Green Preparation and Characterization of Silver Nanoparticles from Amla Extracts and Studying Their Antimicrobial Activity Against Oral Pathogens Manuscript type - Original research

Divij Khullar[1], NABEEL AHMED[2], Subhabrata Maiti[3], Subhabrata Maiti[4]
[1]Post graduate student, Department of Prosthodontics, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences, Chennai, India.
Email: diving1625@gmail.com
[2]Department of prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai
Email: nabeeln.sdc@saveetha.com
[3]Department of prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
Email: drsubhoprostho@gmail.com
[4]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
Corresponding Author
Department of prosthodontics
Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
Email: drsubhoprostho@gmail.com

Abstract
Traditional nanoparticle production procedures involve harmful chemicals, resulting in environmental toxicity. As a result, "green synthesis" will be required. Silver nanoparticles supplemented with amla extract have demonstrated to have strong antimicrobial capabilities in previous research. As a result, this study was conducted to investigate Silver's antimicrobial characteristics. Silver is an effective antimicrobial agent because it stimulates cell wall breakage due to intracellular material leakage, and it was chosen to manufacture a nanoparticle. Aim: Aim of the study was phyto assisted synthesis of Silver nanopaticles from amla and evaluation of its antimicrobial properties. Material and methods: Antimicrobial properties of the nanoparticle were assessed using Bovine Serum Albumin (BSA) and DPPH Assay respectively at 10 µL, 20 µL, 30 µL, 40µL, 50 µL. Result Conclusion: Within the limits of the study, it can be concluded that silver nanoparticles have exceptional antimicrobial properties and further can be incorporated in dental materials or can be used to coat suture materials to improve their properties.
Keywords: Silver, characterisation, green synthesis, nanoparticle, antimicrobial properties

1. Introduction

In the era of antibiotic resistance, there has been growing concern as bacteria rapidly evolve adaptive countermeasures to traditional antibiotics[1]. Bacteria are potentially deadly pathogens that can spread infectious diseases. The use of bacteria as infection causative agents dates back to the 14th century. In 1910, Salvarsan was the first antimicrobial agent to be introduced. Other antimicrobial agents, such as chloramphenicol, nalidixic acid, and macrolides, are widely used soon after. Infectious bacterial infections were temporarily relieved in the twentieth century. [2] Nonetheless, antibiotic usage and the production of successful antimicrobials led to the production of antibiotic resistant bacteria[2, 3] Since then, significant efforts have been made to combat the emergence of these resistant strains by developing new antibiotic drugs with greater chemical diversity, identifying antibiotic-producing bacteria, and discovering additional antibiotics from natural, previously unknown sources. These advances, however, were insufficient to compensate for the rapidly growing number of resistant bacterial strains. The use of nanotechnology is widespread [4]. In the fields of biology and medicine, in the development of a wide range of goods. Nanotechnology has opened up many possibilities in biology, including tissue engineering, drug delivery, diagnosis, imaging, and protection against bacterial infections[5].

doi.org/10.31838/hiv22.02.728 Received: 02.07.21, Revised: 03.08.21, Accepted: 08.09.21

Nanoparticles have been proposed to treat infections as a new antimicrobial agent because they kill bacteria using different mechanisms than standard antibiotics[4, 5] and have low toxicity in human cells. As a result, Nanomaterials could be a feasible alternative to antibiotics in the treatment of bacterial infections[6]. Silver, a nutrient factor that plays an important role in biological processes, is one of the compounds that could be combined with antimicrobial agents. Silver is a trace element that is needed for proper body development and health[7]. The nano form of elemental Se has gotten a lot of attention because it is the least toxic form of Se.

2. Material and Methods

Preparation of amlaextract

Freshly selected organic Amla fruit was washed many times in distilled water. The fruit was chopped into small pieces with a sterilised knife and pounded into fine particles with a mortar and pestle. 1 gramme of amla pulp was combined with 100 ml distilled water to get a 1 molar solution of amla extract.

Synthesis of Silver nanoparticle

Nanocomposite synthesis was done by combining 100 ml of 1M Silver nanoparticle solutions, as indicated in the preceding procedures. Before the colour change was discovered, an orbital shaker was used to mix the nanoparticle solution overnight, followed by a magnetic hot stirrer. To trace the synthesis of silver nano composites, hourly UV-vis spectrometric data were recorded. Silver nanoparticles were obtained after centrifuging the resultant mixture. [8]

3. Results

1 UV-vis spectroscopy

As a result of integrated oscillations of conduction band electrons on the surface of metal nanoparticles in resonance with the light, the Surface Plasmon Resonance absorption band was identified. A UV-vis spectrometer was used to examine the nanocomposite's formation. At a wavelength of 320-350 nm, a colour change was noticed at 1.000 absorption.

2 TEM scan analysis

The size and shape of the produced nanocomposite were examined by transition electron microscopy. The nanocomposite was 12-16 nm in size on average. (Figure 6)

3 Antimicrobial activity of the nanocomposite

As indicated in result table number 1, nanocomposite at 150L concentration exhibited promising results. At 150L concentration, the zone of inhibition for all three spices, Enterococcus faecalis, Streptococcus mutans, and Candida Albicans, was 15, whereas the control group had 14, 27, and 30. (Figure 4)

Figure 1: showing the amla fruit extract used for the green preparation of the silver nanoparticle

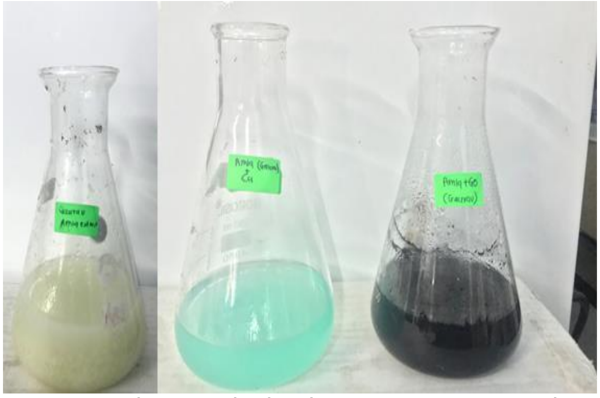

Figure 2: showing the beakers containing 1M solution of amla fruit extract

Figure 3: showing the process of magnetic (auto heated) stirring of Silver nanoparticle extract solution.

A B C

Figure 4: Showing the zone of inhibitions for A. Enterococcus faecalis B. Candida Albicans and C. Streptococcus mutans at 50µL, 100µL ,150µL concentration and the control antibiotic

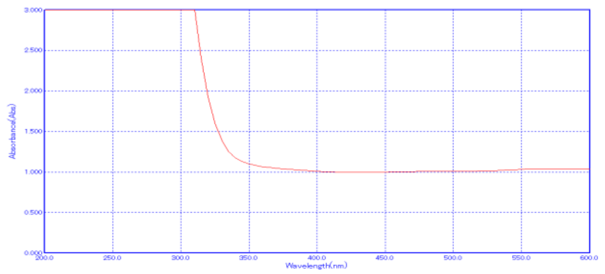

Figure 5: representing the colour change at the 48-hour mark in the solution. UV-Vis spectrometer readings of the nanoparticle.

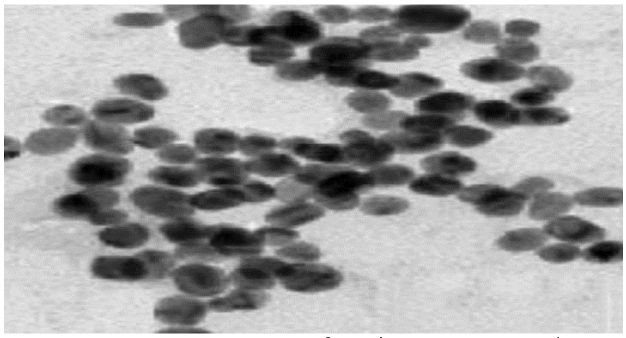

Figure 6: TEM scan image for silver nanoparticle.

Table 1: showing the results of the antimicrobial activity of the Silver nanoparticle from amla extract against Enterococcus faecalis, Streptococcus mutans and Candida Albicans at 50µL, 100µL ,150µL concentration with the help of zone of inhibition.

MICROORGANISM / CONCENTRATION	ZONE OF INHIBITION			
	50µl	100µl	150µl	Control (Antibiotic)
Enterococcus faecalis	9	12	15	14
Streptococcus mutans	9	12	15	27
Candida albicans	9	9	15	30

4. Discussion

Nanotechnology is gaining popularity among researchers because of the following benefits in the medicinal and medical fields: less invasive, reduced risk and adverse effects, faster action with reduced dosage due to increased bioavailability, increased beneficial effects, and unsolved medical problems such as cancer have been one of the most important researches in the field of nanomedicine. Nanomedicines are now being used in local drug delivery in dental disciplines, and not just for fatal systemic disorders like cancer.[9] Incorporating nanoparticles in dental dressings, suture materials, mouthwashes, and local drug delivery media has been a focus of dental nano research[10].

The discovery of effective drug delivery methods that can improve the therapeutic profile and efficacy of therapeutic agents is one of the most pressing concerns in modern medicine. Advances in nanoscience and nanotechnology, which have permitted the creation of novel nanomaterials, have facilitated the development of a number of innovative drug delivery systems.[3]

In comparison to the early half of the century, nanoparticle synthesis has improved fast in recent years.[6] Traditional methods were previously used to create the nanoparticles, and even though these traditional physical and chemical methods for synthesising vast amounts of nanoparticles take less time, hazardous substances are used as capping agents for stability.[11]

These methods resulted in environmental toxicity due to the use of hazardous compounds. The Green Synthesis process was devised to avoid the use of such harmful chemicals, and it is now widely employed all over the world. It is a method that is both ecologically friendly and cost-effective [12]. As a result, we did this study to see how harmful silver nanoparticles containing amlaextract were. antimicrobial activities of the same were proven to be excellent against oral microorganisms in previous studies.[13, 14]

amla extract has been proved in studies to be an excellent antimicrobial[6], and as a result, Silver, which is the main contributor to this feature, was employed in this investigation, with encouraging findings.

When certain bacteria come into touch with an unprotected silver surface, the release of ions from the copper surface causes intracellular oxidative stress in the bacterial cell wall, resulting in bacterial cell lysis[13]. This phenomenon has been known since ancient times, but experts have recently revived their interest in it. For the aforementioned phenomenon, the term "contact killing" was coined. In the year 2008, the United States Environmental Protection Agency (US EPA) designated copper as the first antimicrobial metal [13–15].

Low levels of resistance in bacteria are one of the most prominent advantages of Silver as an antimicrobial agent [13–15]. Because of their large surface area and high charge density, NPs can interact extensively with the negatively charged surface of bacterial cells, resulting in increased antimicrobial action[16]. The Surface Plasmon Method of green synthesis was chosen for the nanoparticle production because it is cost-effective and does not require the use of harmful chemicals[17].

Numerous plant extracts have been accounted for in numerous studies, to synthesize silver nanoparticles. Also, as seen in the results of the current study nano Silver showed promising antimicrobial properties. Further cytotoxicity tests have to be performed to move the nanoparticle into the clinical trial phase and be incorporated in mouthwash and other dental materials to increase the beneficial effects of the materials[18, 19]

5. Conclusion

Within the limits of the study, it can be concluded that silver nanoparticles extracted from amla demonstrated promising antimicrobial properties against Enterococcus faecalis, Streptococcus mutans and Candida Albicans at 150µL. It can be clearly seen that synthesized nanoparticles showed wider zone of inhibition against Enterococcus faecalis than that of the control antibiotic used (figure 4) (table 1) . Further cytotoxicity studies and antimicrobial activity against periodontal pathogens has to be done.

6. Acknowledgment

The authors thank the Director of academics, Saveetha dental college for his encouragement towards research and also, we thank the Chancellor of the university and Dean of the dental college for their valuable support.

7. Conflict Of Interest

The authors have nothing to disclose or any conflicts of interest.

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