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ANTIMICROBIAL EFFECTS OF SILVER NANOPARTICLES SYNTHESIZED USING A TRADITIONAL PHYTOPRODUCT, ASAFOETIDA GUM AGAINST PERIODONTAL OPPORTUNISTIC PATHOGENS

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Abstract

Purpose: Antibiotic resistance is a major problem with inadvertent usage. Thus, there is a need to search for new antimicrobial agents of herbal origin to combat antibiotic resistance. Development of biologically inspired green synthesis of silver nanoparticles is evolving into an important branch of nano-biotechnology. In the present investigation, we report the green synthesis of silver nanoparticles (AgNPs) employing the aqueous extract of a traditional medicinal product consisting of an oleoresin (a combination of macromolecules of carbohydrates and proteins) exuded from the rhizome of the plant *Ferula foetida* (asafoetida gum)

Methodology: The silver nanoparticles synthesized using asafoetida gum (As-AgNPs) were evaluated for its antimicrobial activity. against *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia by* broth dilution method. **Results:** Among the tested microorganisms, P. gingivalis was the most sensitive organism against the biogenic AgNPs with an MIC value of 1.95 mg/ml; while T. forsythia was least sensitive against biogenic AgNPs (15.63 mg/ml).

Conclusion: , The synthesized As-AgNPs were exhibited efficient antimicrobial activities against periodontal pathogens, respectively.

Keywords: Antibacterial activity, herbal extract, asafoetida gum, AgNPs, antimicrobial, periodontal pathogens

Introduction

Periodontitis is associated with dysbiosis of dental biofilm which manifests in chronic inflammation of periodontal lining tissues. This results in damage of alveolar bone and subsequent tooth destruction [1]. Pathogenic microbiota and chronic inflammation in periodontitis contribute in progression and onset of many other systemic diseases, such as cardiovascular diseases [2,3], metabolic syndrome [4], diabetes [5], rheumatoid arthritis [6], respiratory disease [7], chronic kidney disease [8], obesity [8], and cancer [9]. Therefore, shift in content of oral microflora is considered as primary factor that contributes to periodontitis [10]. Studies witness that disturbance of microbiota may activate P. aeruginosa, E. coli, S. pyogenes, and B. cereus [12-14]. There is high Manifestation of multiple drug resistance, prolonged treatment, and increased risk for mortality attributed to extensive use of conventional antimicrobial agents have geared up the nanocomposites research in current decade [15-17]. As a result, recent decade recorded mettlesome development in the field of metal nanocomposites with profound application in microelectronics, photonics, and medicines [18,19].

Among metallic nanoparticles, the high potential of silver nanoparticles (AgNPs) to enhance the antimicrobials and other biomedicine activity always motivate the investigators [20-24]. The AgNPs are reported as powerful tools against multiple drug-resistant bacteria, such as E. coli, erythromycin-resistant S. pyogenes, P. aeruginosa, and B. cereus [25,26]. Investigations assert several methods for metal nanocomposites synthesis like microwave irradiation [27], heat evaporation [28], chemical reduction [29], and electrochemical reduction [30].

The AgNPs synthesis using asserted methods mandates the use of surface passivators to prevent agglomeration. The use of passivators such as thiophenol, mercaptoacetate, and thiourea in synthesis of AgNPs may result in environmental pollution [31]. The AgNPs synthesis by chemical method may lead to toxic entities adsorption over particles surface and manifest many adverse effects during application. Though AgNPs can be formulated by several methods, but the method that offers non-toxicity, high yield, high economy, and environmental safety is a major concern [32].

Nanocomposite synthesis using plant materials is considered as green, as it does not involve hazardous chemicals. The benefits of environmental friendliness, simplicity, cost-effectiveness,

stability, and reproducibility, attached with the biogenic synthesis justify the importance of green synthesis of AgNPs [33,34]. Studies suggest alteration of human gut bacteria by addition of certain vegetables into controlled vegetable diet [35].

The traditional herb Asafoetida is the dried latex extruded from the rhizome of a perennial herb, *Ferula foetida*. Traditionally, asafoetida gum is used for digestive benefits and is a kitchen consumable in many South Indian homes. It gives a pleasant flavor to foodstuffs and is a good antioxidant, with antimicrobial and anticancer properties.[36]The prime constituents of asafoetida latex include resin (above 60%), endogenous gum (above 20%), volatile oil (above 15%) and ash (less than 10%). Asafoetida has been used as a sedative agent for neuropathic patients because it induces sleep without any adverse effects . An antimicrobial study carried out using asafoetida extracts showed good inhibitory action towards both Gram-positive and Gram-negative bacterial pathogens.[37] The bioactive compounds isolated from asafoetida were tested against nosocomial infections, and the result showed that the bacterial strains that were resistant to drugs responded well, confirming the antimicrobial potential of the isolated compounds

Based on the literary research evidences, it was hypothesized that blending of highly efficacious drug Asafoetida into AgNPs would offer a synergistic remedy to combat pathogenic microbiota triggered periodontal disease. Hence, based on complex disorders of periodontitis and expected benefits attached with blend of AgNPs and Asafoetida, the present study was intended to perform the green synthesis of AgNPs using Asafoetida gum aqueous extract (EFLAE), followed by biogenic AgNP's optimization, stability, characterization, and evaluation against common pathogenic periodontal microbiota that triggers periodontal disease.occurrence in periodontal site of chronic patients of periodontitis [15]. Studies consider periodontal disease as the most oral infectious disease that is predominated by microorganism [16]

Material & Methods

Selection of Material

The AgNPs were biosynthesized using EFLAE. The major chemicals, such as silver nitrate, dimethyl sulfoxide, sodium hydroxide (NaOH), potassium bromide (KBr), and Muller Hilton agar were acquired from Sigma Aldrich, SD Fine, Fisher chemicals, and Hi-Media. Prior to antibacterial activity, the glasswares were washed with deionized water, and followed by drying for 2 hours at 160°C (whereas plastic wares were autoclaved).

Preparation and Synthesis of Asafoetida (As)-AgNPs

Asafoetida is an oleoresin exuded from the rhizome of the plant *Ferula foetida*. Five hundred grams of asafoetida gum was purchased from a supermarket in the Riyadh region of Saudi Arabia. The sample was washed with tap and distilled water to remove unwanted fine particles and allowed to dry. The gum was ground into a fine powder. Twenty-five mg of the fine powder was dissolved in 500 mL of Milli Q water and filtered. The filtrate was designated as the aqueous extract of asafoetida and was used for further experiments.

To prepare silver nitrate nanoparticles, 84.94 mg of silver nitrate (1 mM) was dissolved in 500 mL of Milli Q water, to which 20 mL of freshly prepared asafoetida extract was added. The reaction mixture was monitored, and the pale yellow reaction mixture became brown in 24 h. The colored mixture was centrifuged at 18000 rpm for 15 min and then washed. The process was repeated three times. The sediment obtained was dried in a vacuum evaporator. The dried powder was designated as As-AgNPs and stored for further study.

UV-Visible analysis

The biogenic AgNPs synthesis success was confirmed by UV Visible spectral analysis. After dilution of small aliquot of biosynthesized AgNPs in deionized water (1 ml test sample with 4 ml deionized water), the test mixture was subjected to UV-Visible spectral analysis at room temperature to observe surface plasmon resonance (SPR) signal. The analysis was done at 400–800 nm using Shimadzu U-2800 spectrophotometer with a scanning speed of 300 nm/minute. The monitoring of generated UV-Visible absorption spectrum determined reduction of Ag+ ions. The AgNP solution exhibited an absorbance signal at 433 nm. The analysis was carried out as per standard reference procedure with minor modifications [41–43].

Antimicrobial Activity

Source of microorganism

The standard strains of A. actinomycetemcomitans (ATCC 29523), P. gingivalis (ATCC 33277) and T. forsythia (ATCC 43037) were procured from Promochem, Bangalore.

Antibacterial activity

The lowest concentration of the extract/drug that will inhibit the growth of test microorganisms is the Minimum inhibitory concentration (MIC). For the bacteriological suspension, 1.5×10^8 CFU/ml was prepared by comparing the growth turbidity with 0.5 McFarland standards. BHI broth supplemented with horse serum was used as media for all the 3 microorganisms.

For T. forsythia n-acetyl, muramic acid was added to the BHI broth supplemented with horse serum. The stock solution of the biogenic AgNPs extract used for MIC was 500 mg in 1 ml of DMSO 50%. Nine dilutions (50%, 25%, 12.5%, 6.25%, 3.125, 1.562%, 0.781%, 0.390%, and 0.195%) were tested against selected organisms by broth dilution method as per Clinical and Laboratory Standards Institute guidelines. The tubes were kept for incubation for 48 h at 37°C in the bacteriological incubator and observed for turbidity (P. gingivalis and T. forsythia being an obligate anaerobe was incubated in an anaerobic jar while A. actinomycetemcomitans being a facultative anaerobe was incubated in CO_2 dessicator). Turbidity was taken as an indication of growth, and the lowest concentration which remained clear was recorded as the relative MIC. All the tests were performed in triplicate, and the mean value was taken.

Results

The results are summarized in [Table 1]. Among the tested microorganisms, P. gingivalis was the most sensitive organism against the biogenic AgNPs with an MIC value of 1.95 mg/ml; while T. forsythia was least sensitive against biogenic AgNPs (15.63 mg/ml).

Table 1. antibacterial activity of biogenic AgNPs extract	
Periodontal Pathogens	MIC
P. gingivalis	1.95 mg/ml
T. forsythia	15.63 mg/ml
A. actinomycetemcomitans	3.91 mg/ml

Discussion

The mechanism of the antimicrobial activity of As-AgNPs might interact with the microbial membrane, prominently to membrane damage, it will kill the bacteria.[38] The As-AgNPs first attachment to the cell membrane and the interruption and release of cellular constituents, resulting in a loss of permeability.[39] The antimicrobial activity could also be an interaction between small bacterial cells and AgNPs induce a metabolic imbalance in microbial cells after the assimilation of As-AgNPs, leads to the formation of intracellular reactive oxygen species that kills bacteria consequently.[40]

The antimicrobial effect of silver nanoparticles is dominated by their morphological characteristics and concentration. Smaller nanoparticles have the advantage of higher percentages of interactions when compared to larger particles. The smallest nanoparticles displayed maximum activity towards the microbial pathogens, despite being more prone to diffusion than larger particles.[41] The antimicrobial efficiency of nanoparticles is also based on their shape.[42]In particular, triangular nanoparticles have shown more significant inhibitory activity than spherical and rod-shaped AgNPs.[43] In addition, the concentration significantly influences the antimicrobial activities of silver nanoparticles. For example, a 50 to 100 mg of total silver content is required to reduce bacterial colony-forming units. The AgNPs were mainly extracellular, and the resulting nanoparticle size range was 5 to 100 nm, specifically 10–50 nm, with spherical shapes.[36,37,44] The antimicrobial and antitumor potential of As-AgNPs demonstrates the comprehensive development of new antimicrobial particles with an improved antimicrobial mechanism against human microbial pathogens.

Conclusion

According to the objectives of our current work, the As-AgNPs were synthesized using Asafoetida with significant range and size. Further, the synthesized As-AgNPs were exhibited efficient antimicrobial activities against periodontal pathogens, respectively. These outcomes open a new way to the formulation of As-AgNPs for in vivo studies with animal models in our future research investigations.

Reference

1. Lourenco TGB, Spencer SJ, Alm EJ, Colombo AP. Defining the gut microbiota in individuals with periodontal diseases: an exploratory study. J Oral Microb 2018;10:1487741.
2. Tang WW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Cir Res 2017;120:1183–96.
3. El Kholy K, Genco RJ, Van Dyke TE. Oral infections and cardiovascular disease. Trends Endocrinnol Metab 2015;26:315–21.
4. Keller A, Rohde JF, Raymond K, Heitmann BL. Association between periodontal disease and overweight and obesity: a systematic review. J Periodontol 2015;86:766–76.
5. Borgnakke WS, Yl€ ostalo PV, Taylor GW, Genco RJ. Effect of periodontal disease on diabetes: systematic review of epidemiologic observational evidence. J Periodontol 2013;84:S135–52.
6. Fathi HM, Khairy N, Gheita T, Battawy WAE, Taha R. Clinical significance of periodontitis in rheumatoid arthritis patients: association with disease activity and functional status. J Arthritis 2018;7:2.
7. Si Y, Fan H, Song Y, Zhou X, Zhang J, Wang Z. Association between periodontitis and chronic obstructive pulmonary disease in a Chinese population. J Periodontol 2012;83:1288–96.
8. Shultis WA, Weil EJ, Looker HC, Curtis JM, Shlossman M, Genco RJ, et al. Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes. Diab Care 2007;30:306–11.
9. Shillitoe E, Weinstock R, Kim T, Simon H, Planer J, Noonan S, et al. The oral microflora in obesity and type-2 diabetes. J Oral Microb 2012;4:19013.
10. Ahn J, Segers S, Hayes RB. Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. Carcinogen 2012;33:1055–8.
11. Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontol 2011;55:36–47.
12. Elshaghabee FM, Rokana N, Gulhane RD, Sharma C, Panwar H. Bacillus as potential probiotics: status, concerns, and future perspectives. Front Microb 2017;8:1490.
13. Viciani E, Montagnani F, Tordini G, Romano A, Salerni L, De Luca A, et al. Prevalence of M75 Streptococcus pyogenes strains harboring slaA gene in patients affected by pediatric obstructive sleep apnea syndrome in central Italy. Front Microbiol 2017;8:294.
14. Culotti A, Packman AI. Pseudomonas aeruginosa promotes Escherichia coli biofilm formation in nutrient-limited medium. PLoS One. 2014;9:e107186.
15. Colombo AP, Magalhaes CB, Hartenbach FA, do Souto RM, da Silva-Boghossian CM. Periodontal-disease-associated biofilm: a reservoir for pathogens of medical importance. Microb Pathogenesis 2016;94:27–34.
16. Sudiono J, Sandra F, Halim NS, Kadrianto TA, Melinia M. Bactericidal and cytotoxic effects of Erythrina fusca leaves aquadest extract. Dent J Majal Kedokt Gigi 2013;46:9–13.

17. Patra JK, Baek K-H. Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. Front Microbiol 2017;8:167.
18. Yuan Y-G, Peng Q-L, Gurunathan S. Effects of silver nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and Pseudomonas aeruginosa from mastitis-infected goats: an alternative approach for antimicrobial therapy. Int J Mol Sci 2017;18:569.
19. Barros C, Fulaz S, Stanisic D, Tasic L. Biogenic nanosilver against multidrug-resistant bacteria (MDRB). Antibiotics 2018;7:69.
20. Goudarzi M, Mir N, Mousavi-Kamazani M, Bagheri S, SalavatiNiasari M. Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods. Sci Rep 2016;6:32539.
21. Jain S, Mehata MS. Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. Sci Rep 2017;7:15867.
22. Cohen-Karni T, Langer R, Kohane DS. The smartest materials: the future of nanoelectronics in medicine. ACS Nano 2012;6:6541–5.
23. Escarcega-Gonzalez CE, Garza-Cervantes J, Vazquez-Rodríguez A, Montelongo-Peralta LZ, Treviño-González MT, Castro ED, et al. In vivo antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using Acacia rigidula as a reducing and capping agent. Int J Nanomed 2018;13:2349.
24. Mohammed AE, Al-Qahtani A, Al-Mutairi A, Al-Shamri B, Aabed K. Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. Nanomat 2018;8:382.
25. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, et al. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front Microbiol 2018;9:1555.
26. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, characterization and biological applications of biosynthesized silver nanoparticles with chitosan-fucoidan coating. Molecules 2018;23:1429.
27. Rai M, Deshmukh S, Ingle A, Gade A. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 2012;112:841–52.
28. Abdelmoteleb A, Valdez-Salas B, Cecena-Duran C, TzintzunCamacho O, Gutiérrez-Miceli F, Grimaldo-Juarez O, et al. Silver nanoparticles from Prosopis glandulosa and their potential application as biocontrol of Acinetobacter calcoaceticus and Bacillus cereus. Chem Spec Bioavail 2017;29:1–5.
29. Francis S, Joseph S, Koshy EP, Mathew B. Microwave assisted green synthesis of silver nanoparticles using leaf extract of Elephantopus scaber and its environmental and biological applications. Artif Cells Nanomed Biotech 2018;46:795–804.

30. Abbasi E, Milani M, Fekri Aval S, Kouhi M, Akbarzadeh A, Tayefi Nasrabadi H, et al. Silver nanoparticles: synthesis methods, bioapplications and properties. Crit Rev Microbiol 2016;42:173–80.
31. Suriati G, Mariatti M, Azizan A. Synthesis of silver nanoparticles by chemical reduction method: Effect of reducing agent and surfactant concentration. Int J Autom Mech Eng 2014;10:1920.
32. Nasretdinova G, Fazleeva R, Osin YN, Gubaidullin AT, Yanilkin VV. Methylviologen-mediated electrochemical synthesis of silver nanoparticles via the reduction of AgCl nanospheres stabilized by cetyltrimethylammonium chloride. Rus J Electrochem 2017;53:25–38.
33. Mehmood A, Murtaza G, Bhatti TM, Kausar R. Enviro-friendly synthesis of silver nanoparticles using Berberis lycium leaf extract and their antibacterial efficacy. Acta Metall Sin (Eng Lett) 2014;27:75–80.
34. Gomathi M, Rajkumar P, Prakasam A, Ravichandran K. Green synthesis of silver nanoparticles using Datura stramonium leaf extract and assessment of their antibacterial activity. Res Eff Tech 2017;3:280–4.
35. Mittal J, Jain R, Sharma MM. Phytofabrication of silver nanoparticles using aqueous leaf extract of Xanthium strumerium L. and their bactericidal efficacy. Adv Nat Sci Nanosci Nanotech 2017;8:025011.
36. Li F, Hullar MA, Schwarz Y, Lampe JW. Human gut bacterial communities are altered by addition of cruciferous vegetables to a controlled fruit-and vegetable-free diet. J Nutr 2009;139:1685–91.
37. Amalraj A, Gopi S. Biological activities and medicinal properties of Asafoetida: a review. *J Tradit Complement Med*. 2017;**7**:347–359. doi: 10.1016/j.jtcme.2016.11.004
38. Patil D, Shinde S, Kandpile P, Jain AS. Evaluation of antimicrobial activity of asafoetida. *Int J Pharm Sci Res*. 2015;**6**:722–727
39. Zheng K, Setyawati MI, Leong DT, Xie J. Antimicrobial silver nanomaterials. *Coord Chem Rev*. 2018;**357**:1–17.
40. Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim S. Biosynthesis, antimicrobial and cytotoxic effect of silver nanoparticles using a novel *Nocardiopsis* sp. *MBRC Bio Med Res Int*. 2013;287638.
41. Zheng K, Setyawati MI, Leong DT, Jianping Xie J. Antimicrobial gold nanoclusters. *ACS Nano*. 2017;**11**:6904–6910.
42. 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 Interf Sci*. 2004;**275**:177e82.
43. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*. 2009;**27**:76–83.
44. Pal SI, 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**:1712–1720