Green Synthesis of Silver Nanoparticles Mediated by Banana Peel Extract: A Promising Approach for Antimicrobial Biofilm Applications

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Abstract- This is the era of nanotechnology as it has such a big role in almost every field of life these days. In recent times we are facing many food safety and food security problems, especially in backward/underdeveloped countries. Most of the food goes to waste because of no proper handling and their shorter shelf life. In this study, silver nanoparticles are produced with the combination of banana peel extract employing the green synthesis method. The green synthesis method was opted to minimize the risk of toxicity in humans and the ecosystem as well. It also enhances the antibacterial activity as well as antifungal activity of silver nanoparticles. Their characterization was also done with the technique of UV-visible spectroscopy to check their presence in the solution. In the application of this study, a sheet of silver nanoparticles was made with the combination of chitosan and wrapped around the broiler chicken meat to prolong its shelf life and retain its taste and texture.

Index Terms- Nanoparticles; Biofilm; Antibacterial; Banana; Silver

I. INTRODUCTION

Nanoparticles have a very crucial value in recent times. They are being used in so many technologies to achieve desired goals. Major work is being done in so many different industries like data innovation, country security, medication, transportation, energy, food handling, and natural science, among numerous others. There are two approaches to achieve the synthesis of nanoparticles. These approaches are known as the top-down approach and bottom-up approach. The top-down approach is to convert bigger particles into smaller ones. It includes the mechanical strategies to crush/break bulk into smaller parts to create nanoparticles. The bottom-up approach is to convert smaller particles into bigger ones. It includes chemical reactions between ion/atom/molecules to create nanoparticles (1).

In both these approaches, there are mainly three methods (physical synthesis, chemical synthesis, green synthesis) that can be used to achieve our desired nanoparticles. In the physical method, we use different mechanical ways to create nanoparticles. While in chemical methods, we use only chemical ways to get our desired nanoparticles (2). Techniques are usually simple and inexpensive instrumentation is required in these methods. The third method is the green synthesis of nanoparticles. This technique is based on organic ways like plants and animals to get our desired nanoparticles (3)

Reported toxicity is a thing that cannot be ignored. This reported toxicity issue is associated with two of the three main methods i.e., physical method and chemical method, so it is safe for us to go with the third method that is the biological method. This biological method is way more reliable than the other two methods because no harmful chemicals and mechanical ways are used. This method is ecofriendly and organic in nature because different parts of plants or different organisms are being used in this method. Green synthesis is the subgroup of the green synthesis method; in this, we use only plant materials for the process of making nanoparticles (4).

We can use different metallic properties in nanoparticles as per our need. Just like in this study, nanoparticles with silver properties were needed so that it will inhibit the growth of different bacterial and fungal strains and it should not be harmful in any way to the human or ecosystem, so the green synthesis method was used to counter this part (5). Desired properties in nanoparticles were enhanced by the help of green synthesis in which specific parts of plants and fruits were used to combine with metallic properties and use them for our benefit. In this study, silver nanoparticles were used with the combination of banana peel extract to enhance its bacterial inhibition (6).

Nanoparticles production is not enough, we have to do different characterization analysis to check its morphology, size, structure, and chemical nature. Based on these analysis, we can consider nanoparticles' effectiveness in the long run. UV-visible spectroscopy analysis is commonly used to check specific nanoparticles presence in the solution (7).

The application of nanoparticles is the crucial part in which different ways can be used for the benefit of humankind (8). As of recent times, a lot of food preservation issues can be seen all around, so a better way to go is towards food safety and food security. Nanoparticles with antibacterial activity, must be utilized in the food sector to prolong the shelf life of the food products while retaining their original taste and texture. This can be achieved by coating of nanoparticles or by wrapping nanoparticles sheet around the food product will eventually prevent food from bacteria and retain their original texture and taste as well.

II. METHODS

Collection of samples

Freshly ripened banana fruit was taken from the local market to the laboratory under normal conditions. After that they were washed with tap water and double distilled water to clean them from any unwanted or hazardous particles/impurities if present on the skin of the fruit.

Peel separation

Banana fruit was dried at room temperature after cleaning, and their peel was separated manually. The banana peel helps in the process of silver nanoparticles synthesis as a reduction agent.

Banana peel extract (BPE) preparation

Fifty ml double distilled water was taken in a beaker of 100ml, peel with the volume of twenty-five gram (in small pieces) was boiled at 80°C in the beaker for ten minutes, and then filtered twice with Whatman No.1 filter paper.

Synthesis of nanoparticles

Peel extract concentrations on the precursor solution were varied to improve the synthesis route for developing the AgNPs. The volume of hundred ml solution of 10 mM $AgNO_3$ was applied to the varying amounts ranging from 1–5 ml of banana peel extract solution (8).

Characterization of silver nanoparticles

UV visible spectroscopy

The nanoparticles are usually characterized by UV-visible spectroscopy, which is very effective among the other nanoparticles analysis techniques. A Shimadzu UV1650pc spectrophotometer was used to produce ultraviolet-visible spectra (9).

Antibacterial activity analysis

Bacterial cultures

Three bacterial cultures (Staphylococcus aureus, Salmonella, and Escherichia coli) were used in this study to perform the antibacterial analysis.

On nanoparticles solution

Disc diffusion method for nanoparticles sample was used on nutrient agar petri dishes along with swabbing method for bacterial culture spreading on petri plates. After this procedure, the Incubation period for these plates was applied for 20-24 hours to check their inhibition zone.

On nanoparticles film

Disc diffusion method for nanoparticles film sample was used on nutrient agar petri dishes along with swabbing method for bacterial culture spreading on petri plates. After this procedure, Incubation period for these plates was 20-24 hours to check their inhibition zone (10).

Antifungal activity analysis

Fungal culture

Fungal culture (Aspergillus niger) was used in this phase of the study to perform the antifungal activity analysis.

On nanoparticles film

Disc diffusion method for antifungal activity analysis was used on MEA (Malt Extract Agar) petri plates along with spreading method for fungal culture spreading on petri dishes. After this procedure, the plates were incubated for 72-96 hours to check the inhibition zone.

Preparation of nanoparticles film for food products

Two beakers were prepared with distilled water, gelatin (fish skin), glycerol and distilled water, acetic acid, chitosan, glycerol as beaker one and beaker two. The casting solution was prepared (shown in Table 1) by adding solutions from both the beakers and pouring them into five petri dishes having 1- 5ml of nanoparticles sample. After drying, a thin layer of film was separated from the petri dishes.

Table 1. List of reagents used for AgNPS film for coating

Solution A	100ml distilled water, 4g gelatin, 1ml glycerol
Solution B	99ml distilled water, 1ml acetic acid, 2g starch, 0.5ml Glycerol
Solution C	Casting solution (20ml solution A + 20ml solution B) with 1:1 + 5ml of AgNPs solution

Table 2. List of conditions required for different analyses.

Bacterial culture	Incubation period 24hours at 37°C
Fungal culture	Incubation period 72-96hours at 28°C
Shelf life analysis	Incubation period 24hours at 37°C

Analysis for prolonging product's shelf life

PCA (plate count agar) was prepared for plating along with 0.1% peptone water for dilutions to check CFU count on prepared petri plates with meat samples. Total 10 dilutions (5 dilutions for film-coated 1g meat sample, 5 dilutions for control 1g meat sample) were prepared with 0.1% peptone water to check the CFU value of the meat sample. 10 plates were used (5 plates for film-coated meat samples, 5 plates for control samples) each day to perform this analysis.

One gram of meat sample was used for CFU analyses in PCA (plate count agar) while a 10g of meat sample was used for pH analysis. After plating, meat samples were incubated at 37°C for 24 hours to count their colonies.

III. RESULTS

Green synthesis of silver nanoparticles

There are three main methods can be used for the synthesis of nanoparticles, under one of the two approaches (top-down approach, bottom-up approach). After deciding an approach, we usually go with one of the three methods i.e., physical method, chemical method, and biological method for nanoparticles production. The biological method is preferred based on its less or no toxicity reports, and it is organic as well that does not harm the environment and any other organism. Method of green synthesis is one of the most reliable and best methods to produce nanoparticles that are eco and human-friendly because only fruits and plant parts are allowed to use in it (4).

After collection of banana fruit from the market and peel separation, small pieces of the peel were heated at a specific temperature for some time to get its resultant and filtered twice. The resulting filter was held at a cold temperature of 4°C and used as a reduction and stabilization agent.

In the making of silver nanoparticles, silver ion reduction occurs at room temperature within 30 minutes. The change of color of the

solution to brownish-orange color was being detected, suggesting the development of silver nanoparticles (AgNPs) in the solution (8).

UV-visible spectroscopy analysis

Values of AgNPs were observed in AgNO3 solution with banana peel extract were under the range of 400nm to 500nm absorbance and gave us a clear graph of these values in the software. Our changed colored sample gave us the perfect curve in the UV-visible spectroscopy analysis (Figure 1a) with other sample curves (Figure 1 b-e), which proved that our desired silver nanoparticles are present in the solution (8).

Three different bacterial cultures (Escherichia coli, Salmonella, Staphylococcus aureus) were used in this analysis to check that if our nanoparticles were good enough against these bacteria or not. For this, some petri plates with nutrient agar were prepared and spread the bacterial culture on the nutrient agar with the ratio of 1ml revived bacteria into 99ml of sterile water.

After spreading bacterial culture, disc diffusion method was applied after dipping small discs into nanoparticle samples with control (11). After this, plates were wrapped with paraffin film, and incubated for 20-24 hours and checked the growth after the incubation period. Results were satisfactory (as shown in Figures 2a-f), with a clear inhibition zone around the discs. Width of the inhibition zone indicates the affectivity of anti-bacterial analyses. The wider the inhibition zone, grater the anti-bacterial activity.

Antibacterial activity on nanoparticles film

The same three bacterial cultures were used in this analysis to analyze the effectivity of nanoparticles film. For this, same a few petri plates with nutrient agar were prepared and swabbed the bacterial culture on the nutrient agar with the ratio of 1ml revived bacteria into 99ml of sterile water. Revived bacteria, even from last day cannot be used in this process, because it grows very fast and can contaminate or affect our results later on. Every time, fresh revived bacterial culture should be used in the procedure.

After that, disc diffusion method was applied with discs taken from nanoparticles film. Plates were wrapped with paraffin film and incubated for 20-24hours to check the inhibition zone or antibacterial activity around the discs. It also gave us significant results (as shown in Figures 3a-f) with a clear inhibition zone around the discs (12). Width of the inhibition zone indicates the affectivity of antimicrobial analyses. The wider the inhibition zone, grater the antimicrobial activity.

Antifungal activity on nanoparticles film

Fungal culture (Aspergillus niger) was used in this analysis to check the efficacy of nanoparticles film. For this, petri plates were prepared with MEA (Malt Extract Agar), and fungal culture was spread on MEA after making fungal suspension in a test tube.

After that, the disc diffusion method was applied with replicates, and the discs were used in this method were taken from nanoparticles film. Plates were then wrapped with paraffin film and incubated at a temperature of 28°C for about 72-96 hours (3-4 days) to check the inhibition zone or antifungal activity around the discs (Figure 4a-e).

Analysis for prolonging product's shelf life

Results for this analysis were very significant and clear that film-coated meat samples were very much safer against microbes and bacteria than the control samples that were not coated with anything. Results and graphs show that meat samples that were not coated with biofilm, were showing more bacterial growth and spoiled earlier than the film-coated samples. Film-coated samples remained useable when control meat samples crossed the maximum range.

The pH of control, and AgNPs film-coated samples was also observed during this analysis and it showeda slight change in pH during these 4-day analysis.

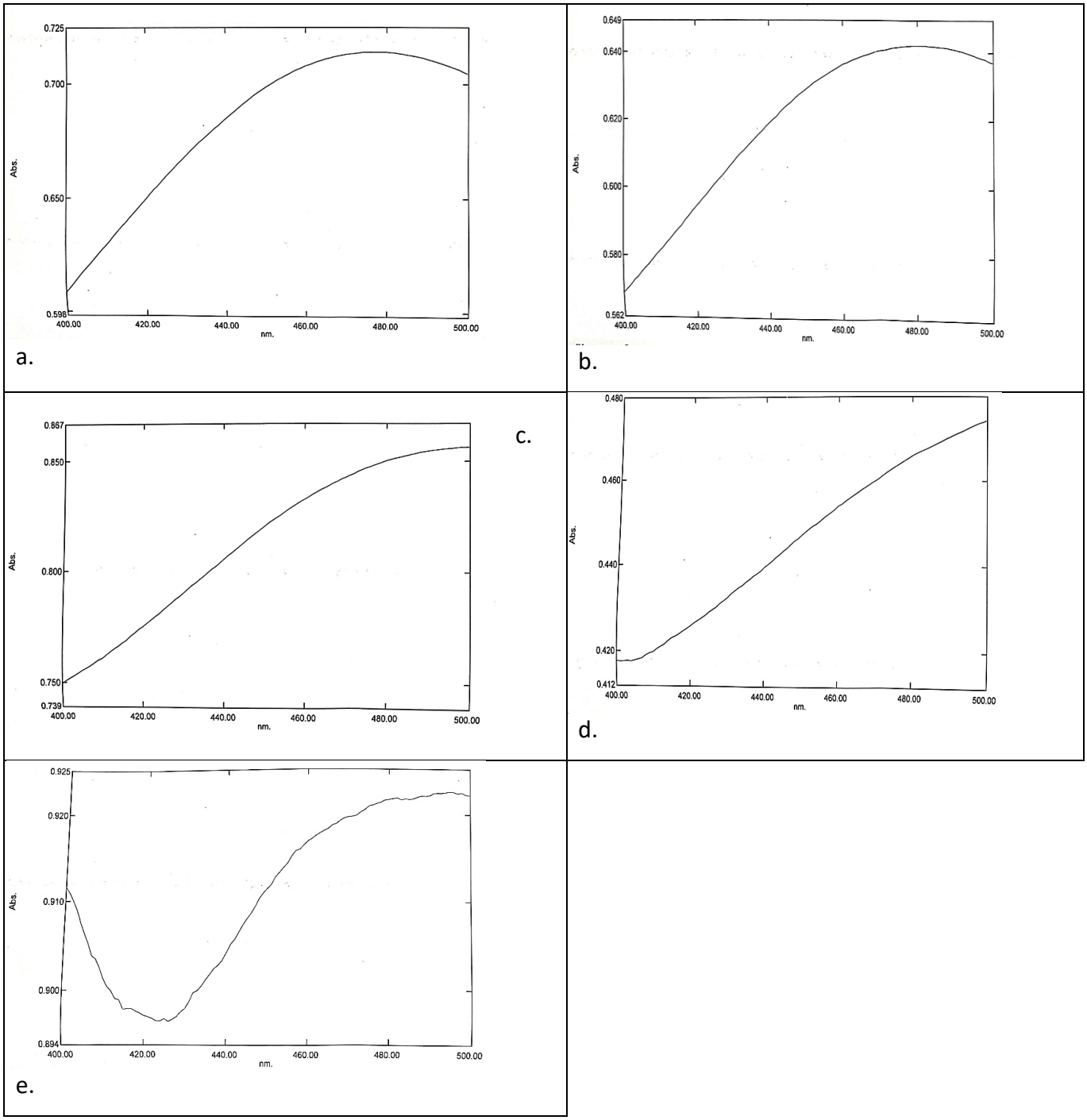

Figure 2 a) Peak at 478nm on UV-visible spectroscopy shows the presence of AgNPs in the solution of AgNO3 and 1ml of BPE. **b)** Peak at 481nm on UV-visible spectroscopy shows the presence of AgNPs in the solution of AgNO3 and 2ml of BPE. **c)** Peak at 498nm on UV-visible spectroscopy shows the presence of AgNPs in the solution of AgNO3 and 3ml of BPE. **d)** Peak at 502nm on UV-visible spectroscopy shows the presence of AgNPs in the solution of AgNO3 and 4ml of BPE. **e)** Peak at 495nm on UV-visible spectroscopy shows the presence of AgNPs in the solution of AgNO3 and 5ml of BPE.

Antibacterial activity on nanoparticles solution

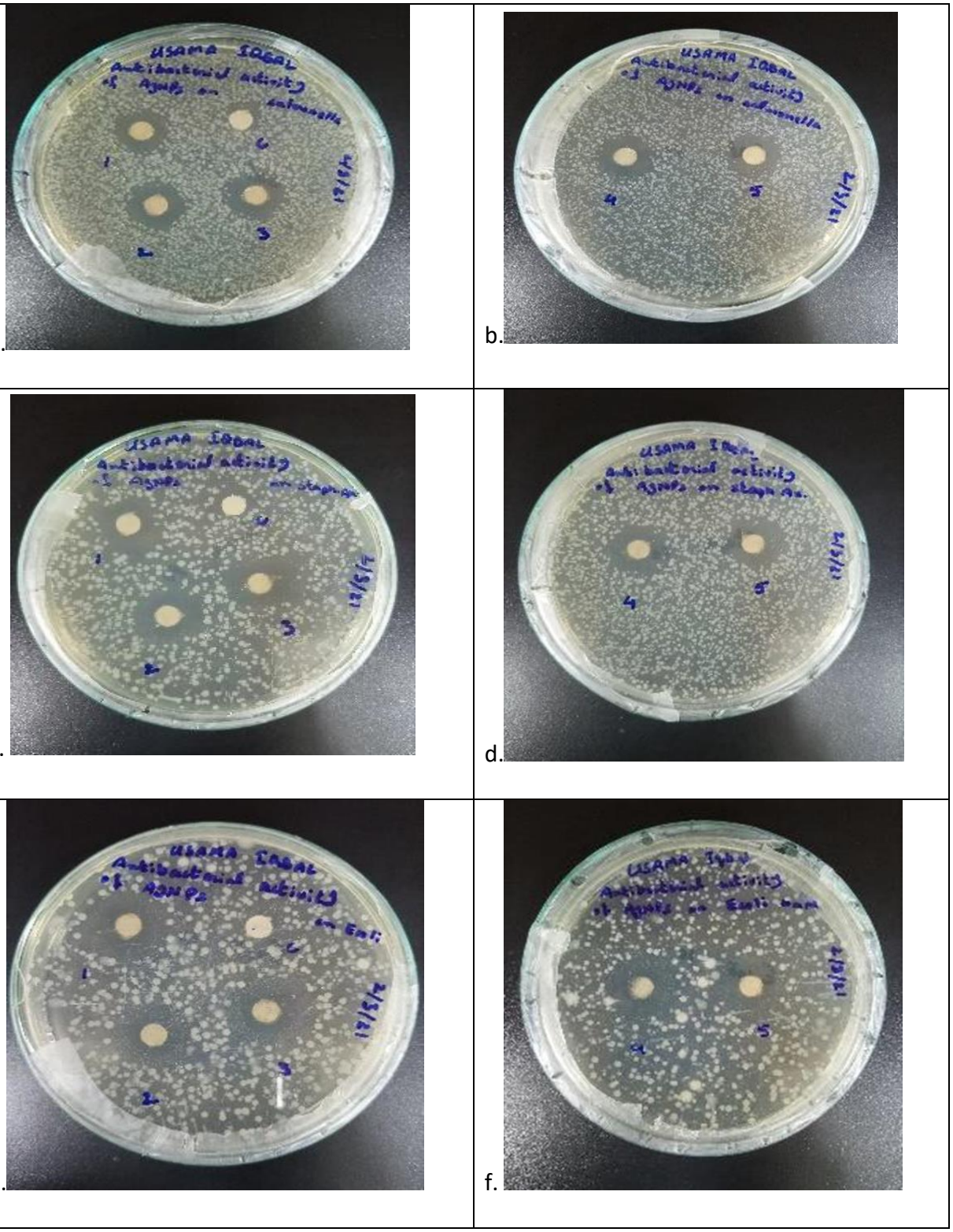

Figure 3 a) Clear inhibition zone around the discs shows antibacterial activity of different concentrations of AgNO3 solution against *Salmonella.* **b**) Clear inhibition zone around the discs shows antibacterial activity **c)** Antibacterial activity of different concentrations of AgNO3 solution against *Staph aureus.* **d)** Antibacterial activity of different concentrations of AgNO3 solution against *Staph aureus***. e)** Antibacterial activity of different concentrations of AgNO3 solution against E. coli. **F)** Antibacterial activity of different concentrations of AgNO3 solution against E. coli.

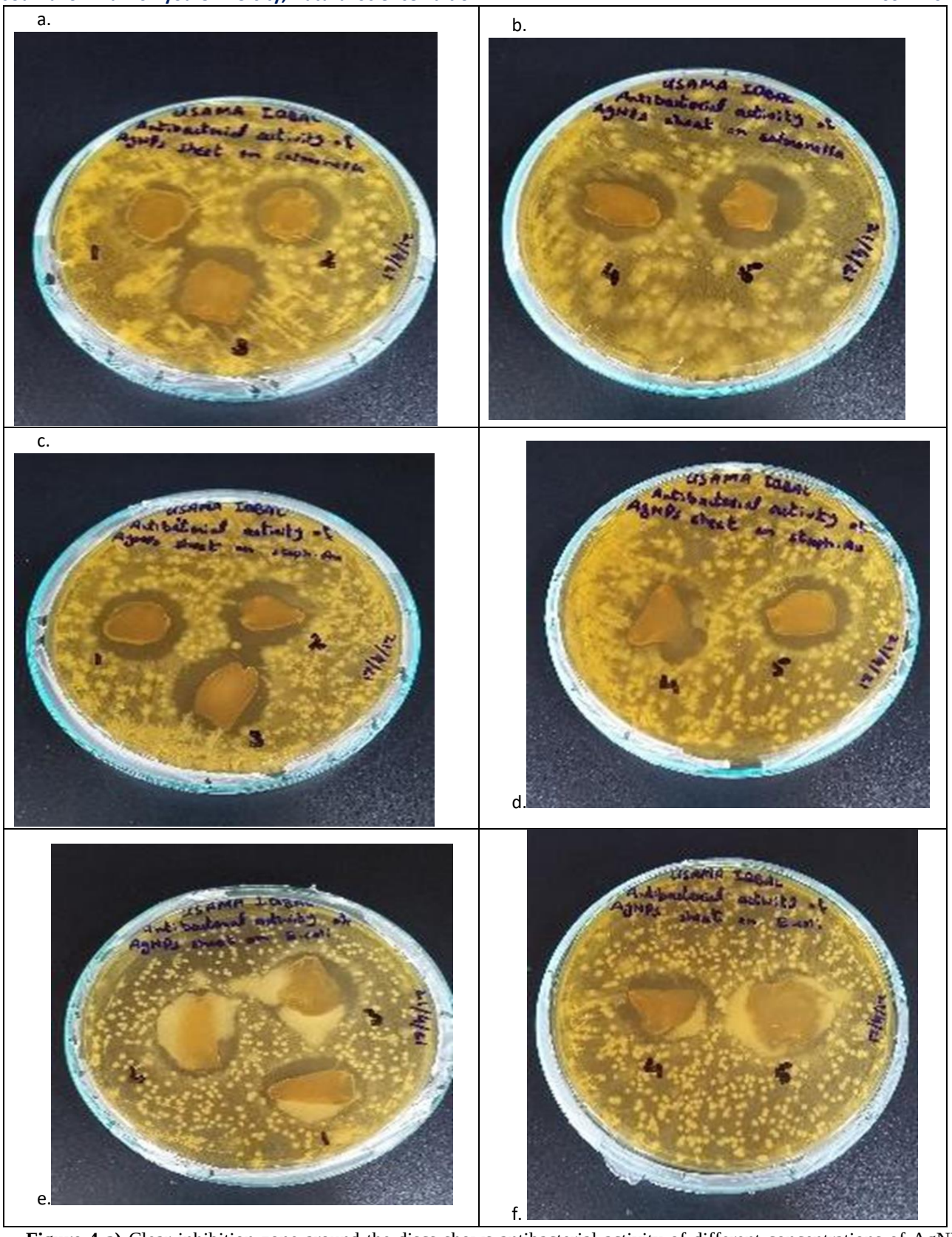

Figure 4 a) Clear inhibition zone around the discs shows antibacterial activity of different concentrations of AgNPs film against *Salmonella.* **b)** Clear inhibition zone around the discs shows antibacterial activity of different concentrations of AgNPs film against *Salmonella.* **c)** Antibacterial activity of different concentrations of AgNPs film against *Staph aureus.* **d)** Antibacterial activity of different concentrations of AgNPs film against *Staph aureus.* **e)** Antibacterial activity of different concentrations of AgNPs film against *E. coli.* **f)** Antibacterial activity of different concentrations of AgNPs film against *E. coli.*

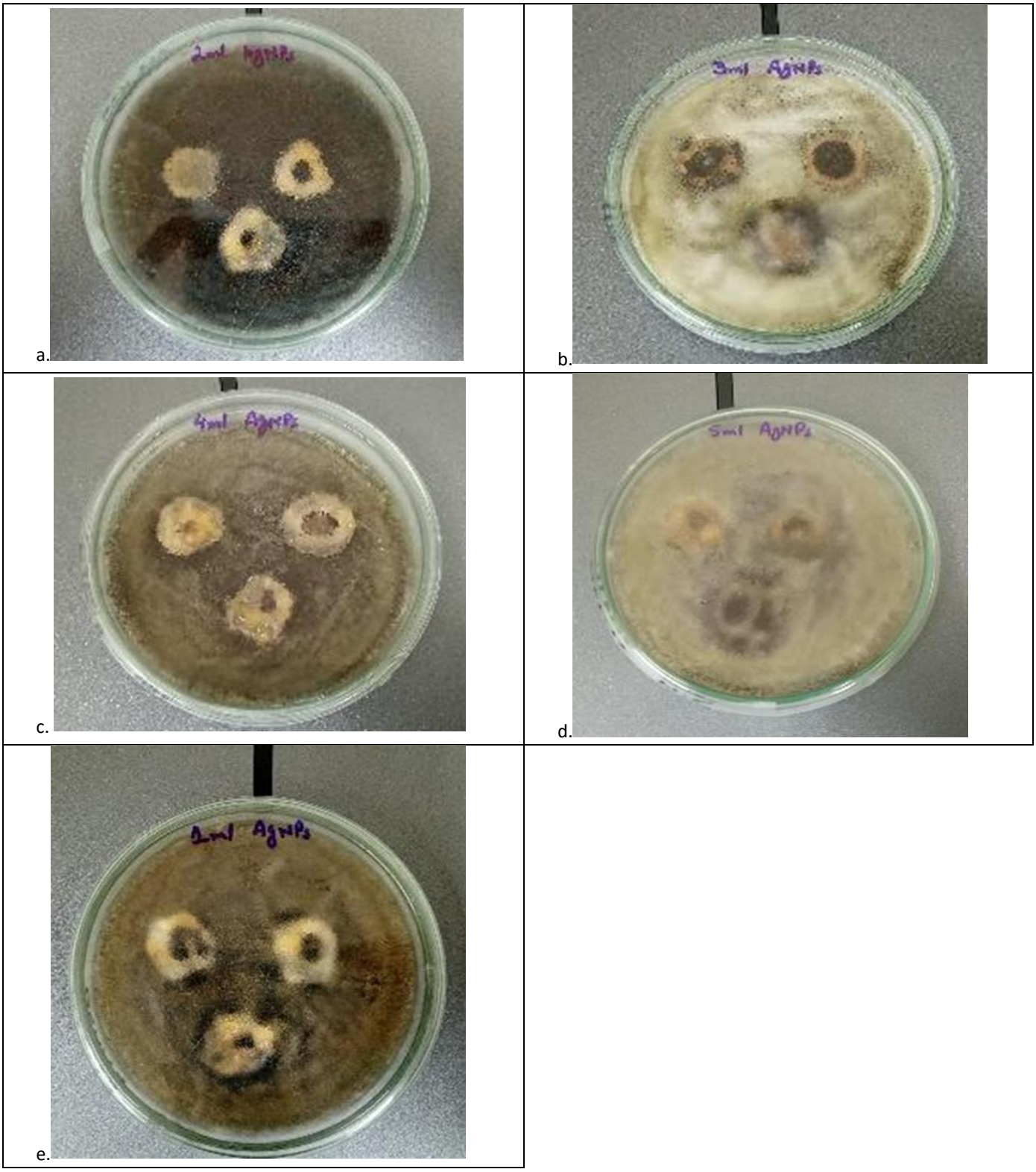

Figure 5 a-e) No inhibition zone around the discs of AgNPs film shows no activity of AgNPs against *Aspergillus niger*.

IV. DISCUSSION

Notably, AgNPs show earthy colored tone (brown) in water, that emerges because of the excitation of surface plasmon vibrations in the silver nanoparticles (8). The blended AgNPs were portrayed utilizing UV-visible spectroscopy and exposed to antimicrobial activity.

Many research articles report that AgNPs can be observed on UV-visible spectroscopy in the range of 400nm to 500nm. Other than that, values either the sample was not well prepared, or desired nanoparticles were not formed in the solution. These should be the reasons for obtaining any other values on UV-visible spectroscopy and graphs obtained by those values. These values could be the impurities (any molecule's absorbance other than desired ones) present in our solution (13).

Different metals show distinctive optical attributes because of surface plasmon- resonance (SPR) (7). Data of AgNO3 solution from this study showed the precise values of AgNPs while using UV-visible spectroscopy with graphs under the range of 400nm to 500nm as mentioned in different research articles that summed up as our AgNPs values and graph are true and silver nanoparticles are present in our solution. The formation of silver nanoparticles was confirmed by visualizing the change in color from transparent to reddish-brown color (8). The UV-visible spectroscopy showed peaks of absorbance at 478nm, 481nm, 498nm, 502nm, and 495nm i.e., in between 475nm-505nm excitation vibrations of the silver nanoparticles. It is because of the depletion of the silver ions (Ag+) into silver nanoparticles (Ag°) in banana peel extract (BPE). In reference to these results, some articles mentioned the similar peaks on UV-visible spectroscopy for silver nanoparticles (14).

Antibacterial action was done utilizing three distinct strains, viz. Escherichia coli, Salmonella and Staphylococcus aureus. The results of the examination showed that AgNPs orchestrated from silver nitrate and banana peel extract have discrete antibacterial activity against harmful microscopic organisms at a 5µg/ml concentration. The AgNP were compared with only banana peel extract as a control sample to check the antibacterial activity difference with the concentration of 5µg/ml (15).

The discs with AgNPs showed better activity than discs applied with only banana peel extract. AgNPs were genuinely harmful to Salmonella, E. coli, and Staphylococcus aureus, with the restraint zone of 26, 22, and 24mm (11). SN connects to the sulfur having proteins of the cell layer, accordingly, causing membrane destruction and exhausting the degrees of intracellular ATP of the microorganism. Silver can likewise communicate with the DNA of microorganisms forestalling cell proliferation(4).

The AgNPs showed a limited range of activity as they effectively forestalled the development of gram-positive microbes more than the gram-negative strains. Banana peel extract and silver nitrate hindered the development of gram-positive strains to the degree of 66.0% at 30°C, though every one of the gram-positive microorganisms were helpless at 20C (5). It is by and large accepted that substantial metals respond with proteins by consolidating the thiol (SH) groups, which prompts the inactivation of the proteins. Microbiological and synthetic analyses suggest that the connection of silver particles with thiol groups assumed a fundamental part in bacterial inactivation. It is undeniably true that the antimicrobial activity of Ag nanoparticles is probably going to be all around associated with its diminished size and shape attributable to the expanded surface region with improved antimicrobial impact.

Management against fungal activity in food products is vital because of its low cost. Not long ago, a better attempt has been given to improve the security control strategies that impart very low risk to human beings and animals, with a perspective of controlling deficiencies of fabricated fungicides (16). Discovering from the ongoing research has proven that the AgNPs with less harmful and a wide range of antimicrobial interest have not been additionally very powerful towards food pathogenic fungi.

The present research is predicated on in-vitro petri dish examination; therefore, the conclusion of the results to additional general cases is limited. But knowledge from this study offers valuable initial effective data on silver compounds against plant infectious agents (12). In this study, we investigated the suppression impact of AgNPs with banana peel extract towards Aspergillus niger fungi in lab examination. The outcomes advise that AgNPs are not able to inhibit those pathogens; however, outcomes vary in keeping with the attention of AgNPs carried out to pathogens from very minor to negligible impact.

The antifungal action of AgNPs against Aspergillus niger was examined utilizing antifungal analysis with a similar control sample. AgNPs did not display any antifungal action against parasitic strains. Various groupings of nanoparticles film, for example, 1, 2, 3, 4 and 5 were checked for antifungal action. AgNPs did not show satisfactory results against fungal activity and did not show any inhibition zone around the discs. This could be because of the non-excessive density at which the solution became capable of saturating and sticking with the fungal hyphae and shut off pathogenic fungi. Reports on the procedure of inhibition activity of silver ions (Ag+) on microbes proved that treatment with silver ions (Ag+), DNA drops its capacity to regenerate, ensuing in the disabled expression of ribosomal subunit proteins, in addition to some different cellular proteins and enzymes vital to ATP production (17).

It has additionally been speculated that silver ions (Ag+) do not affect the characteristic of membrane-bound enzymes. In conclusion, AgNPs had no effective antifungal outcomes on fungi examined in-vitro, possibly via demolition of membrane integrity; therefore, it resulted in such a way that AgNPs do not possess significant antifungal activity. In contrast to this study, some articles reported very efficient antifungal activity of silver nanoparticles (16).

The composite gelatin films containing AgNPs had been decided on for the packaging of raw/uncooked broiler meat and stored at refrigeration temperature (4°C) based on the antibacterial activity. The decided nanoparticles films for packaging were made to assess the life span of raw broiler meat. A boundary has been set by the Food and Drug Administration (FDA) of 6cfu/g for overall plate colony number past which meat ought to now not healthy to eat any longer (18). Samples from broiler meat with no coating of silver nanoparticles (control) surpassed the highest allowable stage inside four days, while meat samples that were packed in gelatin films loaded with AgNPs remained under the most allowable range.

The initial value of pH of the meat sample with no coating at all (control) was 6.8, while the pH value of the AgNPs film-coated meat sample was 6.6 on day one. The pH values (as shown in Figure 7) were keenly observed every single day until the meat

samples got spoiled and crossed the maximum allowable range of 6cfu/g. In these 4 days, the pH values did not show any big change, control samples went from 6.8pH to 6.9pH while film-coated samples ranged from 6.6pH to 6.7pH. A predominant cause for this slight growth in the pH value of broiler meat samples is probably because of the assemblage of metabolites from microbial increase which includes ammonia and amines produced from bacteria (19).

This whole study was performed to keep one goal in mind to take part in preserving food for longer period so that we can fight against hunger and health issues around the world. Silver nanoparticles were produced for this purpose having antibacterial properties of banana peel extract and silver nitrate (AgNO3) solution. These AgNPs showed significant results against different strains of bacterial pathogens, did not affect fungal pathogens. Later on, the biofilm that was produced by the help of AgNPs, wrapped around the meat samples to check if it can increase the life span of meat or not. The outcome was in our favor and the results were satisfactory. In the future, we can use this nanoparticle sheet globally at an industrial scale to preserve chicken meat for longer period. This study takes us one step closer to achieve our goal of fighting against hunger issues and food security problems.

V. CONCLUSION

Banana peels can be used as a protective gel for different food items and have shown antimicrobial activity. These aspects are good for packaging, increasing shelf life of the food and items to be packaged.

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