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Novel eco-friendly synthesis of silver nanoparticles using clove and cardamom extracts and cytotoxic and antimicrobial efficacies against oral pathogens- In vitro study

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ABSTRACT

Background: Green synthesized nanoparticles are perceived as an inexpensive, safe & ecologically beneficent approach to control pathogenic oral microbes compared to conventional methods. In the current study, silver nano particles (AgNPs) were effectively synthesised using clove and cardamom plant extracts.
Methods: In the present study, aqueous silver nitrate was reacted with the clove, cardamom extracts and characterization was analyzed using UV-vis spectrophotometer and TEM analysis. Clove and cardamom reinforced silver nanoparticles were examined at 25 µL, 50 µL and 100 µL concentrations for their antibacterial activity against Staphylococcus aureus, Streptococcus mutans, Lactobacillus species and Candida albicans. Cytotoxicity was assessed using brine shrimp assays.
Results: The formation of AgNPs was observed as shifting in color from orange-red color to dark brown at the end of the day three. Reducing silver ions to AgNPs, as shown by UV-vis spectroscopy, has produced an emission peak at 462 nm. TEM imaging showed that the particles were spherical and varied in size from 5-20 nm. Antimicrobial activity of the AgNPs at 100 µL was superior to that of antibiotics against Streptococcus mutans. Excellent antibacterial activity was shown by a zone of inhibition that was closer to that of antibiotics when examined against Lactobacillus. Staphylococcus aureus and Candida albicans were also moderately impacted. There was no evidence of cytotoxicity.
Conclusion: Silver nanoparticles reinforced with clove and cardamom exhibited no cytotoxicity and showed promise as a potent antibacterial agent against oral pathogensKey words: silver nanoparticles.

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INTRODUCTION

In Dentistry, Nanotechnology has shown immense beneficial effects and is one of the most promising fields of research in the current evidence-based scenario(1,2).Dental infections are better to treat when nanostructure & organic teeth constituents can be mimicked, since this allows for direct interactions with microbes(3).

The oral biosphere is cohabited and colonized by several microorganisms(4). Plaque biofilm development leading to periodontal disorders of the oral cavity complex is encouraged by the fact that teeth do not exfoliate. Pathogenic microorganisms in the mouth may be either Gram-positive (Staphylococcus aureus) or Gram-negative

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(such as Streptococcus mutans, Lactobacillus, Enterococcus, and Staphylococcus species). Among the several microbiological strains linked to caries, S. mutans becomes particularly prominent for onset of caries and progression(5). Oral pathobionts include Gram-positive and negative bacteria such as Streptococcus mutans, Lactobacillus species, Enterococcus faecalis, and Staphylococcus aureus. S. mutans is among the most microbiological strains associated with caries development and progression(5). Treatment regimens are required for oral candidiasis, which arises by commensal yeasts such as Candida albicans(6). The oral microbial balance is disrupted because these bacteria promote infection by fostering the growth of conditional microbes and their virulence factors. Biofilm formation is a risk factor for dental infections, and some dental treatments, such as restorations, prostheses, and orthodontic brackets, may promote their growth(7,8). Therefore, it is essential that dental operations include entire removal of microbes. However, the complex structure of teeth and the increasing rate of resistant strains make this impossible(9). In recent years, nanoparticles are being used as antimicrobials, a revolutionary approach to the prevention and control of infectious illness. The surface-to-volume ratio of silver nanoparticles is quite high, making them far more effective at low concentrations for antibacterial activity than other metallic nanoparticles(10).

The prospective medicinal and dental applications of silver nanoparticles (AgNPs) have garnered a significant amount of interest(11). Inhibition of White Spot Lesion (WSL) production by AgNPs-modified orthodontic resins has been shown against S. mutans and L. acidophilus(12). When combined with acrylic resin, they can be used to create removable dentures; when combined with composite resin, they can be used for direct restorations; when combined with irrigating solutions and obturation materials, they may be used in endodontic therapy; and when combined with a membrane, they can be utilised in guided tissue regeneration during periodontal treatment(13). Silver salts have the potential to inhibit the propagation of bacteria in the human body. Many studies have shown that AgNPs have beneficial effects as antioxidants, anti-inflammatory, anti-viral, anti-fungal and anticoagulants(14). Silver is not hazardous to human cells when present at low concentrations(15). Overuse of antibiotics may lead to resistance. Therefore, alternatives must be safe for human consumption, need no intrusive procedures, and kill pathogens without triggering the development of antibiotic-resistant strains. In the last several decades, physicochemical and green chemistry methods have been extensively used to synthesise metallic nanoparticles. Physico-chemical processes need extreme temperatures, noxious substances, and a high pH, all of which are detrimental and hazardous in biological contexts. However, by using high-energy renewable resources, green synthesis may generate nanoparticles that are safe, clean, environmentally friendly, cost-effective, and non-toxic. Reducing metal ions into zero-valent metal nanoparticles is achieved by using metabolites from microbes, animals, and plants. Stable metallic nanoparticles may be synthesised by biosynthesis, which employs plant extracts with special properties of capping & reducing agents.

However, the feasibility of an environmentally friendly silver nanoparticle production using clove and cardamom plant extracts has not been explored. The plant family Mirtaceae includes the species Syzygium aromaticum (clove), which is endemic to the Maluku Islands in eastern Indonesia(16) and is a popular spice due to its antibacterial and antioxidant properties(17).

Southern India, Sri Lanka, Tanzania, & Guatemala are its natural habitats. Elettaria cardamomum (Cardamom) is an aromatic perennial herb with health benefits including anti-cancer, anti-inflammatory, and antibacterial effects. Streptococcus mutans, the bacterium that causes most occurrences of oral disease, has been proven to be susceptible to cardamom oil's antibacterial effects(18).

In the present study, In the current investigation, silver nanoparticles were synthesised in a room-temperature procedure using plant extracts of clove and cardamom. Synthesis of silver nanoparticles (AgNPs) validated by UV-vis spectroscopy and color change observation. Additionally, AgNPs' cytotoxicity and antimicrobial properties were investigated.

MATERIALS AND METHODS

Plant extracts

About 50 g of clove and cardamom were collected, dried under ambient condition and then milled into powder using electric blender.

Preparation of clove and cardamom extracts

A total of 0.5 grammes of dried powdered clove and cardamom was combined with 50 millilitres of purified water in a heating mantle and boiled for 10 minutes at 80 degrees Celsius. As a consequence of the reactions, red & light-green solutions were produced.

Clove and cardamom extract was made, allowed to cool, and filtered using Whatman No. 1 filter paper. Thirty millilitres of each filtrate was mixed together and then brought to a boil for a full minute. At last an orange-red colour solution was formed. This filtrate was kept in storage before being put to use in the proseri paapomduction of AgNPs.

Green synthesis of Silver (Ag) nanoparticles

A silver nitrate solution of 60 ml was made by dissolving , 1x10-2 M of the salt into 60 ml of distilled water. For three days, a combination of 60 ml of colourless silver precursor solution and 40 ml of cardamom and clove extract was stirred at 600-700 rpm on a magnetic stirrer. The mixture was also left on an orbital shaker overnight to determine if any colour changes would occur.

The gradual shift in colour was tracked over the course of three days, hour by hour. By the end of the third day, the solution

had changed colour from orange-red to a dark brown. The presence of nanoparticles in the processed solution was verified using UV spectroscopy.

Characterization and purification of synthesized Silver (Ag) Nanoparticles using UV-vis spectroscopy

After the nanoparticles had been produced, characterisation of the AgNPs was carried out by taking aliquots of the solution. By the end of the third day, the solution had changed colour from orange-red to a dark brown. The presence of nanoparticles in the processed solution was verified using UV spectroscopy. After placing three millilitres of the solution inside a curette, wavelengths ranging from 300 to 700 nm were utilized to represent the findings graphically.

Characterization of AgNPs was performed using a transmission electron microscope (TEM, JEOL JEM-2100, Tokyo, Japan). A drop of the solution was examined by drying it at room temperature on a copper grid (300 mesh) covered with a carbon sheet. 200 kV was used in bright-field mode for sharp images.

Purification of the AgNPs reaction mixture was accomplished by centrifuging the mixture repeatedly at 8,000 rpm for 10 minutes in a Lark chilled centrifuge. The pellet was then diluted three times in sterile distilled water to remove any remaining reactants before being dried at ambient room temperature.

Antimicrobial activity of Silver Nanoparticles using agar well diffusion method

The synthesised nanoparticles' antibacterial properties were evaluated using the agar well diffusion method. After preparation and sterilisation in an autoclave at 121ºC for 15-20 minutes, Mueller-Hinton agar was used. On the surface of sterile Petri plates, sterile MHA medium was made, and allowed to harden. After the solidification process was complete, sterile cotton swabs were used to collect samples of S. mutans, S. aureus, and Lactobacillus species. In order to make the wells, a well cutter in the form of a T was used. After loading three of the wells on each plate with a solution containing the nanoparticles with range of 25 µL, 50 µL, 100 µL, while last well was filled with a antibiotic which is standard (100mg/mL, Amoxyrite). The plates were then incubated at 37ºC for 24 hours. To cultivate C. albicans, a medium consisting of Rose Bengal Agar was produced, and the inoculation plates were incubated at 37ºC for 48 hours. Inhibitory zone size surrounding nanoparticle-loaded wells could be determined after plates were incubated.

Cytotoxic Effect (Brine shrimp assay)

The first step in setting up was adding 6 litres of distilled water into the artemia tank. 50g of salt devoid of iodine were then added, and the mixture was combined using a spatula. The tank was subsequently given two capsules, each containing 15g of brine shrimp eggs. After inserting the airline's tip into the tank, and the amount of aeration was turned up to its highest setting. Brine shrimp nauplii begin to emerge from the eggs after an incubation period of 24 hrs, at which point they were analysed using a stereomicroscope.

Following the addition of 6–8 ml of seawater to each well of the ELISA plate (containing 12 wells), 10 nauplii were placed in each well. After adding nanoparticles to each well at varying concentrations (3 µL, 6 µL, 12 µL, 24 µL, 50 µL) mixture was incubated for 24 hours. After 24 hours, both living and dead nauplii were counted to determine the mortality rateFigure 4).

% death/ mortality = No. of dead nauplii/No. of dead nauplii – No. of live nauplii × 100

RESULTS AND DISCUSSION

Natural techniques for making high-performance, tiny functional materials are both inventive and intrepid. Due to the increasing demand for metal nanoparticles, there is a pressing need to develop ecological ways of production. The plant extracts used in the creation of AgNPs offers a number of benefits, including reduced production costs and energy use, as well as the promotion of a healthier work and living environment. This offers an alternative to more traditional physio-chemical approaches, which rely on the stability provided by hazardous substances(19). The production of nanoparticles, especially those used in health care applications has advanced rapidly in recent years. Any novel techniques of nano synthesis must always be put through rigorous testing to see whether they are cytotoxic, as well as characterization to establish whether their physical qualities can be justified. In the present study, clove and cardamom were used to generate silver nanoparticles, which were then assessed for their antibacterial and cytotoxic effects.

Green synthesis of AgNPs

When added to the orange-red clove and cardamom extract, the silver precursor solution changed from clear to a dark brown. The formation of AgNPs was mostly extrapolated from the color changes in the reaction mixture (Figures 1 and 2). Researchers have shown that plant extracts may speed up the process of reducing metal ions into nanoparticles(20,21). Clove and cardamom plant extracts were added to silver nitrate solution to catalyse the reduction of Ag+ to Ag0, resulting in the ecologically friendly synthesis of AgNPs. Clove and cardamom extracts include secondary metabolites and phytochemicals that may be used as reducing agents in green synthesis. Here, the oxygen released by broken down phytochemicals acts as a link between the metal ions in their reduced states. Plants rely on these phytochemicals as stabilisers to keep their components from sticking together. A shift in colour from white to brown indicates that the surface plasmon vibrations have been excited, making the AgNPs ready for usage (22). Evidence for the bioreduction of silver ions into silver nanoparticles has been found in a number of studies(23,24). This was the first study employing a new plant extract, comprised of clove and cardamom, to demonstrate bio reduction of silver metal ions to silver nanoparticles.

UV-vis spectroscopy

The change in colour of silver nitrate from an orange-red colour

to a dark brown colour provides a visible confirmation of the bio reduction of silver nitrate to AgNPs. Biosynthesized silver nanoparticle development was tracked using UV-vis spectroscopic spectroscopy. Surface plasmon resonance (SPR) absorption peaked strongly and broadly at 462 nm in the visible region of the spectrum. This peak suggests that silver salts may have been converted into AgNPs by reduction (Figure 3). Several more studies using other plant extracts support the results of these investigations (25).

High-resolution Transmission electron microscope (HR-TEM)

HR-TEM analysis revealed that the freshly synthesised AgNPs were perfectly spherical and ranged in size from 5 to 20 nm, with just a trace amount of NPs aggregating. Silver nanoparticles may be capped by secondary metabolites and phytochemicals found in plant extracts like clove and cardamom. Figure 4)

Cytotoxicity

In the current investigation, a test known as the brine shrimp assay was used in order to assess cytotoxicity. In this investigation, the nauplii's was exposed to green generated silver nanoparticles at concentrations of 3µL, 6 µL, 12 µL, and 24µL. After a period of twenty-four hours, the brine shrimp testing revealed that there was no sign of nauplii mortality at any of the amounts tested. This results shed light on concentration of clove & cardamom reinforced silver nanoparticles that may be safely administered (Figure 5).

Antimicrobial activity

Oral pathogens such as S. aureus, S. mutans, Lactobacillus sp., and C. albicans were used in research to evaluate antimicrobial activity of AgNPs. The experiment used the nutrient agar well diffusion technique. At 100 µL concentrations, the diameter of the zone of inhibition against Streptococcus mutans was 14 mm, which was larger than the zone of inhibition seen with antibiotics (10 mm). Hence, Antimicrobial activity of the AgNPs at 100 µL was superior to that of antibiotics against Streptococcus mutans. . Excellent antibacterial activity was shown by a zone of inhibition that was closer to that of antibiotics when examined against Lactobacillus. Staphylococcus aureus and Candida albicans were also moderately impacted. When the concentration was raised, there was a corresponding rise in antibacterial activity (Figures 6 & 7). Based on the findings, we are able to assert that combining silver nanoparticles with clove and cardamom has a synergistic impact, and that this combination has potential to replace currently existing antibacterial drugs in fight against oral diseases. Penicillin, amoxicillin, and tetracycline have all struggled to eradicate S. mutans, making it one of the most resistant and widespread bacteria. Thus, increased doses of AgNPs were required to achieve the anticipated development inhibition in those with dental caries (26). In these cases, lesser dosages of our silver nanoparticles enriched with clove and cardamom may be beneficial.

CONCLUSION

From the present results, clove and cardamom-mediated AgNPs were reported to produce no cytotoxic effects. Excellent antimicrobial effectiveness against the principal microbes responsible for WSLs, Streptococcus mutans and Lactobacillus species, is also promising. When tested against Staphylococcus aureus and Candida albicans, it showed modest antibacterial efficacy. Developing novel antibacterial medications to treat oral infections is made possible by ecological production of silver nanoparticles. Incorporating silver nanoparticles fortified with clove and cardamom in dentifrices, mouthwashes, orthodontic materials and other fields of dentistry will pave the way for further investigations.

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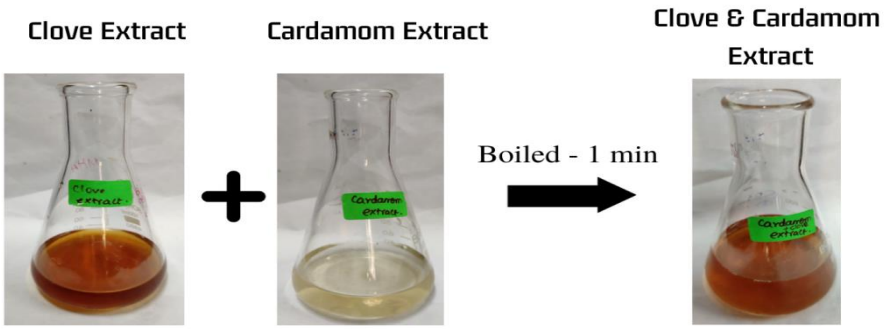

Fig.1: Preparation of plant extract: Exactly 0.5 g of dried clove and cardamom powder was mixed with 50 ml of distilled water and heated at 80 degrees for 10 minutes to make red and light green solutions. Filtration was done using Whatman filter paper no.1. Each filtrate of 30 ml was mixed together and boiled for 1 min and filtrate was stored.

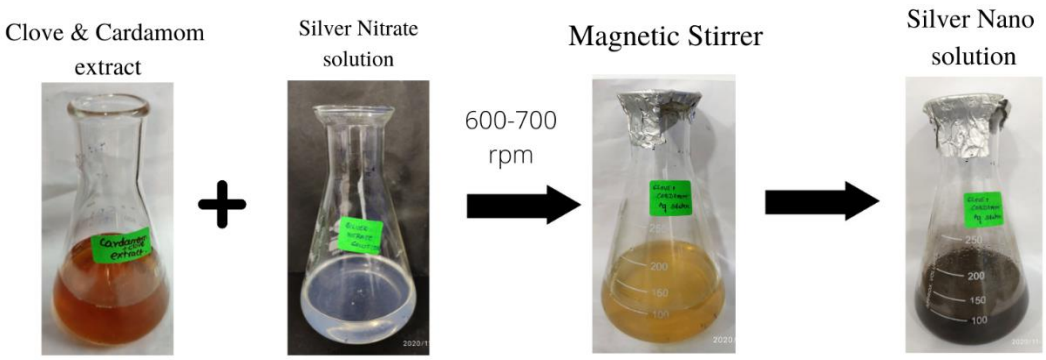

Fig. 2: Preparation of Silver (Ag) nanoparticles and visual observation: 60 ml of cardamom and clove extract were added to 40 ml of Silver precursor solution and mixture was continuously stirred using magnetic stirrer at 600-700 rpm and kept overnight on an orbital shaker till color change was observed from orange-red color had transformed into a dark brown color indicating formation of Ag nanoparticles.

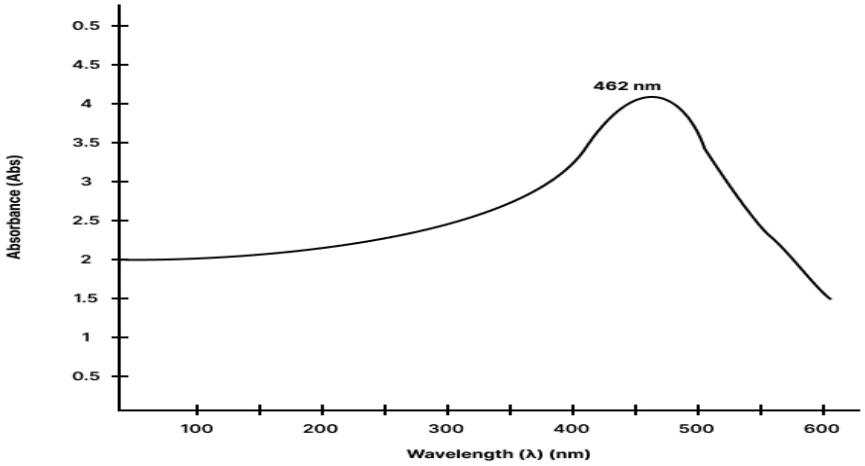

Fig.3: Characterization of AgNPs using UV-visible spectroscopy: SPR peak at 462 nm.

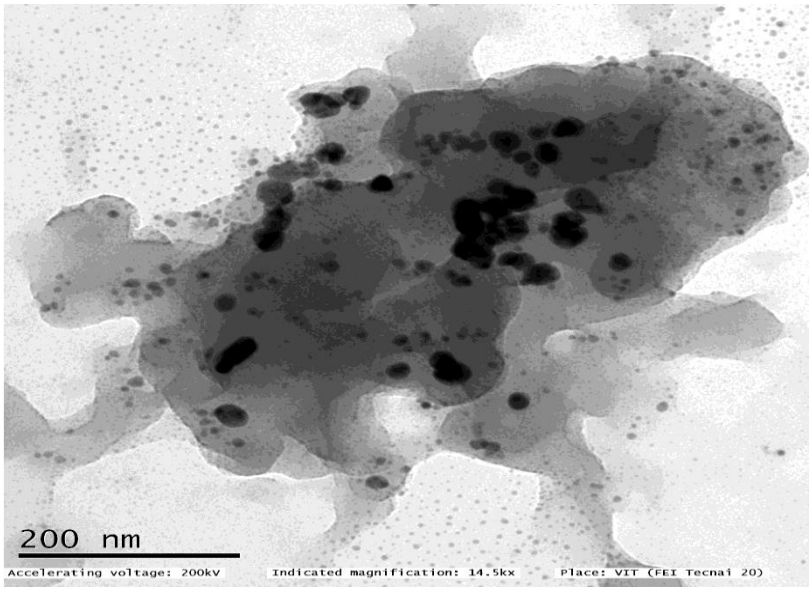

Fig.4: TEM images of biosynthesized AgNPs were taken to confirm the formation of AgNPs. The TEM image reveals that the formed nanoparticles are spherical in shape and 5 to 20 nm in size.

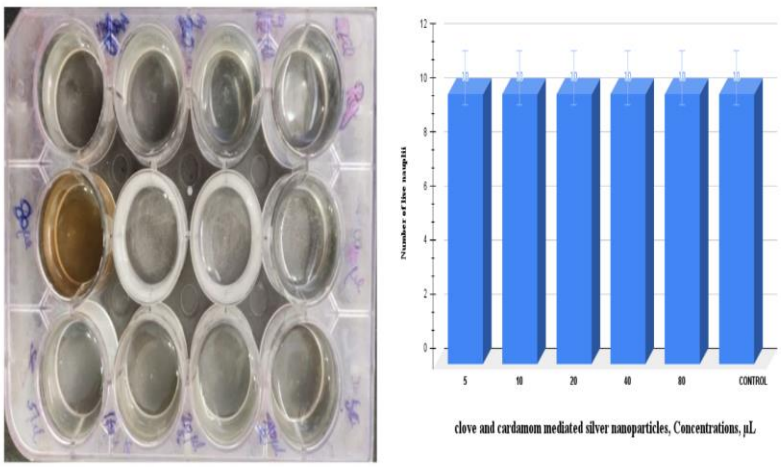

Fig. 5: Cytotoxicity - Brine Shrimp lethality assay of clove and cardamom mediated Ag nanoparticles

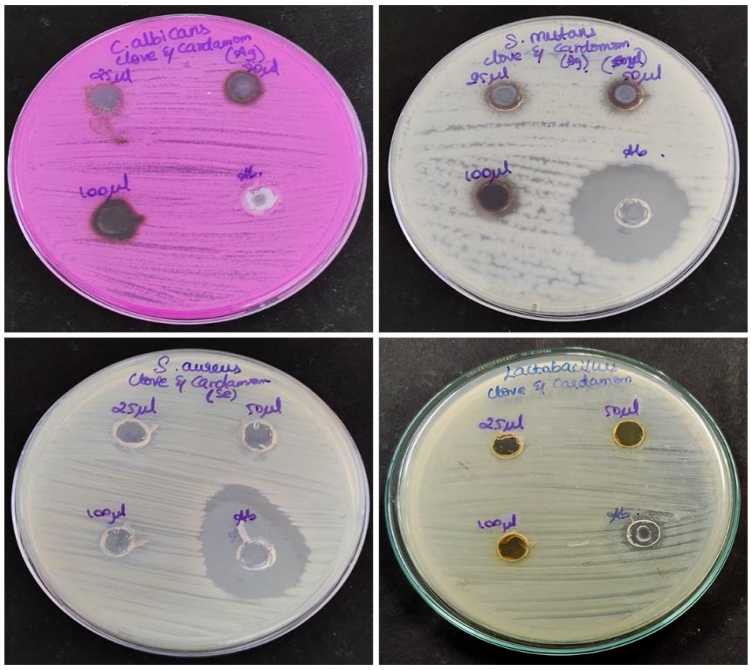

Fig.6: Zones of inhibition for AgNPs at different concentrations against different oral pathogens.

a. Streptococcus mutans

b. Staphylococcus aureus

c. Lactobacillus species

d. Candida albicans

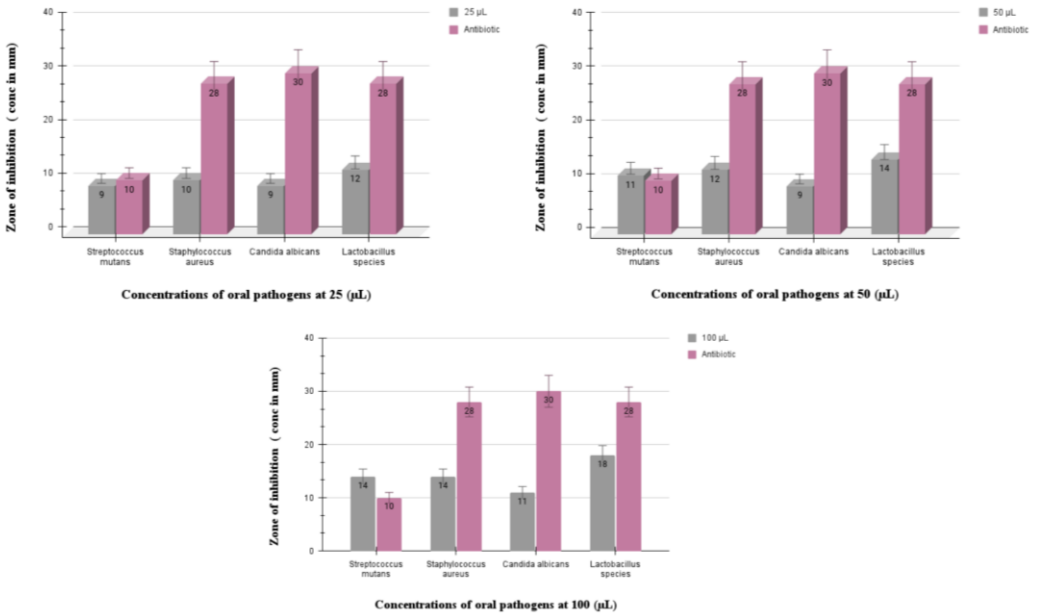

Fig.7: Graph depicts Zones of inhibition for AgNPs at different concentrations such as 25 µL, 50 µL, 100 µL against different oral pathogens such as Streptococcus mutans, Staphylococcus aureus, Lactobacillus species, Candida albicans.