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Biosynthesis Of Silver Nanoparticles From Spices And Herbs, Their Characterization And Anti-Cancer Analysis Against Cancerous Cell Lines

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Abstract

Cancer is considered a metabolic disorder affecting the majority of the world's population. Among the various types of cancer affecting the female population, breast cancer has been the most diagnosed. The use of locally available spices and herbs containing bioactive phytoconstituents has been considered an approach to the prevention and management of cancer. Spices like black pepper and moringa, as well as herbs like onion and garlic, contain bioactive compounds with proven antioxidant, anti-inflammatory, and anticancer properties. These compounds can modulate the pathways involved in the growth and survival of cancer cells. Recently, the green synthesis of silver nanoparticles has been considered an eco-friendly approach in the field of nanomedicine. The present study aims to use natural reducing and capping agents present in the extracts of locally available herbs like onion, garlic, black pepper, and moringa for the synthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray diffraction studies, and Scanning Electron Microscopy. The antioxidant and anticancer potential of the synthesized silver nanoparticles were also evaluated. The results showed the antioxidant potential and anticancer activity of the synthesized silver nanoparticles, especially in the MCF-7 breast cancer cell line.

Introduction

Sensitivity to cancer is an emerging international issue that is of great concern and is growing in nature, as cancer is the second most common cause of death globally (Klement, 2024). According to the International Health Organization, over 18 million people have been diagnosed with cancer, and over 1 million deaths due to cancer have been reported globally (Bray et al., 2018). In the USA, millions of individuals receive a cancer diagnosis every year, and over 1.2 million cancer-related deaths have been reported (Fitzmaurice et al., 2015). Cancer is a multifaceted disease at the tissue level and is difficult to diagnose and treat accurately. Cancer is a disease where normal cells transform into cancerous or malignant cells through uncontrolled growth, division, and genetic changes (Paulmurugan, 2012). Cancer cells develop resistance to treatment through new mechanisms of survival and growth (Ramos et al., 2015). Failure of treatment results from genetic, epigenetic, and microenvironmental factors, resulting in increased drug absorption and enzymatic inactivation (Khalaf et al., 2021).

The aim of the treatment is to increase the life expectancy of cancer-affected individuals by effectively treating the tumor or cancer cells without harming the healthy cells and tissues in the body (Dollinger, 2002). In cancer therapy, drug delivery, and diagnosis, various polymeric and metallic nanoparticles are employed (Desai et al., 2021). The nanoparticles are effective in targeting the cancer cells without harming them and altering the microenvironment of the cancer cells, resulting in cytotoxic effects in the targeted location (El-Seedi et al., 2019). Researchers are working to introduce new therapies in the treatment of cancer by using nanotechnology-based therapies such as nanomedicine, which is effective in dealing with the challenges faced by the traditional therapies in the therapy of cancer (Patra et al., 2018). Researchers are working to introduce new agents in the treatment of cancer, such as plant-based anticancer agents and nanoagents based on nanobiotechnology and improve the effectiveness of chemotherapeutic agents used in cancer treatment.

Nanotechnology is focused on the manipulation of atomic, molecular, and supramolecular structures to create nanostructures with enhanced characteristics (Shanmugam, 2019). The size range of nanoparticles is 1 to 100 nm, and they have a particularly high surface to volume ratio (Zhou et al., 2011). The larger the area used, along with utilizing their conformation and distribution of nanoparticles in a solution, enhances their unique biochemical and appearance features. These properties were beneficial in various fields, including antimicrobial development, biomolecular detection, diagnostics, catalysis, microelectronics, sensing devices, and targeted drug delivery to cancer cells (Moodley, 2017).

Silver nanoparticles represent an attractive opportunity within the nanotechnology industry. A major field using current technology research acts as the development of stable techniques with respect to the synthesis of nanostructures on silver ions (Mukherjee et al., 2012). One promising approach is green synthesis, which offers a method for combining silver nanoparticles using physical, chemical, and biological routes (Vishwanath et al., 2021). Over recent years, there hash been a shift from rapid chemical methods to green synthesis due to concerns over process toxicity and the desire for higher product quality (Li et al., 2015).

The process to create nanoparticles from biologically benign sources such as fungi,h bacteria, and plants (Samuel et al., 2022). These eco-friendly methods avoid the drawbacks associated with conventional synthetic strategies (Chouhan et al., 2013). Plant extracts offer a promising means of producing sustainable nanoparticles on many different scales, so long as they are used for nanoparticle synthesis (Suhag et al., 2023). The use of spices and herbs extracts to synthesize of AgNPs (Otunola et al., 2018). They are the two spices of moringa, black pepper, and two herbs' garlic and onion. Silver nitrate ($AgNO_3$), an often-used precursor in the manufacture of silver nanoparticles, occurs with chemical compounds within these botanical preparations in these investigations, either lowering or at least peaking substances (Mohanta et al., 2023).

Allium cepa L, also known as onion or bulb onion, is an extensively consumed vegetable, especially for its taste and antibacterial activities (Bosekeng, 2012; Burt, 2004). This plant is also famous for its positive biological activities, which consist of complex sulfur compounds, thiosulfates, phenolics, and flavonoids (Międkus et al., 2020). Garlic has been used for centuries, especially for its taste and in herbal medicine, because of its chemical diversity (Morales-González et al., 2019). Garlic is famous for its ability to prevent serious health issues, like cancer, especially when combined with other compounds that prevent the growth of cancer cells (Nobili et al., 2009). The ability of garlic to prevent cancer is attributed to biometabolites containing sulfur, like S-allyl cysteine, diallyl sulfide, and allicin, as supported by other studies (Butnariu et al., 2015).

Piper nigrum (black pepper) is a plant recognized for its high nutritional and therapeutic value, with various parts of the plant containing medicinal compounds (Damanhouri et al., 2014). It has many therapeutic benefits, including antibacterial properties, relief from pain, and antimicrobial qualities (Al-Snafi, 2016). Many researchers have used moringa in the manufacture of nanoparticles. The effects of potentially treatment containing silver nanoparticles that were created to be used as an antibacterial agent and aqueous leaf extract have been compared in studies (Iqbal et al., 2023). It was obtained to produce environmentally friendly silver nanoparticles from fresh leaves of the Moringa oleifera plant. Green nanotechnology is the application of biological processes and environmentally friendly materials to establish particles and materials at nanoscale levels with little energy needed. This environmentally friendly technology is preserved as an innovative field of nanotechnology that combines ideas from biological and physicochemical processes to create functionalized nanoparticles (Srivastava et al., 2022).

For the integration of nanoparticles, the extracts of various plant materials were used, and the size of the nanoparticles was also regulated. Black pepper, a member of the species *Piper nigrum*, and onion, belonging to the species *Allium cepa*, were used for the integration of nanoparticles. Both black pepper and onion are rich in bioactive compounds, which have a considerable part in the integration of nanoparticles (Javed et al., 2020). Black pepper is used for its antimicrobial and anti-inflammatory effects, and the potential of black pepper in the integration of nanoparticles for their use in biomedical applications was demonstrated (Srivastava et al., 2022). Onion is used for its antioxidant activity, which has a considerable part in the integration of nanoparticles. The antibacterial activity of nanoparticles, which are extracted from natural sources, is being investigated (Jain et al., 2017). Significant efforts have concentrated on developing innovative medications made from renewable resources, caused by the development of natural resistance to currently available drugs (Cragg et al., 2020). Biological materials used in medicinal applications have long been sourced from Mother Nature (Clinebell, 2013).

The economical and eco-friendly biosynthesis techniques of herbs and spices can be employed for the cutback and stabilization of silver nanoparticles, which can exhibit inhibitory effects against the growth of cancerous cell lines. Cancer is a multifactorial disease that requires advanced technology-oriented therapies, and the conventional integration of silver nanoparticles entails the use of toxic chemicals, which can constitute a danger to the health of humans and the environment. The green integration of silver nanoparticles can be employed as a sustainable technique by overcoming the drawbacks of conventional techniques, thereby facilitating the development of targeted anticancer therapies. The purpose of this research is to produce silver nanoparticles by utilizing herbs and spices extracts, test them for anticancer potential against cancer cell lines, and confirm the quality and reliability of the produced silver nanoparticles by evaluating particle size, shape, and stability.

MATERIALS AND METHODS

The current research work undertaken by the researcher and titled "Biosynthesis of silver nanoparticles using spices and herbs, its characterization, and anti-cancer analysis against cancerous cell lines" has been conducted in the lab of Biotechnology, Department of Biotechnology, University of Sialkot, Sialkot.

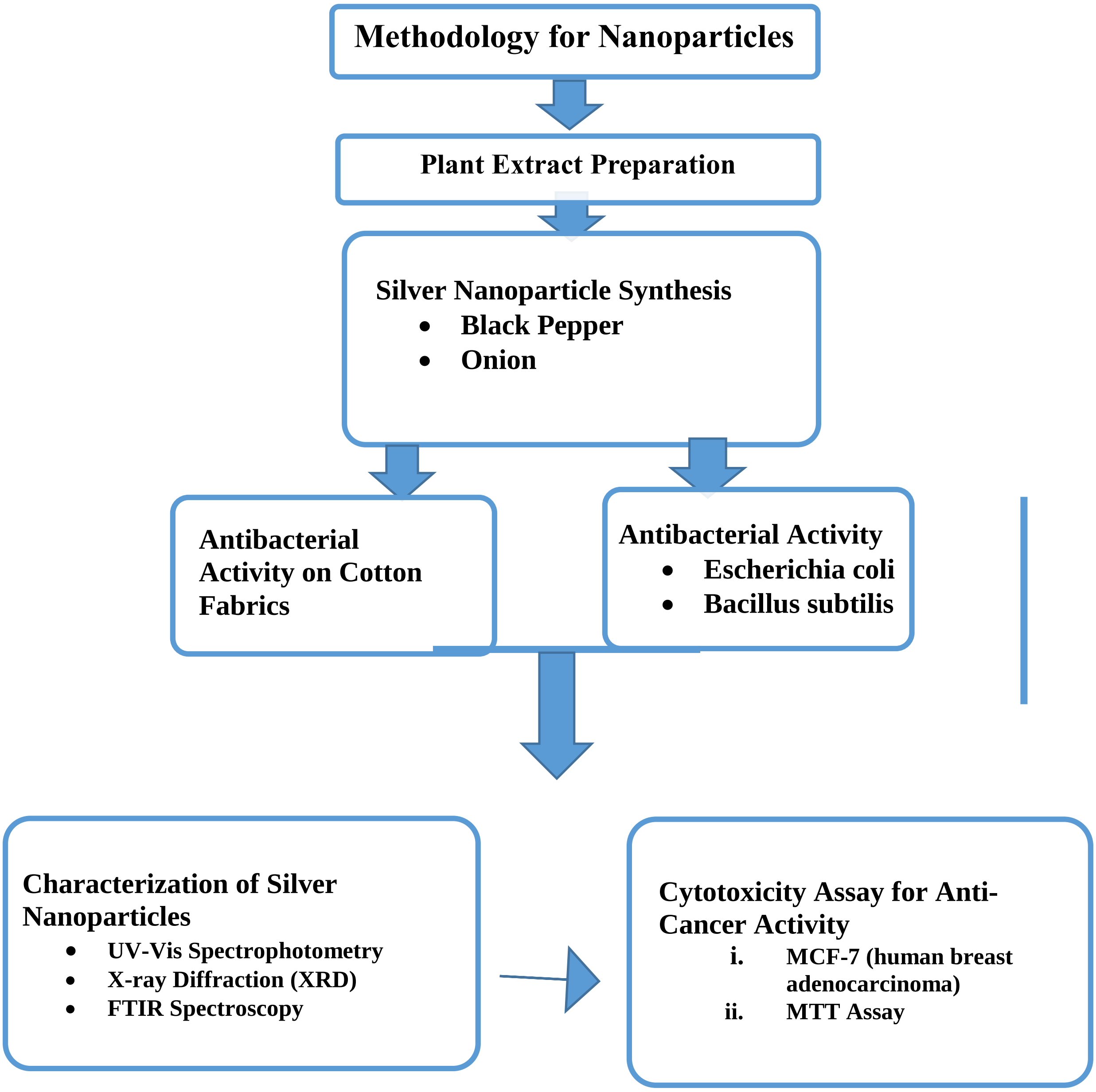
Materials

The silver nitrate solution was arranged, and the silver nanoparticles were synthesized using the following plant materials. The selection of spices and herbs for the biosynthesis of silver nanoparticles is founded on the high content of phytochemicals. Piper nigrum, which is also called black pepper, is selected as the spice, and Allium cepa, which is also called onion, is selected as the herb. All the materials were obtained from local sources and identified before they were used for the experimental procedures.

Chemicals and Reagents

All the experimental procedures were carried out at the Biotechnology Laboratory, Sialkot. Moreover, analytical-grade chemicals were used during the entire study. The chemical requirements for the execution of all the experimental procedures included silver nitrate ($AgNO_3$), sodium chloride (NaCl), distilled water, ethanol (which was used for cleaning as well as sterilizing purposes), tryptone, yeast extract, and agar. In addition, the extracts used for the integration of silver nanoparticles included black pepper, moringa, onion, and garlic.

Methods



Selection of Plant Materials

There are two spices of black pepper (Piper nigrum) and moringa (Moringa oleifera), as well as two herbs of onion and garlic, selected for the production of silver nanoparticles. We bought fresh plant material from a nearby market. Plant materials were collected, and their quality and freshness were carefully verified. The reliability of the identity of the samples that have been collected was then confirmed by an accomplished herbalist. To make sure the plant components were correctly identified, the verification method included morphological analysis and comparison with accepted plant descriptions. The samples were checked and then brought under carefully controlled conditions to the laboratory to avoid contamination or degradation.

Table 3.1 Plants Materials

Plant Material	Scientific Name	Source	Verification Method	Collection Date	Handling Procedures
Onion	Allium	Local	Morphological	May 1, 2024	Kept in cool,

	cepa	Market	examination		dry place; handled with gloves
Garlic	Allium sativum	Local Market	Morphological examination	May 1, 2024	Kept in cool, dry place; handled with gloves
Black Pepper	Piper nigrum	Local Market	Morphological examination	May 1, 2024	Stored in airtight container
Moringa	Moringa oleifera	Local Market	Morphological examination	May 1, 2024	Stored in airtight container

Preparation for Plant Extracts

The process is created by thoroughly washing, drying, and finely powdering the selected plant materials. The plant materials should then be cleaned by washing them in water. In order to further clean the plant materials, it is recommended that after washing the plant materials in water, they should be further cleaned by washing them in distilled water. After cleaning the plant materials, it is recommended that they should be spread on clean and dry paper towels and dried in a fresh area without exposure to the sun, which can destroy the plant materials.

Figure 3.1 Moringa and Black Pepper dried and grind to form powder

For Onion and garlic, a different method was employed. Their extracts were prepared by boiling, which released their active components necessary for the integration of silver nanoparticles. Subsequently, the other plant materials were dried. Once this was done, the plant materials were finely powdered using a clean and sterile grinder. This was done in order to have uniform particles. Furthermore, 10 grams of the powdered plant materials were accurately weighed. The plant materials that had been weighed were subsequently put into various beakers with 100 mL of distilled water. The mixtures were boiled for 30 minutes on a hot plate with continuous stirring to facilitate the extraction of bioactive compounds. During this process careful attention was given to maintaining the temperature within optimal ranges to prevent overheating and degradation of the compounds.

After boiling, the mixtures were cooled to room temperature. Once cooled, the extracts were carefully filtered. Whatman No. 1 was used for the filter. to remove any solid residues, ensuring clear solutions. These filtrates, which comprised the aqueous extracts

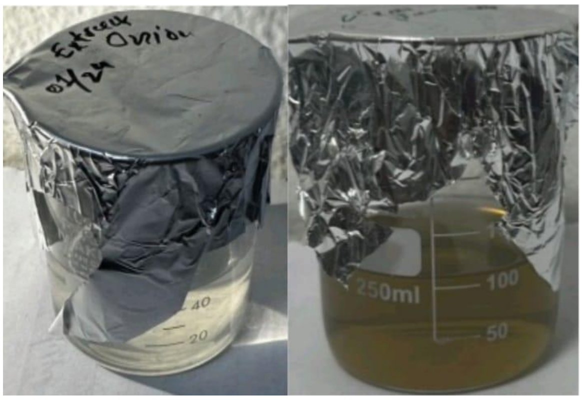


Figure 3.2 Onion and Garlic extract

of the plant materials, were then stored in clean, labeled glass bottles and refrigerated at 4°C until they were needed for the production of nanomaterials.

Preparation of Black Pepper AgNPs synthesis

Piper nigrum (black pepper) was bought at a nearby market and authorized. A 1mM silver nitrate solution was combined with different quantities of Piper nigrum extract (10–40 vol%) to create silver nanoparticles (Ag-NPs). It explains the process of preparing black pepper powder with the help of a mortar and pestle. The powder of black pepper, 20 grams of the powder, is dissolved in 100 milliliters of distilled water. The solution is then boiled at 80 degrees Celsius for 10 minutes. The solution is then filtered with the help of Whatman No. 1 filter paper.

Preparation of onion AgNPs synthesis

Fresh onions were peeled and cleaned with distilled water. The onions were then cut into small pieces to make the onion extract. A fifty-gram fresh onion was mashed into small pieces. The unwanted materials were then filtered out. The pure extract and 100 milliliters of distilled water were then boiled with the help of a hot plate at 60-80 degrees Celsius for ten to fifteen minutes. The solution was then heated and filtered. In another container, 10 mL of the onion extract and 90 mL of the 1 mM silver nitrate solution were mixed to make the silver nanoparticles. The solution turned colorful. The color shows that the silver nanoparticles were made. The color may be brown or yellow.

Preparation of Garlic AgNPs synthesis

Peel the garlic using a peeler, wash with distilled water, and add 10 g. Crush the garlic using a mortar to get a paste. Place garlic in a beaker with 100 mL of distilled water and heat the garlic solution for ten to fifteen minutes using a hot plate at 60 to 70 degrees Celsius. Stir the garlic solution constantly. Allow the garlic solution to cool at room temperature. Filter the garlic solution using filter paper to get a clear solution for garlic extract. Collect the solution in a clean beaker. Add 10 mL of garlic extract and 90 mL of 1 mM AgNO3 and mix in a beaker. Stir the garlic solution and AgNO3 solution constantly for 20 to 30 minutes at room temperature. Color change is expected, which indicates the synthesis of AgNPs. A Brownish or yellowish color is expected, which indicates the synthesis of AgNPs.

Preparation of Moringa AgNPs synthesis

The leaves of Moringa (Moringa oleifera) were sourced from a local market. Using a grinder, the moringa leaves were dried and grounded into powder. Then, a mixture was prepared by combining 20 grams of powdered moringa leaves with 200 mL of distilled water, and the resulting solution was heated to a temperature of 60–80°C for 10 minutes. The solution was filtered using Whatman No. 1 filter paper to eliminate any remaining materials and obtain clarity. Silver nanoparticles (Ag-NPs) were produced by combining 90 mL of a 1 mM silver nitrate solution with various quantities of dried moringa leaf.

Biosynthesis of Silver Nanoparticles

The process to generate a 1mM fluid solution of ($AgNO_3$) was the beginning of the biosynthesis of silver nanoparticles. A suitable quantity of $AgNO_3$ was dissolved in distilled water, and the mixture was mixed before the material was completely dissolved, with the purpose of achieving purpose. In separate vessels for reactions for the synthesis procedure, 10 mL of each plant extract and 90 mL of the 1mM $AgNO_3$ solution was mixed. The mixtures were stirred thoroughly to achieve a distribution of the plant extracts within the solution made with silver nitrate.

To prevent any photochemical reactions, these reaction mixtures were subsequently incubated at room temperature in the dark, which could potentially influence the reduction. To reduce silver ions to silver nanoparticles, the incubation time was increased to 24 hours. The solution's color change confirmed the reduction of silver ions to silver nanoparticles. At first, the solution was colorless. As the reduction reaction proceeded, the change in the color to yellowish brown was evident. The solution’s color change indicates that the silver ions have been reduced to silver nanoparticles. Verification of the synthesis samples from the reaction mixtures was carried out by withdrawing the samples at intervals and subjecting them to UV-visible spectrophotometry. Confirmation of the reduction to silver nanoparticles is evident if there is a sharp absorption band in the region of 420-450 nm.

Characterization of Biosynthesized Silver Nanoparticles

Various characterization techniques are employed to validate the synthesis of these nanoparticles. Characterization, indicating the synthesis of these nanoparticles, is performed using a UV-visible spectrophotometer that operates at a wavelength of 420 nm. XRD analysis is used to confirm the crystalline nature of these nanoparticles. The XRD analysis is performed with an X’Pert Pro Analytical 7602 device, which operates at 40 kV using a Cu target (Kα 1.5405 Å) and covers a 20-80° 2θ range. By employing this method, the crystalline nature of silver is established by identifying its peaks. FT-IR, which identifies hydroxyl, carbonyl, and amine groups, is used to identify the functional groups of the stabilizing agents used in the bio-reduction method.

UV-visible Spectroscopy

The verification of the synthesis of nanoparticles is normally confirmed by the smooth increase in the specific absorption peak according to the reaction time and the concentration of biological extracts with metal ions. The spectrum of the nanoparticles usually exhibits specific peaks according to the surface plasmon resonance.

Fourier Transform Infrared Spectroscopy

FTIR is a widely utilized technique for determining functional groups in either pure substances or mixtures. It can be used to compare various compounds. The principle of this technique is that the vibration of atoms or molecules is associated with infrared radiation.

X-ray Diffraction

Generally, UV-visible spectroscopy is applied to confirm the synthesis of nanoparticles using various methods. XRD is a non-damaging method used for the elemental analysis of materials. It is a useful technique for identifying the essential characteristics of compounds. It helps in identifying the types and nature of compounds. It is a technique used for structural analysis of materials. The structural analysis is performed based on the position and intensity of the X-ray beam.

Antibacterial activity of silver nanoparticles

In the context of understanding the antibacterial activities of biosynthesized silver nanoparticles (AgNPs) from onion and black pepper extracts, Bacillus subtilis, a Gram-positive bacterium, and Escherichia coli, a Gram-negative bacterium, are taken as models to check the antibacterial activities of AgNPs. Bacillus subtilis, which possesses a thick peptidoglycan layer, generally exhibits different patterns of susceptibility compared to E. coli, which possesses a thin peptidoglycan layer along with lipopolysaccharide. The different patterns of bacterial cell wall structure, which include a thick peptidoglycan layer, are the cause of the susceptibility of the bacteria to nanoparticles. The present study verifies the antibacterial functions of silver nanoparticles integrated with onion and black pepper extracts through the disk diffusion method. The presence of an inhibition zone surrounding the bacterial cultures confirms the antibacterial effects of the synthesized silver nanoparticles. The presence of an inhibition zone around the cultures of Bacillus subtilis and E. coli demonstrates the antibacterial effects of the synthesized silver nanoparticles, which alter the structure of the bacterial cells and thus hinder bacterial growth.

Silver Nanoparticle-Coated Cotton Fabrics Antimicrobial Activity

The antimicrobial potential of biosynthesized silver nanoparticles using onion and black pepper extracts was also evaluated against Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* bacteria. The effectiveness of an antimicrobial agent can be tested using these strains of bacteria. These two strains of bacteria are different in their cell walls. In this study, biosynthesized AgNPs with onion and black pepper extracts were used to treat cotton fabric samples. The effectiveness of biosynthesized AgNPs with onion and black pepper extracts in treating cotton fabric samples was tested using these strains of bacteria. After treating cotton fabric samples with biosynthesized AgNPs, these cotton fabric samples were left in contact with these strains of bacteria. A zone of inhibition is seen around these cotton fabric samples, proving the effectiveness of biosynthesized AgNPs.

Cytotoxicity Assay of Green Synthesized Silver Nanoparticles for Anticancer Activity

Cancerous Cell Lines

The human-derived breast adenocarcinoma cell line 'MCF-7' is accessible through our research collaboration network. The cytotoxic activity of these MCF-7 cells is performed by an MTT assay. The transfection of miR34a and silver nanoparticles in MCF-7 cancer cells is carried out with the help of Lipofectamine 3000. Quantitative RT-PCR is used in expression analysis, which is done in the Institute of Biomedical and Genetic Engineering in Islamabad.

Cell Culturing

The human-derived breast adenocarcinoma cell line 'MCF-7' which is an adenocarcinoma cell culture, is maintained in flasks by the use of RPMI medium, 10% fetal calf serum, and four antibiotics collectively known as GPPS. The cell culture is maintained in a 5% CO_2 incubator. After 2-3 days, these cells are then ready for further analysis. These cells are then taken under a microscope after observing them once they have reached 70% confluency (Zhang et al., 2012).

MTT Assay

The MTT assay, which is based on enzymes and uses colorimetry, is employed to evaluate cytotoxicity and metabolic activity in cells. It is user-friendly, secure, and has a high reproducibility rate. This test assesses cell viability through the detection of formazan crystal formation from tetrazolium salt reduction, catalyzed by mitochondrial

dehydrogenase in living cells, resulting in a purple hue. The number of living cells in the wells is directly proportional to the color produced.
For MTT Assay for Anticancer Activity:
Regarding the MTT assay for anticancer activity, four different concentrations of biosynthesized silver nanoparticles (8, 16, 24, and 32 ppm) were created using deionized water through sonication for an hour. The test was performed using MCF-7 cells. Inoculation of cells into 96 wells was done with a volume of 100 µL of the cell suspension. 10 µL of silver nanoparticles is included in the sample solution, which is added to the wells. For 24 hours, the wells were kept at 37°C in an incubator with 5% CO_2. After a 24-hour incubation period, 10 µL of the MTT solution (at a concentration of 5 mg/mL) is introduced into the wells. The wells were once again placed in a 5% CO_2 incubator at 37°C for a duration of 24 hours.
After a 24-hour period, 100 µL of solubilization buffer is added to the wells, and the mixture is incubated at 37°C for 2 hours to dissolve the formazan crystals. The solution is thoroughly mixed to guarantee solubilization. The wells undergo the ELISA plate of reader treatment. Cell viability is recorded by measuring the absorbance at 570 nm. Simultaneously, a control test without silver nanoparticles is also conducted.
The percentage of viable cells was calculated using the following formula.
Cell viability (%) = [A]test/[A]control × 100
Where [A]test is the absorbance of the sample solution. While [A] control is the absorbance of control
The following formula was used to calculate the rate of cell death.
Cell death rate (%) = Cell viability – 100

RESULT AND DISCUSSION
The solvent-based preparations from onions and black pepper were applied to minimize the impact of the quantity of silver nitrate in a 1mM $AgNO_3$ solution. The chemical components present naturally in these plant extracts were used in the bio-reduction process. After mixing the leaves extraction using the solution of silver nitrate for 30 minutes, a distinct difference in color from yellow to dark brown, was observed as shown in "Figure 4.1." The synthesis is responsible for this color modification, which is a solid appearance in the particles surface plasmon resonance (SPR) activity. It is unique in color; The solution is caused by light scattering and absorption; the result involves a surface plasmon (SPR). There was a strong relationship between the color intensity and the duration of the incubation process. Demonstrating was slowly forming AgNPs over time. The effectiveness of black pepper and onion extracts as natural, eco-friendly agents for synthesizing AgNPs is shown in this study, showing their potential for a range of uses in the fields of science, nanotechnology, and healthcare.

a b c

Figure 4.1 A change by color shows the hsynthesis of nanoparticles: (a) Black pepper containing nanoparticles, (b) 1 mM silver nitrate solution, and (c) Onion silver nanoparticles

Antibacterial Activity of Silver Nanoparticles

Onions and black pepper were among the selected plants used to biosynthesize nanoparticles (AgNPs), which were evaluated for antibacterial activity against various pathogenic microorganisms, including Escherichia coli and Bacillus subtilis, using the agar well diffusion method. The Petri plates underwent cleaning and sterilization. Nutrient agar plates were made by autoclaving at 121°C for 15 minutes and then allowing them to solidify. The bacterial cultures were sub-cultured, and separate plates were inoculated with equal amounts of each bacterial strain using sterile cotton swabs. A micropipette was used to add 50 µL of the solution containing the onions and black pepper nanoparticle solution into each hole over the plates. To determine the size of the inhibitory zone, its diameter was measured in millimeters after a one-day incubation at 37°C. This figure is noted as the standard deviation of the outcomes from the duplicate tests. Sterilized disks with a diameter of 6mm, containing Ag-NPs from onion and black pepper extracts, were prepared. The disks were then placed in contact with the surface of the prepared agar plates containing Escherichia coli and Bacillus subtilis bacteria. Afterwards, the plates were put in an incubator at 37°C for 24 hours. The results were observed after the incubation period, and the areas affected by the Ag-NPs were observed. The effectiveness of the onion and black pepper Ag-NPs was observed by using measuring instruments to ascertain the diameter of the inhibitory areas. The areas of the inhibitory zones were observed to be the most effective in the case of silver nanoparticles derived from black pepper and onion. This demonstrates the effectiveness of these nanoparticles in preventing the growth of the tested bacteria, which displays important antimicrobial effects.

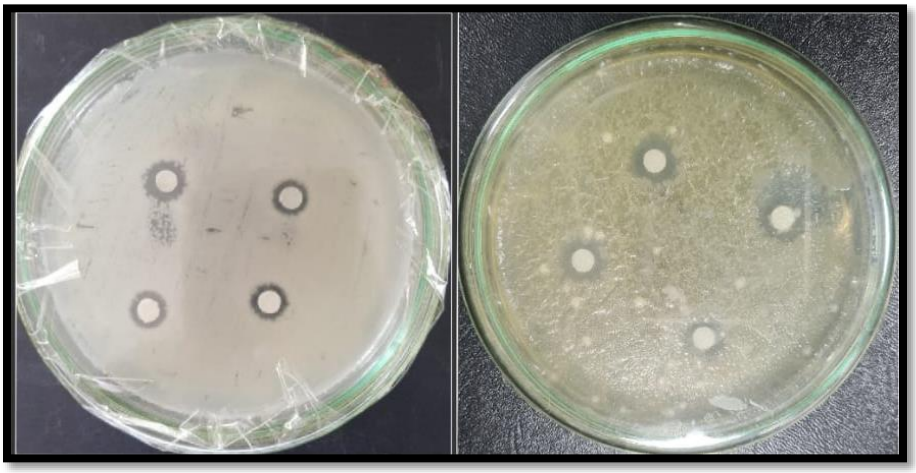


Figure 4.2 Higher zone area of AgNPs synthesized from onion nanoparticles shows antimicrobial activity against (a) Bacillus subtilis and (b) Escherichia coli

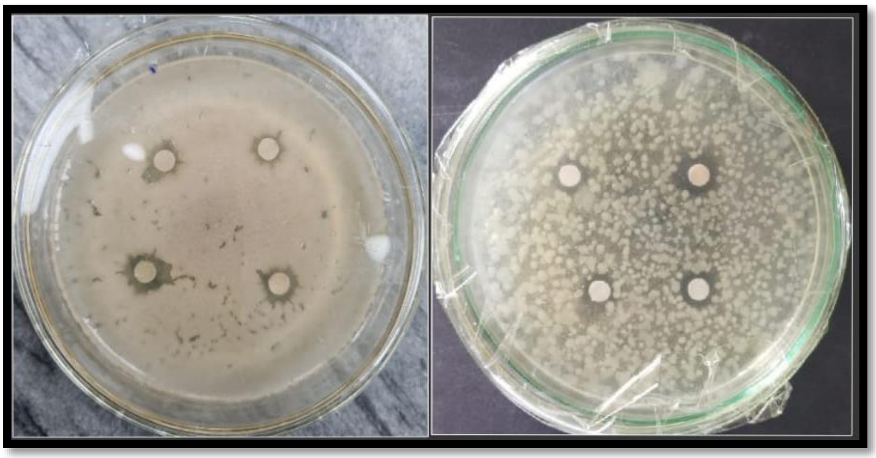


Figure 4.3 Higher zone area of AgNPs synthesized Black Pepper nanoparticles show antimicrobial activity against (a) Bacillus subtilis and (b) Escherichia coli

Silver Nanoparticle-Coated Cotton Fabrics Antimicrobial Activity

It has been observed that silver nanoparticle coated cotton fabrics have gained considerable care due to their excellent antimicrobial activity. The introduction of silver nanoparticles (AgNPs) into cotton fabrics has been observed to enhance the antimicrobial activity of cotton fabrics by either inhibiting or killing microorganisms like bacteria and fungi. A solution of silver nanoparticles was applied to cotton cloths measuring 1 cm^2. On the subsequent day, the cotton samples were put in sterile petri dishes and dried at a temperature of 50°C to eliminate any moisture. After they were dried, the samples of cotton coated with silver nanoparticles were examined for antibacterial characteristics. The negative control consisted of non-coated cotton fabrics that were autoclaved. Zones of inhibition were measured after a 17-hour incubation period. The following day, the cotton pieces that had been treated were put in sterilized petri dishes and heated to 50°C to eliminate excess moisture. While silver-coated cotton fabrics were subjected to antibacterial tests, non-coated, autoclaved cotton served as a negative control. The zones of inhibition were recorded for the silver nanoparticle-coated fabrics after 24 hours of incubation.

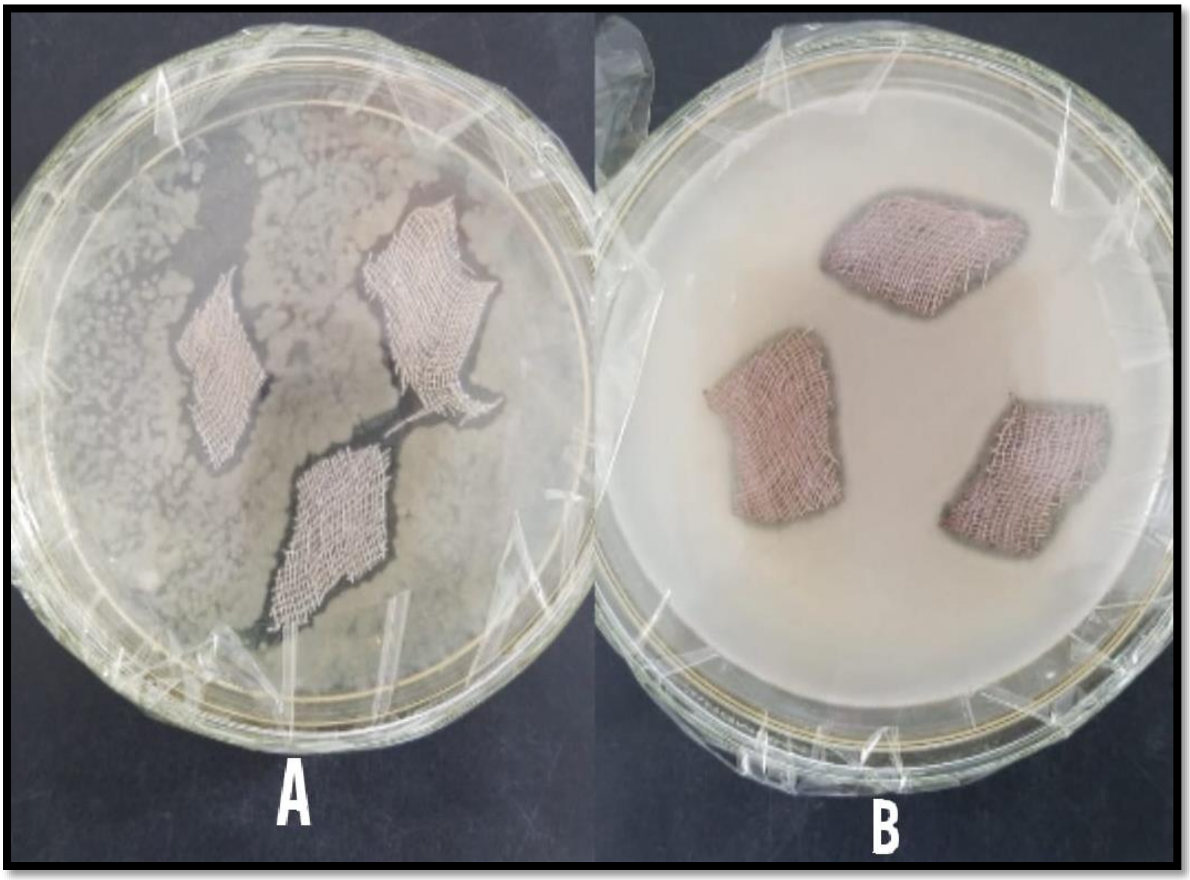


Figure 4.4 Antibacterial activity against (a) Escherichia coli and (b) Bacillus subtilis as evidenced by clear zones of inhibition.

Characterization

The field of catalysis and characterization is a basic process that provides observations on catalyst behavior and the relationship between catalyst structure and catalytic properties.

UV visible Spectrophotometer

An UV-visible spectroscopy investigation is conducted to ascertain the size and characteristics of nanoparticles in aqueous solutions. This study investigated a 1 mM silver nitrate solution through plant extract with biosynthesized silver nanoparticles, analyzing wavelengths ranging from 200 to 800 nm. There were no visible peaks in the silver nitrate solution, showing that there definitely were no particles present.

UV-Visible Spectrophotometry of Onion $AgNO_3$

From the scatter plot of the data of the UV spectrophotometry of the onions, it is evident that there is a positive trend in the data, and the absorbance increases as the wavelength increases from 400 to 800 nm. The aqueous silver nitrate solution did not have a peak in the absorption spectrum, as there were no particles in the solution. The leaf extract showed that it had peaks in the spectrum. This implies that it contains different phytochemicals. The peak in the silver nanoparticle solution was at 417 nm. This is the normal peak for silver nanoparticles that are biologically synthesized. According to the literature, the range of peaks of biogenic silver nanoparticles is between 400 and 475 nm. This may be indicative of some compounds in the onion having a greater absorption at longer wavelengths.

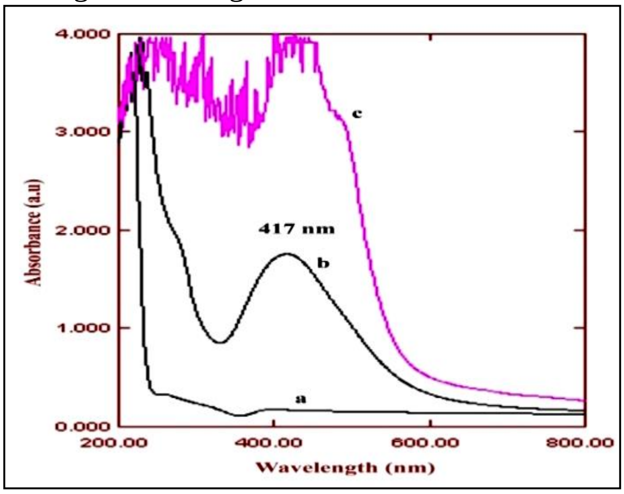


Figure 4.5 UV-Visible spectra of onion nanoparticles

UV-Visible Spectrophotometry of Black Pepper AgNO3

The UV-Visible spectra of silver nanoparticles generated from Piper nigrum extract under varying temperature conditions are shown in the figure. Thus, the color change signifies that silver nanoparticles have developed in the solution of Piper nigrum extract. The UV-Visible spectra illustrate the formation of silver nanoparticles in an aqueous medium at different temperatures: (i) Room temperature (RT) (ii) 40°C (iii) 60°C (iv) 80°C. At these temperatures, the SPR bands of colloidal silver were observed in the range of 420–446 nm. At the higher temperature of 80°C, a more intense SPR band was observed, with a broad peak at 441 nm, indicating successful nanoparticle formation. According to the results, black pepper has better absorption at higher wavelengths and appears to absorb more light in the UV-visible region as the wavelengths get longer.

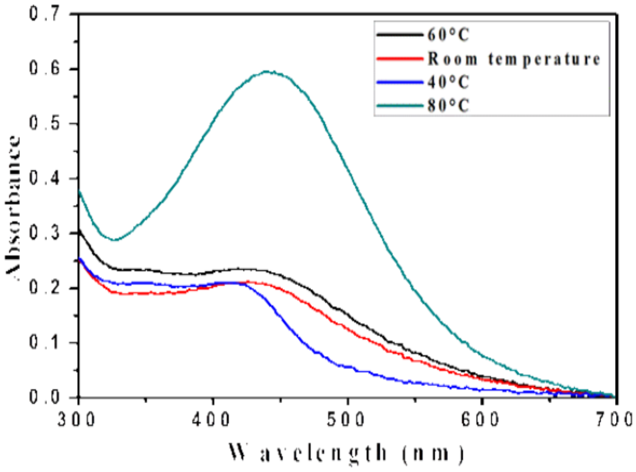


Figure 4.6 UV-Visible spectra of black pepper nanoparticles

We notice that absorbance increases with increasing temperature. This shows that the integeration of silver nanoparticles is favored at higher temperatures. As the temperature increases, the surface plasmon resonance peaks—indicating the integration of nanoparticles shift and become more pronounced. This shows that there are compounds present in black pepper that are more effective in absorbing UV rays of the range 420-446 nm. The results obtained in the experiment are helpful in finding the compounds present in black pepper that are responsible for absorbing UV rays.

This experiment was successfully conducted to prove the biosynthesis of silver nanoparticles by black pepper and onion. The peaks in the spectra of the biosynthesized nanoparticles were clear, which proved the existence of nanoparticles. The peaks in the spectra of the black pepper nanoparticles were found to be around 440-450 nm. This proves the existence of silver nanoparticles, as this is their normal SPR. The effectiveness of black pepper extract in reducing silver ions to generate nanoparticles is evident.

FTIR Results of Specimens

FTIR Results of Onion

An FTIR spectrum of onion would likely show peaks corresponding to these compounds. The Peaks are around 2900-2850 cm^{-1} due to the existence of aliphatic C-H bonds in carbohydrates and other organic molecules. The Peaks are around 1700-1650 cm^{-1} due to the existence of carbonyl groups in sugars and organic acids. A broad peak around 3300 cm^{-1} due to the existence of hydroxyl groups in carbohydrates and organic acids. The S-H peaks around 2550-2500 cm^{-1} due to the existence of thiol groups in sulfur-containing compounds. If onion extract is used for the integration of silver nanoparticles, the organic compounds present in the onion can act as a reducing as well as a capping agent. This implies that they can reduce the silver nanoparticles as well as cap the nanoparticles. The FTIR spectrum of onion-derived silver nanoparticles will exhibit changes in peak positions. The peaks of the organic compounds present in the onion can shift slightly. This is because of their interaction with the silver nanoparticles. The appearance of new peaks can be ascribed to the development of new chemical bonds between organic compounds and silver nanoparticles. The FTIR spectrum of onion-derived silver nanoparticles can give information about their anti-cancer properties. The changes that take place in the FTIR spectrum of the nanoparticles when they are exposed to cancer cells can give information about their interaction with the cancer cells. The appearance of new peaks as well as the disappearance of existing peaks can give information about the response of the cancer cells to the nanoparticles. The FTIR analysis of onion-derived silver nanoparticles can give information about their chemical composition as well as their interaction with cancer cells.

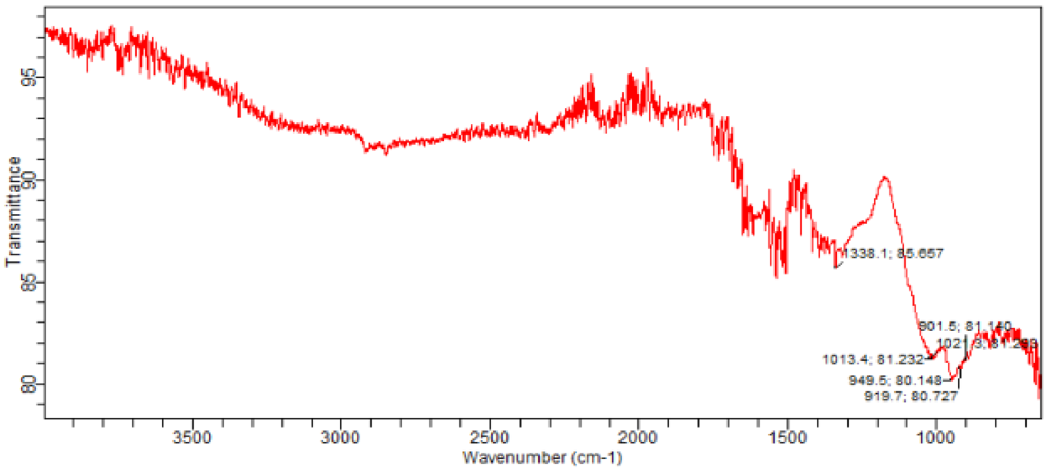


Figure 4.7 FTIR analysis synthesized nanoparticles of onion

FTIR Results of Onion Before Washing

Organic compounds like spices and herbs can act as reducing and capping agents in the synthesis of silver nanoparticles. The interaction between organic compounds and silver nanoparticles can be partially indicated by the FTIR spectrum.

The FTIR spectrum shows peaks that may result from organic compounds on the surface of silver nanoparticles. Organic compounds might be crucial for the stabilization of silver nanoparticles. There may also be peaks present in the FTIR spectrum due to impurities present in the sample if it is not properly washed before taking the spectrum. There may also be a possibility of interaction between organic compounds and silver nanoparticles.

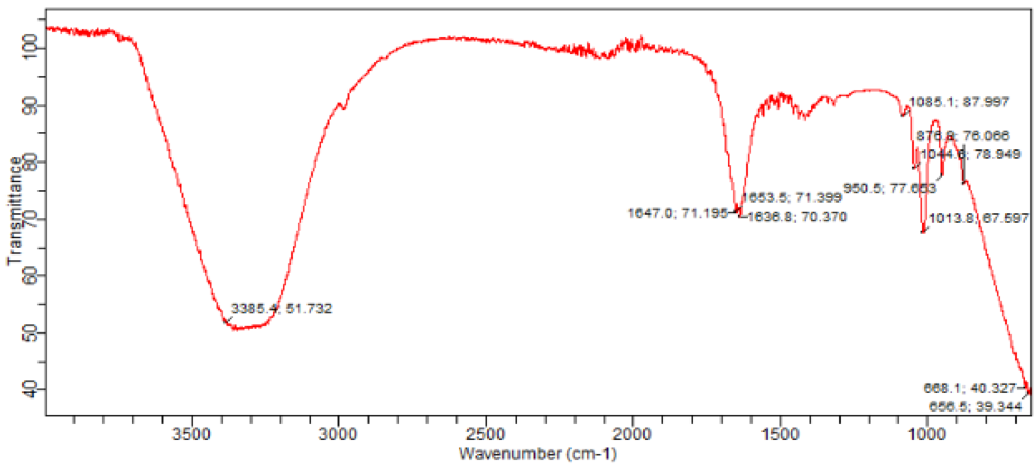


Figure 4.8 FTIR analysis synthesized nanoparticles of onion before washing

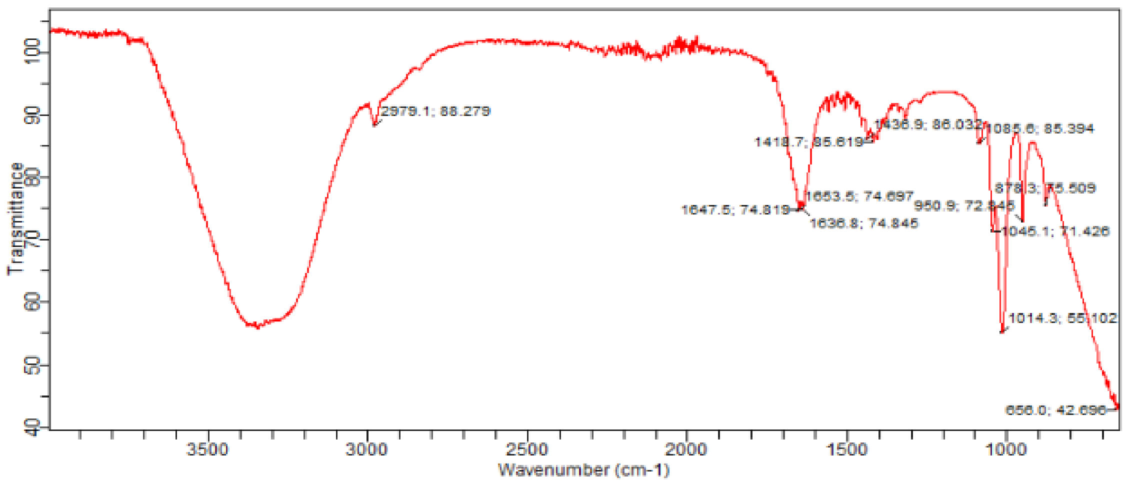


Figure 4.9 FTIR analysis synthesized nanoparticles of onion before washing

FTIR Results of Onion After Washing

The provided spectrum is the FTIR spectrum. The spectrum shows how much infrared light is transmitted at various wavelengths. The existence of different chemical bonds is indicated by the peaks in the spectrum. The wide peak near 3300 cm^{-1} indicates O-H or N-H stretching vibrations, typically linked to alcohols, amines, or carboxylic acids. The peak near 1700 cm^{-1} signifies a carbonyl (C=O) stretching vibration, which is commonly observed in ketones, aldehydes, carboxylic acids, or esters. The peaks found in the 1500-1000 cm^{-1} range correspond to a variety of functional groups, such as C-C stretching, C-N stretching, and vibrations of the aromatic ring.

The peak around 1700 cm^{-1} is due to the presence of carboxylic acids like citric or malic acid. The peaks in the 1500-1000 cm^{-1} region is associated with carbohydrates present in the black pepper. The broad peak around 3300 cm^{-1} is due to the existence of hydroxyl groups in organic acids or other compounds. The black pepper and its compounds have been examined for their possible anti-cancer activity. The FTIR spectrum could give some hints on the organic compounds that could be present and their possible interaction with cancer cells.

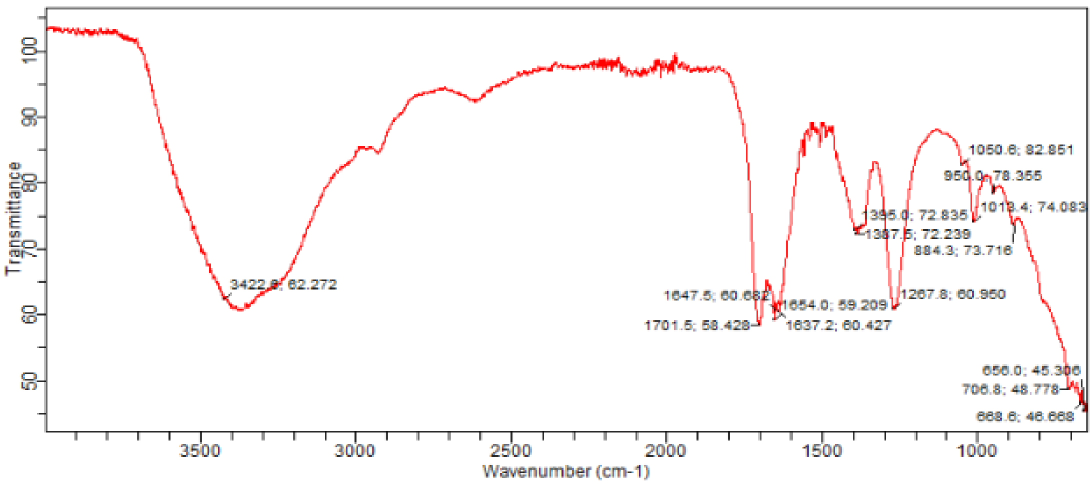


Figure 4.10 FTIR analysis synthesized nanoparticles of onion after washing

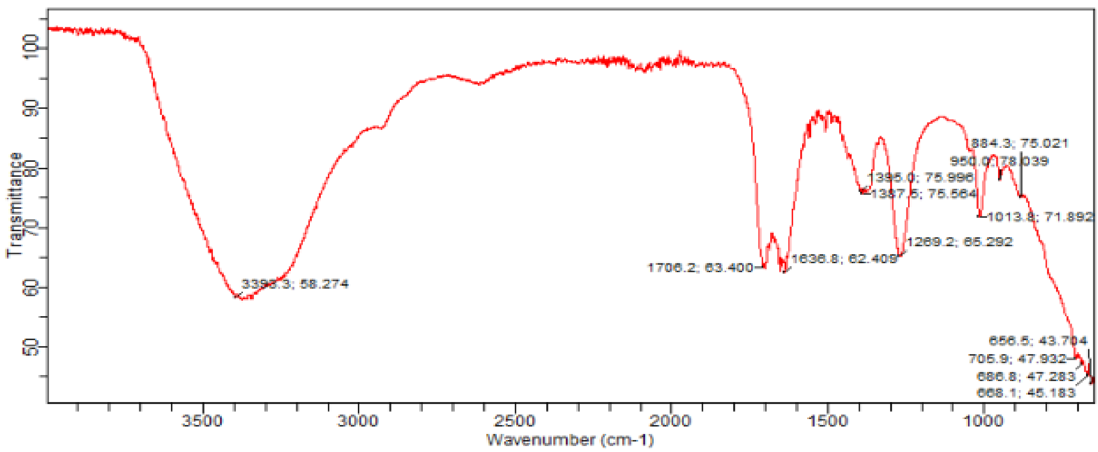


Figure 4.11 FTIR analysis synthesized nanoparticles of onion after washing

FTIR Results of Black Pepper

An FTIR spectrum of black pepper would likely show peaks corresponding to C-H stretching peaks are around 2900-2850 cm⁻¹ due to the existence of aliphatic C-H bonds in carbohydrates, proteins, and essential oils. The peaks are around 1700-1650 cm⁻¹ due to the existence of carbonyl groups in piperine and other compounds. The Peaks around 3300-3200 cm⁻¹ due to the existence of amine groups in piperine. The peaks in the 1600-1500 cm⁻¹ region due to the aromatic structure of piperine. In the integeration of silver nanoparticles using black pepper extract, the organic compounds present in black pepper can be used as reducing and capping agents. They can be used to reduce the silver ions to silver nanoparticles. They also stabilize silver nanoparticles by forming bonds with their surfaces. From the FTIR spectrum of black pepper-based silver nanoparticles, it is evident that the peaks corresponding to organic compounds in black pepper may vary slightly due to their interaction with silver nanoparticles. The new peaks may also appear due to the formation of new chemical bonds with organic compounds. The FTIR analysis of black pepper-derived silver nanoparticles can give information about potential anti-cancer properties. The changes that occur in the FTIR spectrum of the nanoparticles after they are exposed to cancer cells can give an idea about their interaction with the components present in the cancer cells. The occurrence of new peaks or the disappearance of existing peaks can show the interaction with the cancer cells, which can affect metabolism or protein synthesis. The FTIR analysis can give significant information about black pepper-derived silver nanoparticles.

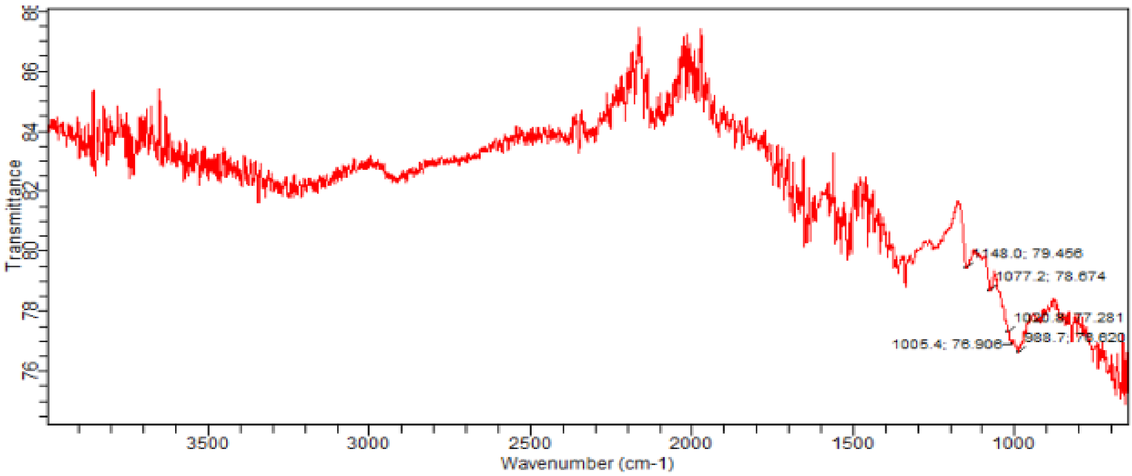


Figure 4.12 FTIR analysis synthesized nanoparticles of black pepper

FTIR Results of Black Pepper Before Washing

The supplied FTIR spectrum depicts the transmittance of infrared light against wave numbers. Specific chemical bonds or functional groups in the sample correspond to peaks in the spectrum. The wide peak near 3300 cm^{-1} indicates O-H or N-H stretching vibrations, typically linked to alcohols, amines, or carboxylic acids. The peaks at approximately 1700 cm^{-1} signify a carbonyl (C=O) stretching vibration, which is commonly found in ketones, aldehydes, carboxylic acids, or esters. The peaks found in the 1500-1000 cm^{-1} range correspond to a variety of functional groups, such as C-C stretching, C-N stretching, and vibrations of the aromatic ring. Spices and herbs can serve as reducing agents in the integration of silver nanoparticles. The FTIR spectrum can give some ideas about the interaction between organic compounds and silver nanoparticles. The peaks in the FTIR spectrum can be due to the existence of organic compounds attached to the silver nanoparticles. These compounds can play a major role in stabilizing silver nanoparticles. If the sample is not properly washed, some peaks in the FTIR spectrum can be due to the presence of impurities in the sample. These impurities can be from the solvents used and from the spice or herb extract. The appearance of new peaks in the FTIR spectrum or the shift in the peaks can be due to the interaction between the organic compounds and the silver nanoparticles. This interaction can be in the form of the formation of new bonds.

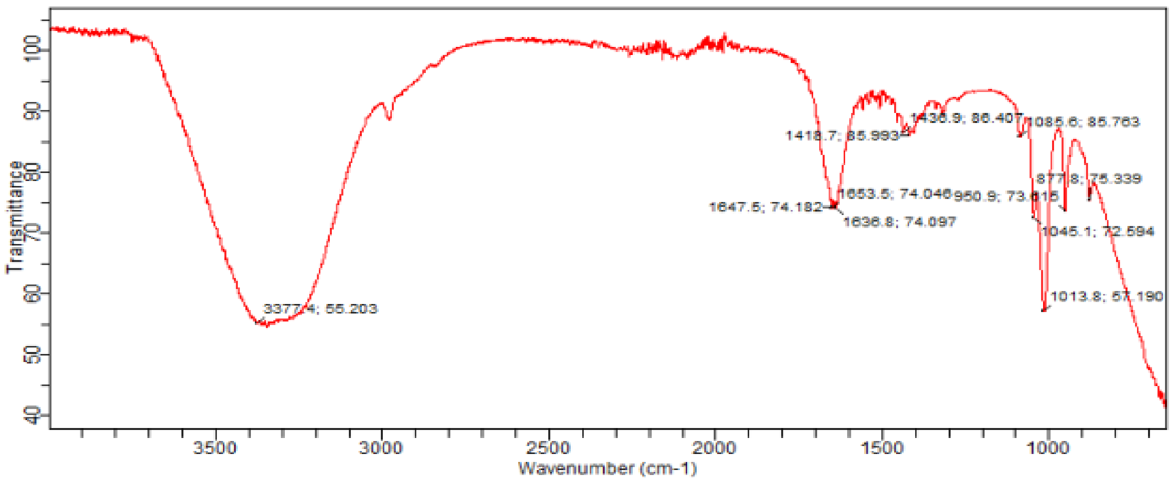


Figure 4.13 FTIR analysis synthesized nanoparticles of black pepper before washing

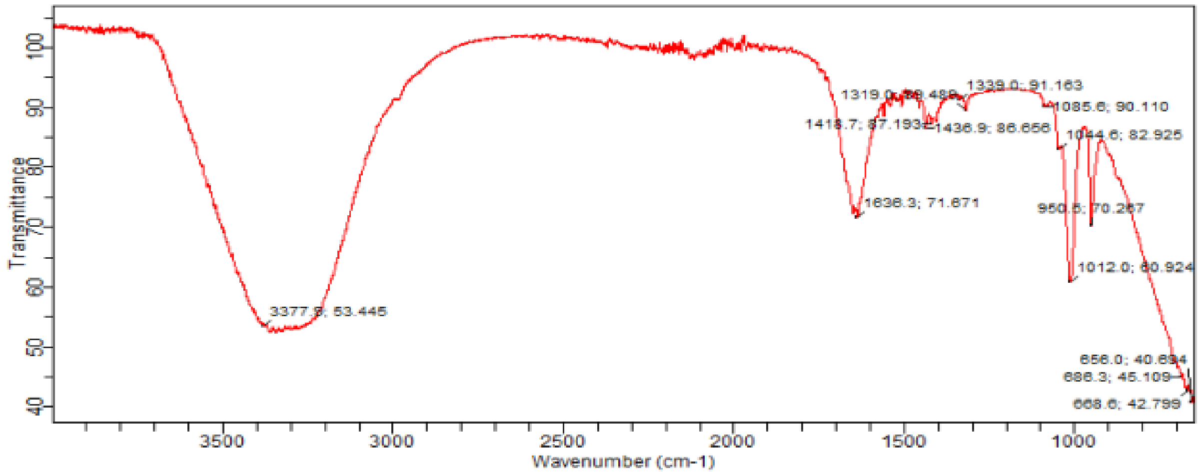


Figure 4.14 FTIR analysis synthesized nanoparticles of Black Pepper Before Washing

FTIR Results of Black Pepper After Washing

The supplied FTIR spectrum depicts the transmittance of infrared light against wave numbers. Peaks in the spectrum indicate specific chemical bonds or functional groups present in the sample. The broad peak near 3300 cm^{-1} suggests O-H or N-H stretching vibrations, commonly linked to alcohols, amines, or carboxylic acids. The peak near 1700 cm^{-1} signifies a carbonyl (C=O) stretching vibration, which is commonly observed in ketones, aldehydes, carboxylic acids, or esters. The peaks found in the 1500-1000 cm^{-1} range correspond to a variety of functional groups, such as C-C stretching, C-N stretching, and vibrations of the aromatic ring.

The peak around 1700 cm^{-1} is due to the presence of carboxylic acids like citric or malic acid. The peaks in the 1500-1000 cm^{-1} region are associated with carbohydrates present in the black pepper. The broad peak around 3300 cm^{-1} is due to the existence of hydroxyl groups in organic acids or other compounds. The black pepper and the compounds in it have been studied for the possible cancer-fighting effects. The FTIR spectrum may give some ideas about the possible organic compounds and the possible interactions with the cancer cells.

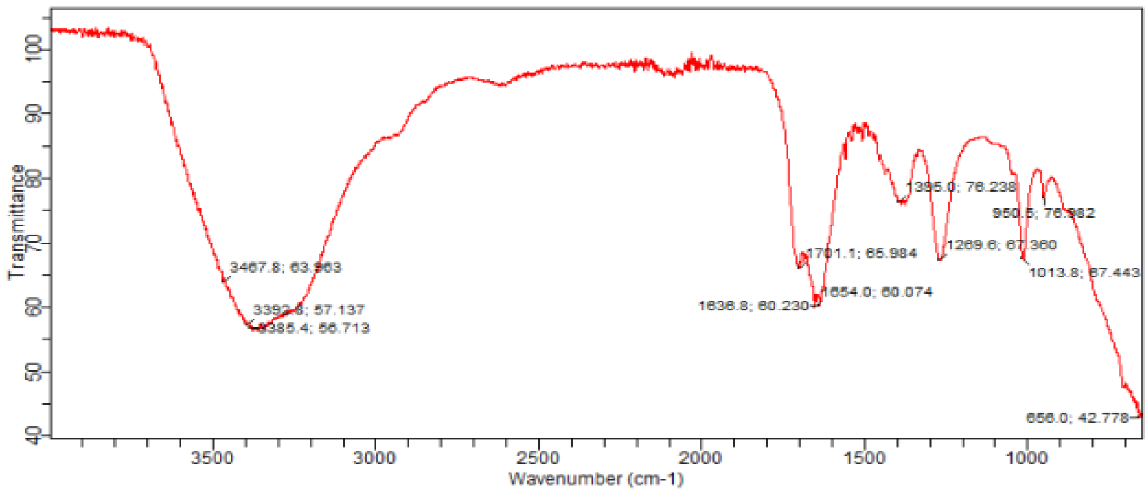


Figure 4.15 FTIR analysis synthesized nanoparticles of black pepper after washing

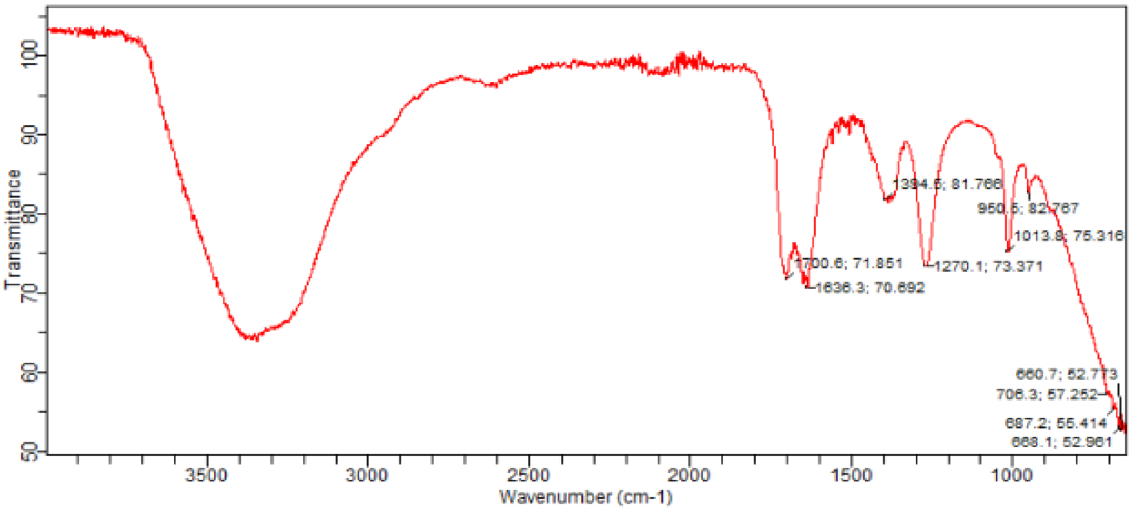


Figure 4.16 FTIR analysis synthesized nanoparticles of Black Pepper After Washing

XRD Results of Specimens

XRD Results of Onion

Analysis of the onion-labeled sample based on the information provided in the XRD spectrum in Figure 4.16 is significant in the analysis of materials. The information provided in the last graph indicates that the diffraction pattern is associated with the integration and characterization of silver nanoparticles from black pepper. The information provided includes an X-ray source that is made up of Cu-Kα (λ = 1.540560 Å), 9.126° to 120.596°, and 3990 resolutions. The experimental diffraction pattern (intensity vs. 2θ) and the peaks in the XRD pattern indicate that the material is of a crystalline nature. Sharp peaks in the XRD pattern are associated with materials that are well-defined and of a crystalline nature. The background is subtracted for a better pattern. In association with the synthesis and characterization of silver nanoparticles from onion extract, it is indicated that the peaks in the XRD pattern show that these peaks are associated with crystal planes. The crystal planes indicate that the material is face-centered cubic in nature. The peaks associated with silver nanoparticles are at 38°, 44°, 64°, and 77°. The broader peaks indicate that the material is small in nature. The peaks associated with other compounds show that other compounds are also present. A clear XRD pattern with peaks associated only with silver indicates that the nanoparticles are pure in nature. XRD helps in understanding the crystal structure of the integeration nanoparticles. In the case of silver, the expected crystal structure is cubic, and the peak positions will confirm this.

The XRD technique helps in comparing the size and structure of the nanoparticles to synthesize. The different extracts can affect the reduction and stabilization process, which can result in different sizes and structures. In the study "Silver Nanoparticles from Spices and Herbs, Its Characterization and Anti-cancer Analysis Against Cancerous Cell Lines," this XRD pattern confirms the crystalline nature of your sample. The presence of well-defined peaks supports successful nanoparticle synthesis, which is crucial for correlating with biological activity and anti-cancer properties.

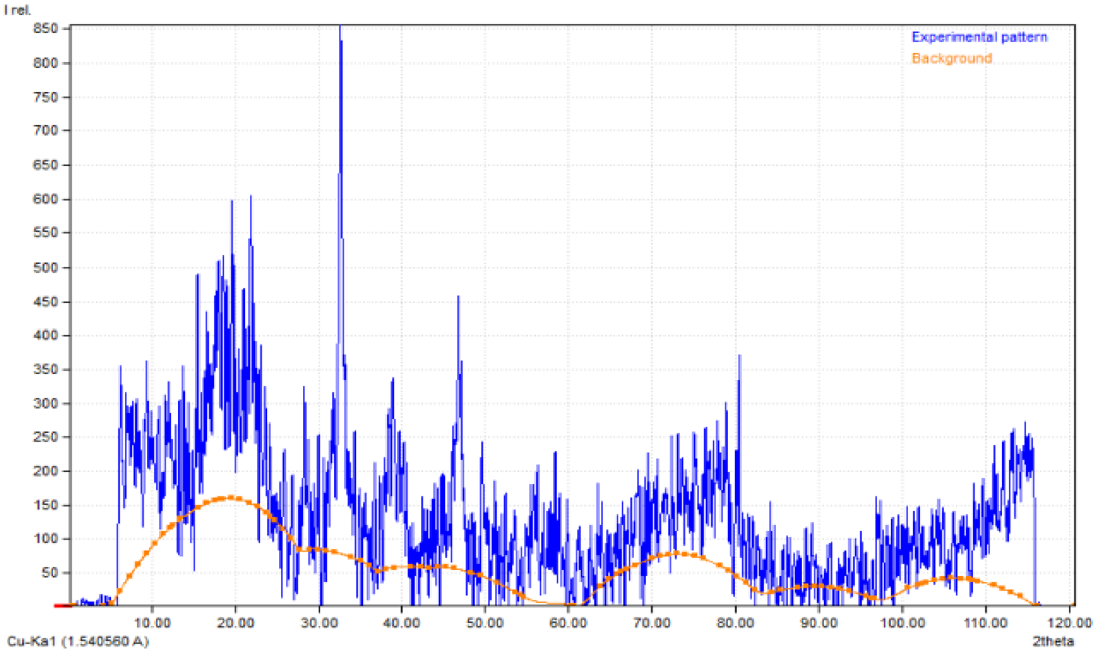


Figure 4.17 XRD analysis of synthesized nanoparticles of onion

XRD Results of Black Pepper

The XRD analysis of a sample labeled "Black Pepper" is shown in Figure 4.17. This sample shows, in fact, an associated with the integration of silver nanoparticles. The process of characterizing their possible anticancer properties includes this analysis. The graph shows 0.030° fine resolution with 9.128° - 120.596° for 3990 high-resolution analyses along with Cu-Kα (1.540560 Å) of wavelength. The above diffraction pattern graph indicates the intensity (I) plotted against the 2θ values. This helps in identifying the phases that are present in the material under investigation. The blue line in the above diagram represents the experimental data. The above diffraction pattern has sharp and distinct peaks. This is an indication that the material under investigation is crystalline in nature. The sharp peaks in the above diffraction pattern are an indication that the material under investigation has a good crystal structure. This is an indication of silver nanoparticles. The above diffraction pattern has sharp and distinct peaks at 2θ values of 38°, 44°, 64°, and 77°. The above diffraction pattern is characteristic of a face-centered cubic structure. The above diffraction pattern matches very well with the JCPDS value for silver nanoparticles. The JCPDS value for silver nanoparticles is around JCPDS No. 04-0783.

There are no major peaks indicating the presence of impurities or other phases apart from the silver phase. This indicates the relatively pure integration of the silver nanoparticles from the black pepper extract. These parameters may vary in the case of other extracts, depending upon the efficacy of the process of bio-reduction during the synthesis of nanoparticles. Silver nanoparticles synthesized by the use of plant extracts have unique properties such as a large surface area and potential biocompatibility. Understanding the crystallinity and phase composition of the silver nanoparticles is very important for the biological application of the silver nanoparticles synthesized by the use of plant extracts. Well-crystallized silver nanoparticles of specific phases and sizes are more effective in the biological application of nanoparticles in cancer therapies. The XRD analysis of the black pepper extract sample confirms the presence of well-defined silver nanoparticles, and the analysis using the crystallite size understands the nanoparticles' effectiveness in biological systems, including their anti-cancer potential.

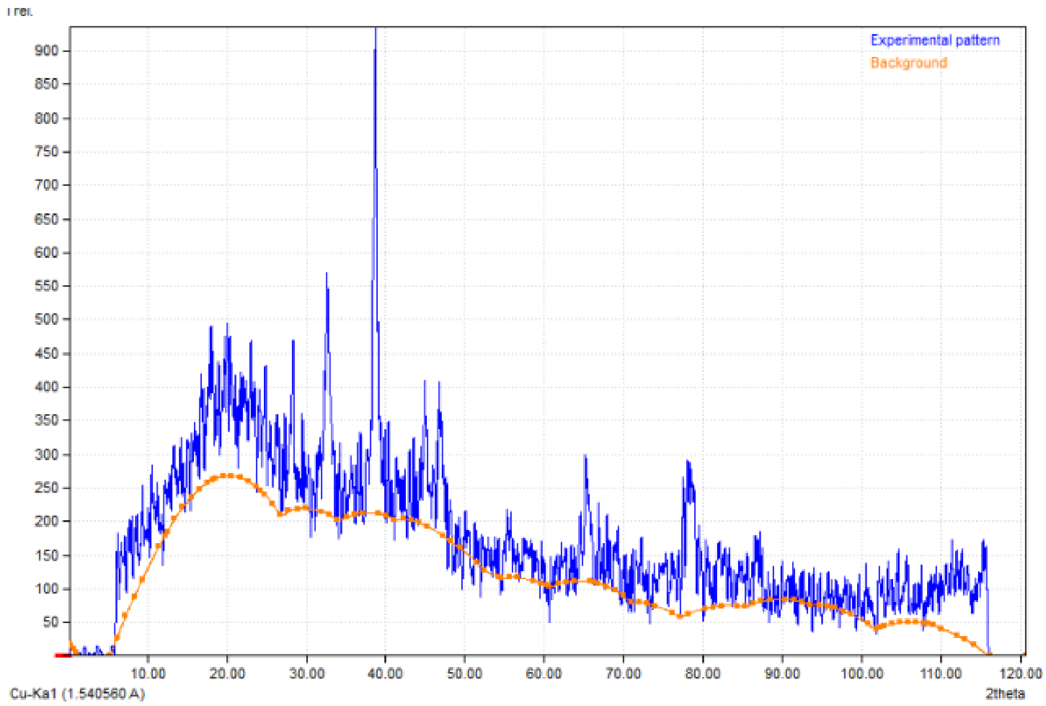


Figure 4.18 XRD analysis of synthesized nanoparticles of black pepper

Antimicrobial Activity Nanoparticles

The antimicrobial activity of nanoparticles using black pepper and onion extracts was evaluated against four bacterial strains, Bacillus subtilis and Escherichia coli. There was use of the diffusion of discs procedure where culture plates with sample discs were incubated for 24 hours at 37°C, and the areas of inhibition were subsequently identified. Its results indicated varying degrees of antibacterial efficacy. The largest inhibition zone was recorded against Escherichia coli with an average inhibition zone diameter of 13 ± 0.65 mm. For Bacillus subtilis, the inhibition zones were recorded at 9 ± 0.45 mm. It was observed that the antimicrobial activities of the nanoparticles were similar to that of silver nitrate but were less effective in comparison to streptomycin.

The specific role of biosynthesized silver nanoparticles in controlling bacterial growth and the possibility of this approach in the face of increasing antimicrobial resistance.

The mode of action involves interaction with the bacterial cell's nuclear content, leading to cell destruction and inhibition of bacterial proliferation. These findings underscore the potential of black pepper and onion nanoparticles as effective antibacterial agents against clinically relevant bacterial strains.

Anticancer activities of nanoparticles by MTT assay

MCF-7 cells were treated with four dilutions of silver nanoparticles derived from black pepper and onions (8, 16, 24, and 32 ppm) using the MTT colorimetric assay to observe their cell death rates. The silver nanoparticles demonstrated significant cytotoxicity for MCF-7 cells. The optimal anticancer activity was observed at 16 ppm, with a cell death rate of 43.7%. The cell death rates for 8 ppm, 24 ppm, and 32 ppm were 37.8%, 18.4%, and 8.24% for each. A negative control experiment was conducted without silver nanoparticles. Only 1.66% of the cells died. The results show that silver nanoparticles possess anticancer properties that are dose dependent. Therefore, they have the potential for treating MCF-7 cancer cells.

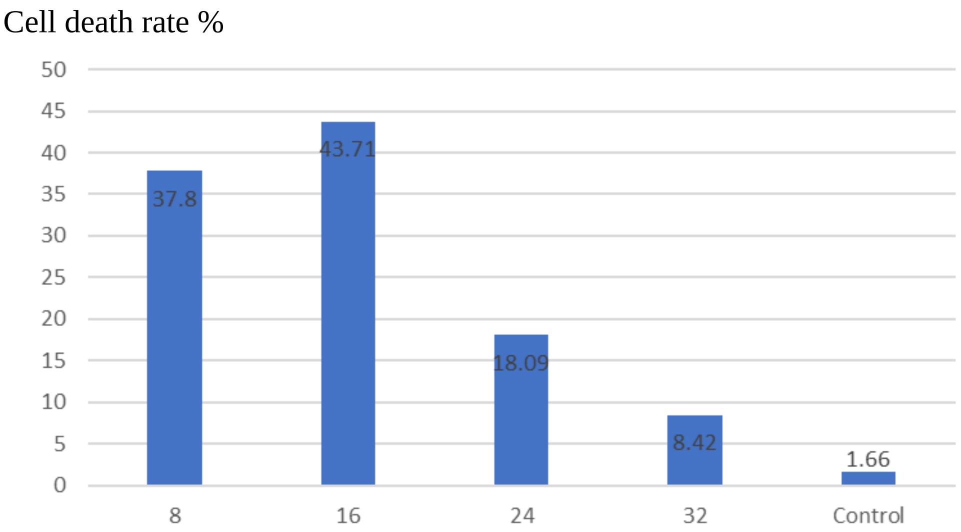


Figure 4.19 Dilutions of Silver Nanoparticles

Previous studies have shown that biosynthesized silver nanoparticles can have strong cytotoxic effects on different cancer cell lines. Nanoparticles made from various plant extracts have demonstrated different levels of effectiveness. Hence, the use of silver nanoparticles from black pepper and onion, in combination with miR34a mimics, could be an effective way of drug delivery in the treatment of human breast cancer cell lines.

CONCLUSION

These environmentally friendly nanoparticles have antimicrobial and anticancer properties and are useful for nanomedical, environmental, and industrial purposes. The silver nanoparticles are integrated by the green synthesis method, which is an environmentally friendly method for the integration of nanoparticles. The green synthesis method involves creating nanoparticles using natural compounds derived from plants like black pepper and onion. These botanical extracts have bioactive components that can reduce metal ions and create nanoparticles, as shown by the solution's color change. The prepared AgNPs can be characterized through various methods, including UV-Vis, FTIR, XRD, and SEM, to evaluate the prepared nanoparticles. The onion contains sulfur, which can form stable nanoparticles with high antimicrobial activity, whereas the black pepper is used to increase the stability of the AgNPs.

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