Green Synthesis of Amla Fruit mediated Silver Nanoparticles And its Antibacterial Efficacy against Clinical Pathogens - an Invitro Study

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ABSTRACT

Background: The overwhelming growing need to generate ecologically benign material syntheses represents a bridge between biotechnology and nanotechnology. This has gained increased attention over the past decade. Many different synthetic approaches are being used to find acceptable biomaterials for the production of nanoparticles. India's indigenous system of healing shows that Emblica officinalis holds a sacred place. Formulation of amla- mediated nanoparticle delivery system could serve an extremely evident use in current medical field.

Aim: The present study aimed to formulate an amla mediated silver nano particles by green synthesis and evaluate its antimicrobial effects.

Materials and Methods: Green synthesis of amla mediated silver nano particles was done by adding appropriate amounts of plant extracts and silver nitrate solutions. They were placed on an orbital shaker at 100rpm and was further centrifuged at 7000rpm for 10minutes to obtain nanoparticles. Antibacterial activity was assessed on three clinical pathogens namely, Streptococcus mutans, Vibrio parahemolyticus and Vibrio harveyi. The bacteria were cultured and two concentrations of the extract and a control (erythromycin) was introduced by disc diffusion method. The zone of inhibition was measured.

Results: The change in colour from green to brown indicated the synthesis of nano-particles. It was found that erythromycin had the maximum activity again *Vibrio harveyi* while 10 µl of amla mediated silver nano-particles had the maximum antimicrobial activity against *Vibriyoparahemolyticus* and *Streptococcus mutans* with a mean and standard deviation of 13.66 ± 0.47 and 8.66 ± 0.47 respectively.

KEYWORDS:*Amla, green synthesis, nanomedicine, antibacterial activity, Nanotechnology, silver nano particle*

INTRODUCTION:

Green nanotechnology is an area of interest that is receiving a lot of attention in the current scenario, with the goal of making nanotechnology-based products that are both eco-friendly and safe for all living things while still being commercially viable.[1-3]Owing to its remarkable optical, chemical, photochemical, and electrical capabilities, the ‘‘green synthesis" of metal nanoparticles has gotten a lot of attention.[4-6] Due to their significant optical absorption in the visible area induced by the collective excitation of the free electron plasma, metal nanoparticles, particularly noble metals, have primarily been researched[7,8]. Silver nanoparticles have a wide range of applications among noble metal nanoparticles, including nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, and antibacterial capacities.[9]

The characteristics of silver nanoparticles (Ag-NPs) are well-defined. This could have a variety of uses in dentistry, clothes, catalysis, mirrors, optics, photography, electronics, and the food industry.[10-15] A wide range of diverse preparation procedures have been created due to the great diversity of uses. However, when developing Ag-NPs preparation methods, regulating size of Ag-NPs must be prioritized. As a result, nano-silver with small particle sizes and no agglomeration between particles is ideal for this application.

G-rays, UV irradiation, heating and electrochemical reduction, and the use of reducing chemicals such as hydrazine, sodium borohydride, polyethylene glycerol, N,N-dimethylformamide, glucose, ethylene glycol, formaldehydeand sodium in liquid ammonia are just a few examples for reducing Ag+. However, a more cost-effective, commercially feasible, and environmentally friendly synthesis approach for Ag-NPs is still needed. Selection of solvent medium, reducing agent, and nontoxic stabilizers for Ag-NPs are three important processes in the green synthesis of Ag-NPs that must be assessed from a green chemistry perspective.[16-27]

Due to the overwhelming growing need to generate ecologically benign material syntheses, the biosynthesis of nanoparticles, which represents a bridge between biotechnology and nanotechnology, has gained increased attention. Many different synthetic approaches are being used to find acceptable biomaterials for the production of nanoparticles.[28] Chemical and physical procedures, as well as the employment of microbes, have gotten less attention than the biosynthetic method employing plant extracts.Considering there is no need to maintain an aseptic environment, the approach is suited for nanoscale metal production.[29]GardeaTorresdey et al (2002, 2003)[30,11]were the first to suggest that plant materials may be used to synthesize nanoscale metals. Bio reduction of various metals to nano size materials of various forms, capable of matching the needs of many industrial uses, was later widely explored.[32]

Amla (Emblica officinalis), often known as Indian gooseberry or Amla, is a deciduous tree.[33] Amla, a member of the Euphorbiaceae family, has antiviral, antibacterial, and anticancer activities.[34] In Ayurveda, India's indigenous system of healing, Emblica officinalis holds a sacred place. It is rich in phytochemicals that works along with the nutrients and fibers to fight against diseases.[35]Flavanoids, ascorbic acid, gallic acid, alkaloids, and hydrolysable tannins are among the active ingredients of Embilica officinalis against microorganisms.[26]Therefore, the present study aims toformulate an amla mediated silver nano particles by green synthesis and evaluate its antimicrobial effects.

MATERIALS AND METHODS:

Plant extract preparation:

The amla fruits were purchased at a grocery store and chopped into little pieces. These were dried for 20 days before being powdered in a mixer grinder. 1 g of amla powder was mixed with 100ml of distilled water and agitated to make the plant extract. This was then boiled for 15 minutes before being strained using filter paper. The solution was kept in a cool site.

Synthesis and characterization of silver nanoparticles:

$AgNO_3$ was purchased from Himedia, Mumbai, India. 1mmol of silver nitrate was mixed with 70ml of distilled water. 30ml of plant extract was added and placed on an orbital shaker at 100rpm for 24hours. This was then centrifuged at 7000rpm for 10minutes to obtain nanoparticles. The nanoparticles obtained were stored at -20℃. The synthesised nano particles were further subjected to antibacterial assays using 2 different concentrations – 5µl and 10 µl. Erythromycin disc was used as control. The synthesis of nanoparticles was observed by the change in colour of the solution which was subjected to UV Spectrometric analysis at 350nm to 550nm for different time intervals. The absorbance and energy were computed.

Antibacterial activity:

The prepared amla-silver nano-particles were evaluated for antibacterial efficacy on various potential pathogenic clinical organisms namely, *vibrio harveyi*, *streptococcus mutans*, and *vibrio parahaemolyticus*. The bacteria were cultured in the appropriate medium on petri dishes. Disc diffusion method was followed to evaluate the antibacterial efficacy. They were incubated for 24 hours and the zone of inhibition was measured.

This method was followed in triplicates to assure reproducibility and reliability. The mean levels of zone of inhibition was calculated. This study did not require ethical committee approval since it was an in-vitro study.

Statistical analysis:

The statistical analysis was done using SPSS software version 25. The measurement of the zone of inhibition was represented as mean and standard deviation in millimetres. T-test was done to compare the mean values of the various concentrations.

RESULTS:

The change in colour from green to brown indicated the synthesis of nano-particles and was observed under UV Spectroscopy. The maximum absorbance was seen at 10 hours reaching more than 1600 at less than 350nm indicating the synthesis of nanoparticles. (Figure 1)

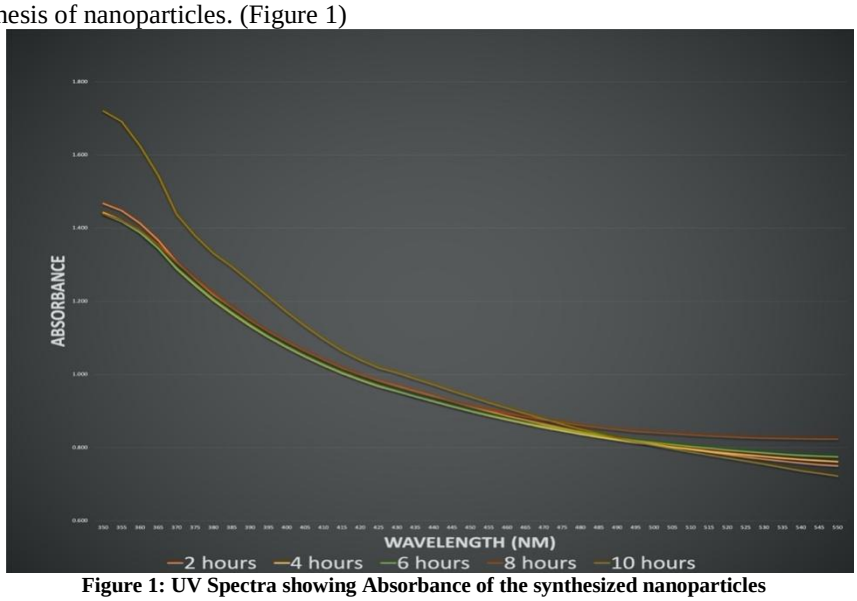

Figure 1: UV Spectra showing Absorbance of the synthesized nanoparticles

The energy reached its peak of 6033 at 490 nm measured at 24hours. The mean and standard deviation of the zone of inhibition is represented as Table 1.

Table 1: Mean and Standard Deviation of the zone of inhibition of the various concentrations on the bacteria.

Bacteria	Control (mm)	5µl (mm)	10µl (mm)	Comparing control and 5µl	Comparing control and 10µl	Comparing 5µl and 10µl
Vibriyoharveryi	21.16 ± 0.62	5.66 ± 0.47	11.66 ± 0.47	0.000*	0.003*	0.004*
Vibriyoparahemolyticus	5	5.16 + 0.25	13.66 ± 0.47	0.211	0.000*	0.001*
Streptococcus mutans	5	6.66 ± 0.47	8.66 ± 0.47	0.018*	0.004*	0.037*

P value <0.05 is significant*

It was found that erythromycin had the maximum activity again *Vibrio harveyi* while 10 µl of amla mediated silver nano-particles had the maximum antimicrobial activity against *Vibriyoparahemolyticus* and *Streptococcus mutans* with a mean and standard deviation of 13.66 ± 0.47 and 8.66 ± 0.47 respectively. These are represented in Figure 2, 3and4.

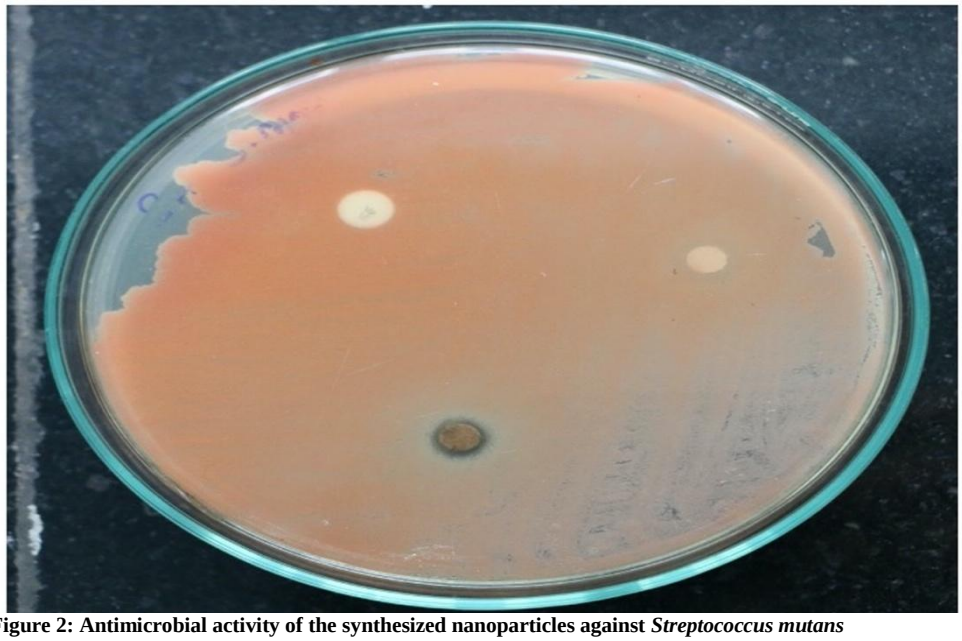

Figure 2: Antimicrobial activity of the synthesized nanoparticles against *Streptococcus mutans*

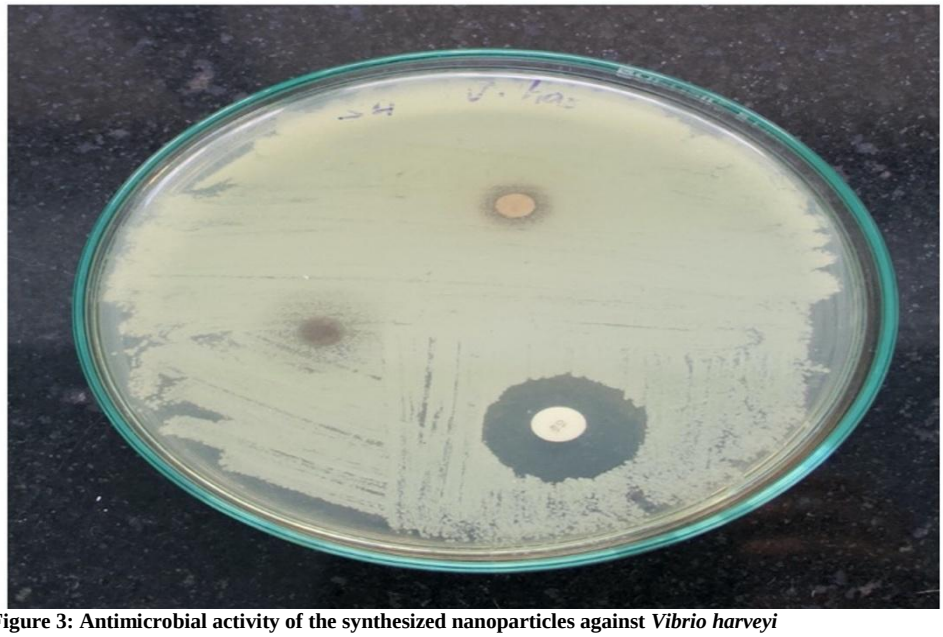

Figure 3: Antimicrobial activity of the synthesized nanoparticles against *Vibrio harveyi*

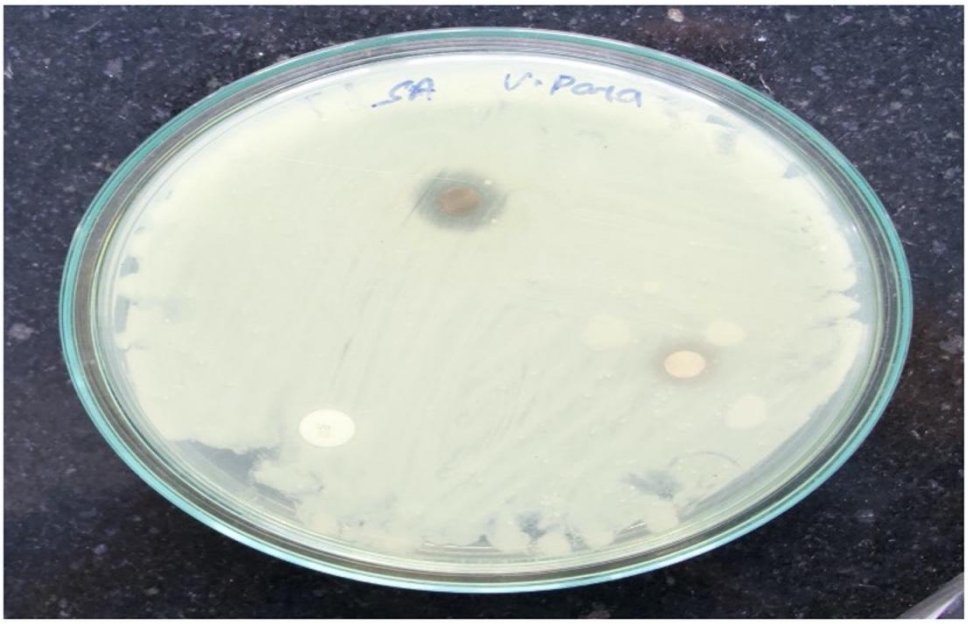

Figure 4: Antimicrobial activity of the synthesized nanoparticles against *Vibrio parahaemolyticus*

DISCUSSION:

Amla mediated silver nano-particles was synthesized and stored in a cool place. The extracted was tested for antimicrobial activity against three clinical pathogens namely *vibrio harveyi*, *streptococcus mutans*, and *vibrio parahaemolyticus*. The synthesized amla mediated silver nanoparticles showed anti-microbial effects against all the three clinical pathogens. However, it was more effective against *Vibrio parahemolyticus* and *Streptococcus mutans.*

AgNPs have been shown to have a broad antibacterial action on both gram positive negative bacteria, as well as drug resistant pathogens. Although several mechanisms for antibacterial action have been postulated, the specific mode of action is yet unknown. According to Jones and Hoek,[36,37] the most prevalent routes of action include free silver ions uptake, which disrupts ATP molecules and prevents DNA replication, AgNPs-induced reactive oxygen species production, and direct silver ions (Ag+) damage to the cell membrane. AgNPs cause enhanced permeability and cell death in gram-negative bacteria by forming pits in their cell walls.AgNPs, in general, produce denaturation and oxidation of the cell wall, resulting in organelle rupture and cell lysis.[36,38]

AgNPs also alter the phosphotyrosine profile of peptides, interrupting signal transduction and preventing proliferation in the organism.[39]AgNPs' antibacterial effect is mostly due to the emission of Ag+ions. When fine AgNPs (10 nm particle size) are used for antibacterial action, the release of Ag+ is higher than when bigger AgNPs are used.[7] For *Fusobacteriun nucleatum*,[10] 0.04 mg/mL for *Streptococcus mutans*,[40] and 0.5 mg/mL for *Actinomyces oris* (, the minimum inhibitory concentration (MIC) of AgNPs is around 0.003 mg/mL. According to Sondi et al., 2004, a concentration of 50–60 g cm3 of AgNPs produces a 100 percent suppression of bacterial growth in *Escherichia coli*.[38]AgNPs' bactericidal properties are also influenced by their size. AgNPs in the 1–10 nm range interact directly with the cell membrane surface, altering permeability and causing cell injury.[41]

AgNPs are thought to bind to the exterior proteins of viruses, preventing them from binding and replicating. Though the antiviral mechanism of AgNPs is yet unknown, there is still need for more investigation.[7]AgNPs have been shown to have antifungal activity against 44 different fungus strains.[42]The action of AgNPs against *Candida albicans* could be the breakdown of cell membrane integrity, which would stop the yeast from growing.[43]AgNPs could thus be one of the treatments used to prevent fungal infections in oral structures. AgNPs included in resins at a concentration of 1 g/ml showed significant antifungal action without cytotoxicity.[44]

Due to the established effects of Amla, incorporation of amla as in a silver nanoparticle base has proved to be extremely effective against clinical pathogens. This is similar to a previous study by Meghana Paramar on 2021[45]where the antimicrobial activity of amla mediated silver nanoparticles was tested against *Staphylococcus aureus* and *Escherichia coli*. The study concluded that amla proved to be a good medicinal remedy and can be used against infectious diseases caused by *S. aureus* and *E. coli*.

Although the study shows promising results on the usage of amla-mediated silver nano-particles, various others aspects related to bio-material characteristics, cytotoxicity and bio-compatibility should be evaluated in order to serve as translational medicine.

CONCLUSION:

In the realm of medicine, silver nanoparticles have the most beneficial effect. Green synthesis of nanoparticles has proven to be a more efficient and effective way of nanoparticle creation. Its antibacterial property can be included into the nanoparticles that are created. Amla mediated silver nano particles have shown to be effective against various clinical pathogens like *vibrio harveyi*, *streptococcus mutans*, and *vibrio parahaemolyticus*. Further in vivo studies are required to substantiate its efficacy and clinical effectiveness.

CONFLICT OF INTEREST: The authors declare no conflict of interest

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