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Green Preparation and Characterization of Silver Nanoparticles from Aloe Vera and Neem Leaf Extracts and Studying their Antimicrobial Activity against Oral Pathogens

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Abstract
The use of toxic compounds in conventional nanoparticle production processes can cause environmental damage. Green synthesis will therefore be a necessary process to minimize the production of these compounds. Previous studies have shown that silver nanoparticles have potent antibacterial properties. Silver was used to create a nanoparticle because it induces cell wall breaking due to intracellular material leakage, which makes it an efficient antibacterial agent. *Aim* : Aim of the study was to test the antimicrobial properties of silver nanoparticles mixed with aloe and neem leaf extracts against oral pathogens. *Material and methods*: Antibacterial activity of the corresponding nanoparticles against the strains of *Enterococcus faecalis, Streptococcus mutans and Candida albicans.* For this experiment, MHA agar was used to identify the zone of inhibition. The zone of inhibition was tested after the incubation period. *Result :* With increasing concentrations, increased values are seen for all three bacteria. *Conclusion*: Within the limits of the study it can be concluded that silver nanoparticles have good antimicrobial properties and further can be incorporated in dentistry either as the drug delivery systems or can be used to coating materials to improve their properties.

1. INTRODUCTION

An expanding field of nanoscience and nanotechnology is the application of materials structures at the nanoscale, which typically ranges from 1 to 100 nanometers (nm). In the fields of catalysis, biology, biomedical science, and water treatment, nanomaterials may offer answers to technical and environmental problems(1) The surface-to-volume ratios of nanoparticles are quite high. When a large surface area is required in science, this attribute can be used. As an illustration, several nanoparticles have really shown to be effective catalysts in the catalytic sector(2). The nanoparticles also exhibit antibacterial properties. Silver nanoparticles have been given credit for these crucial characteristics silver nanoparticles(AgNPs)(2,3). AgNPs have therefore drawn a lot of attention in nano-biotechnological research due to their distinct physical, chemical, and biological characteristics as well as their practicality(1,2). These nanoparticles can be used in the fields of electronics, optics, engineering and even medicine and dentistry(4). Silver nanoparticles can also be employed for a variety of significant applications, such as optical receptors for biolabeling, intercalation

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materials for batteries, and spectrally selective coatings for solar energy absorption(5),(6)

Since the fourteenth century, bacteria have been employed as infection-causing agents. The first antibacterial drug was created in 1910 and was called salvarsan. Following that, antimicrobials including macrolides, nalidixic acid, and chloramphenicol were routinely used(7). Silver nanoparticles can also be employed for a variety of significant applications, such as optical receptors for biolabeling, intercalation materials for batteries, and spectrally selective coatings for solar energy absorption(8). The antibacterial and inhibitory properties of AgNPs are well recognised. Pathogenic bacteria have begun to develop resistance to antimicrobial treatments, nonetheless, in recent years. This has been extensively researched and represents a significant problem for the health care sector.Concern over the rise of antibiotic resistance has grown as bacteria quickly develop adaptive defenses against common antibiotics. Bacteria are pathogens that have the capacity to cause death and spread infectious diseases(5,9).

Since then, numerous efforts have been made to curb the emergence of these resistant strains, including the development of new antibiotic drugs with greater chemical diversity, the identification of antibiotic-producing bacteria, and the discovery of additional antibiotics from previously unknown natural sources. However, these advancements were insufficient to offset the rapidly increasing number of resistant bacterial species(7,10).

Finding a simple, cost-effective, eco-friendly alternative method for the synthesis of Ag NPs is crucial to overcoming these types of challenges(11)(12). Microorganisms and plants are non-toxic reproducible biological resources that are safe for people and the environment, and they may be a better alternative to physical and chemical approaches for the synthesis of Ag NPs(13). In recent times, reports of fungus, bacteria, and plants acting as reducing agents in the production of Ag NPs have surfaced. Plants are a better choice than microorganisms for biosynthesis(11). As a result, this avoids the expansive procedure of cell culture and its maintenance(14).

Silver is an essential trace element for the complete body development and health to be achieved. Because of the fact that this is the least poisonous form of elemental Ag, the nanoparticle form of silver has garnered a lot of attention.

This study was hence conducted to study the antimicrobial efficacy of an extract containing aloe vera and neem leaves.

2. MATERIALS AND METHODS

2.1. Preparation of the aloe vera and neem extract

Commercially available organic preparations of aloe leaf vera leaf powder was procured and used. Fresh neem leaves were obtained and washed multiple times with distilled water and left to dry. A sterilized knife was used to cut the air-dried neem leaf into fine pieces. These pieces were then ground using a mortar and pestle into a powder. 1g of this extract was added to 100ml of distilled water to achieve a 1 M concentration of the aloe vera and neem extract. The Whatmann No. 1 filter paper was used to filter the boiling extract. The filtrates were kept at 5°C for upcoming tests.

2.2 Synthesis of the silver nanoparticle

For the bioreduction procedure, aloe vera and neem leaf extract were employed as aqueous extracts. 0.1mM silver nitrate was dissolved in 100ml of distilled water and stirred for a short period of time to facilitate the biosynthesis of AgNPS. 5ml of filtered neem leaf extract and aloe vera extract was then added drop by drop. For 72 hours, the solution mixture was stirred in a magnetic stirrer at 650–800 rpm. A twin beam UV visible spectrophotometer was used to constantly record the colour changes in the reaction mixture at various wavelengths between 25 and 650 nm. The created neem leaf extract and aloe vera extract-mediated silver nanoparticles underwent a 10-minute centrifugation at 8000 rpm. The reaction colour gradually changed to a yellowish brown colloidal suspension, seemingly indicating that the reduction of Ag^+ ions into metallic Ag NPs was aided by the phytochemicals present in aloe vera and neem leaf extract.

After being cleaned with ethanol and twice-deionized water, the collected Ag NPs were dried at 100 °C for two hours. For the 10 and 15 ml extract volumes, the same process was repeated.

Hourly UV-vis spectrometric data were collected to track the formation of silver nano composites. Centrifuging the resulting mixture produced silver nanoparticles.

TEM analysis was also done to ascertain the size of the silver nanoparticles.

2.3 Determination of the antimicrobial activity

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The antibacterial activity of the corresponding nanoparticles against the strains of Enterococcus faecalis, Streptococcus mutans and Candida albicans was being tested. Muller Hinton Agar was employed in this experiment to locate the zone of inhibition. The agar was prepared and sterilised for 45 minutes at 120 lbs. The medium was placed into sterile plates and allowed to harden. After the wells were cut with the well cutter, the test organisms were swabbed. Concentration was added to several nanoparticle plates, which were then maintained at 37 ° C for 24 hours. After the incubation time, the zone of inhibition was evaluated.

3. RESULTS

3.1 UV-vis absorption study

The figure shows the colloidal solution of leaf extract and aqueous silver nitrate's UV-vis absorption spectra readings. The silver salt solution was colourless at first and then changed to brown when aloe vera and neem leaf extract was added to the reaction mixture. The production of Ag NPs in the aqueous medium is firmly confirmed by this change in colour of the silver solution, which is clearly visible. A larger concentration of Ag NPs with a deep brown solution results from an increase in the reduction rate of Ag+1 ions to Ag caused by an increase in extract volume. In Figure 1.the wavelength is seen between 370-380 nm as in accordance with various studies(15,16).

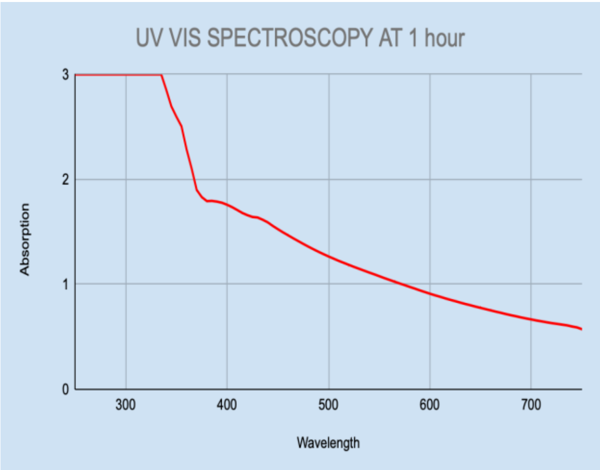

Figure1: The wavelengths are noted between 370-380nm.

3.2 Surface morphological study

From the TEM analysis, the synthesised Ag NPs' size and form were examined, and the results are presented in Fig. 3a. In Figure 2, according to the TEM image, the produced Ag NPs are uniformly disseminated, primarily spherical in shape, and range in size from 20-30 nm. This number can be correlated with the other studies that were done and is in line with the results. Aloe vera and neem leaf extract contains stabilising phytochemicals that might be responsible for the well-dispersion of AgNPs.

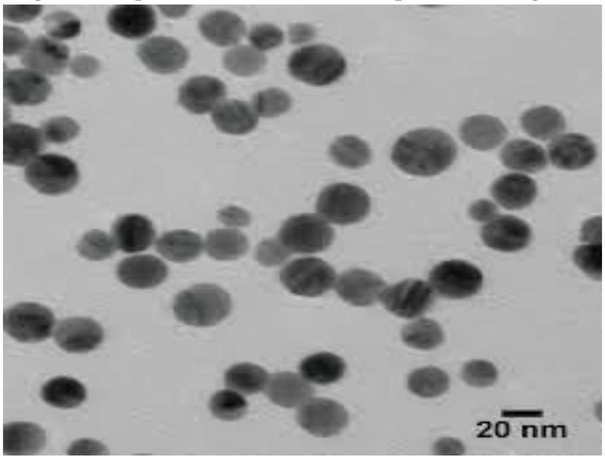

FIGURE 2: SEM analysis revealed the size of the particle at 20nm.

3.3 Antimicrobial Activity

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MICRO ORGANISM CONC.	**ZONE OF INHIBITION**			
	25µ	**50µ**	**100µ**	**CONTROL**
S. Mutans	**20**	**22**	**24**	**30**
E. Faecalis	**12**	**14**	**16**	**28**
C. Albicans	**11**	**14**	**18**	**24**

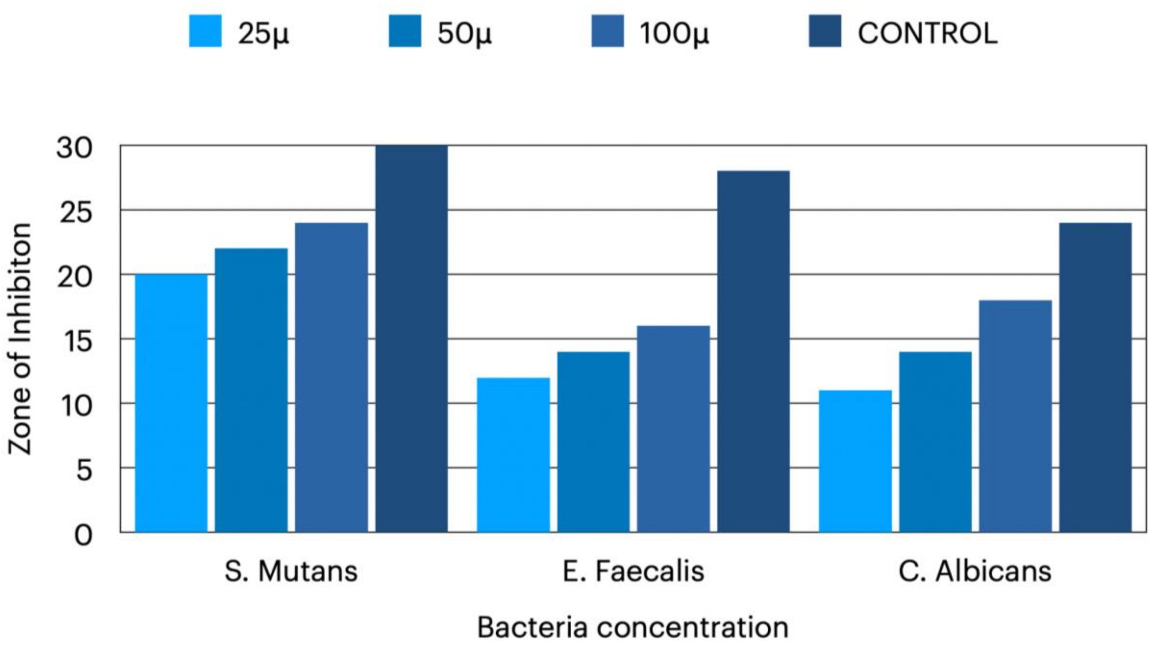

Figure 3 shows the ZOI of *S. Mutans,* Figure 4 shows the ZOI of *E. Faecalis* and Figure 5 shows the ZOI of *C. Albicans.*

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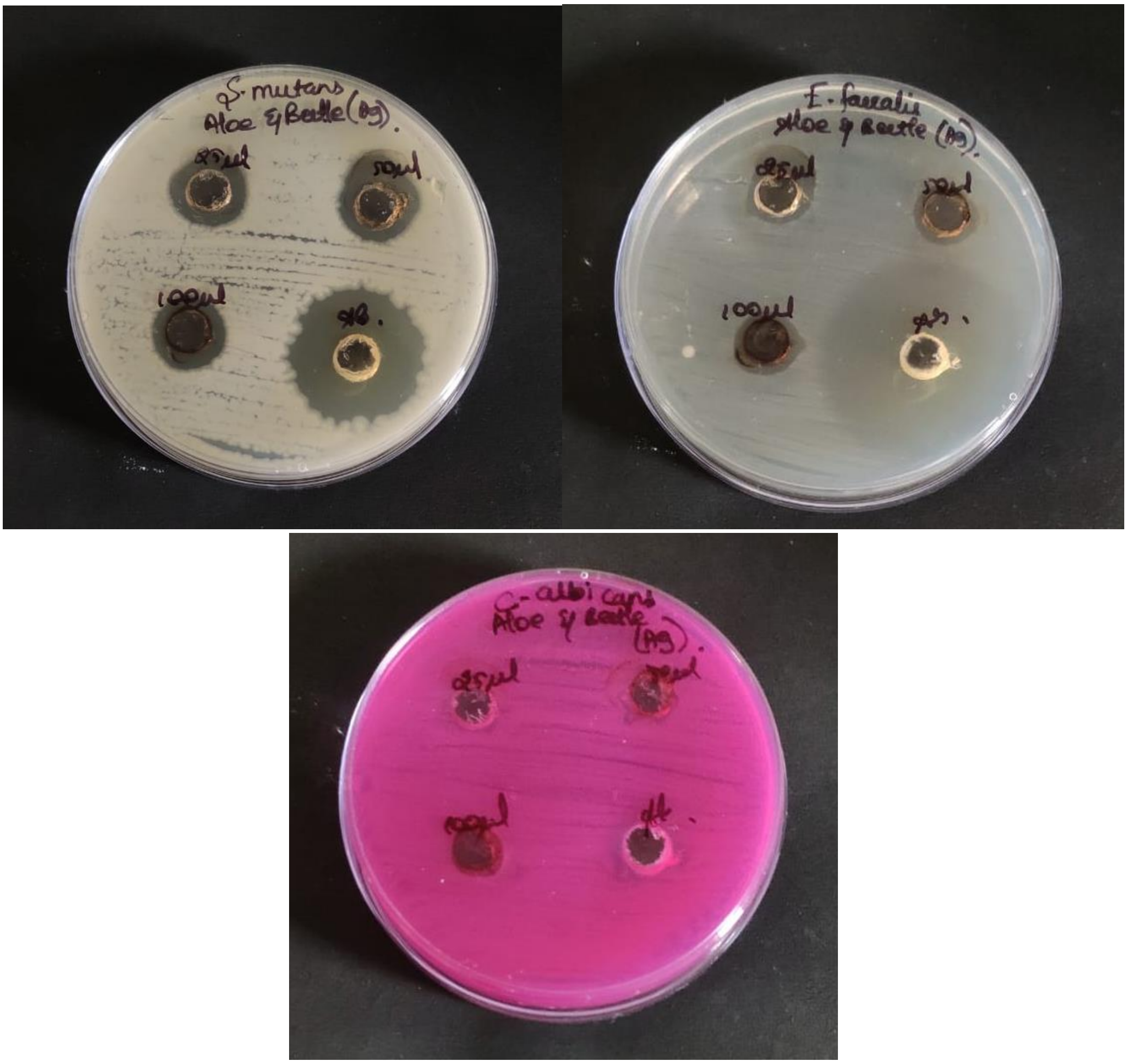

At 100µ, the concentrations of *S. Mutans, E. Faecalis* and *C. Albicans* were 24,16 and 18.

DISCUSSION

One of the most common mechanism of action is that AgNPs can build up on the cell wall and membrane, resulting in visibly noticeable morphological alterations such as cytoplasmic shrinkage, membrane separation, many electron dense pits, and ultimately ruptured membrane(17,18) Gram positive and gram negative bacteria have different cell wall compositions, Ag NPs showed greater growth inhibition of gram-positive bacteria(19). Ag NPs generated from varying extract volumes result in variations in the zone of inhibition, which is due to different particle sizes(20)(21). Because of their increased surface area, researchers found that tiny Ag NPs have superior antibacterial action. Small (nm) sized produced Ag NPs adhere to bacteria's cell wall membrane and even penetrate the bacterium themselves(22). As a result, it hinders the system's normal operation, disrupts the bacteria's respiration mechanism, and ultimately kills them(23). Size of the silver nanoparticles played a very important in the antibacterial properties. In a study conducted by Choi and Hu AgNPs synthesised between 9-21nm showed best antibacterial properties(24,25). Agnihotri et al also concluded that from 100nm to 20nm the best antibacterial properties were seen with the smallest sizes(24). Monroes et al goes on to say that smaller than

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10nm sizes can also be very effective in destroying the bacteria(26). Helmlinger et al. have conducted more in-depth research on the shape-effect on antibacterial activities, synthesising five distinct AgNP kinds with remarkably homogeneous size and shape. He came to the conclusion that, in line with the size-dependent antibacterial activity, the antibacterial activity of variously shaped AgNPs is mostly caused by the production of Ag+ ions on the surface as a result of dissolution(26,27). AgNPs' antibacterial activity was also discovered to be influenced by their form. In order to test their antibacterial efficacy against E. coli in solution and on agar plates, Pal et al. produced spherical, rod-shaped AgNPs and truncated triangular Ag nanoplates(28,29). Truncated triangular silver nanoplates have the highest percentage of exposed facets when compared to silver nanospheres and nanorods. This facilitates direct interaction of the nanoplates with bacterial surfaces, which improves surface binding, cell uptake, and effectively kills bacteria, although both can be effective in killing bacteria(28). Silver nanoparticles' well-known and applied broad-spectrum antibacterial capabilities are employed in wound healing. AgNPs come in a wide variety of sizes, shapes, and surface chemistries; therefore, a thorough understanding of how these structural parameters affect their antibacterial activities can aid in extending the range of biological applications for AgNPs(30).

4. CONCLUSION

Within the limits of this study, we can conclude that silver nanoparticles with the aloe vera and neem leaf extract can be useful against commonly seen oral pathogens. The readings against *S. Mutans* were high at all three concentrations and it was similar to the control. Adequate readings were seen in the case of *E. Faecalis* and *C. Albicans* too. A good antimicrobial activity has been displayed. There is great scope for future research to be done in this field.

5. CONFLICT OF INTEREST

The author claims that there is no conflict of interest.

6. ACKNOWLEDGEMENT

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