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CHARACTERISATION OF SYNTHESIS OF SILVER NANOPARTICLES FROM BARK EXTRACT OF *BUTEA MONOSPERMA VAR. LUTEA* AND THEIR ANTICANCER ACTIVITY ON *HELA* CELL LINE

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ABSTRACT
Butea monosperma var. *lutea* is commonly known as the yellow flame of the forest. It is a medium-sized dry season-deciduous tree growing to 49 ft tall but taller than well-*known Butea monosperma.* The synthesis of AgNPs using bark extract of *Butea monosperma* var. *lutea*, which is used at room temperature as both a reducing and capping agent. The mixture for the reaction turned brownish yellow after about 24 h and an intense surface plasmon resonance (SPR) band at around 406 nm clearly indicates the formation of silver nanoparticles. The presence of stabilizing silver nanoparticles is shown by a UV – Visible spectrophotometry. For nanoparticles, surface. Fourier Transform-Infrared (FT-IR) spectroscopy showed that the nanoparticles were capped with the compounds present in the plant extract. Moreover, these biologically synthesized nanoparticles have also shown an outstanding cytotoxic impact on *HeLa* cells line by MTT assay.

KEYWORDS: Silver Nanoparticles, Bark Extract, UV – Visible Spectroscopy, FTIR, *HeLa* Cell line, MTT

INTRODUCTION
Compared to those of atoms, molecules and bulk materials of the same substance, due to their remarkable difference in structural and physical characteristics, nanomaterials, especially nano-scale noble metals, attract a lot of interest. The advancement of this area has given rise to a new technology known as 'nanotechnology' in recent years, this gives us a technology and process for study and its application to biological nanoscale systems. Different aspects of interesting characteristics, such as optical, catalytic, etc., are shown by nanostructured materials, which greatly depend on the size and shape of nanoparticles. Due to their exceptional electro-catalytic activity[2], metal nanoparticles have tremendous utility in electrochemical, electro-analytical and bio electrochemical applications. Therefore, in the area of interdisciplinary research, nanotechnology is an emerging field with applications in biology. A class of materials in the range of 1-100 nm in size are silver nanoparticles (AgNPs). Due to their distinctive and attractive physical, chemical, and biological properties, the interest in the study of AgNPs concerning their different behaviours has recently increased.[1]

Using plant material as a reducing agent for the synthesis of silver nanoparticles has many advantages. These benefits include: easy usability, handling safety, cost-effectiveness, very low maintenance costs and eco-friendliness.[7] It has different metabolites that enhance the reduction response, resulting in rapid synthesis of Nanoparticles and can serve as a capping agent that for further uses produces very stable nanoparticles.[5] These Phyto-compounds offer the versatility needed for better control over the nanoparticles' size and shape, and finally, it is a one-step procedure that further eliminates the formulation process's complexities. The diverse group of plant extracts have dual properties, such as the reduction and stabilization of phytochemicals and other plant derivatives (e.g. starch, dextran, alginate, cellulose, chitin, etc. Reduction reaction takes place due to the presence of biocatalyst in the form of specific parts plant extract.

Cancer, the one of leading cause of death worldwide, is a group of more than 100 diseases that can affect any part of the body, characterized by uncontrolled cellular growth. chemotherapy is now being used as a standard treatment. The physical and chemical treatments of cancer are limited at different stages. However, currently

available therapies have an adverse effect and affect normal cell functions while giving excess drug and radiation exposures.[18,19] A marginal increase in cancer cases within the last few years ends up mostly, with death.[20] Search for anticancer agents from natural product has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activities a number of plants have been explore. The utility of these plants is increasing day by day. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced.[4] The toxic effect of conventional chemotherapy and drug resistance properties creates an urgent need for the growth of alternative cancer therapy.

Cancer cells are cells that divide relentlessly, forming solid tumors or flooding the blood with abnormal cells. Cell division is a normal process used by the body for growth and repair. A parent cell divides to form two daughter cells, and these daughter cells are used to build new tissue or to replace cells that have died because of aging or damage. Healthy cells stop dividing when there is no longer a need for more daughter cells, but cancer cells continue to produce copies. They are also able to spread from one part of the body to another in a process known as metastasis.[1] To study the biology of cancer and to assess cancer therapies, cancer cell lines are used in science.

The plant *Butea monosperma* var *lutea* is selected in this article for nanoparticle synthesis as our biological material. In the state of Gujarat, primarily in the Ahmedabad district, it is widely accessible. We removed the bark from the plant and used it for the synthesis of silver nanoparticles as a reducing and capping agent. The present investigation deals with *Butea monosperma* var. *lutea* bark extract mediated synthesis and their characterisation of silver nanoparticles as well as their anticancer activity and cytotoxicity against *HeLa* cell line.

MATERIALS AND METHODOLOGY

The present study "Synthesis of silver nanoparticles from *Butea monosperma* var. *lutea* and their anticancer activity and cytotoxicity against *HeLa* Cell Line" was conducted at the Botanical Garden, Department of Botany, Gujarat University.

Plant Materials

The experiment was conducted at Department of Botany, Bioinformatics and Climate Change Impacts Management for all the experimental studies *Butea monosperma var. lutea* was used through the study. The Bark of *Butea monosperma* var. *lutea* were used and its collect from the Botanical Garden of Geer National Park, Indroda Park, Gandhinagar.

Chemicals and Apparatus

In all tests, including silver nitrate ($AgNO_3$), pure and analytical grade chemicals have been purchased from Himedia laboratories Pvt. Mumbai, India, Ltd. For Anticancer activity and Cytotoxicity analysis, the HeLa cell line were obtained from National Canter for Cell Science (NCCS), Pune. Distilled water and Mili – Q water was used throughout for the synthesis. Conical flasks, measuring tubes, beakers, pipettes, glass vials, etc. have all been bought from Borosil, India. Forceps, Scalpels, filter paper, blotting paper, Micro Pipette, (1ml, 0.5 ml) etc. were used during the experiment.

Culture Media for Anticancer activity

The *HeLa* cell line was cultivated for in vitro experiments. The National Centre for Cell Sciences, Pune, India, obtained this cell line. It was grown in medium RPMI 1640, supplemented with 10% fetal calf serum, 100µg/mL penicillin and 100µg/mL streptomycin, 4 mM L-glutamine 5% CO2 and 95% humidified 37℃ atmosphere. Until the number of cells reached 1.0 × 106 cells/mL in the logarithmic growth process, cells were cultured and maintained.

Synthesis of silver nanoparticles from Bark extract of *Butea monosperma* var. *lutea*

Preparation of Bark Extract from *Butea monosperma* var. *lutea*

To extract all impurities, Bark of the collected *Butea monosperma* var. *lutea*, First, under tap water and then in distilled water, the plant was thoroughly rinsed. This was then dried and thoroughly grinding by air to form a uniform mixture used in the analysis. In order to obtain 5 percent (w/v) of concentrated extract, In the 100 mL beaker, about 5 g of the mixture was soaked in Mili - Q water. With the aid of Whattman filter paper no. 1, After 24 h, filtration was carried out and eventually a bright orange-colored extract was collected. It was stored for further use in a refrigerator. For further experiments, the extract was stored at 40℃.

Synthesis of Silver nanoparticles from Bark extract.

The 1mM silver nitrate ($AgNO_3$) aqueous solution was prepared and used for the synthesis of silver nanoparticles. 1 ml extract of bark from *Butea monosperma* var. *lutea* was applied to 10 ml of 1 mM silver nitrate aqueous solution for the reduction of Ag+ ions and held at room temperature for an incubation time of 24 h. The filter serves as a reduction and stabilizing agent for 1 mM of $AgNO_3$ here. The characterisation of silver nanoparticles was done by different analysis. First the formation of silver nanoparticles was confirmed after 24 hours by UV –Visible spectrophotometer. The purified, dried, solid powder of silver nanoparticles was subjected to spectroscopic Fourier Transform-Infrared (FT-IR) Measuring.

The detailed methodology of the analysis is as follow
UV – Visible Spectrophotometer
The Formation of Silver Nanoparticles were confirmed by Shimadzu UV – 1800 an advanced high – resolution spectrophotometer, a precision Czerny – Turner optical system. Operation can be either as a stand-alone instrument or as a PC-controlled instrument with the included UV Probe software. Data processing operations, such as the enlargement or reduction of the spectra obtained, peak detection, and area calculations, were also possible. The scanning range for the samples was 200-1100 nm at a scan speed of 480 nm/min. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "LCD (4.75" × 3.5")". The results show the presence and reduction of silver nanoparticles in the tested sample.

FTIR (Fourier Transforms Infrared Spectroscopy)
FTIR was used to classify the potential functional groups responsible for synthesizing bio-reduced silver nanoparticles for the reduction of the Ag ions and capping. FTIR analysis was performed to determine the functional groups and their potential role in the synthesis of silver nanoparticles. For FTIR analysis, the Liquid Nanoparticles solution was used. In the range of 4000-400 cm^{-1} using KBr pellets, FT-IR spectra were reported on a Bruker TENSOR-27.

Anticancer activity and Cytotoxicity (MTT assay)
For *in vitro* experiments, the HeLa cell line was cultivated. This cell line was obtained from National Centre for Cell Sciences, Pune, India, obtained this cell line. It was cultivated in a medium RPMI 1640 supplemented with 10% fetal calf serum, 100 units/mL penicillin, 4 mM L-glutamine with 5% CO2 and 100 µg/mL streptomycin and 95% humidified atmosphere at 37℃. HeLa cells were cultured and maintained until the number of cells reached 1.0 to 106 cells/mL in the logarithmic growth process. Several doses (0.1, 0.5, 0.10, 1) of AgNPs. From these observations, the Ag NPs have been shown to be stable at pH 7.2. The 10 mg/mL stock of Ag NPs was prepared and sonicated for 15 to 20 min with the dissolution of 10 mg of Ag NPs into PBS. It was then serially diluted with RPMI media in order to prepare working concentrations. For in vitro anticancer activity evaluation in this study, all of these doses were charged against *HeLa* cell lines.

Table I: Culture media for treated Hela Cell line with Different Doses of AgNPs.

Sr. No.	Different Concentration of AgNPs (µg/ml)						
	control	**0.1ml**	**0.5ml**	**0.10ml**	**1ml**	**5ml**	**10ml**
HeLa Cells	Cells + Media for Culture	Cells + Media for culture + 0.1ml AgNPs	Cells + Media for culture + 0.5ml AgNPs	Cells + Media for culture + 0.10ml AgNPs	Cells + Media for culture+ 1ml AgNPs	Cells + Media for culture + 5 ml AgNPs	Cells + Media for culture +10 ml AgNPs

HeLa cells have been treated with various AgNPs concentrations of 0.1,0.0. For 24 hours, 5,0.10, 1, 5, and 10 µg/mL. The cells were broken down into 7 groups. There were seven petri dishes in each group. Cell numbers were preserved at 2 × 106 cells/petridish in any treatment collection. For the experiment, the following groups were considered and cultured for 24 h After 24 h of treatment, the cells were separately extracted from petridis and centrifuged at 2200 RPM for 10 min at 4℃ in order to isolate the cells and the supernatant medium (51). The cells were subsequently washed twice with 1 PBSS (50 mM). Intact cells were used for the assessment of cell viability under different microscopic observations. In triplicates, the experiment was completed.

The cytotoxicity of Silver nanoparticles has been quantitatively tested by a non-radioactive, colorimetric assay method using tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphe nil-tetrazolium bromide (MTT) (51). In short, the 5 mg/mL MTT solution was prepared and filtered by dissolving MTT in phosphate buffered saline to remove the small quantity of insoluble residue present in some batches. Then MTT solution, containing cells (2 × 10 -6/well) and maximum growth medium, with or without the silver nanoparticles, was directly applied to all 96 well plates. To metabolize the MTT to formazan, it was then incubated for 5h at 36℃. The supernatant was subsequently aspirated and added 100 µL of HCl-isopropanolic solution (1:1) to each culture plate and thoroughly mixed to dissolve the dark blue crystals. The optical density (OD) of the samples was measured on the reader using 500 and 700 nm tests and wavelengths, respectively.

RESULTS AND DISCUSSION
The results of each of these aspects are presented and discussed here with considering observations and pooled observations for different characters under study.

Synthesis of silver nanoparticles from *Butea monosperma* var. *lutea*
The colour of bark of *Butea monosperma* var. *lutea* was light yellow in normal milli - Q water due to excitation of surface plasmon resonance. The color was changed from light yellow to dark brown after the addition of $AgNO_3$ solution due to the reduction of Ag+, which indicates the formation of Ag nanoparticles shown in figure – 1.

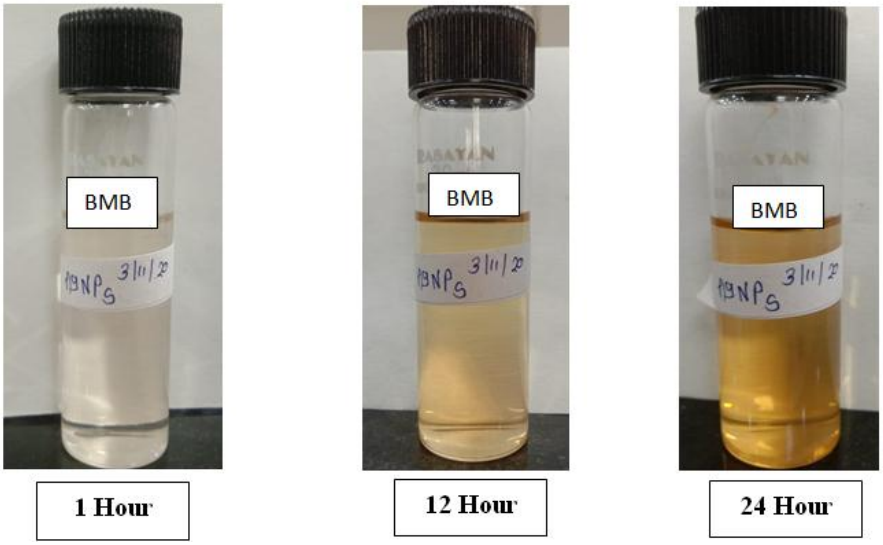


Figure: A. Synthesis of Silver Nanoparticles.

CHARACTERISATION OF SILVER NANOPARTICLES

UV – Visible Spectrophotometry Analysis

Absorbance of silver nanoparticles synthesized from *Butea monosperma* var. *lutea* extract was monitored by UV-visible spectroscopy after 1 h. Light yellow coloured solution of silver nanoparticles was obtained from colourless $AgNO_3$ solution and the Nanoparticle formation was established. The colour of the two solutions could be easily distinguished from the inset picture. The peak at 211 nm in both the spectra in Fig. 1 confirmed the presence of amino acid residues.[56,57] The amino acid residues present in the extract presumably play a vital role for reduction as well as stabilization of nanoparticles.[58] The intense yellow color of the resultant solution and the absorption maxima appeared around 440–433 nm suggest the formation of silver nanoparticles (Fig. 1.b). The inset of Fig. 1.b indicates the optical picture of intense yellow coloured of bark extract. Also observed the maximum absorbance of bio-synthesized silver nanoparticles after 24 h, 30 days, 45 days and 60 days reaction time respectively. The reduction of $AgNO_3$ was assumed to complete at 24 h, as there is no further change of absorbance of the nanoparticles, observed by UV visible spectroscopy. Therefore, 24 h as optimized time point to determine their UV spectrum and the 60 days was maximum optimized time point of stability of silver nanoparticles. (Fig. 1.).

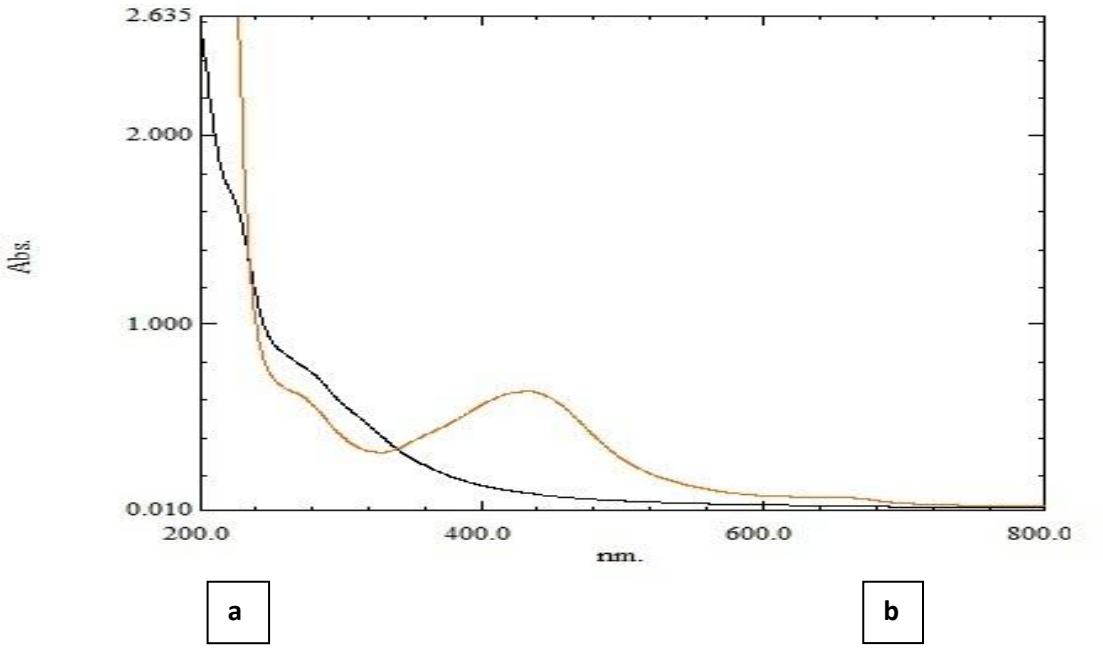


Figure: B. UV–Vis spectra of (a) Butea monosperma bark extract reduced silver nanoparticles (b) Butea monosperma bark extract solution.

FTIR Analysis: (Fourier Transforms Infrared Spectroscopy)

FT-IR spectroscopic analysis was used to classify and recognize the biomolecules that were bound directly to the synthesized Silver Nanoparticles. The obtained spectrum for *Butea monosperma* var. *lutea*, A number of peaks representing its dynamic nature were demonstrated by the bark extract. The peak of 3282.37 The cm1 was the result of stretching vibrations of peptide bonds. The peaks of 3836.08 cm1 are assigned to the stretching vibrations of O-H (Phenolic compound) Classes of functional group. The odd thing was that the Silver Nanoparticles 1637.01 cm1. Therefore, we infer that the lack of the peak is attributable to the fact that in the nanoparticle the group of carboxylic acids is absent and thus plays a role in reducing Ag+ to Ag0. The results set out above are similar to previous studies.[63,64] The fact that tryptophan protein residues also play a role in reducing and stabilizing AgNPs has been revealed by the shifting of these peaks and decreasing band strength. The reasonable changes in the peak positions mean that the various phytochemicals in the *Butea monosperma* var *lutea* are present.

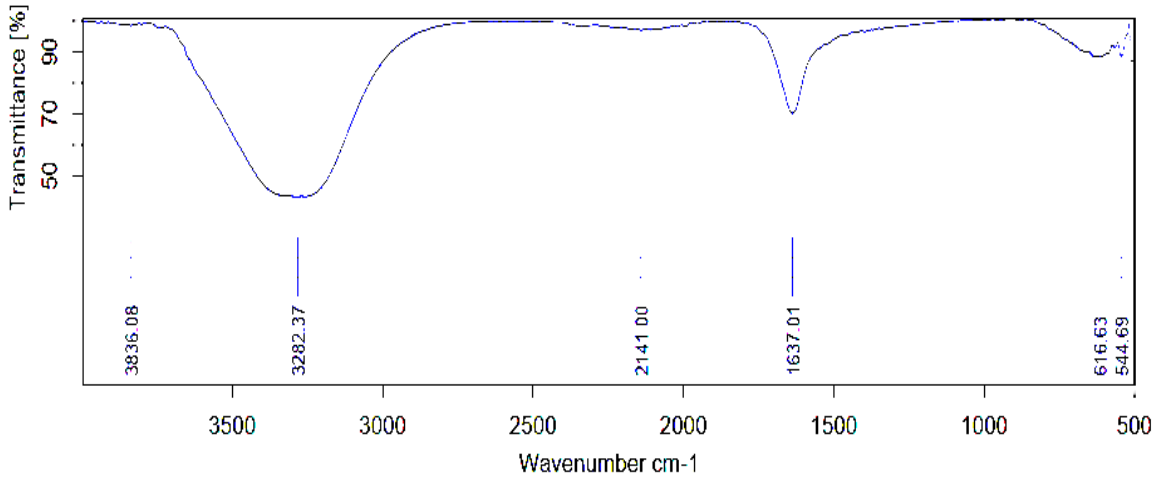


Figure: 3 FTIR spectra of *Butea monosperma* var. *lutea* bark extract stabilized Ag NPs.

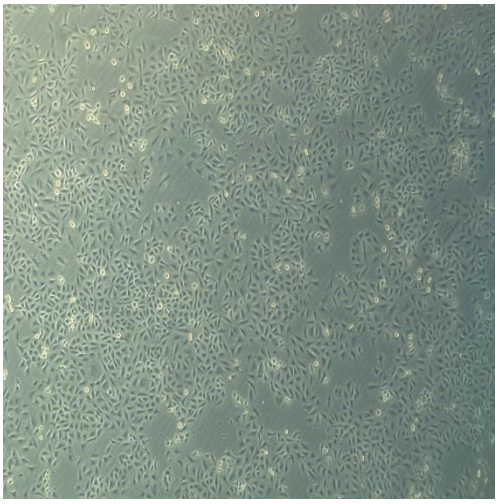
Anticancer activity and cytotoxicity (MTT Assay)

Side toxic effects of the anticancer activity of the silver nanoparticles were assessed in vitro against *HeLa* cell lines. The particles were examined as well. Various concentrations (0.1, 0.5, 0.10, 1, 5 and 10 µg/mL) of *HeLa* cells were exposed to Ag NPs for 24 h and MTT assays have been used to test cell viability. The findings indicate that the Ag NPs lowered the Ag NPs 11.96%, 37.32%, 55.85%, 81.55%, 90.45% and 97.53% viability of HeLa cells at doses of 0.1,0.5,0.10, 1, 5 and 10 µg/mL respectively. The cytotoxicity of aqueous *Butea monosperma* var *lutea* is, however, not reported. A mild cytotoxic effect of *Butea monosperma* var. *lutea* is shown in the present work. The presence of any active anti-cancer or cytotoxic bio-molecules found in the extract may be due to the bark extract. On the other hand, the biosynthesized silver nanoparticles exhibit their biocompatible nature may be due to the formation of silver nanoparticles, the active anti-cancer agents of the bark extract are not conjugating with synthesized nanoparticles and therefore not showing any cytotoxic effect and the active anticancer agents of the bark extract after conjugation with the synthesized nanoparticles cannot be released in the physiological media and hence do not exhibit their cytotoxic activity.

Table: II Reaction of Different doses of Silver nanoparticles on HeLa Cell line.

Sr. No.	Different Doses of Silver Nanoparticles (ml)					
	0.1ml	**0.5ml**	**0.10ml**	**1ml**	**5ml**	**10ml**
Anticancer And Cytotoxicity (%)	11.96%	37.32%	55.85%	81.55%	90.45%	97.53%

Control Cell line



Treated Cell line

CONCLUSION

The bio-synthesis of Silver Nanoparticles using Mili-Q water using *Butea monosperma* var *lutea* bark extract was identified in the present study. This readily available natural, as confirmed by ingredient shaped spherical Silver nanoparticles, was primarily responsible for the reduction and stabilization of Ag ions to Silver Nanoparticles. The formation of silver nanoparticles analysed by UV – Visisble spectrophotometer by the conform spectra band shown at 211nm and 406nm. In the plant material spectroscopic analysis of FTIR, water-soluble organics are present. In this manner, the silver nanoparticles prepared also demonstrated fair anticancer activity. All of these results demonstrate that the bio-ingredients present in the bark extract have been effective in the synthesis of silver nanoparticles that are anticancer-active. The findings showed the powerful anticancer activity against the *HeLa* cell line of silver nanoparticles. In vivo analysis, however, is important to illuminate the effect of silver nanoparticles at the level of the system. Based on these results, the application of Silver Nanoparticles can lead to useful discoveries in anticancer drugs. Such extract-stabilized nanoparticles may therefore be a possible candidate for different biomedical applications.

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