Green-synthesized Silver Nanoparticle as Effective Antibiofilm Agent

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Abstract

Biofilm is the sessile group of organisms that adhere irreversibly with the biotic and abiotic surface with the help of pilli and extracellular polymeric substance [EPS] which comprises of carbohydrates, proteins and nucleic acids that provides nutrition to the developing microcolonies and it also prevents the penetration of drug molecules upto the cells thus rendering antibiotic and antimicrobial resistance. Antibiotics are still considered to be one of the potent antimicrobial agents which helps in removing the biofilm. This results in the overuse of antibiotics thereby developing antibiotic resistance within the microorganisms. Thus an alternative pathway to develop compounds or materials having potent antimicrobial and antibiofilm properties is the need of present hour. Nanoparticles have at least one dimension and are extremely small in size ranging between 1-100 nm so it can easily penetrate in the cells of the microorganisms. But over use of this metallic or non-metallic nanoparticles can cause toxic effect on the living cells. Thus, green synthesized nanoparticles are very useful alternative because it is made up from natural sources and are less harmful to living beings. Green-synthesized silver nanoparticles has provided an alternate pathway in removing the biofilm being formed upon the biotic and abiotic surfaces. This review provides an overall concept upon the synthesis and the mode of action of Ag-NPs.

Key words: antibacterial, antifungal, antiviral, anti-inflammatory, antibiotics.

1. Introduction

A biofilm is a group of sessile organisms that forms irreversible aggregates which adheres on the solid surface with the help of pilli and extracellular polymeric substances [EPS] whose composition varies upon the condition of the physical environment in which the organism is thriving. This EPS is comprised of slimy capsule and is made up of large number of nutrients [1].EPS consists of nutrients like polysaccharides, proteins and nucleic acids that facilitate the growth of the microbial cells. This also helps in maintaining the hydrated condition within the dwelling microcolonies of the biofilm forming cells. The determination of biochemical profile of the EPS is challenging as they are very well hydrated and forms a matrix that keeps the constituents of biofilm together. Some biofilms contain alginate as one of the main components such as in mucoid *Pseudomonas aeruginosa* biofilms [2] The EPS formation is also dependent upon the availability of nitrogen and carbon in their microenvironment. In certain species of bacteria, the synthesis of EPS depends on the availability of carbohydrate sources like glucose, mannose, fructose, xylose, maltose, arabinose, ribose, sucrose and lactose. Studies has shown that the formation of EPS by *Pseudomonas aeruginosa* is controlled by cluster of genes which are responsible for the production of EPS . Genes like algA, algD, algE that codes for GDP-mannose pyrophosphorylase , GDP-mannose dehydrogenase and membrane protein are responsible for alginate export respectively [3]. The EPS provides an important chelating agent that helps in the adherence of the bacterial microcolonies upon the surface of inanimate objects [4].

The overuse of antibiotics and antimicrobial agents in the present day for medical treatment has resulted in the development of antimicrobial resistance that results in the failure of antibiotics to hinder the growth of sessile cells thus causes proliferation of biofilm [5]. Biofilms promote bacterial persistence by resisting host immune responses and antibiotic treatment. The EPS prevents the penetration of drug like molecule thus developing resistance against antimicrobials and antibiotics hence a drift in the course of treatment from the conventional group of drugs to new drug like molecules involving natural systems is necessary to act against the biofilm [7]**.** The field of nanobiotechnology is now-a-days greatly helping in providing new strategies to fight against biofilm. Nanobiotechnology is a promising field of nanotechnology that deals with the generation of new types of biomaterials that have biomedical application. This field deals with the formation and use of nanoparticles whose size ranges from 1-100nm.The nanoparticles has its diverse physicochemical nature that includes magnetic, electronic, optical, catalytic and antimicrobial properties [8]. The synthesis of nanoparticles by simple chemical means generate toxic substances which remain attached to the surface of these nanoparticles thus can have a cytotoxic effect and can be detrimental for human use. This leads to a drift from the chemical synthesis to green synthesis of the nanomaterials by using extracts of fungi, bacteria and natural components [9]. The applicability of these green synthesized nanoparticles ranges from electronic catalysis, drug development, antimicrobial and antibiofilm agent.

Nanomaterials have been observed to have a potent antibiofilm activity against biofilms developing upon medical devices and implants. Metals like silver, gold and platinum have some intrinsic properties such as electrochemical, ultrasonic assisted, photochemical, reverse radiation etc making them an ideal material for the synthesis of nanoparticles. The non-metals like carbon, metallic oxide, polymers-nonporous polymers, metal based polymeric composites, peptides, nanoparticles encapsulating antibiotics or liposomes has antimicrobial effect with minimum damage to the host cell.

The environmental changes and environmental pollution lead to the increase in multidrug resistant bacterial and viral strains which is caused due to rapid mutation taking place in the genome of the bacterium and viruses. Thus, research is continuing to develop drugs for the treatment of bacterial fungal disorders. The green synthesized metallic nanoparticles have an important role in hindering the growth of infectious bacterial organisms. The nanoparticles can act as attractive probes in the form of biological markers as their size ranges from 1-100 nm. The effectiveness of the nanoparticles is due to its large surface to volume ratio. The nanoparticles have different biological and chemical properties that makes it an important utilizable material for several aspects like targeting particular protein, sturdiness of structure due to its atomic granularity, enhanced or delayed particle aggregation, enhanced photoemission and surface modification. Siver nanoparticle [AG-NP] and Gold nanoparticle[Au-NP] has emerged as one of the potent source of nanomedicines that is largely involved in the process of drug delivery, ointments, chemical sensing, nanosensing and as various types of microbial agents [10,11]. This review focuses on the green synthesis of silver and gold nanoparticles, morphological features and its mode of action in inhibiting the formation of biofilm [Fig 1].

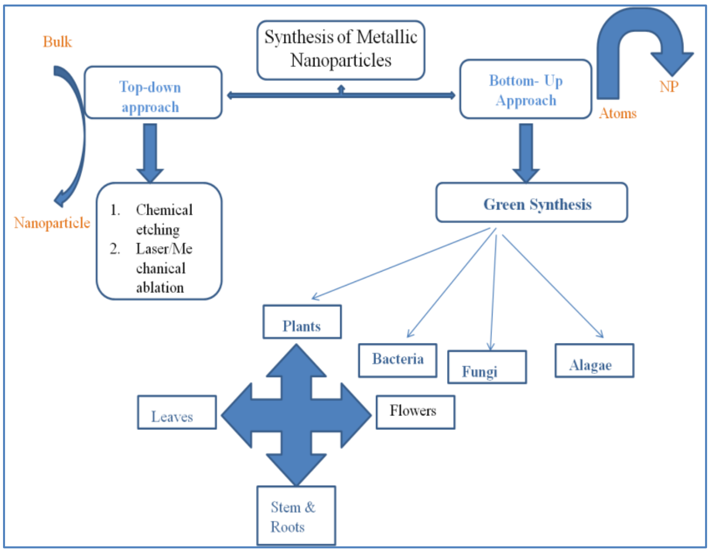

Figure 1: Mode of synthesis of Nanoparticles.

2. Green synthesis of Nanoparticles

The synthesis of nanoparticles by the use of various organic substances can be performed in accordance to the Table 1

Table 1: Synthesis of Nanoparticle from different organic agents.

Sl No	**Name of the Method**	**Type of Nanoparticle**	**Description**
1.	Polysaccharide Method	AG-NP	In this method of preparing nanoparticles, the polysaccharide is used as reducing agent and is also used as capping agent with the NP. It can be easily applied for pharmaceutical uses.
2.	Irradiation Method	AG-NP	This method of synthesizing nanoparticles involves

			the focusing of irradiation through the Ag salt. It results in the formation of nanoparticles of well defined shape and in this technique no use of reducing agent is required.
3.	Tollens Method	AG-NP	The formation of nanoparticles take place by the reduction of silver ions in the presence of polysaccharide.
4.	Biological Methods for the Synthesis of Nanoparticle	AG-NP	The organisms act as a reducing and capping agent for AG-NP.
5.	Polyoxometalates Method	AG-NP	The water soluble polyoxometalates act as potential agent for the purpose of synthesizing silver nanoparticles.

Table 2: Synthesis of Silver Nanoparticles using organic sources.

Type of Nanoparticle	**Reducing Agent Involved**	**Characterization**	**Biological Activity**	**Reference**
AG-NPs	Ascorbic Acid	UV-Vis, TEM	Antibacterial	12

AG-NPs	D-glucose, Hydrazine	UV-Vis, TEM	Antibacterial	13
Polydiallyldimethylammonium chloride	Methacrylic acid	UV-Vis	Antibacterial	14
Chitosan loaded AG-NPs	Chitosan	TEM,FTIR,XRD,DSC	Antibacterial	15

3. Synthesis of Nanoparticle using biological agents

The synthesis of nanoparticles involves use of various types of chemical and physical agents that needs weak and strong reducing agent using sodium citrate, sodium hydroxide and alcohol. These chemicals which are being predominantly used as reducing agent cannot be disposed off into the environment since they are chemically toxic and may bring about degradation to the environment [16-19.]. Thus a drift from the conventional technique is to include the biological agents for the purpose of the production of nanoparticles which mainly involves plant parts, bacteria, fungi, yeast, actinomycetes and algae. These group of organisms produce various types of organic and inorganic compounds in their extracellular fluids that help in reducing the salts of silver. This mode of reduction is a rapid technique than the conventional chemical synthesis of the nanoparticles at ambient temperature and pressure [Figure 2] [20]

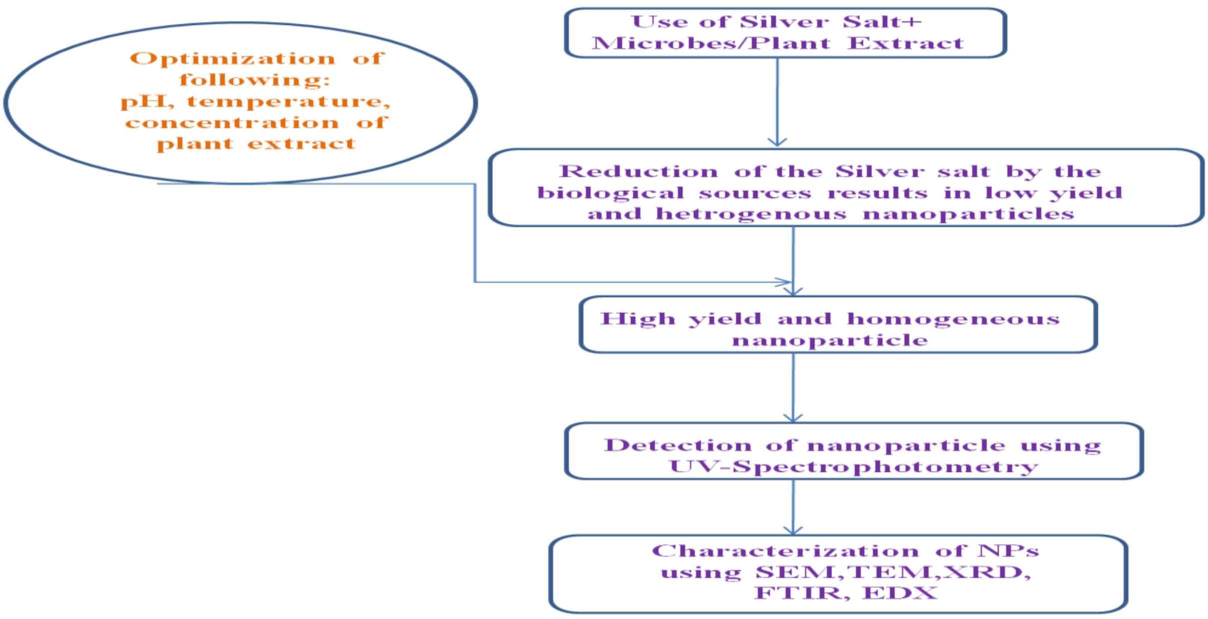

Figure 2: Synthesis of Silver Nanoparticle using Biosources.

3.1. Synthesis of Nanoparticle using plant source

Plants act as a bioagent for the synthesis of nanoparticles as its parts are rich in carbohydrates, proteins, fats, nucleic acids, several secondary metabolites and various types of pigments that act as reducing agent in the formation of the nanoparticles.

Scientific name	**Family**	**Common name**	**Characterization**	**Size**	**Reference**
Ocimum tenuiflorum	Mints	tulsi	UV–vis , XRD, AFM, SEM	28 nm	21
Solanum tricobatum	Nightshade	thoothuvalai	UV–vis , XRD, AFM, SEM	22.3 nm	22
Syzygium cumini	Myrtle family	Java Plum	UV–vis , XRD, AFM, SEM	26.5 nm	22
Centella asiatica	Umbellifers	Gotu kola	UV–vis , XRD, AFM, SEM	28.4 nm	21
Citrus sinensis	Rutaceae	orange	UV–vis , XRD, AFM, SEM	65 nm	21
Prunus yedoensis	Rose family	Yoshino cherry	UV–vis , XRD, AFM, SEM	20–70nm	20
Zingiber officinale	Ginger family	Ginger	UV–vis , XRD, AFM, SEM	10–20 nm	23
Coffee Arabica	Madder family	Arabian coffee		20–30 nm	24
Azadirachta indica	Mahogany	Indian Lilac	UV–vis , XRD, AFM, SEM, ATR-FTIR	50–100 nm	25
Allium cepa	Amaryllidaceae	Onion	UV–vis , XRD, SEM	5.3–10.2 nm	26
Allium sativum	Amaryllidaceae	garlic	UV–vis , XRD, AFM, SEM,	20nm	26

			ATR-FTIR, HPLC,TEM		

4. Anti-Quorum Sensing property of Ag-NPs

During the formation of biofilm, bacteria undergo several phenotypic changes. To understand these changes, one must know about quorum sensing [QS]. Quorum sensing uses the two-component signalling transduction system [TCSTS]. This system consists of an intercellular response regulator, a membrane-bound histidine kinase sensor, and a signal peptide. The quorum sensing is used by both Gram negative and Gram-positive bacteria. The Gram-negative bacteria generally use the Lux I/LuxR-type quorum sensing whereas Gram positive bacteria use the oligopeptide two-component-type quorum sensing system. There is a third pathway of quorum sensing that has recently been described as the most widespread QS system; the *luxS*-encoded auto inducer 2 is used by both the Gram positive and Gram negative bacteria [27].

Efflux pump plays a critical role in cell to cell signalling of biomolecules in formation of biofilm. To combat drug resistance efflux pump inhibitors can be employed, which will block the efflux pump thus ultimately hindering the quorum sensing of the bacteria. The efflux pump extrudes critical components required in quorum sensing. Impairment of this extrusion process of signalling molecules leads to the inhibition of quorum sensing thereby finally leading to the inhibition of biofilm formation.

One other aspect of inhibition of efflux pump leads to accumulation of toxic materials in the cell. The efflux pump exports all the toxic and by products of the cell thus maintaining a healthy microenvironment. But as these pumps gets blocked by the action of the nanoparticles, the waste by products are unable to leave the cell. Slow accumulation of these toxic products leads to cell death. This can be one of the mechanisms of biofilm inhibition.

Silver nanoparticle has anti-microbial and anti-Quorum Sensing activities. It can break the cell membrane of the micro organisms and disturbs the protein synthesis mechanism in the bacterial system thus it is very efficient in antibiofilm like activities of microorganisms. Most of the metallic nanoparticles bind with the bacterial cell membrane and inhibits bacterial cell cycle system. Biosynthesized silver nanoparticle works against *Escherichia coli, Pseudomonas aeruginosa* [Gram-negative] and *Staphylococcus aureus* [Gram-positive]. In case of Gram-positive bacteria, the effect of silver nanoparticles is much lesser as their thin layer of lipopolysaccharides [1–3 µm thick] and peptidoglycans [~ 8 nm thick]. This arrangement facilitates the entry of nanoparticles ions. On the other side, Gram negative bacteria contain much thinner peptidoglycan layer so the nanoparticles easily penetrate through cell wall. Moreover, cell membranes of Gram negative bacteria has negative ions absorbing the positively charged silver ions [28].

Experiments were done to determine the quorum sensing inhibiting activity of silver nanoparticles. The standard bio indicator strain *Chromobacterium violaceium* was used to study this activity. This bioindicator produces pigments of violacein which is regulated by quorum sensing mechanism. Any compound that is able to inhibit the production of violacein is considered to be quorum sensing inhibiting agent. Silver nanoparticles when tested against this bioindicator showed violacein inhibition. The efficacy can depend on a number of factors including the large surface area of the nanoparticles which provides them a better contact with the microorganism. The nanoparticles adhere to the cell membrane and penetrate through them. They might interact with the DNA causing disturbance in the replication process also.

It was also studied that the silver nanoparticles got locked deeply at the active site of various proteins. They inhibit LasI/RhlI synthase which in turn inhibits the synthesis of quorum sensing signalling molecule Acetyl-homolactone [AHL] [29].

5. Mode of action

The exact mode of action of silver nanoparticles on microbial cell destruction is not clearly known and is a debated topic. There are various different theories on the action of silver nanoparticles on microbes to cause the microbial effect.

Silver nanoparticles anchor with the bacterial cell wall and penetrate through the cell membrane, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. These silver nanoparticles form 'pits' on the cell surface, and thus the nanoparticles gets accumulated on the cell surface [30]. The free radicals are also formed by silver nanoparticles which can increase the toxicity level in the living cells of microorganisms. When these free radicals enter in the bacterial cell, it starts to damage the cell membrane and make it porous which ultimately lead to cell death [31,32]. It is also said that the silver ions released from the silver nanoparticles interact with thiol groups of many vital enzymes and inactivate them [33]. The reactive oxygen species are also generated due to interaction of silver ion in cell thereby inhibiting many respiratory enzymes by silver ions and attack the cell itself [34].

In some cases, cells uptake this silver ions from nanoparticles which inhibit the normal metabolism of the cell by increasing the concentration of sulphur and phosphorus content in cell. DNA contain sulphur and phosphorus molecule as the major components. This nanoparticles act on the sulphur and phosphorus of the DNA and disrupt the DNA replication of the bacteria and thus terminating the growth of microorganisms [35].

In living bacterial cell phosphorylation of protein influences bacterial signal transduction. The silver nanoparticles can modulate the signal transduction dephosphorylation in only the tyrosine residues of Gram negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nanoparticles. The nanoparticles dephosphorylate the tyrosine peptide residue which leads to signal transduction inhibition leading to the termination of the cell growth [36].

Table 3: Mode of Action of Green Synthesized Silver Nanoparticles

Name	**Mode of action**	**Reference**
Silver Nanoparticles	Anchor to the bacterial cell wall and penetrate through the cell membrane, thereby causing structural changes in the cell membrane like the permeability of the cell membrane inhibited and death of the cell.	30
	The free radicals are also formed by silver nanoparticles which can increase the toxicity level in the living cells of microorganisms by entering in the cell, it starts to	31

	damage the cell membrane and make it porous which ultimately lead to cell death	
	Silver ions starts to release and binds to thiol group	33
	nanoparticles act on the sulphur and phosphorus of the DNA and disrupt the DNA replication of the bacteria	35
	silver nanoparticles can module the signal transduction Dephosphorylation in only the tyrosine residues of Gram negative bacteria	36

6. Conclusion.

The present review shows that the biofilm can be targeted with the help of nanoparticles which can be synthesized by chemical reaction and can also be synthesized from plants. The reductase enzymes play an important role in converting the metallic salts into nanoparticles. The various types of nanoparticles that are being studied mainly target to block three types mechanisms. First, the nanoparticles blocks the quorum sensing by inhibiting LasI/RhlI synthase which in turn inhibits the synthesis of AHL. Second, most of the nanoparticles penetrate through the cell membranes and binds with the genetic materials thus causeing loss of cell integrity and stops the cell cycle. Third, the nanoparticles inhibits the efflux pumps of the cells. The nanopaticles binds to the efflux pump and blocks the passage thus preventing critical signalling molecules to get secreted from the cell. Since we know that these are the most common ways by which nanoparticles act and prevent the biofilm formation, so we need to work on these mechanisms and try to increase the efficiency of the nanoparticles.

Green synthesized silver nanoparticles has an effective antibacterial and antibiofilm properties which has a pharmacological benefit in the mechanism of drug development.

Benefits of synthesis of silver *nanoparticles* using plant extracts is that it is an energy efficient, cost effective, economical way by which we can help in protecting human health and environment. For the synthesis of nanoparticles employing plants as the source will be very beneficial over other biological entities as this can overcome the time-consuming process of employing microorganisms and maintaining their culture which can lose their efficacy.

References

1.Lopez, D., Vlamakis, H., & Kolter, R. [2010]. Biofilms. Cold Spring Harbor Perspectives in Biology, 2[7], a000398–a000398. doi:10.1101/cshperspect.a000398

2.Flemming, H.-C., Neu, T. R., & Wozniak, D. J. [2007]. The EPS Matrix: The "House of Biofilm Cells." Journal of Bacteriology, 189[22], 7945–7947. doi:10.1128/jb.00858-07

3. K. Czaczyk, K. Myszka. Biosynthesis of Extracellular Polymeric Substances [EPS] and Its Role in Microbial Biofilm Formation. Polish J. of Environ. Stud. Vol. 16, No. 6 [2007], 799-806

4. Hall-Stoodley L, Costerton JW, Stoodley P [February 2004]. "Bacterial biofilms: from the natural environment to infectious diseases". *Nature Reviews Microbiology*. 2 [2]: 95–108. doi:10.1038/nrmicro821. PMID 15040259.

5. Costerton, J. W. [1999]. *Bacterial Biofilms: A Common Cause of Persistent Infections. Science, 284[5418], 1318–1322.* doi:10.1126/science.284.5418.1318

6. Darouiche, R. O., Raad, I. I., Heard, S. O., Thornby, J. I., Wenker, O. C., Gabrielli, A., … Mayhall, G. [1999]. A Comparison of Two Antimicrobial-Impregnated Central Venous Catheters. New England Journal of Medicine, 340[1], 1–8. doi:10.1056/nejm199901073400101

7. Chadha T, Kulsum SN, Adlekha S, Mailapur PC. Comparison of antibiotic susceptibility pattern of community- and hospital-acquired methicillin-resistant Staphylococcus aureus with special reference to inducible clindamycin resistance in a tertiary care hospital in southern India. Med J DY Patil Univ 2014;7:439-42

8. A. Bharathi, V. Janaki, Veenatai. J. MORPHOMETRIC VARIATIONS IN SACRAL HIATUS IN TELENGANA REGION. Int J Anat Res 2016;4[2]:2175-2178. DOI: 10.16965/ijar.2016.170

9. Shankar, S.S.; Rai, A.; Ankamwar, B.; Singh, A.; Ahmad, A.; Sastry, M. Biological synthesis of triangular gold nanoprisms. Nat. Mater. 2004, 3, 482–488. [CrossRef] [PubMed]

10. Basu S, Samanta HS, Ganguly J. Green synthesis and swelling behavior of Ag-nanocomposite semi-IPN hydrogels and their drug delivery using Dolichos biflorus Linn. Soft Mater. 2018;16[1]:7–19

11. Carabineiro S. Applications of gold nanoparticles in nanomedicine: recent advances in vaccines. Molecules. 2017;22[5]:857

12. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Appl Environ Microbiol. 2007;73[6]:1712–1720. doi:10.1128/ AEM.02218-06

13. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 2007;18[22]:225103. doi:10.1088/0957-4484/18/49/495102

14. Dubas ST, Kumlangdudsana P, Potiyaraj P. Layer-by-layer deposition of antimicrobial silver nanoparticles on textile fibers. Colloids Surf A. 2006;289[1]:105–109. doi:10.1016/j. colsurfa.2006.04.012

15. Ali SW, Rajendran S, Joshi M. Synthesis and characterization of chitosan and silver loaded chitosan nanoparticles for bioactive polyester. Carbohydr Polym. 2011;83[2]:438–446. doi:10.1016/j. carbpol.2010.08.004

16. Mohanpuria, P., Rana, N.K. and Yadav, S.K. 2008. Biosynthesis of nanoparticles: technological concepts and future applications. *J Nanopart Res*, 7: 9275–9280

17. Rai, M., Yadav, A. and Gade, A. 2008. Current trends in phytosynthesis of metal nanoparticles. *Crit Rev Biotechnol*, 28[4]: 277–284

18. Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P. and Misra, A. 2009a. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids Surf A: Physicochem Eng Asp*, 339: 134–139.

19. Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., Pyne, S. and Misra, A. 2009b. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. *Colloids Surf A: Physicochem Eng Asp,* 348: 212–216

20. Thakkar, K.N., Mhatre, S.S. and Parikh, R.Y. 2010. Biological synthesis of metallic nanoparticles. *Nanomedicine,* 6[2]: 257–262.

21. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Parishcha, P. V. Ajaykumar, M. Alam, R. Kumar, M. Sastry, Nano Lett. 2001, 1, 515.

22. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R., & Sastry, M. [2003]. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids and Surfaces B: Biointerfaces, 28[4], 313–318. doi:10.1016/s0927-7765[02]00174-1

23. Velmurugan, P., Anbalagan, K., Manosathyadevan, M., Lee, K.-J., Cho, M., Lee, S.-M., … Oh, B.-T. [2014]. Green synthesis of silver and gold nanoparticles using Zingiber officinale root extract and antibacterial activity of silver nanoparticles against food pathogens. Bioprocess and Biosystems Engineering, 37[10], 1935–1943. doi:10.1007/s00449-014-1169-6

24. Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., & Sreedhar, B. [2016]. *Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Materials Science and Engineering: C, 58, 36–43.* doi:10.1016/j.msec.2015.08.018

25. Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. [2004]. *Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem [Azadirachta indica] leaf broth. Journal of Colloid and Interface Science, 275[2], 496–502.* doi:10.1016/j.jcis.2004.03.003

26. Mohammad, F., Aryal, S., Ho, J., Stewart, J. C., Norman, N. A., Tan, T. L., … Claridge-Chang, A. [2016]. Ancient Anxiety Pathways Influence Drosophila Defense Behaviors. Current Biology, 26[7], 981–986. doi:10.1016/j.cub.2016.02.031

27. Beitelshees, M., Hill, A., Jones, C., & Pfeifer, B. [2018]. Phenotypic Variation during Biofilm Formation: Implications for Anti-Biofilm Therapeutic Design. Materials, 11[7], 1086. doi:10.3390/ma11071086

28. Slavin, Y. N., Asnis, J., Häfeli, U. O., & Bach, H. [2017]. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology, 15[1]. doi:10.1186/s12951-017-0308-z

29. Ali, S. G., Ansari, M. A., Sajid Jamal, Q. M., Khan, H. M., Jalal, M., Ahmad, H., & Mahdi, A. A. [2017]. Antiquorum sensing activity of silver nanoparticles in P. aeruginosa: an in silico study. In Silico Pharmacology, 5[1]. doi:10.1007/s40203-017-0031-3

30. Sondi I, Salopek-Sondi B: Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 2004

31. Danilcauk M, Lund A, Saldo J, Yamada H, Michalik J: Conduction electron spin resonance of small silver particles. Spectrochimaca. Acta. Part A. 2006,

32. Kim JS, Kuk E, Yu K, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang C-Y, Kim YK, Lee YS, Jeong DH, Cho MH: Antimicrobial effects of silver nanoparticles. Nanomedicine 2007

33. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T: Mode of bacterial action of silver zeolite and its comparison with that of silver nitrate. Appl. Environ. Microbiol. 2003

34.Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ: The bactericidal effect of silver nanoparticles. Nanotechnology 2005.

35. Hatchett DW, Henry S: Electrochemistry of sulfur adlayers on low-index faces of silver. J. Phys. Chem. 1996

36. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D: Characterisation of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 2007.