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Green synthesis of silver nanoparticles by microorganisms and their antimicrobial activity against human pathogenic organisms

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Abstracts : *The aim of the study was to synthesize five bacterium silver nanoparticles (AgNPs) and their antimicrobial activities with characterization. This study highlights the green, rapid, facile, cost-effective, and eco-friendly synthesis of AgNPs using Staphylococcus spp, E. coli spp, Pseudomonas spp, Bacillus spp, and Aspergillus spp. with their antibacterial mechanisms. The zone of inhibition was used to evaluate the concentration between MIC and bactericidal activity of AgNPs. The antibacterial activity with a zone of inhibition maximum was found in Aspergillus AgNP's e.g. (16, 26, 16, and 23 mm) from the bacterium of Staphylococcus spp, Bacillus spp, E. coli spp, and Pseudomonas spp respectively, followed by Streptomyces (23 and 20 mm). UV-Visible spectroscopy was used to monitor the synthesis of colloidal AgNPs. The AgNP's samples were subjected to optical absorbance measurement using a spectrophotometer between 400-700 nm wavelength. AgNPs exhibited a light yellowish to brown colour. Therefore, AgNPs synthesized by microorganisms can be used as a powerful antimicrobial agent for various therapeutic applications. This study indicates that pathogen-based AgNPs exhibit a strong antimicrobial activity and thus might be developed as a new type of antimicrobial agent for the treatment of bacterial infection including multidrug-resistant bacterial infection.*

Keywords: *Silver nanoparticles, green synthesis, microorganisms, antibacterial activity, a human pathogen.*

1. INTRODUCTION :

Pathogenic bacteria are playing an important role in the creation of unknown diseases and the development of antibiotic resistance which are the major problems in the current scenario. The applications of nanoparticles are gaining an important function in the current scenario as they possess well-defined chemical, visual and mechanical attributes. Nanoparticles of metals are the most potential agents as they show excellent antibacterial activities due to their large surface area-to-volume ratio, which is getting up the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics, and the growth of resistant strains [1].

An expanding field of nanoscience and nanotechnology is important and its applications in various fields. The nanoparticles are of materials structures at the nanoscale, which typically ranges from 1 to 100 nanometers (nm). In the fields of catalysis, biology, biomedical science, and water treatment, nanomaterials may offer answers to technical and environmental problems. Extremely high surface-to-volume ratios can be seen in nanoparticles. When a large surface area is required in science, this attribute can be used. As an illustration, certain nanoparticles have been demonstrated to be effective catalysts in the catalytic sector [2]. The nanoparticles also exhibit antibacterial properties. Silver nanoparticles have been given credit for these crucial characteristics. AgNPs have therefore drawn a lot of attention in nano-biotechnological research because of their distinct physical, chemical, biological, and other features, as well as due to their utility in electronics, optics, and medicine [3,4].

Silver nanoparticles can also be employed for a variety of significant applications, such as optical receptors for biolabeling, intercalation materials for batteries, and spectrally selective coatings for solar energy absorption [5,6]. The antibacterial and inhibitory properties of AgNPs are well recognized. Pathogenic bacteria have begun to develop resistance to antimicrobial treatments, nonetheless, in recent years. This has been extensively researched and is a significant concern for the healthcare sector [6,7].

Antimicrobial nanoparticles offer various distinctive advantages in reducing acute toxicity, overcoming resistance, and lowering cost when compared to conventional antibiotics. Antibiotics in the NPs form may sustain for the long run than in tiny molecules. Physical and chemical synthesis methods, aimed at controlling the physical properties of the particles are mostly employed for the production of metal nanoparticles [8,9]. The use of bacterium in the biosynthesis reaction is a fascinating area of nanoparticle biosynthesis. Recent years have seen the development of green techniques for nanoparticle synthesis into a significant area of nanotechnology [10,11]. The selection of bacterium for this paper was based on their potential for use in medicine.

In this research, the author(s) describe a quick method for making nanoparticles from a bacterium, as well as their characterization and inhibitory impact on both Gram-negative and Gram-positive bacteria. The preliminary findings of the antibacterial activity of biosynthesized nanoparticles for human pathogenic organisms are also included in the prescribed task.

2. Materials and Methods :

Collection of Bacterium spp: In the present study, five bacterium strains were identified, isolated, and purified in the microbiology laboratory (Table 1). The isolates obtained culture were of undoubted monomicrobial origin, and had undergone implant revision procedures in the laboratory. The isolates were characterized using classical microbiological methods. In particular, the staphylococcal species were identified by the Api-Staph test, a biochemical identification kit, and by negativity in the coagulase test.

2.1 Silver nanoparticle synthesis by *Bacillus spp:*

Culture preparation: The overnight culture of *Bacillus* was inoculated in 100 ml of nutrient broth at pH-7 and incubated in an orbital shaker at 30^0C under agitation at 220 rpm. After 48 hours of incubation, the culture was centrifuged and the supernatant was passed through Whatman's filter paper no-1.

Synthesis of silver nanoparticles: The biosynthesis of AgNP′s synthesis was performed in 30 ml of supernatant, 0.003 gm of $AgNO_3$ was added and flasks were incubated in an orbital shaker in dark at 25ºC under the agitation of 120 rpm for 72 hours.

2.2 Silver nanoparticle synthesis of *Pseudomonas spp:*

Culture preparation: The overnight culture of *Pseudomonas spp* was inoculated in 100ml of nutrient broth at PH-7 and incubated in an orbital shaker at 37ºC under agitation at 150 rpm. After 72 hours of incubation, the culture was centrifuged at 12,000 rpm for 5 minutes. The supernatant was used for the synthesis of AgNP′s synthesis [12].

Synthesis of nanoparticles: The biosynthesis of AgNP's was performed in 30 ml of supernatant, and 0.003gm of $AgNO_3$ was added. The reaction between supernatant and silver ions was carried out in bright conditions for 72 hours.

2.3 Silver nanoparticle synthesis by *E. coli:*

Culture preparation: The overnight culture of *E. coli spp.* was inoculated in Luria broth. The culture flask was incubated on an orbital shaker for 48 hrs at 37ºC and agitated at 200 rpm. After incubation, centrifuged at 10,000 rpm for 10 minutes [13].

Synthesis of silver nanoparticles: For biosynthesis of silver nanoparticles, 20 ml of supernatant was mixed with 0.04 gm of $AgNO_3$ and another reaction mixture without $AgNO_3$ was used as a control. The prepared solution was incubated at 30ºC for 96 hours. All solution was kept in dark.

2.4 Silver nanoparticle synthesis by *Streptomyces spp:*

Culture preparation: Overnight culture of *Streptomyces* was inoculated in Starch casein broth and incubated at 28ºC for 6-7 days in a continuous rotatory shaker. After 96 hrs, mycelia were separated from the culture broth by Whatman's filter paper no 1. Mycelium was washed thrice with distilled water. Then 10 gm of mycelia were harvested and then resuspend in 100 ml of sterile distilled water. Allow growing for 3 days on a shaker at 120 rpm [14].

Synthesis of silver nanoparticles: After 3 days mycelia were separated from the filtrate. Then 1mM $AgNO_3$ was added to 100 ml of filtrate. The reaction mixture was put into a shaker at 28ºC and at 120 rpm and maintained in dark.

2.5 Silver nanoparticle synthesis by *Fusarium:*

Culture preparation: The overnight culture of *Fusarium* was sub cultured on PDA plates.

Production of biomass: *Fusarium* inoculated in yeast malt broth. The culture flasks were incubated at 27ºC for 72 hours. After 72 hours mycelial growth was observed, then mycelial growth was filtered by using Whatman's filter paper no.1 and then washing with sterile distilled water [15].

Synthesis of silver nanoparticles: 10 gm of biomass in 100 ml of sterile distilled water for 48 hours at 27ºC & agitated at 120 rpm. After incubation cell filtrate was filtered by Whatman's filter paper no.1. The filtrate was treated with 1mm $AgNO_3$ and incubated in dark for 72 hours. A control containing cell-free filtrate without silver nitrate solution.

2.6. Silver nanoparticle synthesis by *Aspergillus:*

Culture preparation: Overnight culture of Aspergillus was sub-cultured in yeast malt broth at 37ºC for 6 days of incubation. The flask was incubated in a shaker at 200 rpm.

After 6 days of incubation, the mycelium was separated and washed thrice with distilled water. 2 gm of biomass was treated with 20 ml of distilled water for 96 hours at 25ºC and agitated. After incubation cell filtrate was obtained by filtration through Whatman's filter paper no.1 [15].

Synthesis of silver nanoparticles: 0.16gm of $AgNO_3$ in 20 ml of cell filtrate and agitated at 25ºC in dark along with control.

Checking of Antimicrobial Activity: (Disc Diffusion Method)

The antimicrobial activity of AgNP′s against human pathogens *Staphylococcus spp, Bacillus spp, E. coli spp, Aspergillus spp,* and *Pseudomonas spp* was carried out. Overnight culture of pathogenic organisms was swabbed on Mueller Hinton agar plates. Sterile discs were dipped in *Aspergillus* silver nanoparticle solution (showing brown colour) for 10 minutes. Then discs were placed on agar plates. Then plates were incubated at 37ºC for 24 hours. The zone of inhibition was observed and the diameter was measured in mm.

3. Result and Discussion :

Synthesis of silver nanoparticles:

In the present task, five bacterial strains were used for the preparation of $AgNO_3$ particles and also tested against the same bacterial strains (Table 1). Bacteria have been explored in the synthesis of silver NPs. It was reported that highly stable silver NPs (40 nm) could be synthesized by bioreduction of aqueous silver ions with culture supernatant of the nonpathogenic bacterium, *Bacillus licheniformis* [16]. Moreover, well-dispersed silver nanocrystals (50 nm) were synthesized using the bacterium *B. licheniformis* [17]. Saifuddin and co-workers [18] have described a novel combinational synthesis approach for the formation of silver NPs by using a combination of culture supernatant of *B. subtilis* and microwave irradiation in water. They reported the extracellular biosynthesis of monodispersed Ag NPs (5-50 nm) using supernatants of *B. subtilis*, but in order to increase the rate of reaction and reduce the aggregation of the produced NPs, they used microwave radiation which might provide uniform heating around the NPs and could assist the digestive ripening of particles with no aggregation.

Spectrophotometric Analysis:

The absorption spectra of the as-prepared nanosized silver samples were characterized by UV–visible spectroscopy. Biosynthetic nanotechnology is an environmentally friendly technology for the synthesis of nanoparticles. In this aspect, bacterium nanoparticles have proved to be an important biological component for the extracellular biosynthesis of stable AgNPs. It is well known that AgNPs exhibited a light yellowish to brown colour (Fig.1, A to F). The biosynthesis of silver nanoparticles was measured by UV–Vis spectroscopy. UV–Vis spectra of the silver nitrate solutions incubated with five tested bacterial strains as a function of time of reaction.

The surface plasmon resonance (SPR) band of nanosilver occurs initially at 400 nm (3 h). This increases in intensity as a function of the time of reaction. It is observed that the nanosilver SPR band is centred at about 470 nm (Graph 1, A to F). From the spectra, it is clear that when the function of reaction time increased, the SPR band shifted towards a shorter wavelength region which shows a decrease in particle size as a result of the increased band gap from the formula E = hc/k. At lower concentrations, the SPR band is broad and it is due to large anisotropic particles. A smooth and narrow absorption band at 470 nm is observed which is characteristic of almost spherical nanoparticles. The position of the SPR band in UV–Vis spectra is sensitive to particle shape, size, its interaction with the medium, local refractive index, and the extent of charge transfer between the medium and the particles. Meanwhile, similar studies were carried out with marine alga *S. wightii* [19]. and plant extracts were previously obtained [20,21].

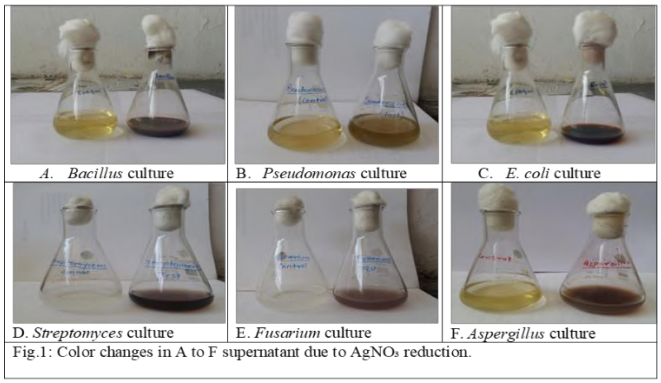
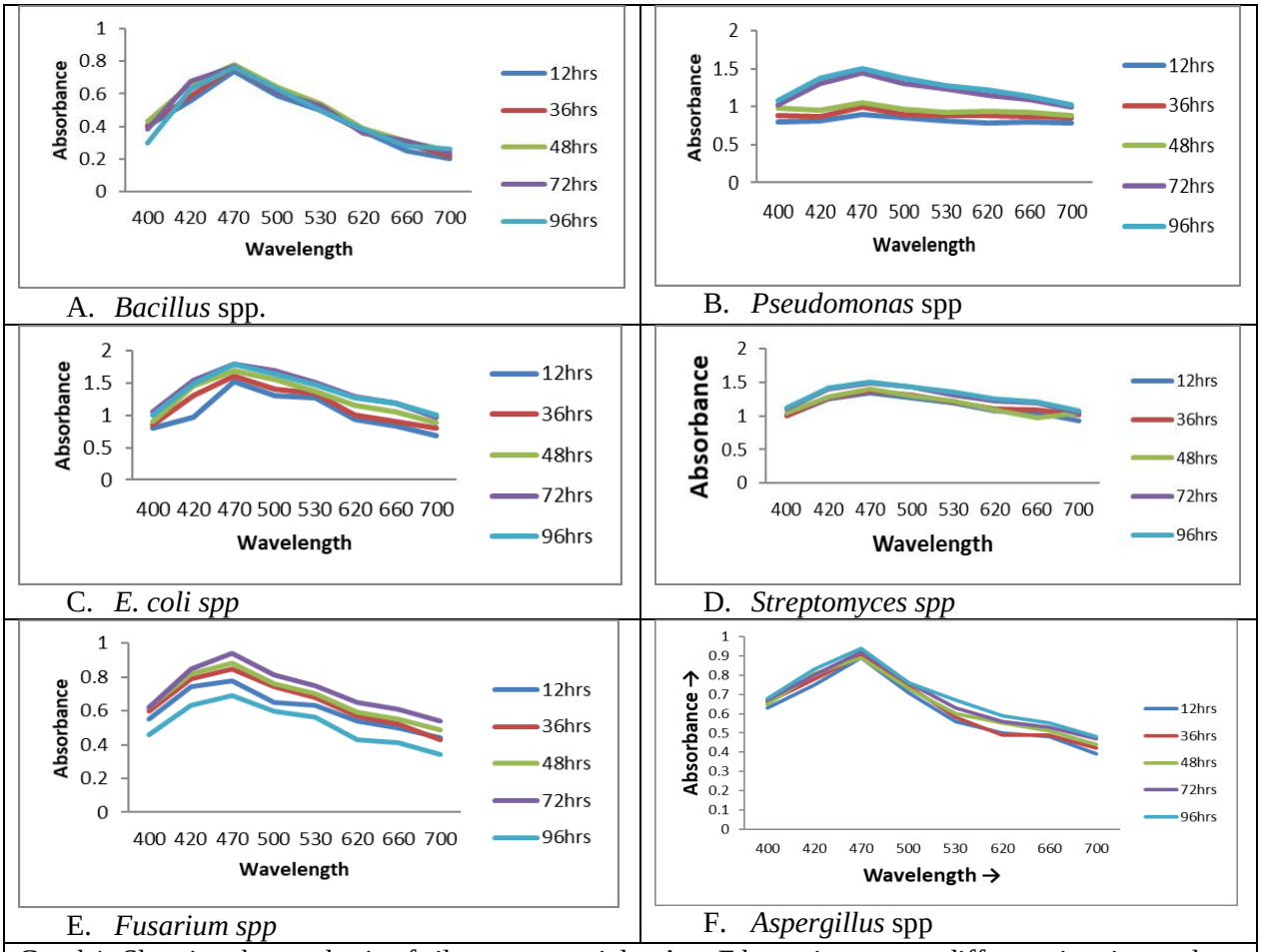


Fig.1: Color changes in A to F supernatant due to $AgNO_3$ reduction.



A. *Bacillus* spp.
B. *Pseudomonas* spp
C. *E. coli spp*
D. *Streptomyces spp*
E. *Fusarium spp*
F. *Aspergillus* spp
Graph1: Showing the synthesis of silver nanoparticles A to F bacterium spp at different time intervals.

Antibacterial activities:

In the present study, the antibacterial activity of green synthesized silver nanoparticles was tested against *Staphylococcus spp, Bacillus spp, E. coli spp, Aspergillus spp*, and *Pseudomonas spp* with the same stain of bacterium silver nanoparticles and the results are shown in Table 1 and Fig. 2. The results of the antibacterial activity with a zone of inhibition maximum was found in *Aspergillus* AgNP's e.g. (16, 26, 16 and 23 mm) from the bacterium of *Staphylococcus spp, Bacillus spp, E. coli spp*, and *Pseudomonas spp* respectively. Also followed by *Streptomyces* AgNP's while, E. *coli* AgNP's not revealed a zone of inhibition any of the bacterium spp.

This enormous difference may be due to the susceptibility of the organism used in the current study. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. When silver nanoparticles enter the bacterial cell [22], it forms a low molecular weight region in the centre of the bacteria to which the bacteria conglomerates thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their

bactericidal activity [22,23]. Several studies propose that AgNPs may attach to the surface of the cell membrane disturbing the permeability and respiration functions of the cell [22]. It is also possible that AgNPs not only interact with the surface of the membrane but can also penetrate inside the bacteria [24]. Drug-resistant pathogenic bacteria have become a major concern for human health, as it is difficult to control these pathogens with available antibiotics. Green synthesized AgNPs can be used to overcome the drawbacks of commercial antibiotics. Biosynthesized AgNPs are well known for their antimicrobial potency against various pathogenic microorganisms [25-27].

Table 1: Antimicrobial activity of silver nanoparticles by disc diffusion method.

Compound	Zone of inhibition (mm)				
	Staphylococcus spp	*Bacillus spp*	*E. coli spp*	*Aspergillus spp*	*Pseudomonas spp*
Bacillus AgNP's	-	-	-	9	12
E. coli AgNP's	-	-	-	-	-
Pseudomonas AgNP's	-	-	8	-	-
Aspergillus AgNP's	16	26	16	-	23
Streptomyces AgNP's	23	-	20	-	-
Control (Silver Nitrate)	9	9	7	7	7

4. CONCLUSION

It has been concluded that the bacterium is capable of producing Ag nanoparticles extracellularly and these nanoparticles are quite stable in solution due to capping likely by the activity present in the bacterium $AgNO_3$. This is an efficient, eco-friendly and simple process. The AgNPs showed potential antibacterial activity against human pathogens like *Staphylococcus spp, Bacillus spp, E. coli spp*, and *Pseudomonas spp* Therefore, nanoparticles of silver in combination with commercially available antibiotics could be used as an antimicrobial agent after further trials on experimental animals.

Future Prospects

In our project, we characterized silver nanoparticles by spectrophotometer. Further size determination can be done by AFM, SEM, TEM, and EDAX. For checking antimicrobial activity, we performed the disc diffusion method. The pure form of silver nanoparticles can be used for the evaluation of MIC and MBC against human pathogenic organisms. By using silver nanoparticles, we performed in-vitro activity, also silver nanoparticles can be used for the in vivo wound healing activity in experimental animal models. We checked the antimicrobial activity of silver nanoparticles in vitro, further, we can also check the antiviral activity against plants virus.

Conflict of Interest

The author(s) claim no war of interest.

REFERENCES:

1. Gong P, Li H, He X, Wang K, Hu J, Tan W, Zhang S, and Yang X, (2007), Preparation and antibacterial activity of Fe_3O_4@Ag nanoparticles. *Nanotechnol.* 18:604–611.
2. Jiang ZJ, Liu CY, and Sun LW, (2004), Catalytic properties of silver nanoparticles supported on silica spheres. *J. Am. Chem. Soc.* 71:2341–2343.
3. Scheringer M, MacLeod M, Behra T, Sigg L, Hungerbuhler K, (2010), Environmental risks associated with nanoparticulate silver used as a biocide. *Household Pers. Care Today*. 1:34–37.
4. Benn TM, and Westerhoff P, (2008), Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol*. 42:4133–4139.
5. Bonsak J, Mayandi J, Thøgersen A, Marstein ES, and Mahalingam U, (2011), Chemical synthesis of silver nanoparticles for solar cell applications. *Phys. Status Solidi*. 8:924–927.

6. Sharma VK, Yngard RA, and Lin Y, (2009), Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv. Colloid Interface Sci.* 145:83–96.
7. Dwivedi P, Narvi SS, and Tewari RP, (2012), Green route to a novel Ag/PLGA bionanocomposite: A self-sterilizing surgical suture biomaterial. *Int. J. Adv. Eng. Sci. Technol*. 2:236–243.
8. Richardson A, Chan BC, Crouch RD, Janiec A, Chan BC, and Crouch RD, (2006), Synthesis of silver nanoparticles: An undergraduate laboratory using green approach. *Chem. Educ.* 11:331–333.
9. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, and Venketesan R, (2010), Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. *Nanomed. Nanotechnol. Biol. Med.* 6:103–109.
10. Badrinarayanan K, and Sakthivel N, (2008), A simple and green method for the synthesis of silver nanoparticles using *Ricinus communis* leaf extract. *Mater. Lett.* 62:4588–4591.
11. Sathyavathi1 R, Krishna MB, Rao SV, Saritha R, and Rao DN, (2010), Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. *Adv. Sci. Lett.* 3:1–6.
12. Jeevan, P, Ramya K, and Rena, AE, (2012), Extracellular Biosynthesis of Silver Nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. *Indian J. Biotechnol*.11: PP 72-76.
13. Kushwaha A, Singh VK, Juhi Bhartariya, Priya Singh, and Khadeeja Yasmeen, (2015), Isolation and identification of *E. coli* bacteria for synthesis of nanoparticles: characterization of particles & study of antibacterial activity. *Euro. J. Exp. Bio.* 5(1):65-70.
14. Saminathan K, (2015), Biosynthesis of Silver Nanoparticles using soil *Actinomycetes, Streptomyces spp. Int. J. curr. Microbiol. App. Sci.* 4(3):1073-1083.
15. Vanmathi Selvi K, and Sivakumar, T, (2012), Isolation and characterization of Silver Nanoparticles from *Fusarium oxysporum. Int. J. Curr. Microbiol. App. Sci.* 1(1):56-62.
16. Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, and Sangiliyandi G, (2008), Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. *Mater. Lett.* 62:4411–4413.
17. Kalishwaralal K, Deepak V, Ramkumarpandian S, Bilal M, and Gurunathan S, (2008), Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids and Surfaces B*: *Biointerfaces.* 65:150–153.
18. Saifuddin N, Wong CW, Nur Yasumira AA, (2009), Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *E-Journal of Chem.* 6:61–70.
19. Govindaraju K, Kiruthiga V, Ganesh Kumar V, and Singaravelu G, (2009), Extracellular synthesis of silver nanoparticles by a marine alga sargassum *Wightii grevilli* and their antibacterial effects. *J Nanosci. Nanotechnol.* 9:1–5.
20. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, and Mohan N, (2010), Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surf B Biointerfaces,* 76:50–56
21. Shrivastava S, and Dash D, (2009), Applying nanotechnology to human health. *J. Nanotechnol.* 12:240–243.
22. Morones JR, Elechiguerra JL, Camacho A, and Ramirez JT, (2005), The bactericidal effect of silver nanopartilces. *Nanotechnol.* 16:2346–2353.
23. Kvitek L, Panacek A, Soukupova J, Kolar M, Vecerova R, Prucek R, Holecova M, and Zboril R, (2008), Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *J. Phys. Chem.* 112:5825–5834.
24. Sondi I, and Salopek-Sondi B, (2007), Silver nanoparticles antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria. *J. Colloid Interface* 275:177–182.
25. Singh N, and Khanna PK, (2007), In situ synthesis of silver nanoparticles in polymethylmethacrylate. *Mater. Chem. Phys*. 104:367–372.
26. Kumar SA, Abyaneh MK, and Gosavi SW, (2007), Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnol. Lett*. 29:439–445.
27. Rajeshkumar S, Malarkodi C, and Paulkumar K, (2013), Intracellular and extracellular biosynthesis of silver nanoparticles by using marine bacteria *Vibrio alginolyticus*. *J. Nanosci. Nanotechnol*. 3:21–25.