



Green Silver nanoparticles as Anticancer and antibacterial agent

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Abstract:

Nanotechnology can be useful in diagnostic techniques, drug delivery, sunscreens, antimicrobial sanitizer and a friendly manufacturing process that reduce waste products. Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The use of nanoparticles of some metals is common and useful in several fields due to the good properties of these nanoparticles. In this study silver nanoparticles were synthesized from aqueous silver nitrate (1 mM) through a simple and eco-friendly method using cinnamon as reductant and stabilizer. The resulting nanoparticles were characterized by UV–Vis spectrophotometer, zeta sizer, transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy. The antimicrobial and cytotoxic activities of the resulting nanoparticles have been investigated. The method can be used for rapid and ecofriendly. Green synthesis of stable silver nanoparticles of size 131 nm possessing antimicrobial cytotoxicity activities suggesting their possible application in the field of medicine.

Acknowledgement

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Introduction

Nanobiotechnology is an advanced technology in the fields of biological activity, for the synthesis of nanosized particles, which has a wide range of application in the field of health and medical applications. Green synthesis of nanoparticle is an important methodology that has been used in the synthesis of metallic nanoparticles such as silver nanoparticles, being an eco-friendly method (less toxic to human and environment), using different parts of any selected plants (having medicinal effect).

This study consists of three chapters, chapter one is an introduction, which provides a brief knowledge nanotechnology such as keywords and their definitions that are closely related to nanotechnology, synthesis and silver of nanoparticles. Chapter two contains the experimental section for green synthesis of silver nanoparticles and their effects in bacteria and cancer cell line. In Chapter three, we present and discuss the results of the present work for synthesized green silver nanoparticles and their characterization using many techniques such as : UV, FTIR, zetasizer, TEM and evaluation their anticancer and antibacterial activities.

Chapter one Background

1.1 Nanotechnology:

Nanotechnology is one of the technology sciences conducted in the nanoscale, which ranges from 1 to 100 nanometers.

Nanoscience and nanotechnology are the study and application of very small objects and can be used in all other scientific fields .

nanotechnology describe extra-high precision and ultra-fine dimensions.

Nanotechnologies are now widely considered to have the potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger and lighter materials. Nanotechnologies involve the creation and manipulation of materials at the nanometre scale.[1]

1.2 Nanoparticles:

Nanoparticles are defined as single particles of no more than 100 nanometers. The unique properties and properties of nanoparticles are due to their small size, chemical structure and surface structure. The characteristic properties and physical changes of different materials in nanoscale have given rise to the development of the properties of industrial products, resulting in a real and impressive increase in industrial and medical applications.

Nanoparticles have begun to find their way into our environment as a result of the unlimited use of nanotechnology products and nanomaterials.[2]

1.3 Silver and green silver nanoparticales:

Silver is a very special element that contains high thermal and electrical conductivity.

The light absorbs at a given wave length, leading to yellow, and silver is the most commonly used material in nanoscale.

Silver nanoparticles are one of the most commonly used nanomaterials in everyday life, and in research laboratories.

In nanoscale, silver exhibits remarkably unusual physical, chemical and biological properties. Due to its strong antibacterial activity.

Green Silver Nanoparticles are anti-cancer and have proven to be more effective and safer. In addition, bio-synthesis of nanoparticles is an environmentally friendly (green chemistry) method without the use of harsh, toxic and expensive chemicals. The production of nanoscale chemical reduction can also absorb

chemicals on the surfaces of nanoparticles that raise the issue of toxicity.

Green chemistry uses a set of principles that reduce or eliminate the use or production of hazardous substances in the design, manufacture and application of chemical products.

Green nanoparticles also use biological methods such as those involving microorganisms, plants, etc. to synthesize nanoparticles.[1,2]

1.4 Previous studies:

In this section we presented some of the studies reported that the green synthesis of silver nanoparticles and evaluation their effects as anticancer agent such as : Saratale et al. developed AgNPs from common medicinal plant dandelion, *Taraxacum officinale* and showed their high cytotoxic effect against human liver cancer cells (HepG2). The AgNPs Synthesized by Kuppusamy et al. Using *Commelina nudiflora* L aqueous extract showed a reduced cell viability and increased cytotoxicity against HCT-116 colon cancer cells. Green silver nanoparticles synthesized within different plant extracts of guava and clove showed the satisfactory anti-cancer effect against four different cancer cell lines; human colorectal adenocarcinoma, the human kidney, human chronic myelogenous, leukaemia, bone marrow, and human cervix. The developed silver nanoparticles using a proteinaceous pigment phycocyanin extracted from *Nostoc linckia* as reducing agent exhibited effective cytotoxic activity against MCF-7. The inhibitory concentration (IC₅₀) was $27.79 \pm 2.3 \mu\text{g/mL}$.³¹ Moreover, the chemically synthesized AgNPs composites possessed promising anticancer activity against the A549 (Human lung carcinoma), HeLa (Human cervical adenocarcinoma), MCF7 (Human breast adenocarcinoma), MDAMB231 (Human breast adenocarcinoma), and SKBR3 (Human breast adenocarcinoma) cells. Kuppusamy et al. successfully bio-synthesized silver

nanoparticles using the ethanolic extract of rose (*Rosa indica*) petals. The Ag functionalized extract proved potential anticancer activity against human colon adenocarcinoma cancer cell line HCT 15.42)[3, 4, 5, 6,7].

Chapter two Experimental

2.1 Synthesis of Silver Nanoparticles:

8 mg of silver nitrate has been added to 50 ml of distilled water under magnetic stirring conditions the temperature is 70 ° C for 15 min. Then 5 ml of cinnamon extract has been added to the AgNO₃ aqueous solution. The cinnamon extract has been prepared by adding 100 ml boiled distilled water to 5 g of cinnamon powder. The bio-reduction of the silver nitrate was then performed within 10 to 15 minutes then it was observed through visual observation that the color changed to dark brown this refers to formation of AgNPs.



Fig.(2.1): preparation of AgNPs.

2.2 Characterization of Silver Nanoparticles :

2.2.1 UV-Vis spectrophotometry:

UV and visible spectroscopy is a very useful technique that allows the estimation of the size, concentration and level of nanoparticles. In the spectroscopy of visible and ultraviolet radiation, molecules are exposed to electromagnetic radiation in the visible and ultraviolet fields, leading to the irritation and

stimulation of valence electrons such as electrons (p or d) In external orbits that is, they acquire energy and undergo electronic transmission within the energy levels of the molecule. The optical absorbance spectrum of the silver nanoparticles suspended in distilled water was obtained in the range of 200–800 nm[8,9].

2.2.2 Zeta-sizer Analysis:

By far the most important physical property of particulate samples is particle size. Measurement of particle size distributions is routinely carried out across a wide range of industries and is often a critical parameter in the manufacture of many products. Dynamic Light Scattering (sometimes referred to as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is a technique for measuring the size of particles typically in the sub micron region. DLS measures Brownian motion and relates this to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. Normally DLS is concerned with measurement of particles suspended within a liquid. The larger the particle, the slower the Brownian motion will be. Smaller particles are kicked further by the solvent molecules and move more rapidly. An accurately known temperature is necessary for DLS because knowledge of the viscosity is required (because the viscosity of a liquid is related to its temperature).

2.2.3 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR (is referred to fourier transform infrared spectroscopy) analysis can identify vehicles and the general type of material analyzed when there are unknown objects. This technique is used to evaluate the purity of certain inorganic and highly reliable samples to determine the composition of the polymer . They are also used to identify potential biomolecules responsible for the reduction of the Ag⁺ ions and the capping of the bio-reduced

AgNPs synthesized by plant extract. The FTIR test relies on infrared light to scan samples, and spectra are usually recorded at a wavelength of 4000 to 400 nanometers. The FTIR spectrometer generates a graph in the form of absorbance spectra, indicating the unique chemical bonds and molecular structure of the sample material. This spectrum will have a peak absorption of existing components. These absorption peaks refer to functional groups (eg alkanes, ketones, and acid chlorides). Different types of connectors, and thus different functional groups, absorb infrared from different wavelengths.[11]

2.2.4 Transmission electron microscopy (TEM):

Developed to achieve the highest picture quality and highest analytical performance, and it is one of the most common methods to obtain accurate data on the average size and distribution of nanoparticle particles, with a maximum magnification of one nanometer and provides a high-resolution image. The TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. Thus, TEMs can reveal the finest details of internal structure - in some cases as small as individual atoms. The screen below the column collides with a fluorescent screen; electrons collide into an electromagnetic wave (light), and the image of the sample appears. The thin sections from which the electrons are carried can be seen in light colors. Thus, the degree of contrast and clarity depends on the amount of electrons in the sample. The sample image can be printed on film by camera. The transmission electron microscope is a very powerful tool for material science. A high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as

the crystal structure and features in the structure like dislocations and grain boundaries. Chemical analysis can also be performed. TEM can be used to study the growth of layers, their composition and defects in semiconductors. High resolution can be used to analyze the quality, shape, size and density of quantum wells, wires and dots.[8]

2.2.5 Evaluation of cytotoxic effect:

The human breast cancer (MDA-MB231) cell will be characterized as adherent and continuous cell when grown in the optimised cell culture. It will be cultured at optimal growth conditions of 5% CO₂ in air atmosphere and 37°C. Cells were regularly checked using an inverted microscope. The total number of cells used in the different experiments was determined by the trypan blue exclusion test (0.4%) using a cell counter. Therefore, cell viability values of untreated cells should be 100% while values of treated cells have values bellow or above 100%. The following equation was used for calculations:

$$\text{Cell Viability (\%)} = \left(\frac{\text{Absorbance of individual treatment}}{\text{Absorbance of the control}} \right) \times 100$$

2.2.6 Microorganisms and antibacterial activity method:

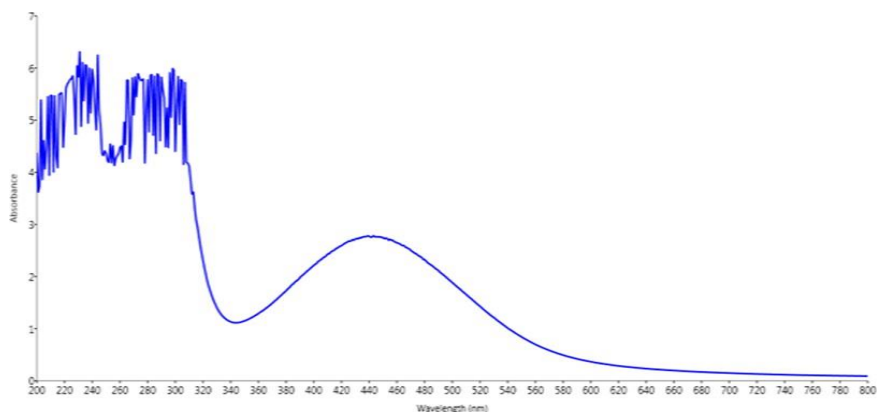
The antibacterial activities of green synthesized AgNPs were carried out by disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification, bacterial cultures were swabbed on these plates. The sterile discs were dipped in AgNPs and placed in the nutrient agar plate and kept for incubation at 37°C for 24 h. Zones of inhibition for control, were measured.

Chapter 3 Results and discussion

3.1 Visual observation and UV–visible spectroscopy:

Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR) [13].

The formation of silver nanoparticles was monitored with color change and UV–Vis spectroscopy. The color of the reaction mixture was changed to reddish after 1 h, indicating the formation of silver nanoparticles, due to the reduction of silver metal ions Ag^+ into silver nanoparticles Ag^0 via the active molecules present in the extract [14]. This color is attributed to



the excitation of SPR. As shown in Figure. 2 characteristic and well-defined SPR band peak for silver nanoparticles was obtained at around λ 440 nm [14].

Fig.(3.1): UV spectroscopy analysis of silver nanoparticles.

3.2 Size distribution analysis by DLS:

Particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution. This technique is commonly known as dynamic light scattering (DLS) was performed in aqueous solution to measure the size of AgNPs. It was found that the average size of AgNPs was 131.8 nm as shown in Figure. 3.

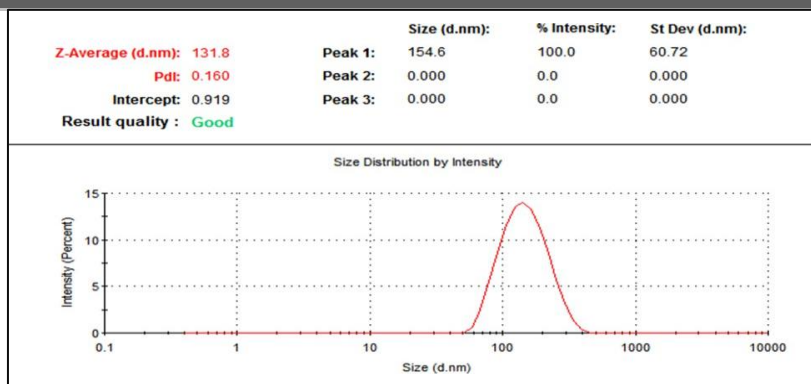
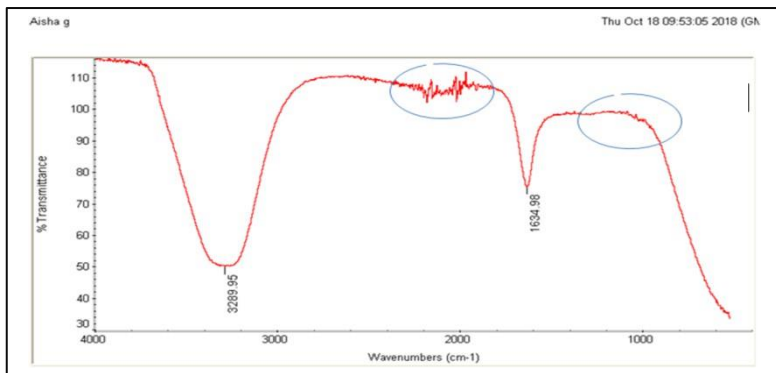


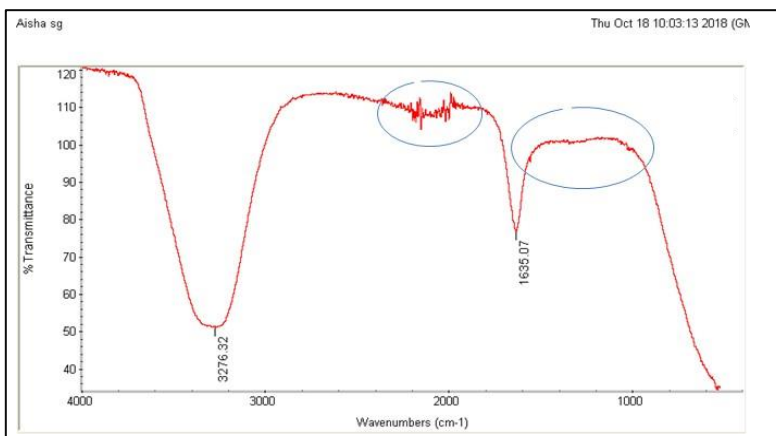
Fig.(3.2): the average size of AgNPs.

3.3 Fourier transform infra red (FTIR) measurements:

FT-IR spectra taken to detect the major functional groups in plant extract and their possible role in reduction process during the synthesis of AgNPs and stabilization of silver nanoparticles and spectrum peaks were compared with standard values to identify the main biomolecules present in the plant extract. From the comparison between the FT-IR spectra in Figure. 4A and Figure. 4B, there is a shift in the peak position of Figure. 4B, which clearly indicates the presence of residual plant extract compounds as the reducing and stabilizing (capping) agent to the Ag NPs, i.e. the spectrum shows that the Ag NPs' banding with some groups in Cinnamon extract compounds may cap the silver nanoparticles and form a layer on the surface of them which could result in reducing and stabilizing of the nanoparticles[15].



(A)



(B)

Fig.(3.3): FTIR spectra of (A): cinnamon extract; (B): green silver nanoparticle.

3.4 Morphology analysis of AgNPs by transmission electron microscopy(TEM):

The transmission electron microscopy (TEM) image of AgNP synthesized by cinnamon extract are represented in Figure. 5 and indicates well dispersed particles which are spherical shape.[16].

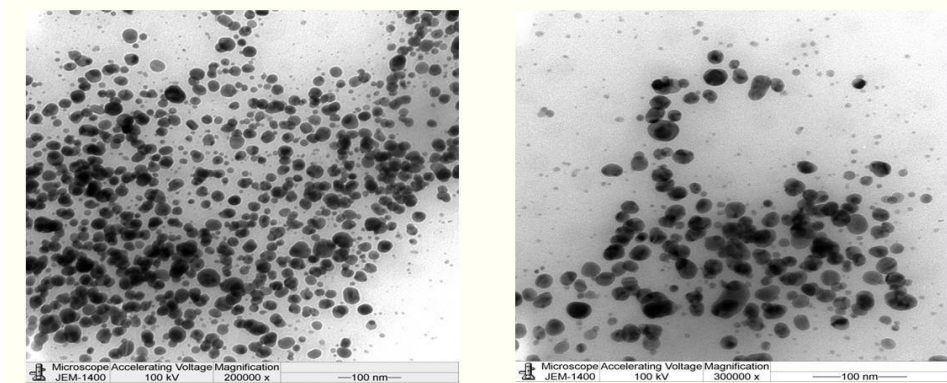


Fig.(3.4): TEM images of the silver nanoparticles.

3.5 Cytotoxicity results:

The in vitro cytotoxic effect of silver nanoparticles against breast cancer cell lines (MCF-7) and cell inhibition (%) was carried out by MTT assay. The anticancer activity increases as the concentration of the nanosilver increases and the cell viability decreases 100% to 41% as shown in figure.6 [17].

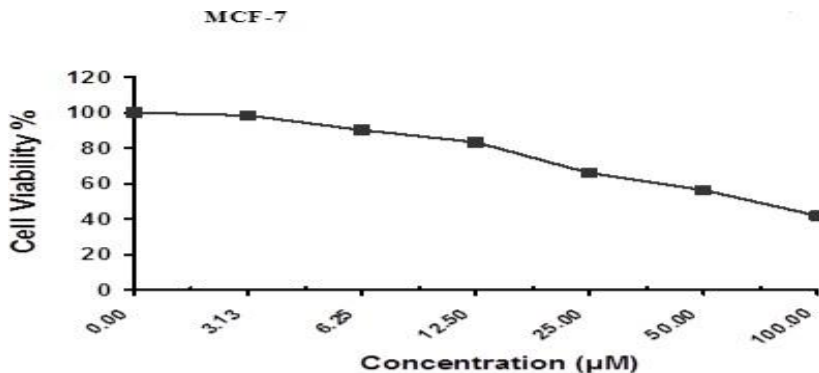


Fig.(3.5): Cytotoxicity effect of synthesized AgNPs on MCF-7 cell line.

3.6 Antimicrobial activity of silver nanoparticles:

Silver nanoparticles displayed antimicrobial activity against studied pathogenic microorganisms, as shown in Figure.7(1,2 and 3). The Gram negative bacteria ((2)E. coli and (3)P. aeruginosa) showed larger zones of inhibition, compared with

the Gram positive bacteria ((1)S. aureus) , which may due to the charge of silver nanoparticles [18].

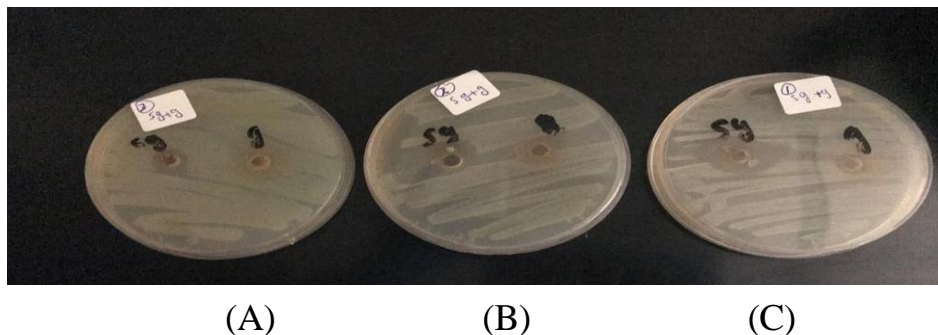


Fig.(3.6): Zone of inhibition of cinnamon silver nanoparticles against various pathogenic microorganisms((A) *Pseudomonas aeruginosa*, (B) *Staphylococcus aureus* (C) *Escherichia coli*).

3.7 Conclusion and recommendations

This study presented the rapid synthesis of green AgNPs using cinnamon extract and their use as an anti cancer and antibacterial agent. The used green method here is non-toxic, environmentally friendly, simple, low cost and has no toxic chemicals. The formation of AgNPs was determined by UV-Vis spectroscopy where surface plasmon absorption maxima can be observed at 440nm from the UV-Vis spectrum. Zitasizer shows the average size of the produced nanoparticles to be 131 nm. The resulting AgNPs were characterized using FT-IR spectroscopic and TEM techniques.

The successfully green synthesized AgNPs showed anti cancer and antibacterial activities this may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like bandages.

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