GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *C.ROSEUS* PLANT EXTRACT ON BREAST CANCER CELL MCF-7

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ABSTRACT

To develop a simple rapid procedure for bioreduction of gold nanoparticles (AgNPs) using aqueous plant extracts of Catharanthus roseus (C. roseus) Characterization were determined by using UV-Vis spectrophotometry, Field Emission scanning electron microscopy (FESEM), energy dispersive X-ray and X-ray diffraction. FTIR, DLS, MCF-7 Brest cancer. The result reveals FESEM showed the formation of gold nanoparticles with an average size of 25 nm X-ray diffraction analysis showed that the particles were crystalline in nature with face-centered cubic geometry. Dynamic light scattering analysis reveals the average particle size 25nm and FTIR Analysis reveal the functional group and biomolecules are present the gold nanoparticles. *C. roseus* demonstrates strong potential for the synthesis of Gold nanoparticles by rapid reduction of gold ions (Au^+ to Au^0). This study provides evidence for developing large scale commercial production of value-added products for biomedical/nanotechnology-based industries.

Keywords---Gold nanoparticles, XRD,FTIR,FESEM,Antibacterial Activity.

INTRODUCTION

Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field are growing rapidly throughout the world. A major output of this activity is the development of new materials on the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension of fewer than 100 nanometers (nm), even the particles could be zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties [1,2]. Nanoparticles exhibit completely new or improved properties compared with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution, and morphology of the particles. Nanoparticles present a higher surface area to volume ratio with a decrease in the size of the particles. Specific surface area is relevant to catalytic activity and other related properties such as the antimicrobial activity of Au NPs [3-5]. The use of nanoparticles is gaining importance in the present century as they possess definite chemical, optical and mechanical properties. Metal nanoparticles are of importance due to their potential applications in catalysis, photonics, biomedicine, antimicrobial activity and optics [6,7].

As the specific surface area of nanoparticles is increased, their biological effectiveness can also increase on the account of a rise in surface energy. Nanoparticles of noble metals, such as silver, gold, and platinum are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. A number of approaches are available for the synthesis of silver nanoparticles for example facile method [7], thermal decomposition of silver compounds [8], electrochemical [9], sonochemical [10], microwave-assisted process [11] and recently via green chemistry route [12]. Unfortunately, many of the nanoparticles synthesis or production methods involve the use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticles synthesis without using toxic chemicals. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Extracts from it have been used to treat numerous diseases, including Diabetes, Malaria, and Hodgkin's disease. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. Previous studies of Shankar and others have shown the rapid synthesis of gold nanoparticles with neem (Azadirachta indica) leaves and sun-dried C. camphor leaves. They attributed the bioreduction capability to a water-soluble heterocyclic compound found in the plants [13]. Chemical tannins have been used as a reducing agent for metallic gold to its nanoparticle size and the presence of phytochemical tannins in the aqueous extracts of this plant suggest their ability to reduce as suggested by [14].

UV- VIS SPECTROMETRY:

OPTICAL STUDIES

The synthesis of the reduced metal nanoparticles (gold) in the colloidal solution was monitored by UV-Vis spectrophotometer analysis. UV-Vis spectroscopic technique is one of the simplest techniques to identify the formation and stability of the gold nanoparticles in aqueous solution. Gold nanoparticles were known to exhibit maximum absorption in the range of 500-600 nm respectively. The synthesis of gold nanoparticles was monitored by observing the UV-VIS spectra after 15 min. of the addition of metal salt solution to an aqueous plant extract and stirring the mixture for half an hour. By marking the difference in the UV-VIS spectra of the plant extract and the colloidal solution obtained after adding the salt solution demonstrated the synthesis of nanoparticles (fig. 1). Maximum absorption at around 540 nm to which Auric chloride solution was added marked the synthesis of gold nanoparticles.

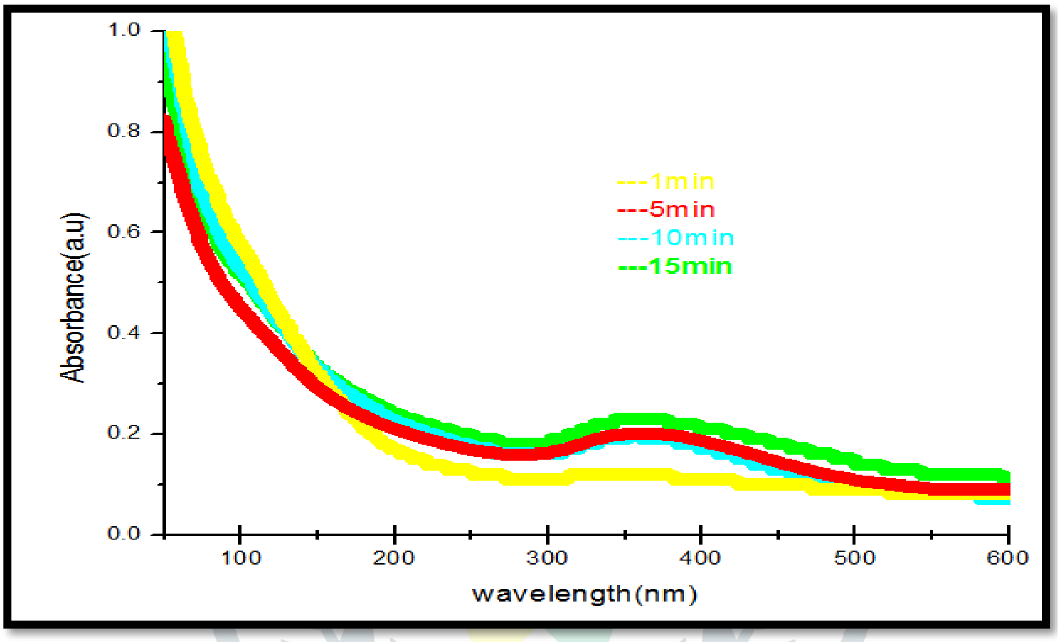


Fig:1 UV-Vis analysis for gold nanoparticles using C.leaf extract in different time duration

FTIR – SPECTRUM OF LEAF EXTRACT AND LEAF EXTRACT MEDIATED AUNPS:

The FTIR spectroscopy profile of leaf extract and AuNPs synthesized using leaf extract is shown in Fig 2 The results of infrared spectroscopy of gold nanoparticle samples using plant Catharanthus Roseus extracts similar peak as that of the plant leaf extract in the ranges around 3444cm^{-1} corresponding to O-H stretching group of Alcohols presence of water molecules. The distinct peaks in the range of 2937 cm^{-1} related to the C–H stretching group of Alkanes in carbonyl groups respectively. The weak band at 2370 cm^{-1} corresponds to C ≡ C stretching group of Alkynes and the peak relating to 1396 cm-1 corresponds to the C – N stretching group of Aliphatic Amines. The IF spectrum of leaf extract also showed two other peaks at 1139 cm-1 and 667cm-1 corresponding to C–O stretching group of Alcohol and C – Br stretching group of Alkyl Halides. Likewise, from the FTIR spectrum of the AuNPs peaks at 1016 cm-1and 572 cm-1. corresponds to C – F stretching group of Alkyl halides and C – Br stretching group of Alkyl halides respectively. The stronger absorption band at 1621 cm^{-1} was recognized and 560 cm^{-1} indicated gold nanoparticles. The presence of amide linkage endorses the existence of the proteins in the reaction mixture. This protein might force the structural variations designated for the reduction and stabilization of gold nanoparticles.

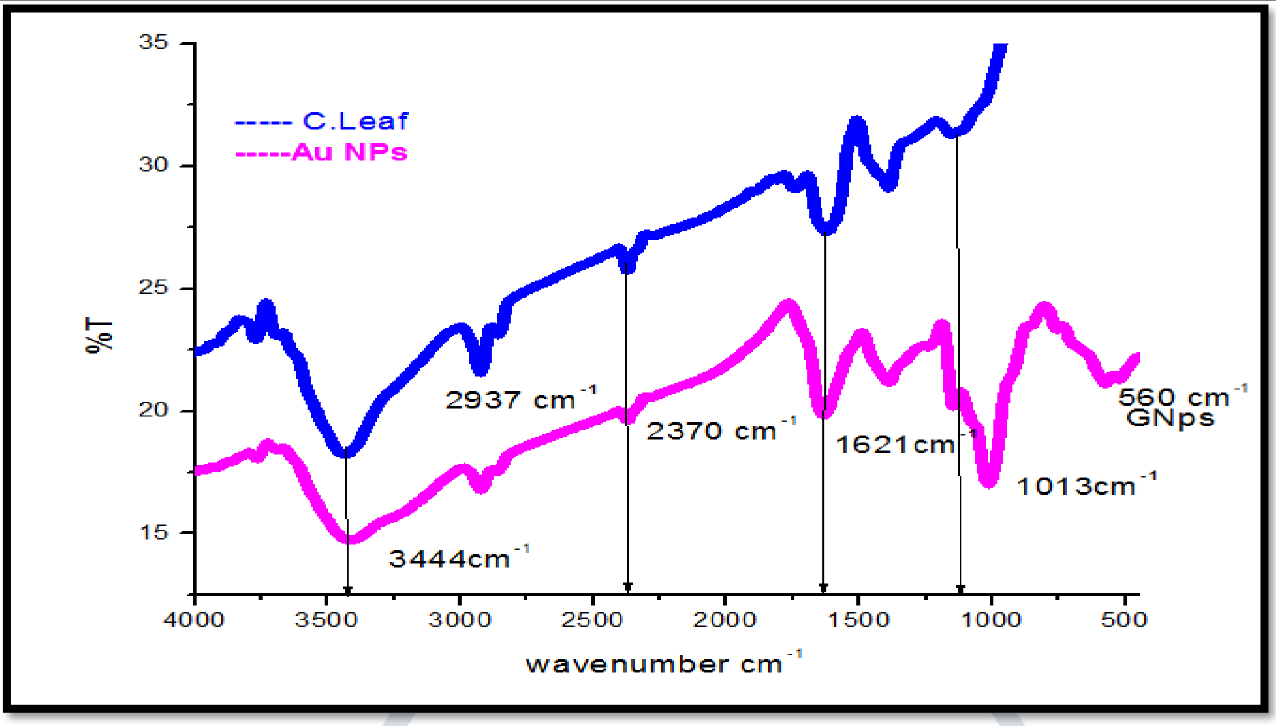


Fig:2 FTIR analysis of gold nanoparticles using C.leaf extract mediated gold nanoparticles.

XRD ANALYSIS

The AuNPs synthesized using leaf extract of C. Roseus plant was reveal to X-Ray diffraction characterization to study the exact size and nature. In Fig :3 The X-Ray diffraction pattern of Synthesized AuNP shows the strong and narrow diffraction peaks which indicates that the sample is well crystalline in nature. The XRD peaks at 38◦, 44◦, 64◦ and 77◦ can be indexed to the (2 0 0), (2 2 0) and (3 1 1), (1 1 1) Bragg's reflections of the cubic structure of gold respectively (JCPDS No. 04-0784). The broadening of Bragg' peaks indicates the formation of NPs. Nearly monodispersed Au NPs with controllable size and uniform shape can be easily obtained in the simple aqueous reduction method. The mean size of Au NPs was calculated using the Debye–Scherrer's equation.

$$D= K\lambda / \beta \cos \theta$$

where,

K – Scherrer formula, Dimensionless shape factor, and its value are close to unity.

A typical value is 0.9

λ – X-Ray wavelength, its value is 1.540598 Å

β – Full-Width Half Maximum (FWHM)

θ – Bragg's angle.

FESEM ANALYSIS AND EDAX ANALYSIS:

The surface morphology of AuNPs was studied through FESEM analysis. The AuNPs are found to be AuNPs are irregular in shape and appears as flakes like structure. The FESEM images of synthesized AuNPs at different magnifications are shown in Figure: 4a FESEM-EDS examination of particle composition of AuNPs was performed by inserting a droplet (~5 μL) of the AuNP colloidal solution onto a clean silicon-wafer surface. In Fig: 4b presents the EDS spectra obtained for AuNPs synthesized using leaf extract of C. Roseus plant. The results clearly show that the particle composition was indeed gold. Indeed, elemental mapping for AuNPs synthesized using leaf extract of C. Roseus plant showed a uniform distribution of gold particles.

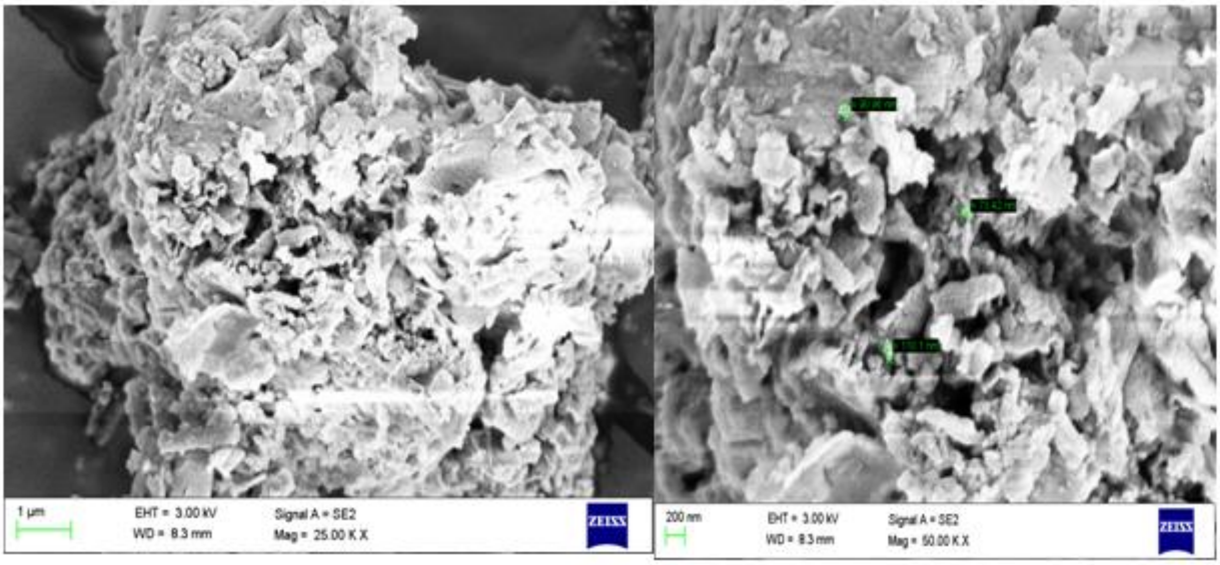


Fig:4a FESEM analysis for Gold nanoparticles in flakes like structure

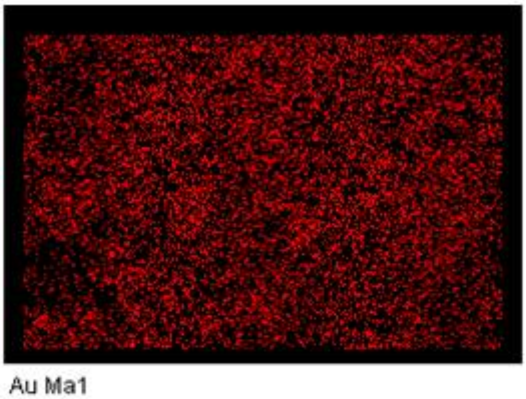
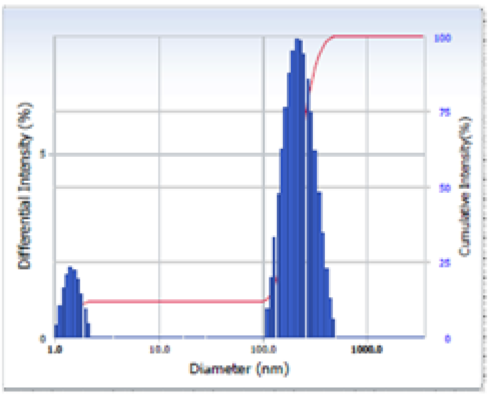


Fig:4b EDAX Mapping analysis for gold nanoparticles

DLS ANALYSIS:

The size-distribution capacity of the hydrodynamic diameters of the colloid gold Nanoparticles synthesized using roseus plant leaf extract was calculated by the light scattering-based method. The DLS apparatus is recognized to measure the thickness of the capping or stabilizing agent encompassing the metallic particles together with the actual size of the metal core. The size distribution vs. intensity graph has been shown in Fig.5 the average size for colloid gold nanoparticles using roseus plant leaf extract was found to have a maximum diameter of 100nm. It can be found from the graph that nearly 25% of the particles are found a diameter as small. Besides, the bulky particle could be formed due to van der Waals forces of interaction that exist in the solution.



IN VITRO ANTIBACTERIAL ACTIVITY:

The antibacterial activity for AuNPs synthesized using leaf extract of C. Roseus plant was determined by the disc diffusion method against the human pathogens (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi*) on Nutrient ager, according to the Clinical and Laboratory Standards Institute (CLSI). The media plates (NA) were streaked with bacteria 2-3 times by rotating the plate at 70˚ angles for each streak to ensure the homogeneous distribution of the inoculums. After inoculation, discs 10 mm film, of the test samples were placed on the bacteria-seeded plates. The plates were then incubated at 36 ˚C for 24 h.

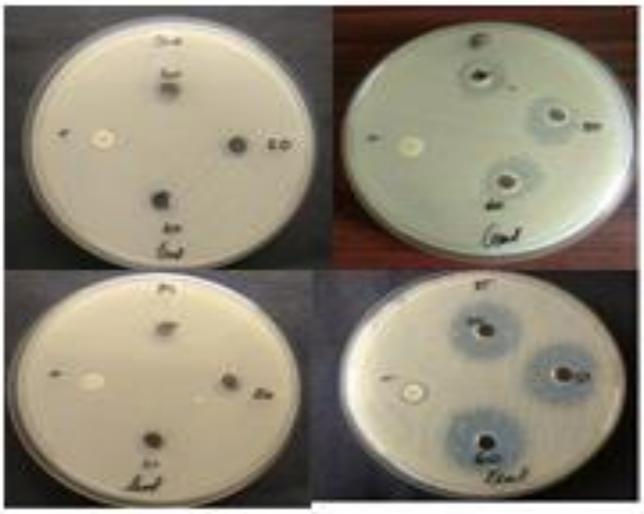
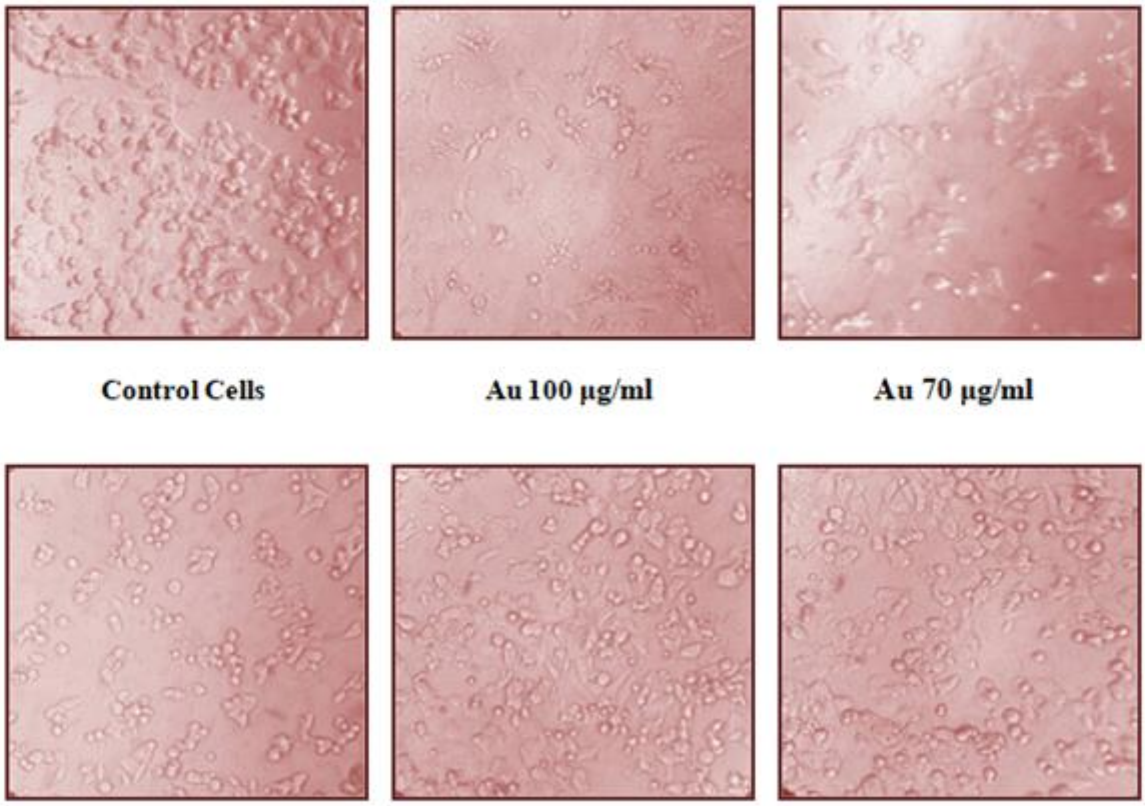


Fig:6 Antibacterial assays for gold nanoparticles.

Concentration	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	Salmonella typhi
40	13	13.5	14	21
50	13	14	14.5	22
60	14	14.5	15	22
Amoxicillin	14	9.5	14	14

MTT ASSAY FOR SCREENING OF ANTI-CANCER ACTIVITY:

The 3-(4,5–dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) dye reduction assay was conducted to diagnose the cytotoxicity study of the synthesized silver nanoparticles. MCF-7 cells were plated onto 48 wells plates, 18 hours before the commencement of the test. Growth medium used was DMEM with 10% Fetal Bovine Serum. The plates were incubated in an animal cell culture incubator, maintained at 370 C with 5% carbon dioxide. The wells achieved 70% confluency at the time of testing. The original growth medium in the 48 well plates was removed and the sample (synthesized silver nanoparticles with varying in concentrations (25 µg/ml, 50 µg/ml and 100 µg/ml) were added to the wells. The plates were returned to the incubator for 24 hours. At the end of 24 hours, the media in the wells were carefully removed and fresh complete growth media was added. To each well, MTT solution (5 mg/ml of MTT dissolved in PBS) was added and replaced in the incubator for 3 hours. After 3 hours, the medium wascarefully removed from the wells, and DMSO was added to each well and kept on a rocking platform for efficient mixing and extraction of formazan dye from the cells by DMSO. After 30 minutes, the absorbance of the DMSO was measured at 570 nm, in a multi-well spectrophotometer.



CONCLUSION

This facile technique aimed at the green synthesis of gold nanoparticles using Catharanthus roseus plant extract has several benefits such as cost-effectiveness, bio- compatibility and is nontoxic. The practice of plant extracts meant for making metallic nanoparticles is economical, non-toxic and eco-friendly. The plant extract-based synthesis can deliver NPs of precise dimensions and morphology. In the existing work, we describe a modest and innovative method for the biosynthesis of AuNPs by the reduction of aqueous $AuCl_4$ ions using Catharanthus roseus plant extracts. Structural analysis by XRD strongly puts forward the formation of elemental AuNPs with an average crystal size of around 25 nm. The FTIR and UV studies similarly confirm the occurrence of AuNPs. The size distributions of the gold NPs measured using dynamic light scattering method confirmed their reduction to nanoscale with particles distributed in the range around approximately 5nm and 100nm respectively. The elemental mapping for AuNPs synthesized using leaf extract of C. Roseus plant showed a uniform distribution of gold particles. Further, the plant-mediated AuNPs have appreciable cytotoxicity against human pathogens such as Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi and showed an excellent cytotoxic effect on MCF -7 breast cancer cells.

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