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

Vibha Bhingradiya[1], Anjali Shukla[2] and Dr. Nainesh Modi[3]*

[1]Department of Botany, Bioinformatics and Climate Change Impact Management, University School of Science., Gujarat University, Ahmedabad (Gujarat) India
[2,3]Associate Professor of Department of Botany, Bioinformatics and Climate Change Impact Management University School of Science, Gujarat University, Ahmedabad (Gujarat) India.

Corresponding Author: Dr. Nainesh Modi
Associate Professor of Department of Botany, Bioinformatics and Climate Change Impact Management University School of Science, Gujarat University, Ahmedabad (Gujarat) India.

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ABSTRACT

Butea monosperma var. *lutea* (*Papilionaceae*) is differs from *Butea monosperma* var. *monosperma* in presence of ivory-white flower buds and bright yellow flowers. the synthesis of silver nanoparticle using *Butea monosperma* var. *lutea* bark extract, which is used at room temperature as both a reducing and capping agent. The mixture for the reaction after around 24 h, it turned brownish yellow clearly indicates the formation of silver nanoparticles. The presence of stabilizing silver nanoparticles is shown by a larger DLS data of 46 nm. 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.

KEYWORDS: Silver Nanoparticles, Bark Extract, DLS, FTIR, *HeLa* Cell line.

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 ones, 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.). Due to the oxidation of the-OH groups and the carbonyl group of phytochemicals, it can act as a reducing and capping agent for the stabilization of metallic nanoparticles. Some studies use an external source of energy for incubation to promote the reaction. 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 method 3, 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. All cell lines were cultured at 37 °C in a humidified atmosphere containing 5% CO2, as the normal growth condition.

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 DLS and FTIR analysis 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.

Brief description of procedures used for synthesis of silver nanoparticles their characteristically analysis and anticancer activity and materials viz, plant materials, chemicals and glassware's used in this study is presented in this chapter.

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

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.

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 100 µg/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.

Apparatus

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.

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. To measure the size of a nanoparticle, including its environment, Dynamic Light Scattering (DLS) measurements were carried out. 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:
DLS (Dynamic Light Scattering) Particle Size Analyser Method:
Particles size analysing experiment were carried out by laser diffractometry which is analyse particle size using e extremely compact optical bench, the CILAS 2 sequenced laser sources pointed at 0 and 45 measurements were taken in the range between 0.04 into 500 mm. through the software, the particle distribution curve represented. Size and Zeta potential of the silver nanoparticles were determined by Metrohm Nanotrac instrument allows the measurement of particle sized distribution in the range 1nm to 100nm.

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.

For in vitro experiments, the HeLa cell line was cultivated. The 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.

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:

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 + 1 ml AgNPs	Cells + Media for culture + 5 ml AgNPs	Cells + Media for culture +10 ml AgNPs

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.

In vitro cytotoxicity assay
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 (1 × 105/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.

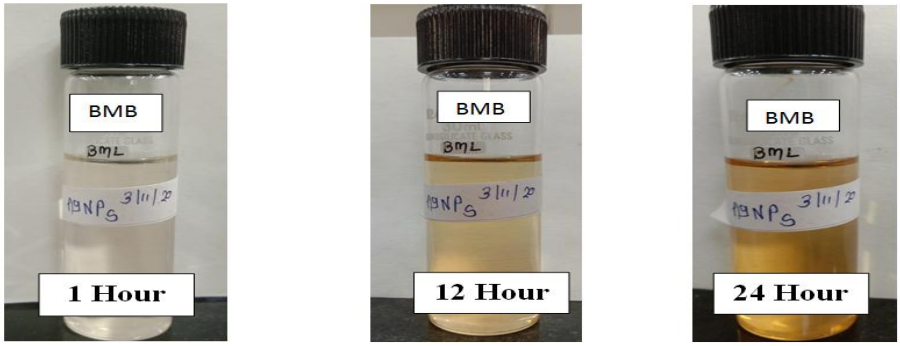


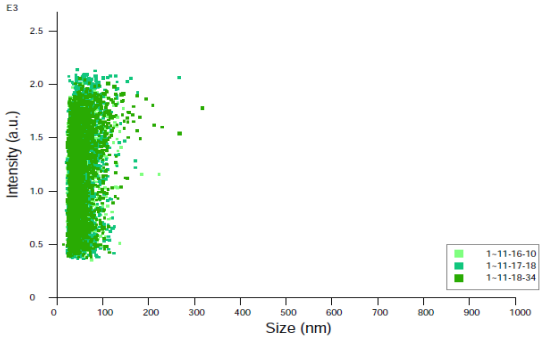
Figure 1: Synthesis of Silver Nanoparticles.

Characterisation of Silver Nanoparticles

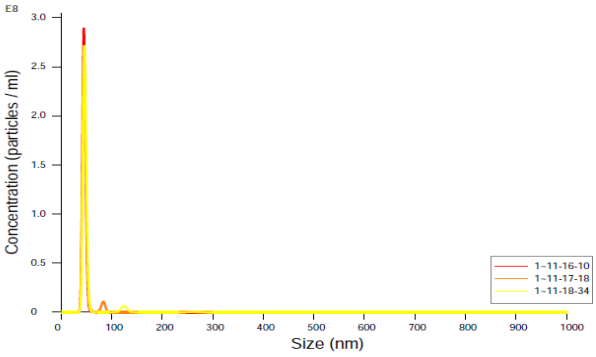
DLS: (Dynamic Light Scattering) particle size analyser

The zeta potential of nanoparticles gives an important idea regarding surface charge and stability of nanoparticles.[19] After 24 hours of Nanoparticles synthesising the size of Silver Nanoparticles was analysed by DLS. The size of synthesised nanoparticles was found in the range of 1 nm to 125nm. The average size of the Nanoparticles was found 46nm. The mean, mode, Standard deviation and Standard Error was 48.3nm +/- 1.5nm, 45.5nm +/- 0.4nm and 14.8nm +/- 4.4nm respectively. (figure – A, B, and C).

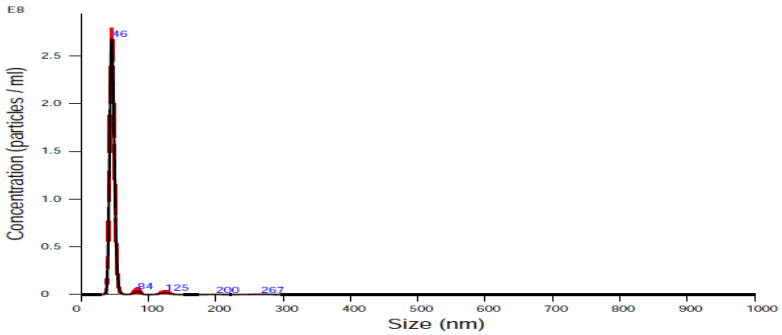
Size Of AgNPs	Mean +/- SE	Mode +/- SE	SD +/- SE
46nm	48.3nm +/-1.5nm	45.5nm+/- 0.4nm	14.8nm +/- 4.4nm



Intensity / Size graph for Experiment:
1 2019-01-10 11-15-13



FTLA Concentration / Size graph for Experiment:
1 2019-01-10 11-15-13

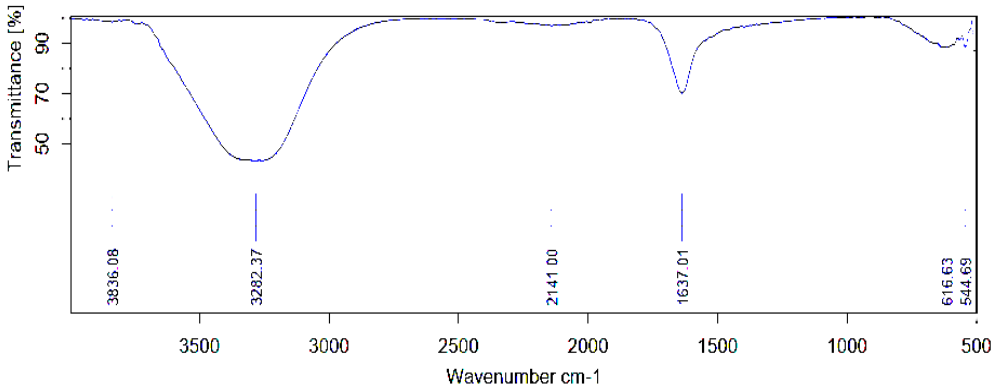


Averaged FTLA Concentration / Size for Experiment:
1 2019-01-10 11-15-13
Error bars indicate + / -1 standard error of the mean

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. bark extract is present in the stabilized nanoparticles of the extract.



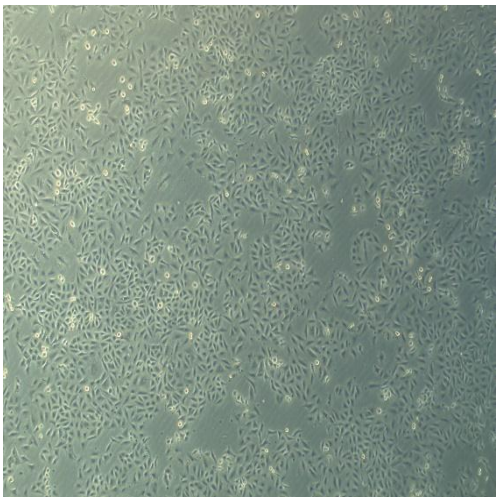
FTIR

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 percent viability of HeLa cells, 37.32 percent, 55.85 percent, 81.55 percent, 90.45 percent and 97.53 percent 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 as synthesized nanoparticles cannot be released in the physiological media and hence do not exhibit their cytotoxic activity.

Sr. No.	Different Doses of Silver Nanoparticles					
	0.1	0.5	0.10	1	5	10
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. In the plant material spectroscopic analysis of FTIR, water-soluble organics are present. DLS measurements have been used to study the size of Silver Nanoparticles. 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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