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ECO-FRIENDLY SYNTHESIS OF NANO-PARTICLES AND EXPLORATION OF THEIR BIOCHEMICAL APPLICATIONS

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ABSTRACT

Our eco-friendly approach aims to provide solutions that not only advance scientific knowledge but also address the need for sustainable nanoparticle synthesis. This green synthesis strategy will address relevant social challenges by reducing the impact on the environment, reducing the use of dangerous chemicals, aligning with social ideals, and many other ways. In the present study, *Carica papaya* leaf extract acts as a reducing and capping agent for the synthesis of silver nanoparticles. Once the synthesis process is completed, the antibacterial activity of silver nanoparticles is tested against gram-positive and gram-negative *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* as well. This test involves observing the formation of a clear zone around nanoparticles, indicating the inhibitory effect on specific microorganisms. Additionally, HPLC (High Performance Liquid Chromatography) was employed to assess the purity and concentration of silver nanoparticles, offering a detailed analysis of their characteristics. Following characterization, the nanoparticles are characterized using UV spectroscopy. The optical density values gradually increase, the absorbance peaks were seen at 410 and 640nm for *Carica papaya* indicating successful nanoparticle production. Then FTIR (Fourier Transform Infrared Spectroscopy) was performed to determine the functional groups present in our silver nanoparticles. These peaks indicate the presence of aliphatic primary amine, carbodiimide, and alkene functional groups. To study shape and size, nanoparticles were seen under a scanning electron microscope (SEM). The size of nanoparticle between 50 to 500 nm. Research on the potential uses of these nanoparticles is ongoing while keeping all of these considerations in mind.

KEYWORDS Green synthesis, Silver nanoparticles, Antibacterial activity, High Performance Liquid Chromatography, Fourier Transform Infrared Spectroscopy.

INTRODUCTION

Nanotechnology is the branch of science which deals with the examination of materials in nano range, generally between 1 to 100 nm. It is a science that works at the nanoscale and gives various focal points to the diverse fields of science like dentistry, pharmaceuticals and bio-engineering [1]. NPs can be categorized broadly as inorganic and organic NPs. Inorganic NPs incorporate semi-conductor NPs (like ZnO, ZnS, CdS), metallic NPs (like Au, Ag, Cu, Al), and magnetic NPs (like Co, Fe, Ni), while organic NPs incorporate carbon NPs (like fullerenes, quantum dots, carbon nanotubes). There is a growing enthusiasm for Gold and Ag (noble metal) NPs as they furnish superior characteristics with useful flexibility [1]. Ag-NPs have a substantial surface zone which results into noteworthy biochemical reactivity, catalytic activity, and atomic behavior compared with bigger particles having same chemical composition [2]. The formation of Ag-NPs has received significant interest because of their potential applications in catalysis [3], plasmonics [4], optoelectronics [5], biological sensors [6,7], antimicrobial activities [8], DNA sequencing [9], Surface-Enhanced Raman Scattering (SERS) [10] , climate change and contamination control [11] , clean water technology [12], energy generation [13] , information storage [14], and biomedical applications [15]. The formation of NPs has provided us remarkable developments in the field of nanotechnology by demonstrating its potential from the last decade [16]. A number of techniques are available for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc.[17,18,19].unfortunately, many of the nanoparticle syntheses methods involve the use of hazardous chemicals or high energy requirements, which are rather difficult and including wasteful purifications [20].Therefore, green synthesis has been considered as one of the promising methods for synthesis of nanoparticles because of their biocompatibility, low toxicity and eco-friendly nature. The plants or plants extract, act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes [21,22]. because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis [22]. Different parts of plant materials such as extracts, fruit, bark, fruit peels, root and callus [23-28] have been studied so far for the synthesis of silver, gold, platinum and titanium nanoparticles in different sizes and shapes [29]. In the present study leaf extract of Carica papaya act as bio reducing agent for the synthesis of silver nanoparticles .*Carica papaya* belongs to family of caricaceae which is commonly known as Papaya, Paw Paw, Kates, Papaw. The papaya fruits, bark, leaves are being used as medicine to treat various diseases such as warts, corns, constipation, amenorrhoea general debility, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, cancer cell growth, diabetes, malaria, expel worms and stimulate reproductive organs, syphilis and gonorrhea [30].The extract of Carica papaya leaves and fruit are rich in vitamins, phenols, proteolytic enzymes which acts as a good antioxidant and an excellent antimicrobial agent [31,32].Therefore, the present study aims to synthesize silver nanoparticles by a green biological route using an extract derived from papaya leaf extract, purification using high-performance liquid chromatography (HPLC), and characterization of the synthesized nanoparticles utilizing UV-visible spectroscopy, scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR) analysis. Besides, their antimicrobial activity against pathogenic microorganisms was investigated.

MATERIALS & METHODS

SAMPLE COLLECTION

Carica papaya leaf extract was used to prepare silver nanoparticles on the basis of cost-effectiveness, availability, and medicinal properties. Fresh leaves of *Carica papaya* were collected from Ch. Sambhajinagar, Maharashtra, India, in the months of August and September 2020, respectively. They were washed with running tap water to to remove dust particles and other unwanted materials accumulated on the leaves. Further, they are also washed with double-distilled water for dual sterilization and allowed to air-dry at room temperature.

PREPARATION OF PLANT EXTRACT

Carica papaya leaves were diced into fine pieces and transferred into sterile distilled water. About 10 grams of finely cut leaves were kept in a beaker containing 100 ml of double-distilled water. The mixture was heated at 60 °C for 30 minutes to facilitate aqueous extraction. The extract was colled down and then filtered using filter paper, and the filtered extract was stored at 4 °C for further analysis [33].

SYNTHESIS OF AgNPs

Silver nitrate (AgNO3) was taken from the laboratory of the Institute of Biosciences and Technology at MGM University, Ch. Sambhajinagar.10 ml of plant extract was mixed with 90 ml of 1mM (16.987 mg) AgNO3 solution, resulting in a pale yellow color mixture. It was incubated for 24 hours at a rotatory shaker (180 rpm) at 37 °C. The reaction mixture's color changed from pale yellow to dark brown, signifying the reduction of Ag+ ions to Ag nanocrystals. [33].

ASSESSMENT OF ANTIBACTERIAL ACTIVITY

The bacterial strains used during the study *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* were obtained from the laboratory of the Institute of Biosciences and Technology at MGM University,Ch.Sambhajinagar,Maharashtra,India. The test strain was then inoculated on the nutrient agar plate and spread with a sterilized swab to obtain a lawn culture. By Using the slightly modified agar well diffusion and disk diffusion method [34].100 µl of synthesized AgNPs were placed in the wells made in the nutrient agar and potato dextrose agar plates. After incubation for 14–16 hours at 37 °C, the diameter of the zone of inhibition was measured.

CHARACTERIZATION OF *C. papaya* LEAF SILVER NANOPARTICLES

UV-SPECTROSCOPY

UV-Vis spectroscopy measures the absorption of light by nanoparticles. Silver nanoparticles exhibit a characteristic absorption peak in the UV- Vis spectrum, known as the surface plasmon resonance (SPR) peak [36]. The SPR peak position and intensity provide information about the size and concentration of the nanoparticles. The absorbance at different wavelengths was recorded for the reaction solution of reduced silver nitrate by leaf extract of the maximum Carica papaya. Previously, the absorbance peaks were seen at 410 and 640nm for Carica papaya.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY

To characterize the surface morphology of the synthesized product, we employed field emission scanning electron microscopy (FESEM) analysis [35]. This technique utilizes a focused electron beam to scan the sample surface and generate detailed images of its topography and structure. In our study, we utilized a field emission-scanning electron microscope with an acceleration voltage of 20 kV. A higher acceleration voltage enhances the resolution and clarity of the obtained images, allowing us to capture fine details of the surface morphology.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

FT-IR spectra of the synthesized Ag NPs were performed at a wavelength of 100 nm. This analysis provided valuable information about the chemical composition and molecular structure of the silver nanoparticles.In the FT-IR analysis, a beam of infrared light was passed through the sample, and the resulting absorption spectrum was recorded. By analyzing the peaks and patterns in the spectrum, we could identify the functional groups present in the Ag NPs and thus gain insights into their surface chemistry and potential applications [35].

RESULTS & DISCUSSION

SYNTHESIS OF AgNPs

As reported earlier, a change in the reaction solution (dark brown color) confirmed the formation of AgNPs. indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag°. Figure shows the AgNO3 and AgNP solutions before and after synthesis, with the color changing from bright green to a stable dark brown, indicating a complete reduction of Ag+ into AgNPs as shown in following figures. (fig-1,2,3).

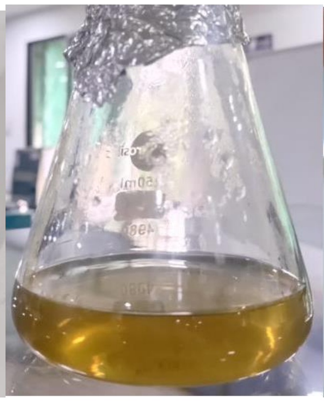
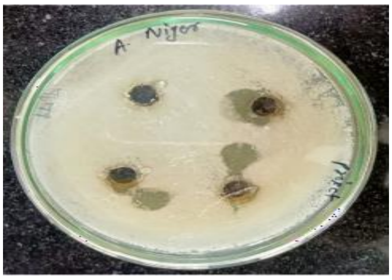




Fig-1 Papaya leaf sample Fig-2 Papaya leaf extract Fig-3 Synthesized AgNPs

ASSESSMENT OF ANTIBACTERIAL ACTIVITY

Antimicrobial activity of the *Carica papaya* leaf extract and synthesized nanoparticles was tested against different bacterial strains such as *Staphylococcus aureus and Escherichia coli,* as well as fungi such as *Aspergillus niger*, by agar well diffusion and disk diffusion methods. A mixture of *Carica papaya* leaf extract and silver nitrate, which is AgNPs, showed good activity. Out of the three organisms we utilized, *A.niger* displayed a larger clear zone area (2cm) than *S. aureus* (1cm) and *E. coli (1 cm).* This indicates that silver nanoparticles are acting more effectively against fungus as shown in following figures and table (Fig-3,4,5 and Table no -1).

fig-4 antifungal activity fig-5 antibacterial activity fig-6 antibacterial activity using *A.niger*



using *E.coli*

using *S.aureus*

Sample	***A.niger***	***E.coli***	***S.aureus***
AgNPs sample of *Papaya*	1cm	2cm	1cm

Table no-1 The diameter of zone inhibition

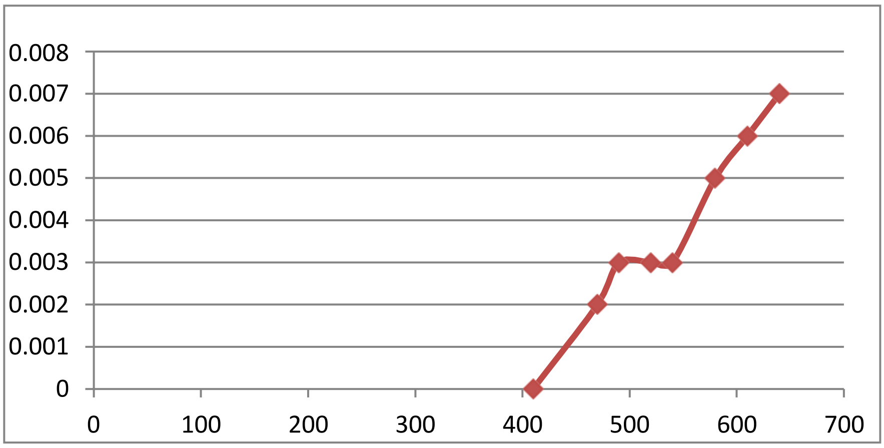
CHARACTERIZATION OF *C. papaya* LEAF SILVER NANOPARTICLES UV-SPECTROSCOPY

AgNPs exhibit a high surface plasmon resonance in an aqueous solution and emit light between 400 and 500 nm, depending on their size, shape, and morphology. In the case of your silver nanoparticles, it has been observed that the absorbance is gradually increasing. (Table no-2; Graph-1)

Wavelengths	**Absorbance**
410	0.000
470	0.002
490	0.003
520	0.003
540	0.003
580	0.005
610	0.006
640	0.006

Table no-2 Peak values of Agnps

silver nanoparticles exhibit a progressive rise in absorbance with higher wavelengths, as demonstrated above.



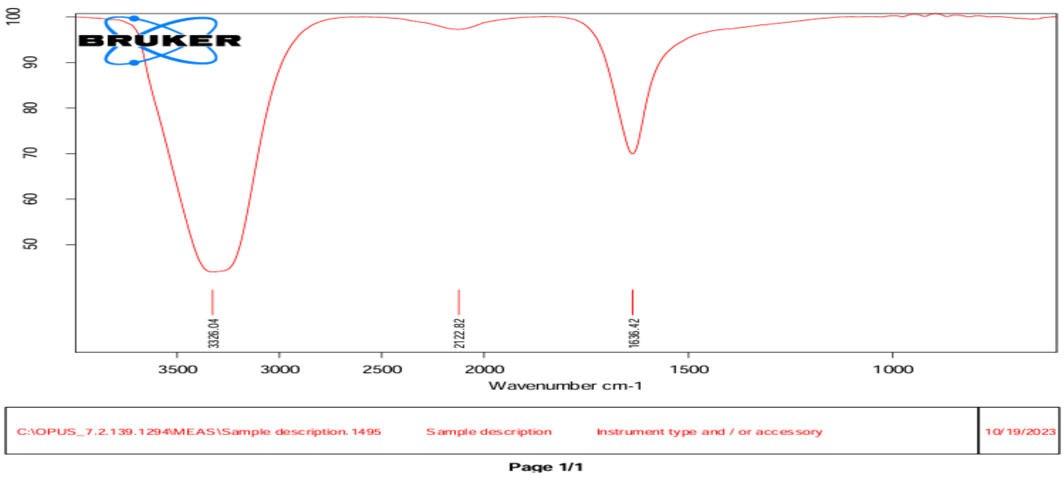
Graph-1 Optimal Density (OD) of AgNPs From 410nm-620nm.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

FTIR analysis elucidates the functional groups within the synthesized silver nanoparticles, offering insights into their evolution from basic inorganic AgNO3 to elemental silver through the coordinated action of various phytochemicals. These compounds serve multifunctional roles, acting as both reducing and stabilizing agents, while also capping the nanoparticles. The synthesis of silver nanoparticles (Ag NPs) from Carica papaya resulted in distinct peaks at 3326.04, 2122.82, and 1636.42 cm-1, corresponding to N-H, N=C=N, and C=C stretching, respectively. These peaks indicate the presence of aliphatic primary amine, carbodiimide, and alkene functional groups, each exhibiting medium, strong, and medium intensity absorption, respectively. These characteristic peaks reflect the unique frequencies required for absorption by each bond and functional group, providing a clear signature for their identification. (Table no-3, Graph-2).

Range	Appearance	Group	Compound
3326.04	Medium	N-H stretching	Aliphatic primary amine
2122.82	Strong	N=C=N stretching	Carbodiimide
1636.42	medium	C=C stretching	Alkene

Table:3 Functional Groups Present in AgNPs

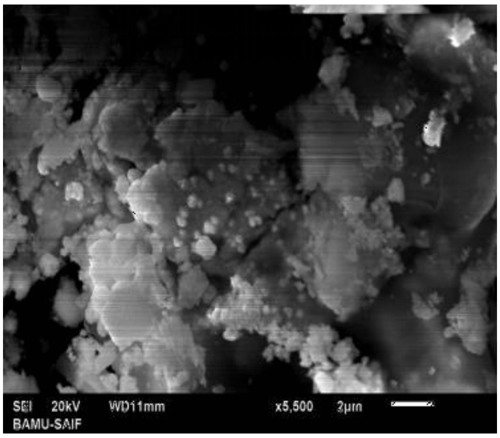


Graph:2 FTIR Analysis of AgNPs

FIELD EMISSION SCANNING ELECTRON MICROSCOPY

FESEM provided further insight into the morphology and size details of the silver nanoparticles. The scanning electron microscope works on the same principle as an optical microscope, but it measures the electrons scattered from the sample rather than the protons. Figs. 8 and 9 showed that the diameters of the prepared nanoparticles in the solution have sizes of several µm. The size of the prepared nanoparticles was greater than the size of the nano dimensions, which should be between 50 and 500 nm. The size was greater than the desired size as a result of the proteins, which were bound to the surface of the nanoparticles.

In the present study, we collected *carica papaya leaves* for a green synthesis method. The leaves were diced into fine pieces and transferred into sterile distilled water. It's color change to brown indicates successful synthesis of silver nanoparticles, similar to Rafique M.et al.(2017) synthesized silver nanoparticles from Carica papaya leaves. After that, the antimicrobial activity of silver nanoparticles was tested against *E. coli*. *S.aureus*, *A. niger*, and *S. aurus* showed larger clear zones, indicating the action of AgNPs against them, whereas Padalia H et al. (22014) showed *A. niger* fungi showing a larger clear zone. After that, to study characterization, U.V. spectroscopy, FESEM, and FTIR were performed, whereas Mathur P et al. (2017) performed TEM and FTIR. According to our study, our nanoparticles range between 50 and 500 nm, and their range is 100 nm. After a successful study of silver nanoparticles, our study continued to study their applications in water purification and crop yielding, as mentioned by Rafique et al. (2017). In water purification, we are able to purify the segregated water. Whereas we are able to produce nanofertilizer, which is a good source of nitrogen for the healthy growth of plants, as mentioned in the review article by Rafique et al. (2017).



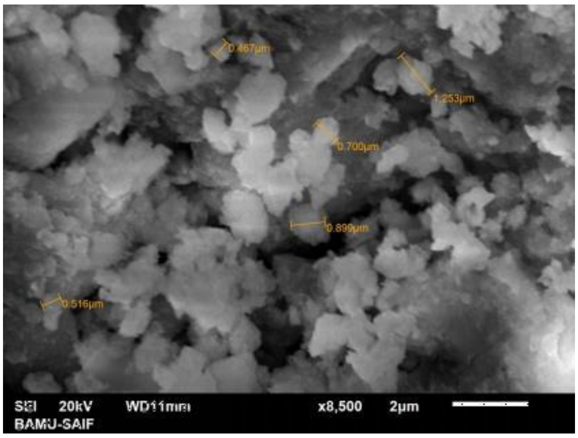


Fig -8 Structure of silver nanoparticle Fig -9 Image of silver Nanoparticles

CONCLUSION

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. In the present study, we have demonstrated that use of a natural, low-cost biological reducing agent and *C. Papaya* leaves extracts can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of toxic solvents and waste. The biosynthesized silver nanoparticles using leaves extract proved *C. Papaya* to be excellent against microorganisms. The present study showed a simple, rapid, and economical route to synthesize silver nanoparticles

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