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ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED BY ALOVERA GEL EXTRACT AGAINST RHIZOPUS STOLONIFER

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Abstract

Silver nanoparticles have gained popularity in the world. The aim of this study is formation of AgNPs by aloe Vera gel and to check out the antifungal activity of AgNPs against Rhizopus sp. Aloe Vera is reducing and stabilizing agent that have 75 components which include sugars, minerals, vitamins, saponins, lignin, salicylic, enzymes and amino acids. For the characterization of AgNPs three different techniques were applied firstly Fourier Transform Infrared, secondly UV-Visible spectroscopy and finally X-Ray diffraction. The changing in colour from colourless to reddish brown indicated the formation of AgNPs at room temperature within 48-72hr. UV-Visible absorption spectrum shows maximum peak of AgNPs at 400nm approximately. The functional group and possibility of protein-silver nanoparticle interaction of biosynthetic silver nanoparticles were examined and determined with the help of the FT-IR spectra. FT-IR of AgNPs, Spectra exhibited transmission peaks at 3,270, 3690, 1,592 and 1,471 and 1,805 cm1 because of FT-IR analysis. The X-Ray Diffraction was used to determine the crystallization of AgNPs. SEM shows the size and shape of silver nanoparticles. Scan Electron microscopy shows that at room temperature, the shape of AgNPs is spherical and its size is 2um. AgNPs has antimicrobial effects. AgNPs show maximum results against Rhizopus sp. Rhizopus is saprophytic fungus that grows on plants and parasitic for animals. AgNPs inhabit the growth of Rhizopus.

INTRODUCTION

For hundreds of years, the Aloe Vera plant and its extract has been used for many purposes including health care, beauty agent and skin protector due to its therapeutic, beauty and skin care benefits. It has long been a component of Egyptian Queens Nefertiti and Columbus' routine beauty regimen (1). Aloe Vera was used to cure wounds. Due to its nature, the aloe Vera mostly grows in dry regions of different

parts of world including Asia Europe, Africa and America. It has trigonal chubby leaves with disperse corners (1). Aloe Vera has 75 different components that are sugars, minerals, vitamins, saponins, lignin, salicylic, enzymes and amino acids. It acts as antioxidant because it has different kinds of vitamins, folic acid and choline. It has anti-inflammatory properties due to enzymes that are present in it such as amylase, phosphatase, lipase catalase and many more (1). Hormones, which are present in it, are Auxins and Gibberellins that play role in wound healing process and anti-inflammatory actions. Aloe Vera acts as Analgesic, Antibacterial and antivirus because it has almost 12 anthraquinones also Called Laxatives. It has different amino acids that act as anti-inflammatory, antibacterial, cleansing and antiseptic properties. When applied directly to skin, aloe Vera may be used to treat a variety of skin diseases, including wounds and eczema (2).

Nanoparticle applications have demonstrated to be effective in the treatment of endodontic infections and oral cancers. AgNPs became the main focus of intensive research because of their wide selection of applications in areas like catalyst, optics, antimicrobials, and biomaterial production (3-7). Biomaterials containing AgNPs have been produced to either prevent or reduce the formation of biofilms in the environment. Silver nanoparticles (AgNPs) have been employed in medicine and dentistry for a long time because of their antibacterial and antifungal characteristics (8-10). As a result of their greater surface-to-volume ratio and small particle size, which do not degrade the mechanical properties of the material, they have shown enhanced antibacterial and antifungal activity (8, 11, 12).

Fungus is small organism that is present everywhere in water, air, surfaces, soil and dead plants and animals. Many species of fungus exists that are harmful or causes diseases in humans, animals and plants (13). Rhizopus stolonifer are the members of phylum Zygomycota that have filamentous growth habitat. Their threads like objects are coenocyte (13). It is also known as black mold fungus. Fungi cause many infections in plants, animals and humans. In humans fungus causes skin infection, athlete's foot, ring worm, Aspergillosis and so many other infections (13). Almost 8000 different types of fungus cause infections in plants. Rhizopus specie also causes leak disease in strawberries and tomatoes, in a similar way. Rhizopus species are also responsible for soft rot and ring rot in sweet potatoes, as well as fruit rot in papayas (13). In this research, silver nanoparticles were prepared by aloe Vera extract and antifungal activity of silver nanoparticles against Rhizopus stolonifer was assessed for its affectivity.

Material and methods

Preparation of Plant Extract

Aloe Vera leaves were collected from University Garden and washed by pure water. After cutting leaves into small pieces, leaves were mixed with 100ml of distilled water then this solution was heated for 10-12 minutes. By Whitman filter paper this extract was filtered, collected and stored in refrigerator. The preparation is shown in figure 1.

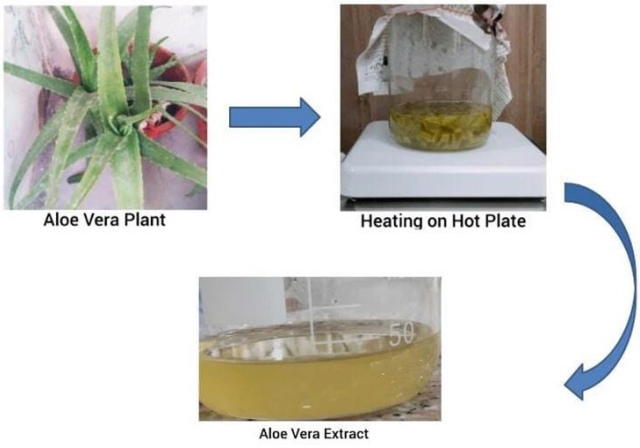

Figure 1: Formation of Aloe Vera Extract

Preparation of silver nitrate solution and silver nanoparticles:

About 0.69 mg of silver nitrate was dissolved in 100ml distilled water for making 10mM solution. The 10mM silver nitrate and extract of aloe Vera was used for the formation of silver nanoparticles. Solution of aloe Vera and silver nitrate after mixing kept at room temperature. After 48h colour of solution was changed from colourless to brown that indicates formation of silver nanoparticles.

Agar preparation

The 7.8g of PDA agar (potato dextrose agar) was mixed with 200ml distilled water. The conical flask was cotton-wrapped and autoclaved for 15 minutes at 14.5 1b/inch2 pressure and 120 °C. After chilling the broth medium, fungi (Rhizopus sp.) were inoculated with a needle from a pure culture media into the broth medium and kept at 28 Celsius.

Medium preparation and antifungal activity test

For antifungal action, 1 mL silver nanoparticles and fungus were combined in 20 mL distilled water. After that, the agar medium was put into sterile petri plates. After solidification, the culture was placed on each dish using a spreader. The leaf extract was then poured separately and then with the salt solution. The culture media was then kept at 28°C for 48 hours to allow for additional investigation.

Plate Count Method

The fungus was cultivated on potato dextrose agar plate at room temperature. The medium was autoclaved after 19 g of agar was dissolved in 500 mL of distilled water. A total of 250 microliters of manufactured Ag-NPs were applied to a sterile petri plate, followed by pure Aloe Vera extract. Gentle mixing and pouring of sterilised medium after that fungal disc with a diameter of 6 mm were put aseptically into plate's centre. After 7 days of growth from cultures of the fungus described above, we assessed the number of colonies in each plate by CFU/ml method that reveals the influence of silver nanoparticles using plate count technique (14).

Characterization of silver nanoparticles

The confirmation of formation of AgNPs by using green synthesis Aloe Vera leaf extract was obtained visually by examining the colour change and spectrophotometrically by identification of absorption peak using the UV-Vis spectrophotometer.

FT-IR study and XRD Analysis

To investigate the functional components that may be involved in the production of nanoparticle, the FT-IR analysis was performed on SHIMADZU. The leftover solution containing the silver nanoparticles was centrifuged at 4800 rpm for 30 minutes before being dispersed in 15 mL sterile distilled water. Before being dried at 400°C, procedure of re-dispersing and centrifuging was performed three times. XRD spectroscopy technique was used for the shape and nature of silver nanoparticles (15).

Scanning Electron Microscopy

AgNPs were dried and sent for scan electronic microscopy (SEM). An electronic simulation was used to perform the SEM (Hitachi, S-530). A thin image of the sample was made by placing a small portion of the sample on a carbon coated copper foil, wiping the excess solution with filter paper, and allowing the image to be on SEM and dry grid for 5 minutes under mercury lamp (16).

Results

Silver nanoparticles preparation confirmation

When silver nitrate solution that was colorless was mixed with Aloe Vera extract, the color of solution was changed due to the surface Plasmon resonance and monitored by using a UV-visible spectrophotometer. With increasing the exposure time from 10 min to 10 h, the colourless AgNO3 solution was converted to yellowish golden and subsequently to reddish brown. An increase in the response time, such as 24 and 48 hours, resulted in a slight increase in colour, indicating that reaction is completed.

UV-Visible Spectral Analysis

Periodic sampling (0.5 ml) of the solution of silver nitrate and Aloe Vera extract was examined on Bio-Base BK-D560 UV-Visible spectrophotometer. UV-Visible spectroscopic studies of silver nanoparticles were recorded. From 190 to 1100 nm, the absorption spectrum displayed depending on

wavelength range. The presence of silver nanoparticles was confirmed by a maximum absorbance at 450 nm wavelength as seen in figure 2.

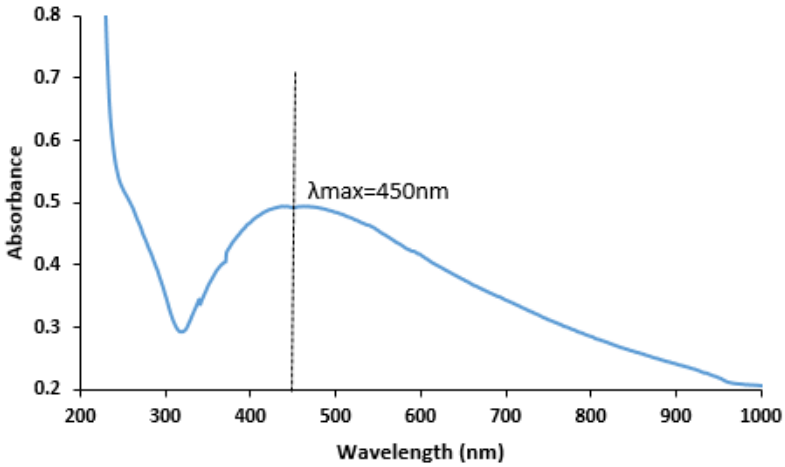

Figure 2: UV-visible Analysis of AgNPs

Scanning Electron Microscopy

The shape and size of nanoparticles were determined using scanning electron microscopy (SEM). Figure 3 shows the high AgNPs produced by Aloe Vera plants, confirming the presence of AgNPs. The interactions between biological nanoparticles, such as hydrogen bonds and electrical connections, are the reason for the formation of silver nanoparticles using plants. Figure 3 shows that silver nanoparticles have a uniform distribution and are arranged, rectangular, triangular, and spherical in shape. However, the aggregation of particles seen in most cases seems to be due to the presence of a weak adhesive that reinforces the nanoparticles. Agglomerated nanoparticles were measured to be 20nm.

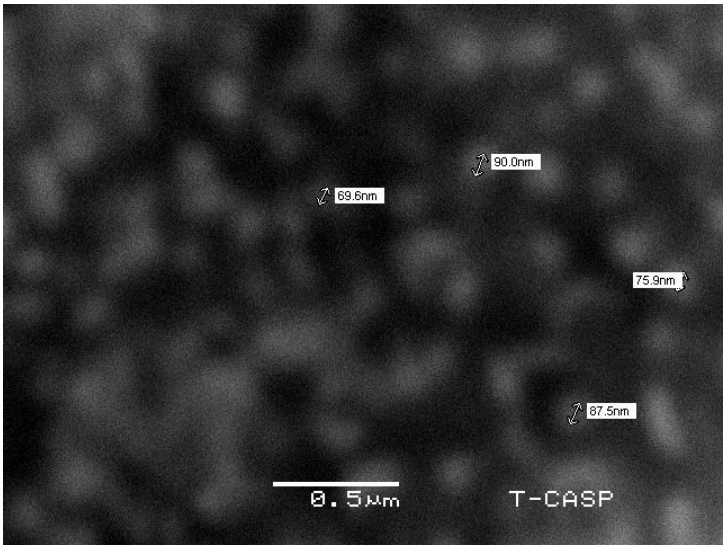

Figure 3: Scanning Electron Microscopy Analysis of synthesized AgNPs

FTIR-Analysis

Results of FT-IR analysis is shown in Figure 4 sharp peaks of AgNPs formed at 648,960, 1060, 1116,1632, 2008, 2972, and 3287 cm−1. Primary amines are shown at 1632cm-1 peak and 3287cm-1 peak showed O-H, nitriles group (-C=N) in AgNPs shown at peak of 1598cm-1. Found that transmission peaks were seen at 3,355, 1,636 and 1,507 cm while performing the FT-IR spectra of AgNPs. While the main amines (3,355 cm1 peak) to O-H in AgNPs are represented by the 1,636 cm1 peak, nitrile groups (-C = N) are represented by the 1,507 cm1 peak. The 1,507 cm1 peak correlates to the involvement of nitrile (-C = N) groups in AgNPs is represented by the 1,636 cm1 peak. In addition, the components of carbonyl proteins have a high affinity for metallic ions, which ensures that they interact with biosynthesized nanoparticles while also guaranteeing that their secondary structures are not altered during the reaction with Ag + ions. This is indicated in table 1.

Table 1: **T**he result of FT-IR analysis of AgNPs

Frequency (cm-1)	**Chemical bond**	**Phytoconstituent Present**
3500-3200	O-H stretch	Alcohols and Phenols
3300-2500	O-H stretch	Carboxylic acid
3000-2850	C-H stretch	Alkanes
1600-1585	C-C stretch (in-ring)	Aromatics
1500-1400	N-O stretch	Nitro group
1390-1350	C-H rock	Alkanes
1360-1290	N-O symmetric stretch	Nitro Compounds
1320-1000	C-O stretch	Esters, ethers
1250-1020	C-N stretch	Aliphatic amines
910-665	N-H stretch	1,2 amines
900-675	C-H oop	Aromatics
690-400	C-Br stretch	Alkyl halides

The FT-IR analysis of silver nanoparticles is shown in figure 4.

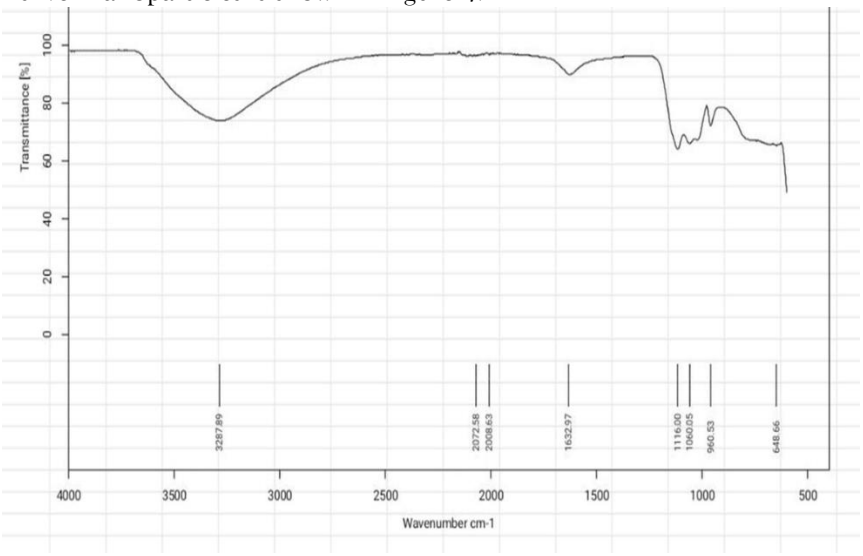

Figure 4: FT-IR analysis of AgNPs

XRD-Analysis

Using a Bruker d8 Advance X-ray diffract meter with CuK radiation (= 1.5406), 40 kV- 40mA, 2/ scanning mode, the X-ray diffraction (XRD) pattern of the sample was prepared of silver nanoparticles were sealed. Data were collected for 2 lines of 30 to 70 degrees, with 0.0202-degree levels. Because of the silver metal, four peaks at 2 values of 38.2901, 44.5583, 64.8185, and 77.4383 degrees are confirmed, corresponding to (111), (200), and (210), (220). The XRD analysis confirmed that the assembled samples consisted of silver nanoparticles with a shaped cubic crystal structure. Exhibited peak indicated the Nano crystalline shaped silver particles. This is shown in figure 5.

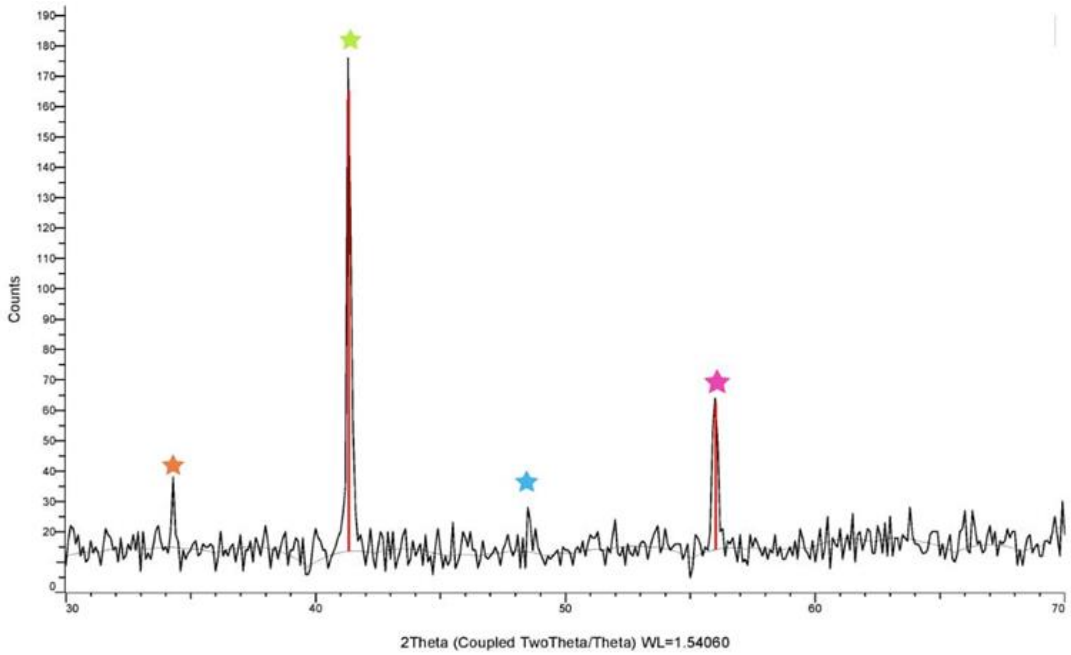

Figure 5: XRD analysis of AgNPs

Antifungal Activity

Many diseases can be treated by Aloe Vera. As technological advances have made their production more affordable, Nano-sized AgNPs have become increasingly popular as antibacterial agents. Silver has many potential uses, including the treatment of plant diseases. Silver can be used to control many plant infections in a safer way than computerized fungicides because there are many ways to prevent plant infections. Nano silver's chemical properties have a greater effect on ion exchange, and as a result, Nano silver has a more potent chemical reaction.

Here antifungal activity of silver Nano particles by Aloe Vera against Rhizopus sp. has been done. Rhizopus is saprophytic specie can be grow on dead materials, breads and different fruits. Sample of AgNPs and fungus colony mixed in 20ml distilled water, serial dilution of this solution is done as shown in figure 6. Synthetic AgNPs made from Aloe Vera leaf extract have antifungal efficacy against Rhizopus sp. Antifungal activity was determined by counting the number of colonies on each plate. AgNPs' antifungal activity is dependent on the kind of fungus and their size. The number of colonies on each plate was also counted as a control. As seen in figure 6, there are more colonies on plates that are controlled and that are treated by aloe Vera than the plates that are treated by AgNPs. Although the mechanism of AgNPs' fungicidal impact is unclear, it has been proposed that AgNPs impede budding by creating holes in the fungal cell membrane, which may result in cell death.

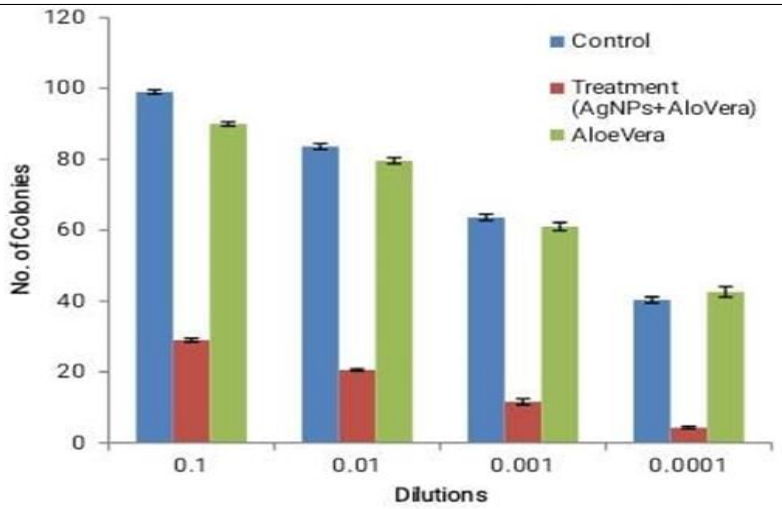

Figure 6: Graph shows the antifungal activity of Rhizopus against AgNPs and Aloe Vera

The figure 6 was plotted between the dilutions and number of colonies of fungus that are found on each plate. Four dilutions were prepared for each group (control, treated with AgNPs+ aloe Vera and with aloe Vera just). In this graph blue bar shows the number of colonies that are in control group without any antifungal agent. Red bar shows the number of colonies that were treated with AgNPs and green bar shows the number of colonies that were treated with just aloe Vera. Number of Colonies in each group was decreased by decreasing the dilutions. Hence results shows that AgNPs shows high antifungal activity against Rhizopus.

Discussion

Silver nanoparticles have become more popular across the globe (8). The goal of this research is to see whether aloe Vera extract may produce AgNPs and if they have antifungal action against Rhizopus sp. Sugars, minerals, vitamins, saponins, lignin, salicylic, enzymes, and amino acids are among the 75 components of Aloe Vera, which act as a reducing and stabilizing agent (1).

Three separate approaches were used to characterise AgNPs: first, Fourier Transform Infrared, then UV-Visible spectroscopy, and lastly X-Ray diffraction. Within 72 hours, the colour changed from colourless to reddish brown, indicating the production of AgNPs at ambient temperature. The greatest peak of AgNPs in the UV-visible absorption spectrum is about 400nm. FT-IR spectra were used to evaluate and identify the functional group and potential of protein-silver nanoparticle interaction in biosynthetic silver nanoparticles. FT-IR investigation of AgNPs revealed transmission peaks at 3,270, 3690, 1,592, and 1,471 and 1,805 cm1, respectively.

The crystallisation of AgNPs was determined using X-Ray Diffraction. SEM images of silver nanoparticles illustrate their size and form. Scan AgNPs are spherical and 2um in size at room temperature, according to electron microscopy. Antimicrobial properties of AgNPs had the best anti-Rhizopus sp. results. Rhizopus is a parasitic fungus that grows on plants and is saprophytic. Rhizopus grows in the presence of AgNPs. Logaranjan, Raiza (17).reported that at 420 nm,plasmon peak of UV-Visible absorption spectra of AgNPs was formed.

Medda, Hajra (18) showed that within 72 hours of incubation at room temperature, the colour of the embedded AgNPs changes to reddish brown, and the detection of UV-Visible exposure of AgNPs is recorded. The Plasmon resonance on the obtained silver nanoparticles showed greater applicability at 400 nm in the spectra of AgNPs. SPR's interest in the incorporation of silver nanoparticles resulted in colour change. Similar results were found in my work, a UV-Visible absorption spectrum shows maximum peak of AgNPs at 350-400nm approximately. The changing in colour from colourless to reddish brown indicated the formation of AgNPs at room temperature within 48hr.

Medda, Hajra (18) found that transmission peak were seen at 3,355, 1,636 and 1,507 cm while performing the FT-IR spectra of AgNPs. While the main amines (3,355 cm1 peak) to O-H in AgNPs are represented by the 1,636 cm1 peak, nitrile groups (-C = N) are represented by the 1,507 cm1 peak. The 1,507 cm1 peak correlates to the involvement of nitrile (-C = N) groups in AgNPs is represented by the 1,636 cm1 peak (18). In addition, the components of carbonyl proteins have a high affinity for metallic ions, which ensures that they interact with biosynthesized nanoparticles while also guaranteeing that their secondary structures are not altered during the reaction with Ag + ions (18). Kamala Nalini and Vijayaraghavan (19).reported XRD study of AgNPs obtained by bio reduction and aloe Vera stabilization revealed a Bragg reflection peak at (111), (200), (220), and (311), confirming the face-cantered cubic lattice structure of the nanoparticles. Same results were found in my research in XRD analysis peaks at 111, 200, 220 are found.

Alwhibi, Soliman (20).discovered that the AgNPs generated had substantial antifungal effect against bipolar heterothallic fungi. AgNPs were also shown to have antifungal action against Fusarium oxysporum among other pathogens. In B. heterothallic, the AgNPs generated suppressed the majority of fungal growth, which was followed by F. oxysporum. Alwhibi, Soliman (20).also found that Aloe. vera extract was used to cure the fungal growth. The antifungal activity of the AgNPs generated was substantial against all of the fungus strains tested (20).

Conclusion

According to the findings of the present research, an aqueous extract of Aloe Vera gel was discovered to be a good option for the synthesis of Nano-sized silver particles in the laboratory. The stimulation of surface Plasmon resonance resulted in a change in the hue of the synthetic medium. That conclusion was supported by optical and spectral studies between 350 and 400 nm. After being synthesized, it was discovered that the silver nanoparticles were spherical in form and had an FCC structure, which demonstrated inhibitory activity against Rhizopus. The number of colonies on plates that have been treated with AgNPs is reduced.

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Author's contribution

All authors contributed equally in the manuscript

Conflict of interest

None

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