Research Article

Green Synthesis of Fucoidan-Chitosan Coated Silver Nanoparticles Biocojugates and their Antimicrobial Activity

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ABSTRACT

Reliable and environmentally friendly methods for the separation of nanoparticles are urgently needed in the field of nanotechnology. Traditional NP synthesis approaches are costly, harmful and detrimental to the atmosphere. To overcome these challenges, scientists have turned to natural sources such as plants, microorganisms, fungi and biopolymers for synthesizing Silver Nanoparticles (AgNPs), with these sources acting as both reducing and capping agents. Avocado leaves, known for their abundance in antioxidant compounds that protect against free radical damage and disease, are particularly noteworthy in this regard. This study aims to establish the synthesis and formulation of Fucoidan-Chitosan-coated silver nanoparticles using plant extract, with characterization conducted via SEM, EDX, FT-IR and UV-Visible Spectroscopy, along with the evaluation of the antibacterial activity of Ag nanoparticles. Nanoparticle-based therapy has garnered significant attention in the pharmaceutical and biomedical sectors. Functional nanoparticles were produced by coating fucoidan-chitosan onto silver nanoparticles loaded with avocado extract. These nanoparticles were synthesized through green synthesis using avocado leaf extract. The findings revealed that the fucoidan-chitosan coating enhances the stability and dispersibility of AG nanoparticles in aqueous environments, while also conferring antibacterial properties. Notable antibacterial activity of the avocado plant extract-coated AGNPs were observed against various organisms *in vitro*. The obtained sample holds promise for treating a range of disorders such as cancer, diabetes, cardiovascular diseases and Polycystic Ovary Syndrome (PCOS). The hybrid nanoparticles represent a potential therapeutic agent in cancer therapy, with the active extracts of avocado leaves offering promising medicinal applications.

Keywords: *Persea americana*, Fucoidan, Chitosan, Sliver Nanoparticles.

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INTRODUCTION

Persea americana is a moisture-loving plant belonging to the Lauraceae family. It is widely acknowledged that the leaves of *Persea americana* are rich in dietary and therapeutic compounds. Compared to the rest of the leaves, which can heal wounds in a quick manner and contain many monounsaturated fat acids like palmitoleic acid, phosphoritic acid, strachic acid, olitic acid, Linoleic acid and lactic acid, Avocado leaf composition has an oil content that is high.[1] Marine green growth contains adequate measures of polysaccharides, to be specific, cell wall primary polysaccharides, mucopolysaccharides and capacity polysaccharides.[2] These are polymers of basic sugars monosaccharides linked by glycosidic bonds and have a wide range of applications in areas such as thickeners, gelling specialists, stabilizers, emulsifiers, foodstuffs, feed additives, drinks etc.[3]

Extraction of fucoidan from microalgae is completed in major difficulties, followed by precipitation with natural solvents or salt. In any case, alterations of these strategies are involved in light of their additional utilize and the prerequisite of additional significant and cleaner polysaccharides. In favor of this intention, its

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taking out technique has been carefully contemplated to grow financially and economically practical frameworks. The process consists of three main phases: processing aid, extraction and purification, which result in a number of extensive extracts using aqueous or acidic solutions, including the addition of calcium to facilitate alginate precipitation; drying and storage management. Fucoidan, ranging from 0.26 to 20% of the algal biomass dry weight, can be produced through these extraction methods.[4] The physiochemical characteristics of isolated fucoidan are dependent on the degree to which extraction is performed, such as reaction times, chemical concentrations, temperature and algae's intrinsic properties. Fucoidan's subload changes from 13 to 950 k Da, depending on a number of factors such as source, extraction technique, atomic season and time during which it is harvested.

Chitin and chitosan are drawing in extraordinary interest as a result of their useful organic properties, like biodegradability, biocompatibility, non-antigenicity and non-poisonousness.[5] Given their flexibility as biopolymers, extensive exploration into their potential applications across various modern fields has been conducted. For instance, chitin and chitosan have been recorded to be helpful as antimicrobial, emulsifying, thickening and balancing out specialists in the food business.[6] They have likewise shown striking bioactivity in biomedical fields, including wound recuperating advancement, safe framework upgrade and hemostatic, hypolipidemic and antimicrobial movement.[7] N-acetyl-D-glucosamine units are transcended in the polymeric chain and form a copolymer in chitin when they are joined by a â-(1-4) glycosidic link. Chemically described as a copolymer of á-(1,4) glucosamine (C6H1104N)n, with various quantities of N-acetyl groups, chitosan is the deacetylated derivative of chitin. It looks like fine, solid, pale to light reddish granules that are soluble in organic acids but insoluble in water. Chemical processing can be used to extract chitin from the exoskeletons of shellfish.[8]

Nanoscience, from an overall perspective, in very normal in natural sciences thinking about that the sciences thinking about that the size of numerous bio particles manages (Like catalysts, infections and so on,) Fall inside the nanometer range.[9] Silver nanoparticles stand out as one of the most notable varieties in the field of nanotechnology. These nanoparticles, which usually have sizes between 1 and 100 nm, have attracted a lot of scientific interest. It is widely acknowledged that silver nanoparticles can adhere to cell walls, potentially disrupting their permeability and cellular respiration. Furthermore, these nanoparticles have the ability to enter cells and cause harm through interactions with substances that contain phosphorus and sulfur, such as proteins and genetic material. By and large silver not unfavorably influence practical cells and doesn't handily incite microbial opposition. Thus silver containing materials were likewise utilized in material textures, as food added substances and in bundle and plastics to kill microorganisms. Due to the wide range of applications, many methods for producing silver nanoparticles and other silver-based compounds that contain metallic silver (Back) have been developed.[10]

MATERIALS AND METHODS

Plant extract preparation

The plants were dried in air, crushed and an aqueous extract was obtained by placing the crushed leaves in a Soxhlet apparatus. After a 12 hr extraction operation, the resultant solution was dried with a rotary evaporator set at 40ºC, yielding a residue labeled AEPA, which was then stored in clean vials until needed.[11]

Preparation of Chitosan solution and fucoidan extract

The chitosan solution is prepared by, 0.005 g of chitosan is added with 50ml of prepared 1% acetic acid and 1 mL of TPP is added for dissolving.[12] The brown seaweed was collected from the shore regions, Tamilnadu, India. The brown seaweed is rinsed with distilled water to eliminate any sand particles. Once cleaned, the seaweeds are dried in the shade and then ground into powder.

Preparation of Chitosan- Fucoidan complex with leaf extract

The fucoidan is added to the plant extract in the concentration of 1 mg/mL. They are subjected to the stirring process for 24 hr in the shaker. Then they made into complex using the chitosan. Now the Chitosan-fucoidan complex is made with the leaf extract.

Preparation of Plant extract and Complex coated with AgNPs

The Chitosan- fucoidan complex with the avocado leaf extract is coated with the silver nitrate at the concentration of 0.0169 g of silver nitrate in 100 mL of milliq water. Then the silver nanoparticles synthesized are observed with the colour change in the solution after kept in shaker for 48 hr. The colour change is due to the metal reacted with the complex (Figure 3).[13]

Biomimetic Synthesis and Characterization of the Chitosan Fucoidan Complex coated AgNPs using *Persian Americana*

UV - visible spectral analysis

Ultraviolet-visible Spectrophotometry is employed to monitor the characteristics of silver nanoparticles. Ultraviolet (UV) spectroscopy encompasses absorption and reflectance spectroscopy within the light and ultraviolet regions of the electromagnetic spectrum. One useful method for examining colloidal particles is UV-visible spectroscopy. Noble metal particles, like silver nanoparticles, have a strong surface plasmon resonance absorption in the visible spectrum and are highly sensitive to surface alterations, making them ideal for UV-Vis spectroscopic research. UV-Vis spectroscopy was used to evaluate $AgNO_3$'s optical absorption characteristics. A Shimadzu UV 3600 UV-Vis-NIR spectrometer (Shimadzu Corporation, Kyoto, Japan) was used to record the samples' UV-Vis absorption spectra in the 200-800 nm wavelength range in diffuse reflectance mode, with BaSO4 serving as a reference. The Kubelka-Munk function was used to process the data after the spectra were collected at room temperature.

SEM analysis

A concentrated electron beam is used to scan a sample in a Scanning Electron Microscope (SEM), a specialized kind of electron microscope that creates images of the material. These electrons' interactions with the sample's atoms produce a variety of signals that reveal details about the sample's makeup and exterior characteristics. To form an image, the electron beam is usually scanned in a raster pattern and its position is correlated with the signals that are detected. SEM has the capability to achieve resolutions finer than 1 nanometer. The operation of a scanning electron microscope involves directing electrons onto the surface of the sample and forming an image from the reflected electrons. SEM allows for the collection of a significant amount of analytical data in addition to the visual image. In the analysis of silver nanoparticles, the nanoparticles are first obtained in powdered form and SEM is used for examination. The SEM analysis is conducted at various magnifications, typically ranging from 15,000 X to 35,000 X, providing an estimate of the size of the silver nanoparticles.

FTIR Analysis:

Fourier Transform Infrared (FTIR) spectroscopy stands as the favored method in infrared spectroscopy. During infrared spectroscopy, IR radiation traverses through a sample, wherein the sample absorbs some of the infrared energy while the rest is transmitted. The resulting spectrum provides the sample with a molecular fingerprint by illustrating the absorption and transmission of molecules. Much like fingerprints, no two unique molecule configurations yield the same infrared spectrum. Consequently, infrared spectroscopy serves various analytical purposes. An FTIR spectrophotometer is used to examine the chemical makeup of silver nanoparticles that have been produced. Using the KBr pellet technique, the dried powders were characterized in the 4000-400 cm range. During a model, the FTIR instrument emits infrared radiation in the range of about 10,000 to 100 cm^{-1}, with some of the emission being absorbed and the remainder being transmitted. The model molecules then convert the fascinated emission into turning and/or vibrational power. The subsequent signal that the detector picks up manifests as a spectrum, usually ranging from 4000 cm^{-1} to 400 cm^{-1}, which is the sample's molecular fingerprint. Since every particle or substance structure produces a unique spectral fingerprint, FTIR analysis is an invaluable method for identifying elements.

EDX Analysis

EDX is utilized alongside SEM. An electron beam, with energy ranging from 10 to 20 keV, impacts the surface of the conducting sample, resulting in the emission of X-rays from the substance. The power of these emitted X-rays depends on the substance being examined. EDX does not belong to surface science techniques since the X-rays that are produced within a range is approximately 2 microns deep. An image of every element contained in the sample can be obtained by moving the electron beam across the material. However, acquiring these images typically requires extended durations due to the low intensity of the X-rays. Energy dispersive X-ray analysis (EDX, EDS, or EDXA), also known as energy dispersive X-ray spectroscopy, is an X-ray technique employed to ascertain the fundamental masterpiece or chemical categorization of materials. EDX ray analysis was performed using a high-resolution transmission electron microscope to confirm the presence of silver in the particles and to identify other elemental compositions of the particles.

Antibacterial properties of silver nanoparticles

The test organisms used in the study were obtained from the Department of Microbiology:

- *Salmonella typhi*
- *Escherichia coli*
- *Pseudomonas aeroginosa*

- *Klebsiella pneumonia*
- *Proteus vulgaris*

By evaluating the zone of inhibition, the synthetic Sliver particles' antibacterial activity was determined.[15] 100 mL of sterile distilled water was used to measure and dissolve 3.6 g of nutritional agar. After adjusting the pH to 7.2, the mixture was autoclaved for 15 min at 121ºC. The disinfected petri dish was then filled with 30 mL of molten agar and let to harden.

DISC DIFFUSION ASSAY

Using Filter paper Whatman No. 1, 4mm discs were prepared, punched out and placed in vials, then sterilized in a range at 150ºC for 15 min. These discs were then coated 10 µL of concentrated crude extract, while the next set of discs were coated with 10 µL of synthesized Ag nanoparticles with a coated complex. After impregnation, the discs were left to evaporate at 37ºC for 24 hr. Following this, the prepared discs that consist of various fractions were carefully positioned on inoculated plates using sterile forceps. Gentamycin served as a control antibiotic. The plates were then inverted and incubated at 37°C for 24 hr in an incubator. The zones of growth and inhibition of the microorganisms surrounding the test fractions were observed in order to acquire the results. The diameter of the Inhibition Zone (IZ) surrounding each disc was measured in order to assess the antibacterial activity.

RESULTS

Biosynthesis of fucoidan-chitosan complex with leaf extract coated silver nanoparticles

The reaction mixture, consisting of silver nitrate and a fucoidan-chitosan complex coated with plant extract, underwent periodic color changes over a 48 hr period. The observed color change was indicative of the reduction of silver ions into silver particles upon exposure to the extracts. This change in color is attributed to the surface plasmon resonance phenomenon, resulting in the silver nanoparticles displaying a dark brown hue in aqueous solution. Based on the findings, the synthesis of silver nanoparticles by the extract was verified through the observed transition in color from dark green to brown during the various time.[14] reported that the distinctive brown color exhibited by silver solutions serves as a convenient spectroscopic indicator of their formation.

Characterization of silver nanoparticles

UltraViolet-Visible spectrophotometric examination

UV-Visible spectroscopy is among the most widely utilized techniques for structurally characterizing silver nanoparticles. Following the development of the dark brown color, the absorbance of the nanoparticles was assessed across wavelengths ranging from 200 to 800 nm, with intervals of 1 nm, utilizing a UV-Visible spectrophotometer.The common peak values lie between the range of 250-400 nm.The highest peak value lies between the range of 330 nm.

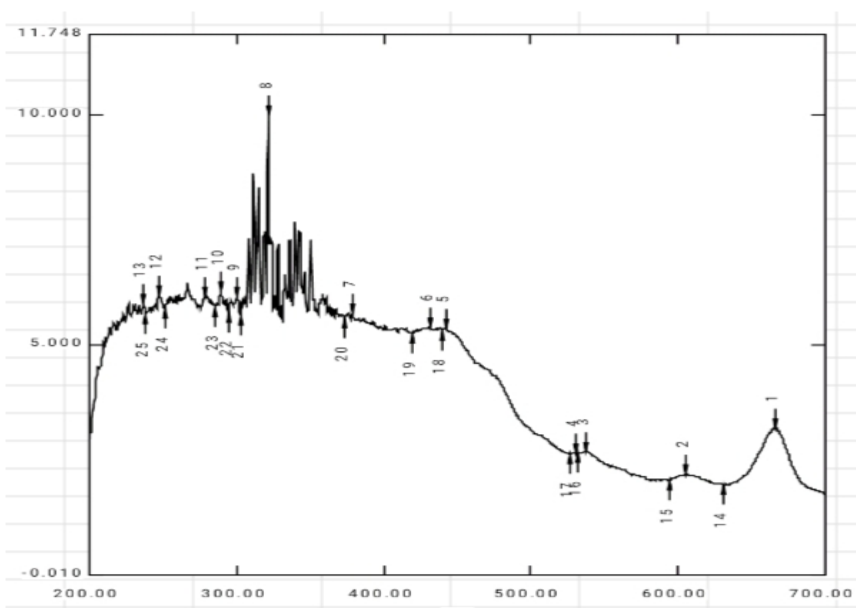


Figure 1: UV-vis spectroscopy analysis.

FTIR analysis

FTIR analysis is employed for the identification of molecular compounds. This technique operates by quantifying the absorbance of infrared radiation by a sample. The resulting spectrum can then be utilized to identify the functional groups present in the compound.

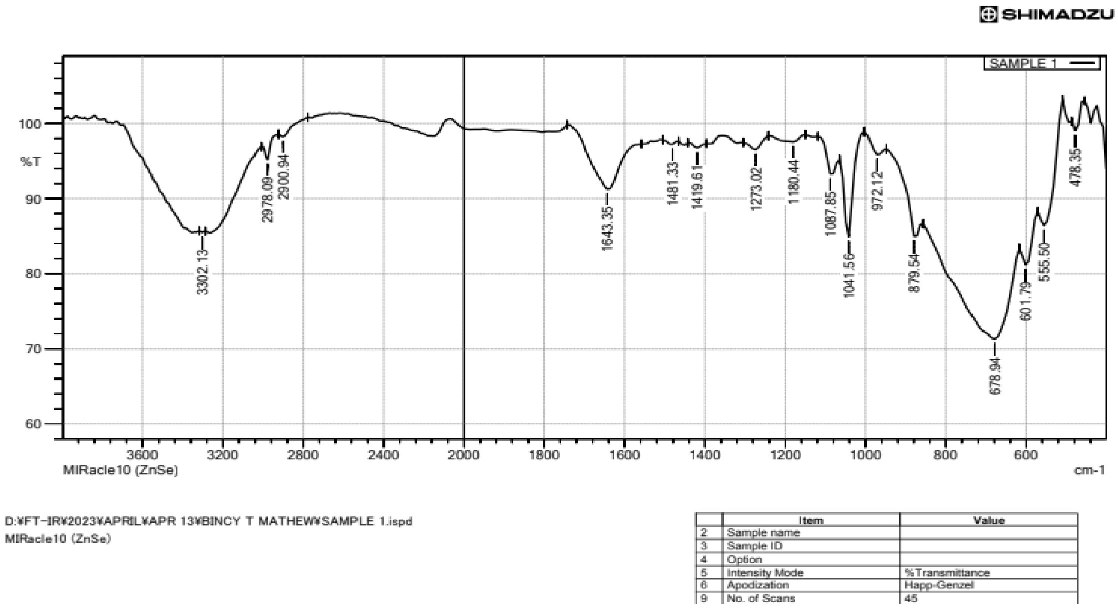


Figure 2: FTIR analysis of the sample.

FTIR spectrum which are displayed in the following figure Fucoidan-Chitosan complex coated with silver nitrate along with avocada leaves. In the extract, the peak at 3302 cm^{-1} revealed aliphatic primary amine (N-H Stretch); the peak at 1643 cm^{-1} revealed alkene (C=C Stretch); peak at 1041 cm^{-1} revealed fluro compound (C-F Stretch), peak at 678cm^{-1} revealed alkene (C=C Bend).The FTIR analysis spectroscopic studies revealed the presence of aliphatic primary amine, alkene, fluro compound. In the similar studies, the FTIR spectrum exhibits the emergence of absorption characteristics peaks at 1111cm^{-1} corresponds to C-O Stretching bonds.[16] The peak at 1303 cm^{-1} corresponds to C=C Stretching bonds.[17] The peak 2353 cm^{-1} corresponds to C-H stretching bonds.[18]

SEM analysis

The biosynthesized silver nanostructure by employing *Persia americana* extract with complex coated with AgNps was further demonstrated and confirmed by the characteristic peaks and the structural view under the scanning electron microscope. The surface morphology of the was confirmed by using FESEM (ZEISS). In the scale of 1μm under 915X. The SEM analysis includes the formation of non uniform sized particles.[19]

EDX Analysis

The energy dispersive spectrum of synthesised nanoparticles is shown in graph. The metallic silver nanoparticles considerably shown siganl peak at 1.14kev which is due to surface plasmon resonances.[20] The figure also dispays the qunantitative information of biosynthesised AGNPs such as C, Ag, Cu, Ca, K, Cl, Si, Al, Mg, Na and O. In the present study the synthesised nanoparticles show strong absorbance with range of 3.9 kv.

ANTI-MICROBIAL ACTIVITY

Antimicrobial activity of Synthesis and Characterization of the Chitosan Fucoidan Complex coated AgNPs using *Persian Americana*

The antimicrobial effectiveness of biologically synthesized plant extract using Ag nanoparticles coated with a complex was assessed against both diderm bacteria *(Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Proteus vulgaris)* and monoderm bacteria (*Staphylococcus aureus)* bacteria. Additionally, the region of inhibition produced by Ag nanoparticles against diderm bacteria and monoderm bacteria was measured. The findings demonstrated that Ag nanoparticles synthesized from the extract exhibited significant antibacterial activity against both diderm bacteria and monoderm bacteria. Inhibition of bacterial growth by Ag nanoparticles could be credited to harm of the bacterial cell membrane and extrusion of the cytoplasmic contents thereby resulting in the death of the bacterium.[21]

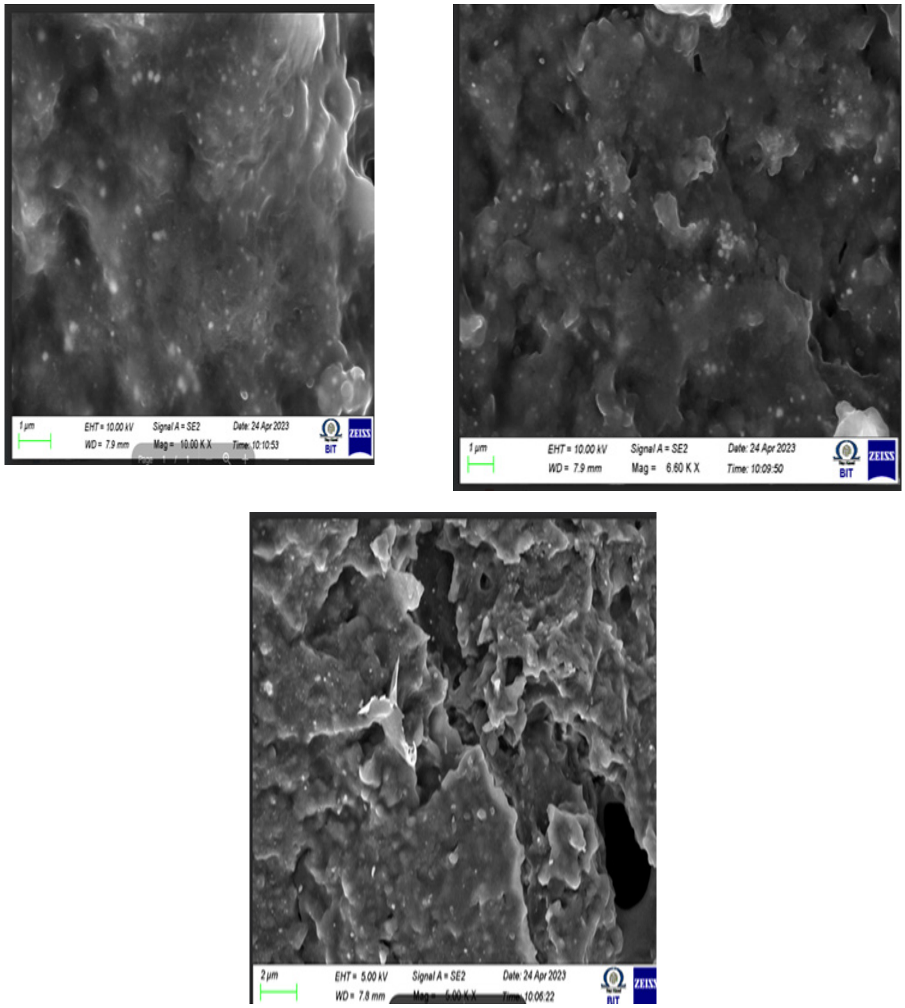


Figure 3: SEM analysis of silver nanoparticles.

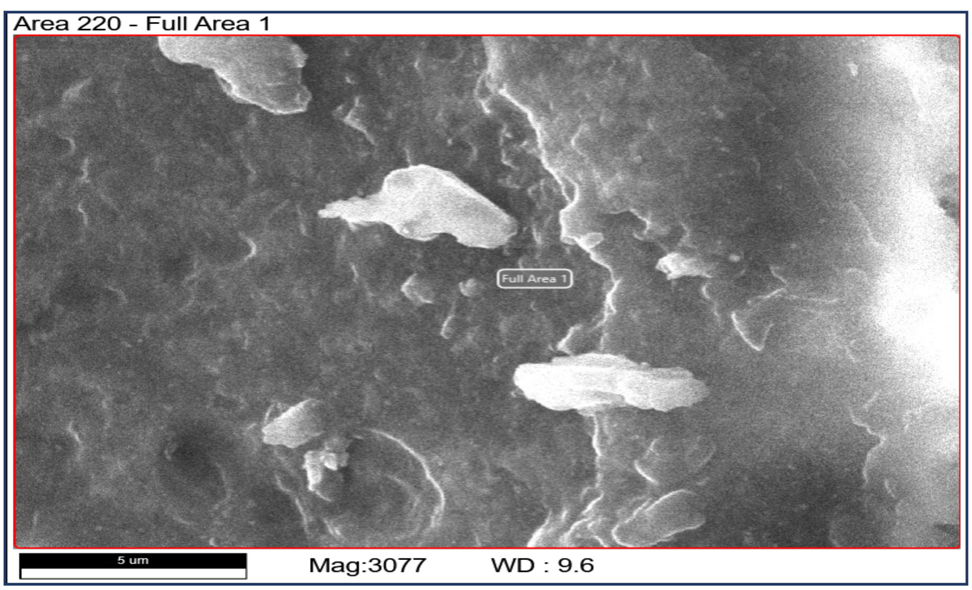


Figure 4: EDX analysis of silver nanoparticles

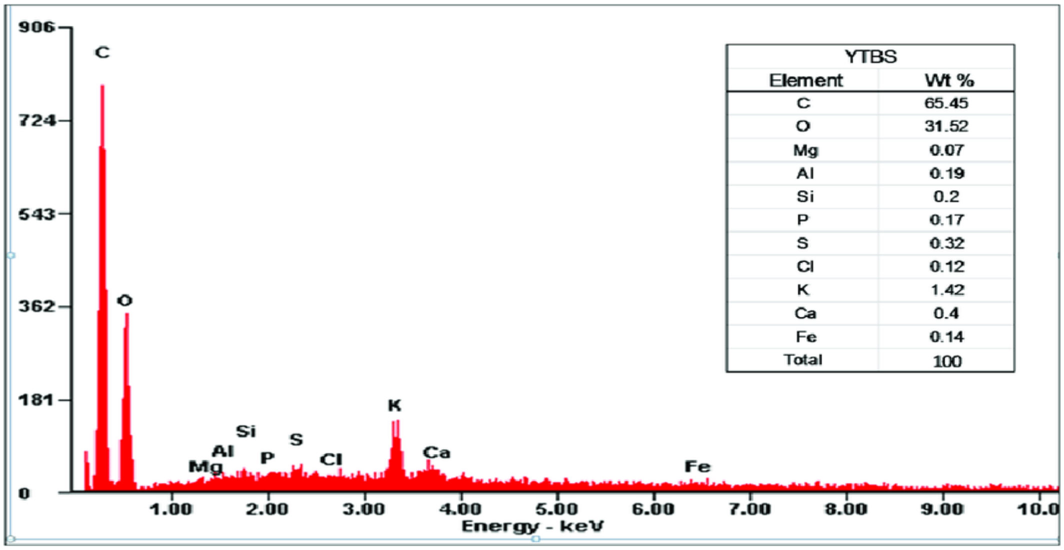


Figure 5: EDX analysis of silver nanoparticles plotted graph.

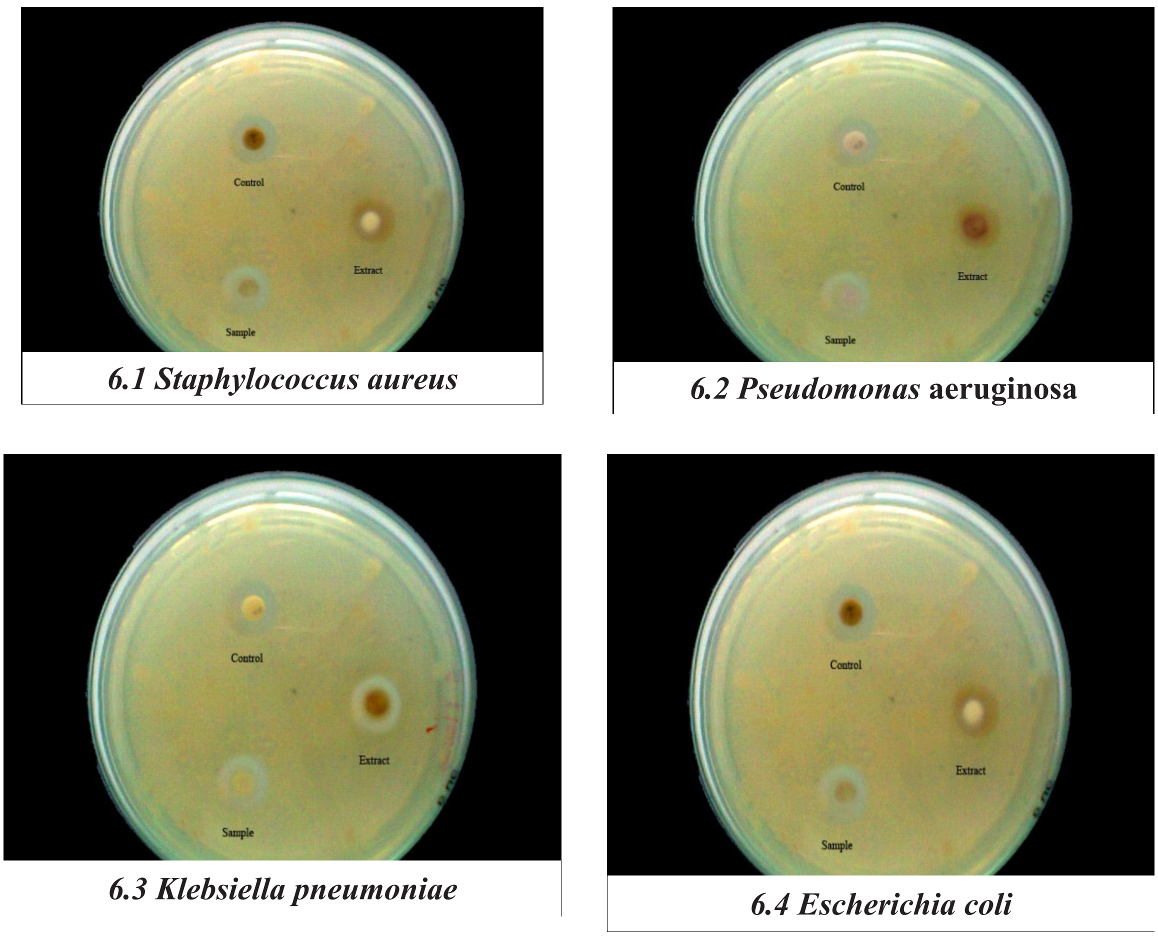
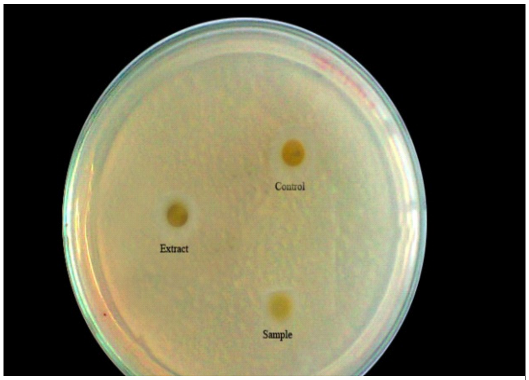


Figure 6: (Continued)



6.5 Proteus vulgaris

Figure 6: Antibacterial study of Plant extract and Complex coated with AgNPs against selective bacterial pathogens.

Proteus vulgaris

Table 1: Antibacterial activity of Sliver nanoparticles of *Persea Americana* Coated with complex.

Sl. No.	Organism	Plant extract (Persea Americana) (mm)	Control-Gentamycin	Sample-Nanoparticles of Persea Americana (mm)coated with complex
1	Staphylococcus aureus	10 mm	12 mm	15 mm
2	Pseudomonas aeroginosa	14 mm	12 mm	15 mm
3	Klebsiella pneumoniae	9 mm	11 mm	14 mm
4	Escherichia coli	7 mm	8 mm	10 mm
5	Proteus vulgaris	11 mm	9 mm	12 mm

DISCUSSION

Even though the use of silver and silver salts as uncontaminated agents is a prehistoric method, Silver Nanoparticles (AgNPs) fabrication has only recently been developed. Silver nanoparticles are well known for their antimicrobial activity and there are also reports of their acute and chronic toxicity.[22,23] Considering this approach, nanoparticles were produced through environmentally-friendly synthesis, incorporating various compounds, peptides, or chemical groups onto their surfaces. This method effectively mitigates toxicity, enabling the utilization of nanoparticles for combating human infectious diseases. Fucoidan-chitosan coated silver nanoparticles stand out among potential coatings, garnering considerable interest in biomedical applications due to their distinct characteristics of being biodegradable, biocompatible, non-toxic and possessing antimicrobial properties.[24] Many scientists have reported that fucoidan- chitosan has a strong affinity for metal ions as a result of a large number of amino and hydroxyl groups. According to the study conducted by Nate *et al*, the role of the fucoidan-chitosan ester and amino group was defined as being crucial for metal ion release and the formation of FU-CH-AgNPs.[25] Plants are considered as the primary source for NP synthesis. Several studies have demonstrated that the polyphenols present in plant extracts play a major role in the reduction of Ag^+ ions.[26] In the present study, the antibacterial effect of FU-CH-AgNPs prepared using Persea Americana leaf extract was assessed. The multitude of bioactive compounds present in Persea Americana makes it an excellent candidate for the synthesis of nanoparticles.[27] Moreover, the synthesis of metal nanoparticles using Persea Americana has not been explored to any great extent.

The results of UV-visible spectroscopy obtained in our study revealed a difference in size FU- CH-AgNPs. The FU-CH-AgNPs were larger than uncoated AgNPs since layers of fucoidan- chitosan were observed to be wrapped around AgNPs, as in the work described by Lim *et al.* (2013).[28] The zeta potential on the surface of nanoparticles indicates the particles are stable. Nanoparticles possessing higher negative or positive zeta potentials will tend to repel each other and do not form aggregates.[29,30] Our synthesized nanoparticles were found to be more stable than those previously reported in studies.[31] The current study reports that the FT-IR analysis depicted that numerous amino groups act as capping agents. The results clearly indicate that these identified amino groups are responsible for the interaction with the metal surface, acting as capping sites for AgNP stabilization.[32] From the results, it can be concluded that capping agents permit proteins to attach to AgNPs by the electrostatic attraction of negatively charged carboxylate groups contained in the protein secreted by plants.[33] Many of the peaks observed in the IR spectra of FU- CH-AgNPs are closely associated with flavonoids and terpenoids, suggesting the adsorption of these molecules on the nanoparticle surface. The existence of multiple functional groups may impact the conversion of metal ions into their respective metal nanoparticles. Using SEM analysis, we observed the presence of polydisperse nanoparticles.[34] The SEM images clearly indicated a difference in average size, which were 32 and 50 nm for AgNPs and CH-AgNPs, respectively. The cytotoxicity of synthesized FU-CH-AgNPs was screened in order to define the optimal concentrations for antimicrobial assays. The antibacterial potential of FU-CH-AgNPs was evaluated against standard and clinically isolated Gram-positive and Gram-negative isolates. The FU-CH-AgNPs efficiently inhibited bacterial growth compared to AgNPs. Our findings show that the values of FU-CH-AgNPs were lower than those of plant extract. Within the similar species, the coated nanoparticles showed a distinguished effect between the various strains. The present antimicrobial evaluation studies agreed with the reports of Dell *et al*. 2020.[35] Therefore, it could be assumed that the use of FU-CH-AgNPs at their value can effectively inhibit the growth of bacteria while not being toxic to normal cells. The mechanism of action for the antibacterial activity of fucoidan-chitosan might come from the contact-inhibitory mechanism between negatively charged teichoic acid in the peptidoglycan layer on the bacterial surface and the positively charged protonated amine moieties on the chitosan backbone.[36] Similar studies reported that the enhanced antibacterial activity of FU-CH-AgNPs is due to the synergistic antibacterial potential of AgNPs with chitosan.[37] It is notable that metal NPs have more relevant bactericidal effects due to their high affinity with the active surface groups of microbial strains.[38] Similarly, it has been suggested that fucoidan and chitosan reacts with both the bacterial cell wall and the cell membrane.[39,40]

CONCLUSION

The present study presents the characterization and antimicrobial activity determination of fucoidan-chitosan covered Silver Nanoparticles (AgNPs) with Persea Americana leaf extracts which provide a promising strategy for developing effective antibacterial agents. These results indicated that the Ag Nanoparticles have improved stability and better dispersion in water as a result of coating them with mucopolysaccharide-conjugate; this is contributed by the fucoidan-chitosan film, it also imparts anti-bacteriality. The avocado plant extract-coated AGNPs demonstrated potent inhibition on different pathogens tested *in vitro*. Moreover, the synthesized nanoparticles showed good antimicrobial potentials against several pathogenic microorganisms thereby indicating their potential application in fighting microbial infections. The positive outcome of combing Ag NPs with fucoidan-chitosan and Persea Americana leaf extract shows that it is important to use natural substances in preparing nanotechnology-based drugs for curbing infections. Consequently, this research adds to existing literature on green synthesis methods for nanoparticle production and underlines how these new materials can be utilized in medical science as well as ecological concerns though further studies must be conducted to investigate additional therapeutic and industrial applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

The present study was carried out by synthesizing Silver Nanoparticles (AgNPs) from Avocado leaves, coated with Chitosan-Fucoidan polysaccharides. The characterization was conducted via SEM, EDX, FT-IR and UV-Visible Spectroscopy, along with the evaluation of the antibacterial activity of Ag Nanoparticles. The results indicate the formation of stable nanoparticles with distinct physicochemical properties suitable for antimicrobial applications. The size and shape of the leaf extract with fucoidan chitosan complex coated AgNps was confirmed using SEM. The presence of various elements like C,Ag,K,Cl,Fe,Si,S,P was confimed using EDX analysis. The FTIR analysis spectroscopic studies revealed the presence of aliphatic primary amine, alkene, Fluro compounds. Along with silver nanoparticles, the avacoda leaves has numerous effect on the biological terms. The antimicrobial activity was assessed against both diderm and monoderm bacteria and results demonstrated that the synthesized AgNPs from the avocado extract showed a exemplilary antibacterial activity against both monoderm and diderm bacteria. They can be used to cure various number of disorders such as anticancer, diabetes, cardiovascular system, prostate cancer and PCOs. So the final product such as caspsules can be formed using the complex mixture which are rich in promoting beneficial cancers studies.

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