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Formulation and Characterization of Chitosan Coated Silver Nano Herbal Mouthwash Against Oral Pathogens

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Abstract

The oral antibacterial properties of silver nanoparticles made from Piper betel leaf aqueous extract are the subject of this investigation. Silver nanoparticles have been created with a spherical form and a size range of 11 to 19 nm. Because of this, silver nanoparticles are becoming a popular medication delivery method for the treatment of several oral infections. FTIR, SEM, XRD, and UV spectroscopy have all been used to confirm the synthesized nanoparticles. The current project is to create an herbal mouth rinse with chitosan coated silver nanoparticles and assess its effectiveness against oral bacteria.

Keywords: Silver Nano Particles, Chitosan, Piper Betel Leaves, Mouth Rinse

Introduction

Many people use mouthwash as an addition to good oral hygiene. Globally, dental caries is the most prevalent non communicable illness. Severe dental caries can lead to discomfort, infection, and even the need to pull teeth. It also has an adverse effect on overall health. Dental caries is a localized chemical degradation of teeth brought on over time by metabolic activity inside the biofilm. Together with fluoride prevention, other factors taken into account are better dental care and controlling the amount of time spent in sugar. The study's objective was to create an alcohol-free, antimicrobial mouth rinse with S-NP$_s$ that is effective, non-irritating, and beneficial for patients' dental health. We tested the solution against Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans, which are typical causes of fungal infections. We also employed this treatment to combat tooth caries-causing Streptococcus mutans. Betel vine, sometimes referred to as Piper betel, is a member of the Piperaceae family. In Asia, this herb is widely used as medicine. The betel vine's leaves are the most utilized and researched component. Chewing betel leaves is a common habit in many nations, where it is said to help prevent bad breath, strengthen gums, protect teeth, and stimulate the digestive system. Because of its great abundance and low cost, it encourages more industrialization and research, especially in the food and pharmaceutical sectors.

This study aims to provide current findings about the antibacterial and antifungal characteristics of betel leaves. XRD, FTIR, and UV spectrophotometry are frequently used techniques for characterizing nanoparticles. The size of the nanoparticles may be inferred from their absorption spectra. Because of quantum confinement effects, absorption peaks in nanoparticles are often shifted with respect to bulk material. Researchers can determine the size distribution of nanoparticles in a material by examining the UV spectrum. When figuring out a nanoparticle's crystal structure, XRD is quite helpful. Researchers can determine the arrangement of atoms within the nanoparticles by examining the diffraction pattern generated when X-rays contact with the crystal lattice of the nanoparticles. Understanding the characteristics and actions of nanoparticles in many applications requires knowledge of this kind. Information on the chemical makeup of nanoparticles may be obtained via FTIR. By monitoring the absorption of infrared light that corresponds to the vibrational modes of chemical bonds, it is possible to identify the functional groups that are present in the nanoparticles. This makes it possible for scientists to determine the kinds of molecules or ligands that are present on the surface of the nanoparticles as well as any potential chemical alterations or functionalizations that happened during synthesis or post-processing. When synthesizing silver nanoparticles, chitosan—a naturally occurring biopolymer produced from the deacetylation of chitin—is frequently utilized as a stabilizing and reducing agent. Because chitosan is non-toxic and biocompatible, it can be used in biomedical applications.

Chitosan aids in the production of biocompatible nanomaterials that may be employed for a range of biomedical applications, including antibacterial agents and medication delivery, when it is utilized in the synthesis of silver nanoparticles.

Silver nanoparticles are stabilized by chitosan, which keeps them colloidally stable by avoiding agglomeration. Functional compounds found in chitosan, such as hydroxyl and amino groups, can work as reducing agents during the creation of silver nanoparticles. These functional groups help to produce silver nanoparticles (Ag^0) with regulated size and shape by facilitating the reduction of silver ions (Ag^+) to silver nanoparticles. Silver nanoparticles with precise size, shape, and dispersity may be synthesized thanks to chitosan. Chitosan is more ecologically friendly than synthetic polymers since it comes from renewable natural sources and is made from sustainable materials like fungal and shellfish cell walls. The ideas of sustainable materials and green chemistry are in line with the usage of chitosan in the manufacture of silver nanoparticles.

Silver nanoparticle

With their unique optical, electrical, and magnetic properties, silver nanoparticles, which range in size from 1 to 100 nm, are widely used in industrial applications such as photonics, electronics, and catalysis. They can also be used as antimicrobial agents, biosensor textiles, cosmetics, composite fibers, electronic components, and to extend the shelf life of food ingredients. The primary goal of this study was to concentrate on silver nanoparticle formation techniques, including current developments and potential future applications. Nanomaterials' physical and chemical characteristics can differ from those of the same material in the massive bulk class; nano subatomic particles have a single characteristic that ranges from 1 to 100 nm. These find use in the management of nourishment, surgery, advertising materials, bandaging, computer gadgets, memory aids, water purification, textiles, makeup, and contact lenses. The one substance that producers can identify as being the most versatile in nanotechnology goods is silver nanoparticles. To extend the shelf life of food, they can be included into food packaging polymers. Because of their extensive antibacterial action, silver nanoparticles (AgNPs) are now the most often employed nanoparticles. A minimum of 383 out of 1628 items utilizing nanotechnology include silver nanoparticles in them. Milk contains silver nanoparticles to reduce the multiplication of microorganisms. Silver nanoparticles bind to the cell wall and membrane of bacteria when they interact with them, preventing them from replicating and ultimately causing cell death. Silver ionizes to produce nanoparticles that boost the bactericidal action when it dissolves in the cytosol. Therefore, the most important thing to do to increase the number of biomedical applications for multifunctional silver nanoparticles is to design and develop superficial, one-step, dependable, low-cost, nontoxic, complex fibers, cryogenic superconducting materials, electronic components, and an eco-friendly technique for prevarication (D. Subhadip, K.R.P. Biswas, et al. 2015).

Medicinal uses of piperbetel leaves

Originating in Southeast Asia, the Piper betel is a kind of flowering plant belonging to the Piperaceae family of peppers. This dioecious vine grows evergreen and has glossy, heart-shaped leaves with white catkins. Betel leaves, commonly referred to as "paan" in various cultures, have been utilized for ages in traditional Chinese and Ayurvedic medicinal systems.

Oral Health

The antibacterial qualities of betel leaves can aid in maintaining good dental hygiene and avoiding infections. Chewing betel leaves is said to improve gum health, lessen plaque buildup, and freshen breath.

Respiratory Health

Betel leaves are used in some traditional medical practices to treat respiratory conditions like bronchitis, asthma, and cough. The expectorant chemicals found in the leaves can aid in thinning mucus and improving breathing.

Antimicrobial Properties

Betel leaves have antibacterial and antifungal properties against certain microorganisms. They are used topically to small cuts, wounds, and infections of the skin.

Anti-inflammatory Effects

Compounds found in betel leaves have anti-inflammatory qualities. Topical use of betel leaf paste or oil has been shown to help reduce inflammation and relieve pain in illnesses such as arthritis.

Materials and Method

Sample collection

The fresh leaves of Piperbetel were collected from Tirupur district. Identification of the specimen was authenticated in the Botanical Survey of India, TNAU campus as Piperbetel leaves.

Figure 1: Piperbetel leaves

Preparation of the extract

Leaves of Piper betel was collected and washed under running water and once in distilled water. Then leaves samples was shade dried and powdered by using mixer grinder. The powdered sample was then mixed with the double distilled water. The sample was then filtered using whatman No.1 filter paper.

Green synthesis

Phytochemicals of the plant is used as the reducing agent to convert metal oxide into metal nanoparticles. Green synthesis, also known as eco-friendly synthesis or sustainable synthesis, refers to the process of designing and developing chemical reactions or procedures that minimize the use of hazardous substances and energy, reduce waste generation, and employ renewable resources wherever possible. This approach aims to mitigate the environmental impact associated with traditional chemical synthesis methods while still achieving desired product outcomes. This approach minimizes pollution and contributes to the sustainability of chemical processes. Green synthesis aims to minimize or eliminate the generation of waste products during chemical reactions. Piperbetel leaves plants extract powder was taken. plant powder was weighed10g and mixed in 200ml of distilled water. Autoclave at 121˚Celsius / 15lbs for15minutes.After Autoclave, bring the medium into warm condition. Pour into falcon tube. Then go for cooling centrifuge. Centrifuge at 4500rpm for 30 minutes. Discard the pellet and collect the supernatant. Filter the collected supernatant solution through filter paper. Piperbetel leaves aqueous extract was collected in different conical flask.

Calculation and metal oxide preparation:

Prepare Silver nitrate of 5mM for 25 ml Formula to calculate Molarity for Silver nitrate.

$$M = \frac{\text{Molecular weight of metal oxide} \times \text{Volume of distilled water} \times \text{no. of molarity}}{1000}$$

$$M = \frac{169.89 \times 25 \times 5}{1000}$$

M= 0.42

Titration

Take 25ml of silver nitrate in burette. Titrate with Piperbetel aqueous extract with the help of magnetics beats through magnetic stirrer which ensure the uniform mixing of bimetallic.

Green synthesis of silver nanoparticles

Keep the plant aqueous extract solution in rotatory shaker for 24 hrs. Take that plant extract in Eppendorf tube markitas(T0). After 24 hrs. colour changes takes place. Then mark it as (T24) to check UV. Pour the solution in falcon tube for cooling centrifuge. Centrifuge at 4500 rpm for 30 minutes. Discard the supernatant and add equal amount of distilled water to pellet and mix with vortex to avoid cell debris. Again, go for centrifuge at 4000 rpm for 15 minutes. Collect pellet which contains metal nanoparticles and pour it in sterile petri Plate. Allow it to dry in hot air oven, Scrape the dried nanoparticles and store in Eppendorf tube.

Antibacterial Activity of Green Synthesised AgNPs

The antibacterial activity of green synthesized AgNPs was evaluated against oral pathogens like Streptococcusmutans, Porthyromonas gingivalis and Candida albicans by well diffusion method

Uv- Vis spectroscopy analysis

UV-vis spectrophotometer is an analytical instrument that measures the amount of ultraviolet (UV) and visible light that is absorbed by a sample. It is a widely used technique in chemistry, biochemistry, and other fields, to identify and quantify compounds in a variety of samples. Double distilled water is water is used as reference and 1ml of the colloidal silver and copper solution is poured in a quartz cuvette and subjected for wave length scanning between 450 nm and 600 nm.

Formulation of mouth rinse

Take a 10g of Piperbetel leaves in 100ml of distilled water and autoclave it 121**°C** Centrifuge it 2500 rpm for 15 mins and collect the supnent. Take 70 ml of distilled water, add 5 ml of supernent and Add 5ml of Glycerine, 0.15g of Sodium saccharine, 0.5g of Nacl, 1ml of Peperment oil, 0.05 g of Sodium benzoate, 0.3 g of Sorbitol then make up into 100 ml. Add 0.1 mg of Chitosan coated silver nanoparticles and mix well.

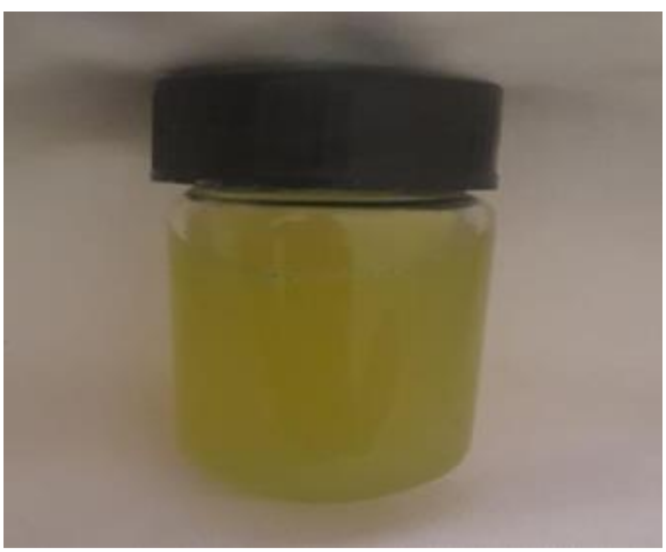


Figure 2:

Results and Discussion

Antimicrobial susceptibility test

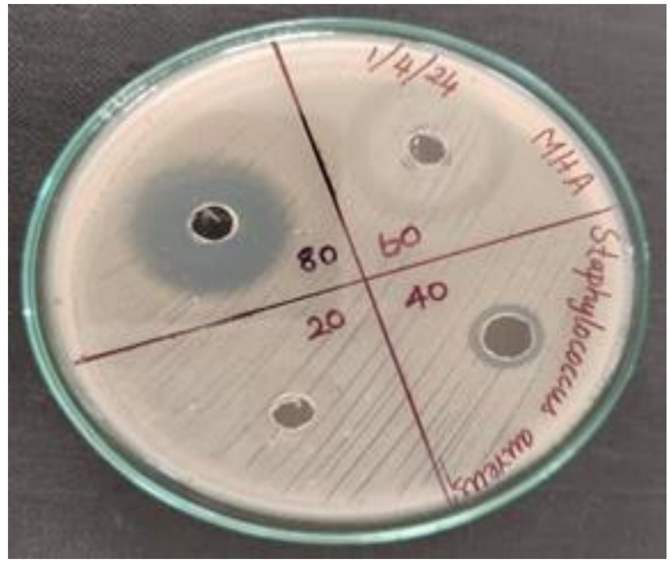


Figure 3: Staphylococcus aureus strain

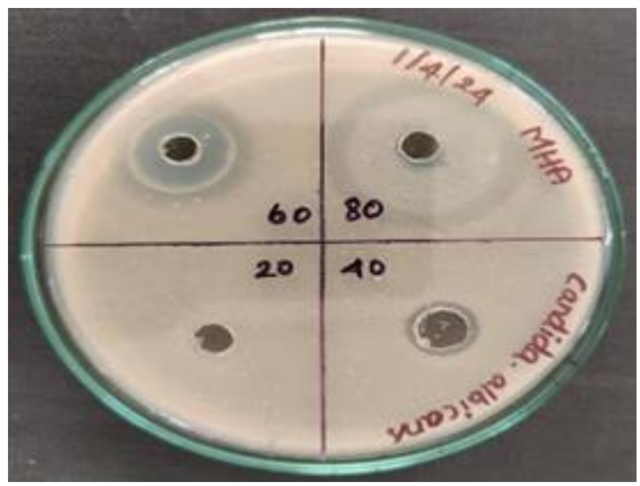


Figure 4: Candida albicans strain

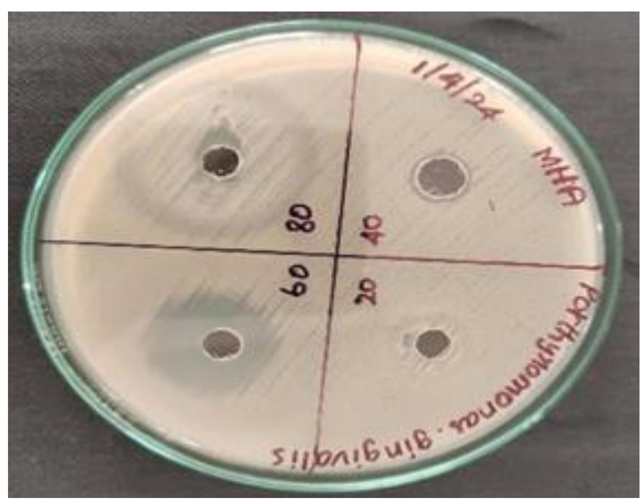


Figure 5: Porthyromonas gingivalis strain

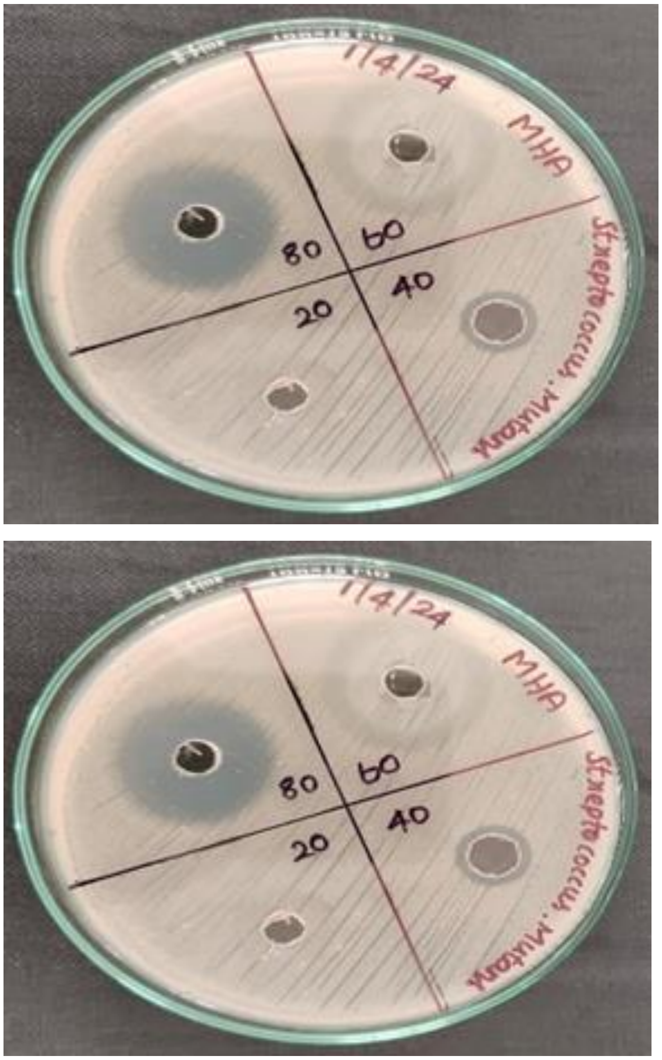


Figure 6: Streptococcus mutans strain

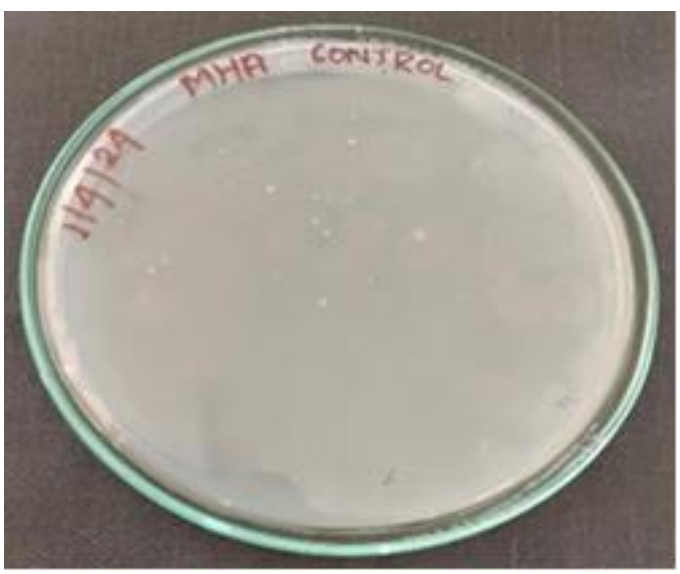


Figure 7: Control Plate

Zone of inhibition shown by the Ag nanoparticles against different microorganisms.

Table 1: Analysis synthesized Ag nanoparticle

S. No	Organisms	Level of inhibition
1.	Staphylococcus aureus	Moderate
2.	Candida albicans	Higher
3.	Streptococcus mutans	Moderate
4.	Porthyromonas gingivalis	Higher

The synthesized Ag nanoparticles from leaves extract of Piper betel showed higher level of inhibition against Porthyromonas gingivalis and Candida albicans. Hence it has a potential for mouth rinse against to kill oral pathogens.

Characterization of Nanoparticles

Ultraviolet-visible Spectrophotometer

Figure 8: UV wave length: Silver Nitrate–456nm

Graph 1: Absorbance and Transmittance wave length for Ag nanoparticles(After24hrs).

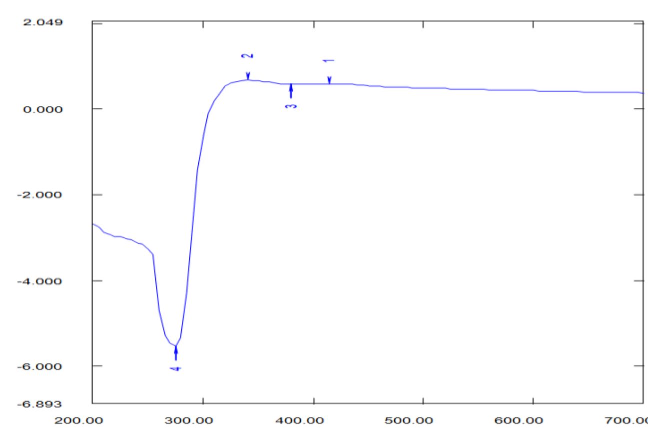


Table 2: Analysis of UV wave length for synthesized Ag nanoparticle

Wave length	nm
375wv	280nm
455wv	390nm
560wv	400nm
645wv	456nm

UV-visible absorption spectra of synthesized silver nanoparticles, showing the surface Plasmon resonance peak of silver nanoparticles at 456 nm. Like the same result was reported by Moustafa Zahran et al., 2016,and maximum absorption of silver nanoparticles was obtained at wavelength 300-600nm.

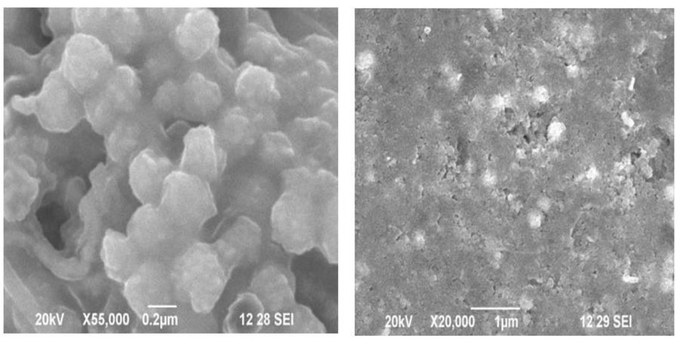
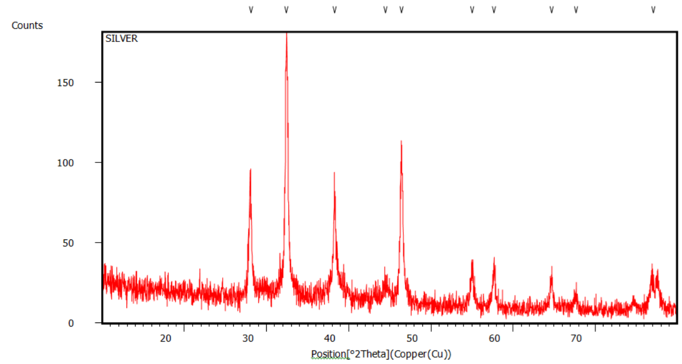


Figure 9: SEM image of the silver nanoparticles from Betelleaves

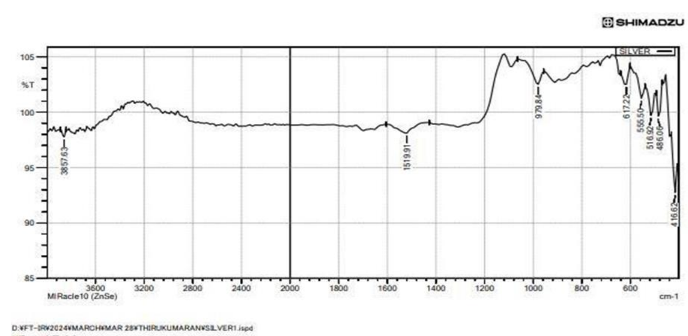
The surface morphology, size and shape of the silver nanoparticles were analyzed by Scanning Electron Microscope. shows the SEM image of silver nanoparticles synthesized from leaves extracts. The SEM images show individual silver nanoparticles which are predominantly spherical in shape as well as number of aggregates with no defined morphology. The presences of biomolecules in the leaves extracts have resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaves extracts. The SEM image shows the size of the silver nanoparticles ranging from 40 to 50 nm. Similar result of the silver nanoparticles size was reported by using Piperbetel leaves extract (KeroJemalet.al,2017).

Graph: 2



Analysis of structure and crystalline size of the synthesized silver nanoparticles were carried out by XRD. The XRD analysis of synthesized silver nanoparticles from leaves extract showed diffraction peaks at = 28°, 32°, 38°, 45.5°, 55° and 57.4° respectively. When compared with the standard, the obtained XRD spectrum confirmed that the synthesized silver nanoparticles were in nano crystal form and crystalline in nature. The high peaks in the XRD analysis indicated the active silver composition with the indexing (Figure 5.2.1). The same result was reported by Kero Jemal et al 2022, and indicates that the silver nanoparticles are face-centered, cubic, and crystalline in nature. The Full Width at Half Maximum (FWHM) values were used to calculate the size of the nanoparticles. The average sizes of the synthesized nanoparticles from leaves and callus extracts were found to be 42 nm and 44 nm, respectively.

Graph 3: Fourier-transform in frared spectroscopy measurements were conducted to identify the G-AgNPs



The FTI R spectra of G-AgNPs are shown in Figure Comparing the FTIRG-AgNPs, the high broad peaks for G-AgNPs appear at 2884.93 (asymmetric and symmetric C-H stretching, or secondary a mines), 6172.22 [carboxyl groups (-C=O), and carbonyl group (-C=O)-stretching vibration of proteins], 555.50 (C-H bending of COO–orcarboxylate groups), 516.9.2 which are identical to the supernatant spectrum. The FTIR results indicate the presence of carboxyl groups (-C=O), and amine groups (−NH) which represents the presence of proteins, amino acids, and other biomolecules originating from the supernatant on the surface of the produced G-AgNPs, (Priyanka Singh et al.,2022). The FTIR spectrum proved that the reaction medium contains reducing and stabilizing agents such as sugar, proteins, and amino acids responsible for the green synthesis of G-AgNPs.

Conclusion

The oral antibacterial properties of silver nanoparticles made from Piper betel leaf aqueous extract are the subject of this investigation. Ag nanoparticles that were generated using Piper betel leaf extract exhibited a greater degree of inhibition against Candida albicans and Porphyromonas gingivalis. Therefore, mouthwash has the ability to eradicate oral infections. The microorganisms (Candida albicans and Porphyromonas gingivalis) are inhibited by the produced Ag nanoparticles derived from Piper betel leaves when present in high concentrations.

However, Streptococcus mutans and Staphylococcus aureus are inhibited by it. a mediocre degree of focus. Using a scanning electron microscope, the silver nanoparticles' size, shape, and surface morphology were examined. The SEM pictures display a variety of aggregates with an unclear morphology in addition to individual silver nanoparticles, most of which have a spherical form. The creation of spherical silver nanoparticles has been facilitated by the presence of biomolecules in the leaf extracts, and the aggregation may be caused by the presence of secondary metabolites in the extracts. It has been demonstrated that silver nanoparticles have potent antibacterial properties. They are effective against a variety of microorganisms, such as viruses, fungus, and bacteria. This may contribute to improved oral hygiene and a lower risk of dental infections by lowering the number of germs in the mouth.

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