Molecular mechanism of formulations mediated (amla, neem) silver nanoparticles in e. Faecalis

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Abstract: Aim : The aim of the study is to evaluate the molecular mechanism of herbal formulations mediated silver nanoparticles against different organisms .
Materials and methods : Azadirachta indica and Amla leaves were dried and powdered , which were made into herbal formulation . Silver nitrate (1 millimolar) was dissolved in 80 ml of distilled water , to that 20ml of filtered Amla , Neem plant extracts were added and kept in an orbital shaker for 2 days . To determine the antibacterial activity of silver nanoparticles Agar well diffusion method was done . Bacterial suspensions were dispersed with various organisms on the surface of plates containing Muller -Hinton agar . Readings were taken using U.V spectrophotometer at (250 -750 nm) and Centrifugation done (8000 rpm for 10 mins) Pellets were collected .Statistical analysis done using One way ANOVA and post Hoc test . P value was analysed.
Results : After the synthesis of silver nanoparticles color change was observed , the particles collected were then characterized and the peak value was seen at 425nm using UV-Spectroscopy . As the concentration of silver nanoparticles increases the zone of inhibition also increases in size.
Conclusion: Dental materials' antibacterial capabilities might be improved by adding silver nanoparticles to them. The Amla and Neem extracts used to synthesise the silver nanoparticles have also demonstrated anti-inflammatory properties. As a non-toxic substitute for traditional antibacterial treatments, silver nanoparticles may be utilised.

Keywords: Silver nanoparticles , E.Faecalis , Antibacterial activity , Azadirachta indica.

1. Introduction

Nanotechnology has recently emerged as a rapidly growing field with numerous biomedical science applications. Silver nanoparticles were one of the most common research items in recent decades, among numerous nanoparticles(1). Silver has been used since ages in the treatment of burns, wounds and several bacterial infections.

Because of their strong antimicrobial properties, silver nanoparticles (AgNPs) have earned considerable recognition and interest. Silver nanoparticles contain between 20 and 15 000 silver atoms and are typically less than 100 nm in diameter. Due to a high volume-to-surface ratio of silver nanoparticles, antimicrobial activity is important, even at low concentrations(2). Many studies on the physical, chemical and biological synthesis of colloidal AgNPs are available.

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It is possible to create metallic nanoparticles by physical, chemical, or biological processes. However, biological synthesis has drawn interest since it is dependable and environmentally friendly. Actually, a variety of bacteria and fungal species have been found to decrease metal ions and create metallic nanoparticles having antibacterial properties.(3),(4) Furthermore, metallic nanoparticles can be produced using plant extracts(5,6).

It is considered to be of intrinsic cytotoxic activity to silver nanoparticles and ions(7,8) to have a sustained antimicrobial effect upon silicon structures . Microbiological experiments have shown that the association between silver ions(9) and extracellular lipoprotein matrix molecules increases the permeability and ends up leading to their death(10). They can increase the permeability of cell membranes, produce reactive oxygen species, and interrupt replication of deoxyribonucleic acid by releasing silver ions.

High hydrogen ion concentrations are resistant to E. faecalis. By virtue of the living substance's ability to act as a buffer, it keeps the hydrogen ion concentration at a certain level. It also includes a nucleon pump, which it uses to provide additional physiological state. According to studies, this organism is unable to withstand hydrogen ion concentration levels above, however (Venugopal et al. 2008). Nanoparticles were synthesised using silver nitrate to get the benefit of incorporation of the silver particles. Azadirachta indica (neem) and aloe vera were mediated by the herb. Even if silver nanoparticles can be synthesised conventionally using physical and chemical processes, biosynthesis proves to be a safer alternative as it does not need the use of hazardous chemicals(11,12). Silver nanoparticles of biological synthesis are also highly resilient and soluble(13).

Because of its large number of biologically active compounds, Neem is considered a wonder in the modern medicinal world. Its constituents have anti-inflammatory, antiviral, antibacterial, antimalarial and anti-oxidant properties(14). Aloe vera is commonly used in the dermatology and cosmetology fields and is renowned for promoting wound treatment with antiseptic and anti-inflammatory properties. It has a laxative potential and can be used as an anti-aging material(15). Hence this study was done to study the possible molecular mechanisms behind the antimicrobial properties governing the nano silver particles made from Aloe vera and Azadirachta indica against E. faecalis.

2. Materials And Methods

It was an in vitro study conducted in the month of November 2021– December 2021 in the city of Chennai, Tamil Nadu.

Ethical approval

The study was approved by the Saveetha Institute of Medical and Technical Sciences Institutional Review Board, Chennai, Tamil Nadu, India. The institutional review board of the SIMATS has received ethical clearance.

Plant material and characterisation of the silver particles

The methodology was in accordance with the previous study by Chithralekha et al(16). Aloe vera and Azadirachta indica leaves were collected from Chennai, Tamil Nadu, India from the University Campus in November. Flowing water carefully cleaned the leaves, to clear the pollution and dust from the leaf surface. Then, the well-dried leaves with mortar and pestle were pounded into powder. In airtight jars, the accumulated leaf powder was stored.

Around 1 g of indica powder of Azadirachta leaf has been dissolved in distilled water and heated at 60–70 °C for five to ten minutes. Similar procedure was followed for the Aloe vera powder. The solutions were then filtered with the filter paper Whatman No. 1. For further use, the diluted extract was obtained and placed in 4 °C.

In 90 ml of double distilled water, approximately 1 mmol silver nitrate has been dissolved. With a metal solution, the plant extracts Azadirachta indica and A. vera have been added and made of a 100 ml solution. The change of colour was visually noticed and photos were recorded.The solution was then held for synthesis of nanoparticles in a magnetic stirrer/orbital shaker.
Nanoparticle synthesis is principally distinguished by UV-visual spectroscopy. The solution measures 3 ml in a cuvette and screened under 350 nm to 550 nm in UV-vis-spectrometers.For graphical analysis, the results were reported.

Silver Nanoparticle Powder Preparation

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Centrifuged with a lark-refrigerated centrifuge was the Ag-NP solution. Centrifugation was performed at 8000 to ten minutes, and two purified water pellets were extracted and cleaned. At 60 °C is extracted and dried the final filtered pellet. The nanoparticles were finally powdered in an airtight Eppendorf tube and deposited in it.

Inhibition of Albumin Denaturation Assay

Bovine serum albumin (BSA) was used as a reagent for the assay. BSA makes up approximately 60% of all proteins in animal serum. The pH for the reaction mixture was recommended to 6.8 using 1N HCL and the 2 ml 1% bovine albumin fraction was combined with 400 µL of plant crude extract at different levels (500-100 µg/mL). At room temperature, the reaction blend was incubated for 20 minutes and then pumped in a water bath at 55 °C for 20 minutes.

The mixture has been refrigerated to room temperature and the absorption value has been recorded at 660 nm. An equivalent volume of extract from a plant was substituted for control by bitter melon seed oil. As a normal, diclofenac sodium was used at various concentrations. Triplicate experiments were conducted.

% inhibition was calculated using the following Formulae:

%inhibition = (Control OD - Sample OD)/ Control OD × 100

Statistical Analysis

The results of the test were described as means ± the standard deviation and analyzed using the UV-Vis Spectroscopy.

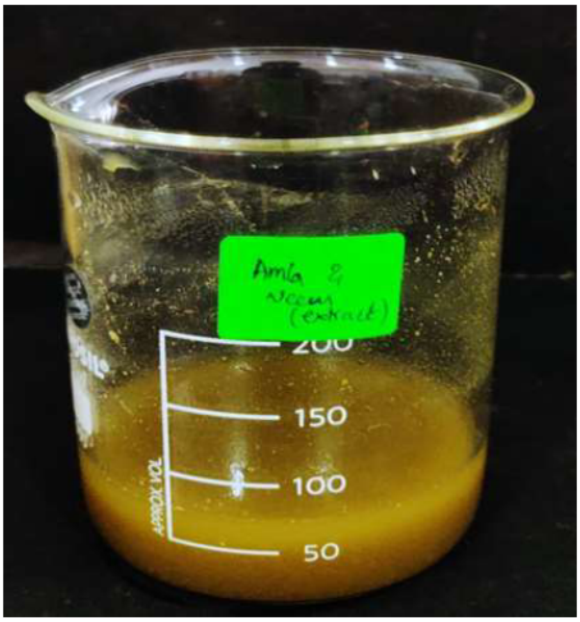

FIGURE -1

FIGURE -2

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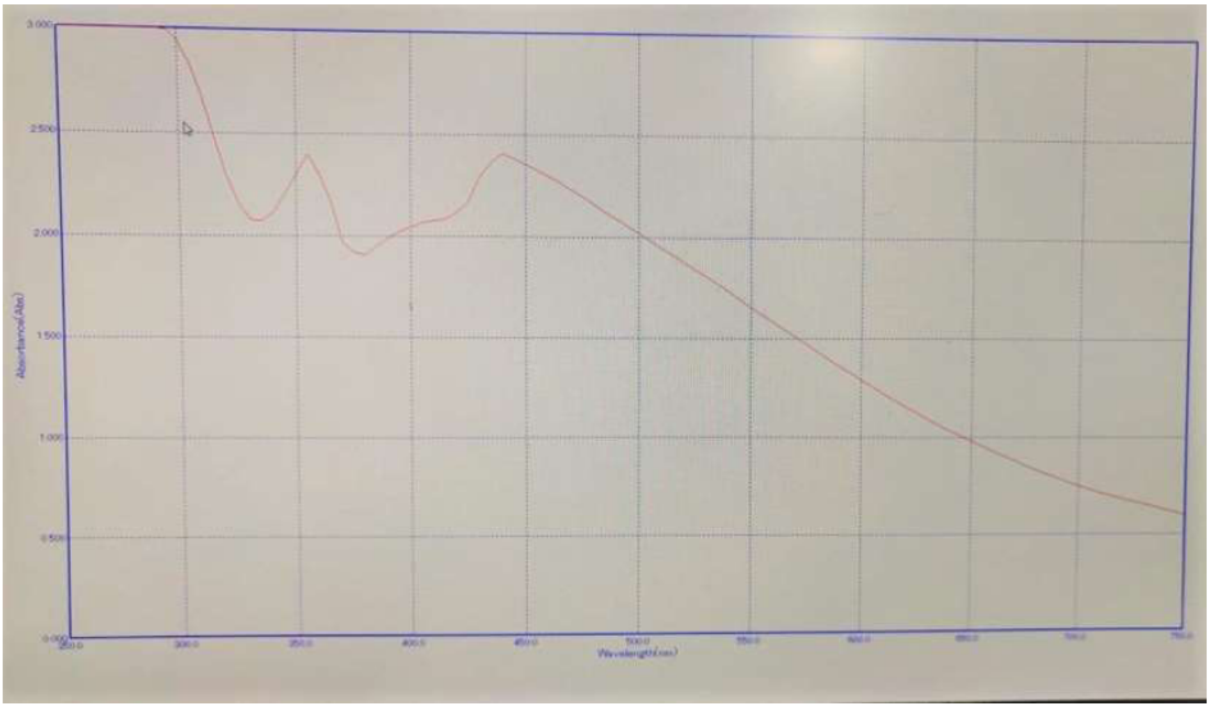
FIGURE -3



Graph -1

Graph 1 represents , the UV- spectrophotometric analysis of the synthesized amla and neem silver nanoparticles .

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3. Results And Discussion

Ag-NPs with A. indica and A. vera were prepared, which were evidenced by UV absorption spectra [Figure 4]. As the concentration of leaf extract increased the number of biomolecules available in the leaf extract that is needed for the metal reductive process also increased.Kamdar et al in 2019 found that Ag-NPs incorporated into the extract C. gingantea against E. faecalis are less effective than tetracycline. This could be because of the lower concentration of the extract(17).Recent results suggest that silver nanoparticles undergo a shape-dependent interaction with the bacterial cells. Pal et al(18). demonstrated that truncated triangular silver nanoplates displayed the strongest biocidal action especially against E. coli, when compared with spherical and rod-shaped nanoparticles and also with silver ions. Similar conclusions were reached by Sharma et al(10).

The antibacterial activity was particle size dependent. Small particles exhibited higher antimicrobial activity than big particles(19). Owing to electrostatic attraction and affinity to sulphur proteins, silver ions can adhere to the cell wall and cytoplasmic membrane. The adhered ions can enhance the permeability of the cytoplasmic membrane and lead to disruption of the bacterial envelope(20). Moreover, silver ions can inhibit the synthesis of proteins by denaturing ribosomes in the cytoplasm(21). Owing to its complex architecture, Biofilm is tolerant to silver nanoparticles. The diffusion coefficients of silver nanoparticles, usually related to size and physicochemical characteristics, establish their mobility in biofilm and bioavailability. These coefficients are diminished with increasing molar weight, so that it is more difficult to penetrate biofilm for larger silver nanoparticles(22). For particles larger than 50 nm, transport through biofilm can be significantly obstructed. The chemical composition of nanoparticles will lead to a decrease in adsorption and the aggregation of silver nanoparticles in a biofilm and the electrostatics can affect the penetration of charged nanoparticles through biofilm through the interaction between bacteria and silver nanoparticles(23).

Moreover Ag NO3 is employed as a reducer as silver has distinctive properties such as sensible conduction, chemical action, and chemical stability(24). The liquid silver ions once exposed, its extracts were reduced in resolution, thereby resulting in the formation of silver hydrosol. This will further increase the hydrogen ion concentration thereby inhibiting the growth of E faecalis cells.Microbiological analyses affirm the synergistic antibacterial action of the combination of amoxicillin with silver nanoparticles on E. coli cells. The quantity of 5 mg mL-1 silver nanoparticles in this sample did not have a clear impact on bacterial growth and a small pause in growth of 0.15 mg mL-1 amoxicillin, although a mixture of these drugs at those same doses resulted in a considerable growth reduction that could possibly be clarified by their synergistic activity(25). Seetharaman et al in his study also concluded that the synergistic effect ofCHX-AgNP solution has better antimicrobial effect against *E. faecalis*, *K. pneumonia*, and *C. albicans* when compared to each of those solutions used individually(26).Luna et al. [12] who evaluated the effect of using AgNPs as a final irrigant on E. faecalis and found a positive bactericidal effect from the AgNPs solution as an endodontic irrigation with the same effect occurred by using sodium hypochlorite at 2.25% which was also in accordance with a study done by batool et al(27). Wu et al. highlighted that irrigation with 0.1% AgNP solution did not disrupt E. faecalis biofilm structure or produce significant killing of the resident biofilm bacteria(28).

Some researchers are concerned about the toxicity of silver nanoparticles. The toxicity of silver nanoparticles is directly associated with the free silver ions(29). Because of the nanoscale size of silver nanoparticles, they can readily disturb biological molecules, cells and human organs.

4. Conclusion

Incorporating silver nanoparticles into dental materials may enhance the antibacterial properties of the dental materials. The silver nanoparticles synthesised using the extract of Amla and Neem have also shown anti-inflammatory activity. Thus, silver nanoparticles could be used as a non-toxic alternative to conventional antibacterial agents.

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Conflict of interest
There are no conflicts of interest.

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