used to synthesize silver nonoparticles and it was then characterized by using UV-Vis spectroscopic studies, dynamic light scattering test, XRD measurement, FTIR analysis, AFM, EDX, and TEM. The antibacterial activity of fungus mediated silver nanoparticles (100µg/ml) was done by agar well diffusion method against six indicator bacterial strains such as *Bacillus cereus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, and Micrococcus*

luteus. The size of the silver nanoparticle varies between 10-60 nm. The activity was shown in all the tested organisms (Saha *et al.*, 2010).

There are different studies which revealed that, the plant and fungus can also produce silver nanoparticles. Using *Penicillium purpurogennum*, Rati rajan *et al.*, (2011) successfully produced silver nanoparticles. They found that, increase in concentration of silver nitrate solution increases the formation of silver nanoparticles. They have also reported that, the change in pH of the reaction mixture lead to the change in the shape and size of the silver nanoparticles.

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Another study was reported on biological synthesis and characterization of silver nanoparticles from *Trichoderma harzianum*. The fungus species were collected from culture collection centre (CAS in Botany, University of Madras, India) and was maintained in potato dextrose agar medium. The silver nanoparticles which are produced from *Trichoderma harzianum* found to be high stable in nature. UV-Visible spectroscopy and TEM was carried out in order to characterize the silver nanoparticles. And the nanoparticles were found in the range of 30-50nm in size and spherical in shape (Prashant Singh and Balaji Raja, 2011).

In 2011, a study was performed on antimicrobial activity and the characterization of silver nanoparticles

from *Spinacia oleracea* and *Lactuca sativa* leaves. The leaf extracts were reacted with silver nitrate solution and it resulted in the formation of a brownish colour which showed the presence of silver nanoparticles due to the reduction of phytochemicals present in them. It was then characterised with UV-visible spectra, TEM, SEM, FTIR which showed the shapes and structures of the silver nanoparticles. The antimicrobial studies were done against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by agar disc diffusion method which resulted that the silver nanoparticles had stronger potential against gram negative rather than gram positive bacteria (Amarnath *et al.*, 2011).

Yogeswari Rout *et al.,* (2011) demonstrated the synthesis of eco-friendly silver nanoparticles from the leaf extract of *Ocimum sanctum* and also checked its antifungal and antibacterial activity. In this present study *Ocimum sanctum* was taken from local garden and its crude extract was diluted in DMSO. Silver nitrate solution was added to the plant extract and the formation of silver nanoparticles was observed. It was characterized by using UV-Vis spectroscopy, SEM analysis, and X-ray diffraction method. Antimicrobial susceptibility test was performed by disc diffusion method against *E.coli, E.cloacae, E.faecalis, P.vulgaris, K. pneumoniae, S.aureus, S.saprophyticus.* Three antibiotics namely cefotaxime (30mcg/disc), streptomycin (10mcg/disc) and ampicillin (10mcg/disc) was used as a positive control. The synthesized nanoparticles showed better antibacterial activity against *S.saphrophyticus.* The antifungal activity was done by agar well diffusion method against *Candida albicans, Candida tropicalis, Candida krusei, Candida kefyr, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*. The fungicidal activity was showed more in *Candida albicans*. Minimum Inhibitory concentration was done with micro dilution method and the lowest MIC value was observed for *S.saphrophyticus*.

The methanolic extract of *Vitex nigundo* was treated with silver nitrate solution and observed for the colour change. The synthesized silver nanoparticles were characterized using X ray diffraction, Transmission Electron Microscope and UV- Visible spectroscopy. The antibacterial activities of the silver nanoparticles were checked against *Staphylococcus aureus* and *Escherichia coli* by Kirby Bauer method. The zone of clearance was observed in both the organisms (Mohsen Zargar *et al*., 2011).

Nagajyothi *et al*., (2011) performed the synthesis of silver nanoparticles by treating *Dioscorea batatas* rhizome extract with silver nitrate solution. The formation of silver nanoparticles was confirmed by the colour change in the extract. It was then characterized by UV Vis spectrophotometer, SEM, EDX, XRD and FTIR analysis. The antimicrobial activity was tested against pathogenic bacteria such as *Bacillus subtilis, Staphylococcus aureus, E.coli* and pathogenic fungi such as *Candida albicans* and *Saccharomyces cerevisiae* by agar disc diffusion method on Muller Hinton agar and potato dextrose agar. It showed maximum effect against *Candida albicans* and *Saccharomyces cerevisiae* and intermediary effects on *Staphylococcus aureus* and *Bacillus subtilis* and lowest activity against *E.coli*.

Aditi *et al*., (2011) demonstrated the synthesis of silver nanoparticles from fresh extract of *Anthoceros* by adding 1mM solution of silver nitrate solution. The formation of silver nanoparticles was confirmed by the colour change from light green to red. It was then characterized by UV-Vis spectra analysis, SEM and EDX analysis. The antibacterial activity was checked by standard agar well diffusion method against human pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumoniae.* The good antibacterial activity was observed against all four species by the formation of zone of inhibition.

Naheed Ahmad *et al*., (2012) synthesized silver nanoparticles using extracts of *Ananas comosus* treating it with the aqueous solution of silver nitrate. The colour change confirmed the formation of silver nanoparticles. It was then characterized by UV-Vis spectrophotometer at regular intervals. The XRD and TEM analysis was performed and the size of the nanoparticles ranges between 10-15 nm.

Several researchers synthesized silver nanoparticles from the leaves; they reported the production of silver nanoparticles from the stem bark extraction of *Callicarpa maingayi.* For the study, the authors were used methanolic extracts of *Callicarpa maingayi*. The plant species of beauty berry pants and mainly found in Malaysia and Singapore. The authors successfully achieved the production of silver nanoparticles from the barks of the plant. The colour change found in the sample confirmed the production of the silver nanoparticles. The synthesized silver nanoparticles were characterized by UV- Visible spectroscopy, X-ray diffraction (XRD),Transmission electron microscopy (TEM),Scanning electron microscopy

(SEM),Energy dispersive X-ray fluorescence (EDXF) Spectrometry, Zeta potential measurements and Fourier Transform Infrared(FT-IR) spectroscopy. The XRD study revealed the particle nature and it found to be in crystalline nature (Kamyar Shameli *et al.*, 2012). Microbial synthesis of AgNp was synthesized using soil fungi of high altitudes of eastern Himalaya. Soil samples were collected; serial diluted and plated on potato dextrose agar plates. Fifty three fungal isolates were screened for biosynthesis of Ag NPs. The cell free supernatant was treated with silver nitrate for the formation of Ag NP. Out of 53 isolates only three isolates such as SP5, SF1and MP5 reduced silver salt to the nanparticles. The 3 fungal strains was identified as *Aspergillus terreus SP5*, *Fusarium species* MP5 and *Paecilomyces lilacinus* SF1 based on the morphological, microscopic observations and also by molecular methods. The characterisations of silver nanoparticles were done by using UV visible spectroscopy analysis, and TEM. The antimicrobial activity of the synthesized AgNPs were done against four test microorganism such as *Staphylococcus aureus, Strepotococcus pyogenes, Salmonella enterica* and *Enterococcus faecalis* by agar well diffusion method. All the three fungal strains showed highest activity against *Streptococcus pyogenes*. The synergistic effect of extracellularly synthesized Ag NP was done with a commonly available antibiotics and standard disc coated with synthesized Ag NP against four human pathogenic bacteria. The increase in fold area was calculated by the mean surface area. The diameter of inhibition zones for antibiotics alone and in combination with Silver nanoparticles showed significant increase in fold area in all the cases with erythromycin, methicillin, chloramphenicol and ciprofloxacin. The synergisitic activity of Ag NPs with antibiotics was highest against *S.aureus* and *S.pyogenes* when compared with *Enterococcus faecalis* and *S.enterica* (Lamabam sophiya Devi and Joshi, 2012).

Xuetvan Wei *et al.,* (2012) investigated the synthesis of silver naopartcles from *Bacillus amyloliquesfaciens* extract reacted with Silver nitrate solution. The synthesised Ag NP was determined by UV- Visible spectrophotometer, TEM, FTIR. The antibacterial activity of Ag Np was done in a liquid medium containing AgNP concentration of 0, 3, 6 and 9 µg/ml inoculated with *E.coli* and *Bacillus subtilis*. Compared to *E.coli*, *Bacillus subtilis* showed highest inhibition at 9µg/ml. In solid medium antibacterial activity was done by agar well diffusion method. The bacterium was mixed with LB agar and solidified, wells are cut and silver nanoparticle was added. It was checked for the zone of inhibition. *Bacillus subtilis* showed highest inhibition to the silver nanoparticle.

Gnanadesigan *et al.,* (2012) performed a study on synthesizing silver nanoparticles and estimating their antibacterial activity of *Avicennia marina* mangrove plant extract. The silver naoparticles were synthesized by silver nitrate solution which produced changes in colour of the extract to show the presence of silver nanoparticles. It was further characterized by UV-Visible spectroscopy, FTIR and X-ray diffractometer. The biosynthesized silver nanoparticles were studied for antibacterial activity by agar well diffusion method. It was tested against *E.coli, Pseudomonas aeuroginosa, Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilis* which showed maximum inhibition with *E.coli* followed by *Pseudomonas aeruginosa*, and showed minimum inhibition against *Staphylococcus aureus* species.

Dipankar *et al.,* (2012) demonstrated the green synthesis and biological activities of silver nanoparticles from *Iresine herbstii* leaf aqueous extracts. In this current study, the silver nanoparticles were synthesized and checked for antibacterial, antioxidant and cytotoxic activity. The leaves were disease free leaves and collected from west Bengal. Biosynthesis of silver nanoparticles was done with silver nitrate solution and filtered extract. It was characterized by using UV-Vis spectroscopy, XRD, SEM and EDX, FTIR analysis. The size of silver nanoparticles ranges from 44-64nm. *Staphylococcus aureus, Enterococcus faecalis*, *Eschericia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used as the bacterial pathogens for antibacterial screening and for minimum inhibitory concentration. It was done by agar well diffusion method. Total phenolic content, DPPH free radical scavenging assay, reducing power assay are the antioxidant tests carried out. Trypan blue assay was performed by HeLa cell line for the cytotoxic activity.

The extract of *Gelidiella species* used for the synthesis of silver nanoparticles and its anticancer activity was checked. The seaweed extract was mixed with Silver nitrate solution and incubated until the colour change was observed. The reduction of silver ion into silver nonoparticles and due to the surface Plasmon resonance phenomenon Ag Np exhibited dark yellowish brown colour. Then it was confirmed by using UV-visble spectroscopy, FTIR, X-ray diffractometry, extended dispersive analysis X-ray spectroscopy, SEM analysis. The absorption spectra of Ag Nps are dependent on the shape of the particles.

Renugadevi *et al.,* (2012) described the biosynthesis of Ag NP from green young stem of *Cissus quadrangularis*. The stem extract was used for the synthesis of Ag NP by using silver nitrate solution. It was then characterized by UV-Visible spectra analysis at 422nm, TEM and EDAX analysis. The size of the Ag NP was 15 nm. The synthesized silver nanoparticles was checked for antibacterial activity against various pathogenic bacteria such as *Escherichia coli, Enterococcus faecalis , Bacillus subtilis , Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Vibrio cholera* by disc diffusion method. The zone of inhibition was observed highest against *Vibrio cholorae*. The *invitro* cytotoxicity assay was done by MTT assay using Hep2 cell line and Vero cell line at different concentrations (20, 40, 60, 80,100, 120,140,160 µg). 50 % of cell death for HEP2 and Vero cell line required the concentration of 64µg and 90µg. IC50 value of Hep2 cell line was less when compared with Vero cell lines.

A comparatively different study was reported on synthesis of silver nanoparticles from *E.coli*. The researchers isolated a peptide from *E.coli* and the molecular weight of the protein was determined by SDS-PAGE. From that protein silver nanoparticles were synthesized. UV- Visible spectroscopy, FTIR, TEM and XRD analysis was carried out to characterize the silver nanoparticles. These *E.coli* silver nanoparticles showed antibacterial activity against *Bacillus subtilis, Klebsiella pneumonia, E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Silver nanoparticles showed high activity against *Klebsiella pneumoniae* (20nm). The antioxidant activities of protein capped nanoparticles were examined by DPPH radical assay. The protein capped silver nanoparticles showed better activity when compared to protein sample (Veeraapandian *et al.,* 2012).

Tile Vaishali arjun *et al.,* (2012) demonstrated the biosynthesis of silver nanoparticles from the microorganism and its antifungal activities. In this study the silver nanoparticles was produced from extracellular and intracellular extract of *E.coli* and *Bacillus subtilis*. The colour turned from pale yellow to brownish and it indicates the formation of silver nanoparticles. It was characterized by using UV-Visible spectrophotometer, Transmission Electron Microscope, Scanning Electron microscope. The size of the synthesized nanoparticles varies from 16-20nm. The antifungal activities of silver nanoparticles were checked against the pathogenic fungal cultures such as *Candida albicans, Aspergillus fumigates Trichophyton rubrum.* The silver nanoparticles showed highest antifungal activity against all fungal pathogens by disrupting the structure of cell membrane and mycelia extensions.

Dhawal *et al.,* (2013) demonstrated the biosynthesis of silver nanoparticles from microorganism isolated from soil sample collected from silver mines in South Korea. 16sRNA sequencing of the isolated microorganism was done and confirmed as Exiguobacterium Sp.KNU1. Extracellular synthesis of silver nanoparticles was synthesized from the supernatant of the culture using silver nitrate solution. The synthesized silver nanoparticle was observed with a colour change from pale yellow to brown. It was then characterized by using UV-Visible spectrophotometry, X-ray photoelectron spectrophotometry, FTIR, TEM. The antimicrobial activity of the silver nanoparticles (50-100µg/ml) against different gram negative and gram positive organisms such as *Salmonella typhimurium, Pseudomonas aeruginosa*, *E.coli* and *Staphylococcus aureus* was checked by agar well diffusion method. Minimal inhibitory concentration was carried out by micro dilution method with varying concentration of silver nanoparticles ranging from 0.005 to 100 µg/ml. Among the clinical pathogens silver nanoparticles showed highest activity against gram negative *E.coli*, and least activity against gram positive *S.aureus*. Arun *et al*., (2013) studied on the synthesis of silver nanoparticles from *Corynebacterium species* and checked for its antimicrobial activity. The culture was collected from micro lab Coimbatore. The bacterial filtrate was reacted with Silver nitrate solution and incubated for 3 days at room temperature for the formation of silver nanoparticles. The colour change was observed from pale yellow to brownish colour. The reduction of silver ions was confirmed by UV-visible spectrophotometer and a strong peak was observed at 420nm. The nitrate reductase assay was carried out and the presence of nitrate reductase enzyme was determined. It was then characterized by SEM analysis and EDAX analysis. The SEM image showed an average size range of about 24.55 nm-32.05nm. EDAX analysis confirmed the presence of elemental silver in the synthesized silver nanoparticles. The bactericidal activity of silver nanoparticles was examined against few pathogenic bacteria such as *Escherchia coli, Corynebacterium diptheriae, Klebsiella pneumoniae and Staphylococcus aureus* by agar well diffusion method. The synthesized Ag Nps were diluted in DMSO and ethanol. The highest activity was showed against *Staphylococcus*

aureus in both the dilutions and no activity was observed in case of *Pseudomonas aeruginosa*.

Tokeer Ahmad *et al.,* (2013) reported that the biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. In this present study silver nano particles were synthesized by the fungus extract of *Candida albicans*. It was cultured in Macconkey agar. Silver nanoparticles were synthesized by silver nitrate solution, the colour was observed as pale yellow, wine red and dark brown at some time intervals. Gold nanoparticles were synthesized by using $HAUCI_4$ solution, the colour turned from light yellow to light purple. It was then confirmed by using XRD, TEM, UV-VIS, FTIR, Thermo gravimetric analysis (TGA) and B.E.T surface area analysis. In XRD no impurity peak is observed. TEM has the particle size ranges from 4nm to 10nm. But TGA analysis was confirmed by the bio molecules present in cell free extract of gold and silver nanoparticles. The ranges spreads in the B.ET surface area analysis from 96 $A^0$to $17A^0$respectively. The result from the antimicrobial activity for the gram positive bacteria is less susceptible than gram negative bacteria.

The silver nanoparticles were synthesized from *Nocardio species*. The bacterium was isolated from the marine sediment sample from the Busan Coast. The organism was confirmed by 16s rRNA gene sequences. The supernantant of the bacterium was used for the biosynthesis of silver nanoparticles. The productions of silver nanoparticles were confirmed by the occurrence of colour change. The characterization of nanoparticles was done by UV-Visible Spectroscopy, TEM, FE-SEM, EDX, FTIR and XRD Spectroscopy. The synthesized silver nanoparticles have showed antimicrobial activity against *Bacillus subtilis, Enterococcus species, Pseudomonas aeruginosa, Shigella flexneri* and *Staphylococcus aureus* and the anti fungal activity was carried out against *Aspergillus niger, Aspergillus brasiliensis, Aspergillus fumigates* and *Candida albicans*. The cytotoxicity was checked against *invitro* human cervical cancer cell line (HeLa). The authors reported that the synthesized silver nanoparticle showed dose-response activity against HeLa cancer cells (Panchanathan Manivasagan *et al.,* 2013).

The green synthesis of silver nanoparticles from *Origanum vulgare* and its anticancer activity was reported. In that study the researchers achieved silver nanoparticles from aquous extract of *Origanum vulgare* (oregano). The formation of reddish brown colour was confirmed the presence of silver nanoparticles. The biosynthesized silver nanoparticles were characterized by UV-Vis Spectroscopy, Fourier Infrared Spectroscopy (FT-IR), Field emission – scanning electron microscopy (FE-SEM), X-ray diffraction (XRD) and Dynamic light scattering measurements. The nanoparticles were found to be spherical in shape with an average particular size distribution of 136±10.09nm. The antibacterial study was carried out against bacterial strains namely *Aeromonas hydrophilla, Bacillus species, Escheirchia coli, Klebsiella species, Salmonella species, Salmonella paratyphi, Shigella dysenteriae,* and *Shigella Sonnei*. In that the *Bacillus species* and *Klebsiella species* showed comparatively less zone of inhibition. The *invitro* cytotoxicity was evaluated against human lung cancer A_24_9 cells. They have found that 500µg/ml of silver nanoparticles successfully inhibits the cell growth by more than 85%. So the authors concluded that the silver nanoparticles synthesized from *Origanum vulgare* have showed impressive antibacterial as well as anticancer activity. There for it can be used in the medical field (Renu Sankar *et al.,* 2013).

The supernatant of the *Pseudomonas aeruginosa* was treated with Silver nitrate solution solution and the colour change from greenish to brown indicates the formation of silver nanoparticles. It was then characterized by using UV visible spectroscopy between 415 and 425 nm, SEM, XRD and FTIR analysis. The size of the silver nanoparticles varies between 13 to 16nm. The antibacterial activity was done against gram positive and gram negative organisms such as *E.coli, V.cholerae, Salmonella species, Klebsiella species, Aeromonas species, Proteus species, Corynebacterium species, Bacillus species and Staphylococcus species* by paper disc method. It showed highest inhibition against *E.coli, V.cholorae, Aeromonas species* and *corynebacterium species.* The cytotoxic assay was carried out against human cervical cancer cell line by MTT assay method. Depending on the size of the Ag NP the sensitivity of the cancer cell lines varies. The concentration of Ag NP (0-100µg/ml) decreased the proliferation capacity of the cells and at 40µg/ml cell death was observed (Ramalingam *et al.,* 2013).

Abishek kaler *et al.,* (2013) reported the green and rapid synthesis of anticancerous silver nanoparticles by *Saccharomyces boulardii*. In the present study the yeast, probiotic strain was collected from local market. The cell free extract was used for the synthesis of silver nanoparticles by silver nitrate solution. It was

then characterized by using UV –Visible spectrophotometer in the range of 200-800nm, TEM, FTIR, EDX. Various physiochemical parameters of synthesized Ag NP optimized such a pH, time, temperature, cell age and concentration. The cytotoxicity assay was carried out against breast cancer cell line named MCF-7 cells. At low concentration, it showed highest inhibition on MCF-7 cells but no much difference in the higher concentration (10-100µg/ml) of silver nanoparticles. The IC50 value of synthesized silver nanoparticles is less than 10µg/ml.

The silver nanoparticles were synthesized from *Bacillus subtilis* and the anticancer activity was checked against THP-1 cancer cell line. The supernatant of the bacterial cultures was treated with Silver nitrate solution for the synthesis of silver nanoparticles. It was then characterized by UV-Vis spectroscopy, SEM, EDX, and XRD. The EDX spectra showed peak around 1.3kev, 2.2kev, 3kev, 3.20 kev that are corresponding to the binding energy of AgLsec, AgLa, AgLb1, AgLb2, it indicates the presence of AgNp. The *invitro* cytotoxicity study was done against Acute Myeloid Leukemia cell lines (THP – 1). Combination of chemotherapeutic drugs such as mercaptopurine and busulfan with synthesized AgNP showed more effectiveness when compared with individual drugs and Ag NP, whereas some drugs were not effective when used in combination (Rebecca *et al.*, 2013).

Niramathi *et al.*, 2013 suggested the biosynthesis of silver nanoparticles from fresh leaves of *Alternanthera sessilis* using silver nitrate solution. The colour change was observed from yellow to reddish brown colour indicates the formation of silver nanoparticles. It was then characterised by UV- Visible spectra analysis, FTIR analysis, SEM analysis, TG-DSC analysis, Particle size measurement was carried out using laser diffractometry. The size of the nanoparticle observed in SEM image varies from 20nm to 30nm. The particles average diameter was found to be 344.4 and 5272 nm. The antimicrobial activity was screened against *Staphylococcus aureus* and *Escherchia coli*. Both the pathogens were sensitive for the Ag NP. The antioxidant activity was determined by DPPH assay measured at 517nm. The purple colour of the freshly prepared DPPH disappears while adding synthesized Ag NP due to the presence of antioxidant. The free radical scavenging activity is purely close dependent showing a maximum of 62% at 500µg/ml. The IC50 value was observed as 300.6µg/ml.

Kelly Ishida *et al.*, (2013) have worked on the silver nanoparticles production from *Fusarium oxysporum* filamentous fungus and its antifungal activity against some pathogenic yeasts. The fungal filtrate was reacted against silver nitrate solution for the synthesis of silver nanoparticles. It was then characterized using TEM, AFM and SEM analysis. The highest absorbance of silver nanoparticles was observed between 340-560 nm. With different concentration of silver nanoparticles the antifungal activity was checked against *Candida species* and *Cryptococcus species* by disc diffusion method. It exhibited the fungicidal activity against *Cryptococcus species* followed by *Candida.*

Periera *et al.*, (2013) demonstrated the synthesis and characterization of silver nanoparticles from *Penicillium chrysogenum* and *Aspergillus oryzae* and checked the antifungal activity. Biologically synthesized silver nanoparticles were produced by reacting the cell free filtrate with silver nitrate solution. It was characterized by using UV-Visible spectroscopy, SEM, XRD, FTIR analysis and dynamic light scattring analysis. They compared the effect of chemically and biologically synthesized silver nanoparticles with commercially available antifungal drugs against *Trichophyton rubrum* and observed that the antifungal activity was higher than flucanozole drugs.

Ritika chauhan *et al.*, (2013) demonstrated the synthesis of silver nanopaticles from *Streptomyces species* and its antibacterial activity. The species were isolated from the soil sample collected from Kodaikanal hill station. From the isolate silver nanoparticles were successfully synthesized. The nanoparticles were characterized using UV-Visible spectral analysis, FT-IR analysis, XRD analysis and atomic force microscopy. The size of the nanoparticles was found to be 68.13nm. The antimicrobial activity was checked against *E.coli, Pseudomonas aeruginosa, Salmonella species, Staphylococcus aureus, Ganoderma species, Scedosporium species, Fusarium species* and *Candida tropicalis*. In that, *Fusarium species* and *E.coli* showed high zone of inhibition against silver nanoparticles.

Saravanan *et al.*, (2014) has proposed that the biolologically synthesized nanoparticles have a good antibacterial activity against various pathogens. In this study the *Bacillus subtilis* was obtained from National Collection of Industrial Microorganism, Pune, India for the biosynthesis of silver nanoparticles using silver nitrate solution. The supernatant was used for the extracellular synthesis of silver nanoparticles. It was

then characterized by UV visible spectroscopy, AFM, TLC, FTIR. The colour change of the solution to yellowish brown indicated the formation of silver nanoparticles due to excitation of surface plasmon vibration. The antibacterial activity of Ag NPs was checked against various pathogenic organisms such as *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi. Klebsiella pneumoniae* and *Vibrio cholerae* using agar well diffusion method. The synthesised silver nanoparticles showed highest antibacterial activity against all the pathogens except *Vibro cholerae.*

Green synthesis of silver nanoparticles using *Millingtonia hortensis* was done by adding silver nitrate solution to the extract. The colour change from light yellow to dark brown was observed due to the surface plasmon excitation and it confirmed the formation of silver nanoparticles. It was then characterized by UV spectrophotometer, SEM, EDAX, XRD and FTIR. The antibacterial activities of synthesized silver nanoparticles were performed by disc diffusion method. The zone of inhibition was observed against *Bacillus subtilis* and *Klebsiella planticola* (Gnanadhas *et al*., 2013)

Phototrophic bacteria mediated synthesis of silver nanoparticles and its antibacterial activity was studied. A purple non sulphur bacterium was collected from stagnant water and it was confirmed as *Rhodopseudomonas species* by morphological and cultural characteristics. The biomass was mixed with water and agitated for 24 hrs and then filtered through whatmann no1 filter paper. The supernatant with silver nitrate solution was used for the synthesis of silver nanoparticles and incubated at room temperature. Nanoparticle formation was observed with a colour change due to excitation of surface Plasmon vibration. It was then characterized by UV-visible spectrophotometer from 400nm to 800 nm, FTIR spectra in the range of 750-4000 cm, TEM analysis. The antibacterial assays of Silver nanoparticle were done on *Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas putida, E.coli, S.aureus* by agar disc diffusion method. Varying concentration of synthesized Ag NP was used to prepare a sterile paper disc with a standard ampicillin. The sizes of the nanoparticles were spherical in shape with the range of 6-10nm and showed highest activity against *E.coli* and *S.aureus* (Manisha *et al*., 2014).

Ropisah *et al.,* (2014) performed a study by synthesizing and characterizing the silver nanoparticles obtained from lichen *Parmotrema praesorediasum*. The silver nanoparticles were synthesized using 1mM silver nitrate solution, which resulted in the formation of a yellowish brown colour solution occurring due to excitation of surface Plasmon resonance in the Ag Np. The characterization was performed using UV spectrophotometer, TEM, energy dispersive spectroscopy and X-ray diffractometry. It showed the morphology and size distribution of the obtained Ag Np. The nanoparticles were tested for antibacterial activity against gram negative bacteria (*Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens)* and gram positive bacteria (*Staphylococcus epidermidis, Bacillus subtillis, Staphylococcus aureus*) by disc diffusion method which showed that it had great activity against gram negative bacteria.

Nazeruddin *et al.,* (2014) evaluated antimicrobial activity of synthesized Ag NP from *Coriandrum sativum*. The seed extract was reacted with Silver nitrate solution. Formation of Ag NP was observed from the colour change. It was then confirmed by UV-spectroscopic analysis, XRD, particle size distribution, SEM analysis, TEM analysis. The antimicrobial activity of Ag NP was screened against a gram positive *Bacillus subtilis* and gram negative pathogenic bacterium by agar well diffusion method. It showed highest activity against gram positive bacteria. The combination of penicillin with Ag NP was more effective with a zone of inhibition (17mm) when compared with standard antibiotics (13mm). Hence these would be new perspective towards nanomedicine.

Gravita singh *et al.,* (2014) described the green synthesis of silver nanoparticle from *Anabaena doliolum* and its antibacterial and antitumor activity was screened. Synthesis of silver cyanobacterial nanoparticles was carried out by using silver nitrate solution and the cell free extract. It was then incubated at 25ºc for 72 hours and the formation of AgNp was monitored for every 2 hours. The colour change was observed from reddish blue to brown and the bioreduction of silver ions was monitored by the spectroscopic analysis in the range of 20-700 nm in a UV-visible spectrophotometer. The strong and broad peak was observed at 420 nm. The size and the morphology of Ag-CNPs were confirmed by transmission electron microscopy. The size distribution and Zeta potential value were examined by particle size analyser. It was also then characterized by X-ray diffraction and FTIR analysis. The antibacterial activity of Ag-CNp was tested on two gram negative and one gram positive bacteria such as

Klebsiella pneumoniae, Escherchia coli and *Staphylococcus aureus* using disc diffusion method. Varying concentration of 5, 50, 100, 250 and 500µg/disc were tested for bactericidal effect the zone of inhibition was observed in all the concentration against all the three multidrug resistant bacteria. Hence it indicates, there is no correlation between the concentration of the disc and size of zone of inhibition. The antitumor activity was carried out on Dalton's lymphoma and colo 205 cells. The cell viability was determined by the standard MTT assay. Different concentrations of 10µg/ml and 20µg/ml of Ag CNp were exposed to DL cells, colo205 cells and normal cells. In 10µg/ml concentration of Ag-CNp, DL cells showed 47-50% loss of survival, in colo205 cells showed 62-65% and in normal cells it showed 10% loss of survival.

Hala yassin and Azza Ahmed, (2014) demonstrated the bactericidal and cytotoxic activity of biosynthesized silver nanoparticles with an extract of the red seaweed *Pterocladiella capillacea*. The Silver nitrate solution solution and the extract of the red seaweed were used for the green synthesis of Ag Np and were observed for the brown colour. It was then characterized by UV-visible spectrophotometer, TEM, EDX, FTIR. The absorption spectra were measured at the wavelength of 300-700 nm and confirmed the synthesis of Ag Np. The shape of the Ag Np was spherical and the presence of elemental silver is confirmed by EDX spectrum. The bactericidal activity of synthesized Ag Np was checked against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginnosa* and *Escherchia coli* by agar well diffusion method. Different concentrations of AgNp (5, 10 and 20µg/ml) were added to each wells. The activity was showed more in *Bacillus subtilis* followed by *Staphylococcus aureus* at higher concentration and it also revealed that the activity is highly dose dependent. The anticancer activity of biosynthesized silver nanoparticles was evaluated *invitro* against human carcinoma (Hep G cell line) at different concentrations such as 5, 2.5, 1.25, 0.625 and 0.312 µg/ml. The neutral red assay was carried and confirmed the viability of tumour cells. The tumour cell death was observed at higher concentration (5µg/ml) and the cytotoxicity was decreased at lower concentration. Hence it revealed that the anticancer activity is purely dose dependent.

Mohamed Abdel-Aziz *et al.*, (2014) had done a study on antioxidant and antibacterial activity of silver nanoparticles biosynthesised using *Chenopodium murale* leaf extract. The plant belongs to chenopodiaceae family; the leaves were collected from the Dekernis district, Egypt. In the study, the fresh leaves extracts were used for the biosynthesis of silver nanoparticles. The formation of dark brown colour was indicated the synthesis of silver nanoparticles. The characterizations of nanoparticles were done by U-V Visible absorbance spectroscopy and TEM analysis. In U-V Vis analysis the nanoparticles showed maximum absorption at 440nm. The TEM analyses were confirmed the size of the nanoparticles and it was ranged between 30 and 50nm. The determinations of antibacterial activity were performed by the cup- plate agar diffusion method. The antibacterial activity was carried out against *Staphylococcus aureus* and it showed zone of inhibition. The antioxidant activities of silver nanoparticles were evaluated by DPPH scavenging and β- carotene bleaching assay. The nanoparticles were showed significant antioxidant activity, and the values were increased in a dose dependent manner.

Authors reported the biosynthesis of silver nanoparticles from *Garcinia mangostana* fruit extract. The plant belongs to clusiaceae family. It grows mainly in Southeast Asia and also in tropical South American Countries.The paper reported the successful synthesis of silver nanoparticles from *G. mangostana* fruit. Characterizations of silver nanoparticles were done by UV-Visible spectra analysis and Transmission electron microscopy (TEM). The synthesized silver nanoparticles showed antibacterial activity against *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antioxidant activities of biosynthesized silver nanoparticles were evaluated by DPPH scavenging assay. The recorded highest value was 71+0.58. Hence, the biologically synthesized silver nanoparticles have applications in medical filed, especially in the drug discovery and delivery process (Subashini Rajakannu *et al*., 2014).

The leaf extract of *Canthium coromandelicum* was used for the synthesis of silver nanoparticles by silver nitrate solution. It was then characterised by UV Visible spectra analysis and SEM analysis. The size of the silver nanoparticle ranges from 10-40nm. The antibacterial activity was done against *Staphylococcus aureus, E.coli, Bacillus subtilis*. The synthesized Ag NPs showed highest activity against *E.coli*. DPPH radical activity of synthesized Ag NP was determined and the absorbance was measured at 517 nm. The Ag NP showed antioxidant activity based on the concentration as compared with plant extract (Chandra *et al.*, 2014).

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