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# *Butea monosperma* Silver Nanoparticles Anticancer Activity Against MCF 7 Human Breast Cancer Cell Line

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**ABSTRACT**

**In this research, silver nanoparticles were synthesized from *Butea monosperma* for *in vitro* cytotoxicity efficacy against MCF-7 cells. Silver nanoparticles are deemed the most positive, considering their strong volume surface region, and are of concern for study because of the improved microbial tolerance to antibiotics and medicines. Therefore, green synthesis of nanoparticles of silver using biomolecules derived from various plant sources in the form of extracts can be applied for the screening of different diseases which trigger microorganisms and for the physical and biological characterization of plant-derived silver nanoparticles. The experiment involved the green synthesis of silver nanoparticles (AgNPs) from *Butea monosperma* leaf extract. Biosynthesized *Butea monosperma*-AgNPs were characterized by UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The intensity of peak broad range 200-800nm in UV-vis spectra, EDS test. The SEM shows the actual size of the nanoparticles. The MTT assays were carried out for cytotoxicity of various concentrations of biosynthesized silver nanoparticles. The biosynthesized silver nanoparticles showed a significant anticancer activity against both MCF-7. Our study thus revealed an excellent application of greenly synthesized silver nanoparticles. At the Concentration 80µg/ml, Sample Code A, B, C, D samples showed good percent inhibition MCF7cell line as compared to standard drug.The study also suggested the potential therapeutic use of these nanoparticles in cancer study.**

***Keywords-*** *Butea monosperma*, UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM), Silver nanoparticles, Anticancer activities, MCF 7 human breast cancer cell line, MTT assay.

## I. REVIEW OF LITERATURE

Cancer is an abnormal uncontrollable cell cycle disease characterized by the rapid proliferation of normal cells. Cancer has been ranked as the second leading cause of death all over the world, preceded only by cardiovascular diseases Metals such as metals and metal oxides, silicates, non-oxide ceramics, polymers, organic materials, biomass and biomolecules may be used for producing nanoparticles. In various morphologies, nanoparticles occur, including balls, cylinders, platelets, tubes etc. Inorganic nanoparticles such as golden and silver metal nanoparticles have superior material properties with mechanical flexibility, with broad availability, comprehensive mobility, strong compatibility, selective therapeutic products and regulated drug release capabilities (Xu *et al*., 2006). For the synthesizing and stabilisation of silver nanoparticles, many physical, chemical and biological methods were used (Senapati*et al*., 2005). The word biofilm has been

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used to refer to the thin coated condensations of microbes (for example bacteria, fungi, protozoa, etc.) which can appear in different types of surface structures. Antifungal performance may be calculated by means of well diffused methods on various fungal strains. (Chitte *et al*.,2016) Free floating bacteria, classified as planktonic microorganisms in an aqueous climate, are a requirement for the development of biofilms. Thus, such films may be formed on every organic or inorganic substratum where planktonic microorganisms prevail in a water solution (Choudhary*et al*., 2012). Because of its unusual physical and chemical properties, silver nanoparticles (AgNPs) are progressively being used in numerous fields, including medical, fruit, patient treatment, consumption and industrial uses. This involves visual, electronic, thermal, heavy electrical and biological characteristics (Gurunathan *et. al.* 2015). Because of its unusual properties, it has been used for many applications in the medicinal, food processing, surgical, orthopedic, medication distribution, anticancer industries, as well as for numerous applications such as non-bacterial agents, automotive, domestic and health goods, electronic products, medical equipment jackets, optical sensors and cosmetics AgNPs have been widely used lately in numerous textiles, keyboards, wound dressings and biomedical instruments. The nanosized metallic particles are peculiar and, because of their surface to volume ratio, can greatly alter physical, chemical and biological properties; thus, nanoparticles have been used for different purposes. In order to satisfy the AgNPs criterion, different methods for synthesis have been introduced. In general, current approaches of physics and chemistry appear rather costly and risky. It is important to notice the high yield, solubility and high stability of biologically prepared AgNPs (Gurunathan*et al.*, 2015). Biological methods for AgNPs seem simplistic, quick, nontoxic, reliable and green among. A range of analytical methods are used, including UV spectroscopy, Xraydiffractometry (XRD), Fourier infrasound transform spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), DLS scanning, SEM, transmission electron microscope (TEM), atomic force microscopy (AFM) (K Venugopal*et.al.*,2017) (Raju Vivek, *et.al.*,2012),( V. Kathiravan*et.al.*,2014),( Shahnaz Majeed, *et.al.*,2019),( Sabina Yeasmin*et.al.*,2017),( R.M. Genga*et.al.*, 2013),( R.R. Remya*et.al.*, 2015). Several competent books and studies have identified different styles of methodological methods for characterisingAgNPs. A highly effective and accurate technique for the primary characterization of synthesised nanoparticles used for tracking the production and stabilisation of AgNPs is UV-Visible Spectroscopy. (Sastry *et al.*, 1998).

## II. MATERIALS AND METHODS

***2.1 Sample preparation****:* The young and disease-free leaves of *Butea monosperma* were selected.

**Drying of leaves:** Leaf samples were dried in room temperature for more than two weeks, so that they may be converted into fine powder.

**Preparation of fine powder**: after proper drying of leaves, thick mid ribs of the leaves was removed, dried leaves were grinded into fine powder using a grinder.

**Preparation of extracts**: aqueous extracts using distilled water, 50% ethanol, 50%methanol & 50% acetone were prepared.

***2.2 Synthesis of Silver Nanoparticles from Butea monosperma extracts:***

AgNPs were synthesized by the following method. 10mM $AgNO_3$: plant extracts in different solvent in 9:1 ratio in a reagent bottle mixed thoroughly, forming a uniform mixture. The mixture was then rested at room temperature for 24 hours at 37°C, with continuous monitoring. After about few minutes, the mixture was observed to start changing from pale green to yellowish brown. After about 24 hours, the mixture had completely changed colour to brown in all solvents. This color change is visual evidence of formation of AgNPs. *(*Kasthuri *et al., 2009).*

2.3 ***Characterization of silver nanoparticles:***

For determination of the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken200–8000 nm using a UV–vis spectrophotometer. The silver nanoparticles were synthesized by novel green chemical route. The nanoparticles were characterized by UV-spectral analysis, SEM -EDAX analysis (Scanning Electron Microscopy) was performed for studying the surface morphology & to predict the size of the nanoparticle. Also, FTIR analysis was conducted for identifying the presence of functional groups. (Anuja *et al. 2020)*

***Anticancer Activity:***

1. CO2 Incubator- ThermoFisher,USP
2. Multimode micro plate reader- Bene SpheraE21 Avantor USP.
3. Refrigeraterated centrifuge- Eppendorf Germany.
4. Cell : MCF-7 (Human breast cancer cell line) NCCS Pune.
5. MTT.(3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazoliumbromide)).
6. Fetabovineserum (Gibco,Invitrogen) (CatNo.-10270106)
7. Trypsin.
8. Penicillin.
9. DMEM With high glucose (CatNo.-11965-092).
10. Antibiotic- Antimycotic 100X solution

***(Thermo fisher scientific)- (CatNo.-1524006)***

Media: DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106 Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062 Activity: MTT Assay: Experimental procedure:

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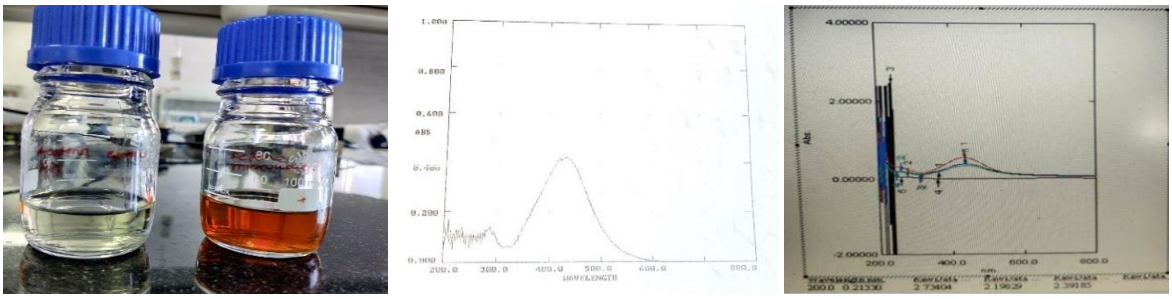
1) Cells were incubated at a concentration of 1 × 10 4 cells/ml in culture medium for 24 h at 37°C and 5% CO2.
2) Cells were seeded at a concentration (70µl) 104 cells/well in 100 µl culture medium and 100µl sample of Sample A to D in (10, 40.80 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells).
3) Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.
4) Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientific BB150)
5) After incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS).
6) After addition of MTT, cells incubated for 4 hrs at 37°C in CO2 incubator.
7) Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only.
8) After removing the medium completely. Added200µlof DMSO (kept for 10 min) and incubate at 37°C (wrapped with aluminum foil).

## III. RESULT

3.1 The detailed study on biosynthesis of silver nanoparticles by natural *Butea monosperma* extracts such It was observed that the color of the solution turned from yellow to bright yellow and then to dark brown after 1,24 and 48 h of the reaction, which indicated the formation of silver nanoparticles (fig 1).

3.2 The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra show maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract. The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for silver is a characteristic of these novel metal particles (Nestor *et al*., 2008). The synthesis of AgNPs from the ethanolic, aqueous, methanol & acetone extract of leaves of *Butea monosperma* was further confirmed by ultraviolet - visible spectroscopy (UV/VIS) in the range of between 200 nm to 800 nm and solvents were used as a blank. The spectrum has a maximum absorption peak at a which is reported to have an absorption maximum of between about 400nm to about 450nn. The presence of the maximum peak absorption peak at 400nm to about 450nn is therefore an indication and confirmation that the AgNPs were present. (Fig 1)



a) b) c)

**Fig.1. a) Synthesis of nanoparticles using *Butea monosperma* b) and c) presence of the maximum peak absorption peak at 400nm to about 450nn**

3.3 Fourier Transform Infra-Red Spectrometer (Equipped With ATR) Model Tensor 600 Brucker. As seen in figure given below, FTIR spectra of all samples shows similar pattern.

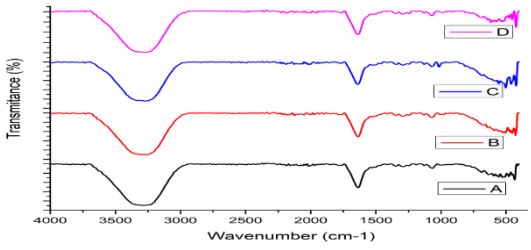



**Fig. 2. FTIR spectra of all four solvents of *Butea monosperma* synthesized Sliver AgNPs**

FTIR spectra depicts bands at ~3200-3300 corresponding to alcoholic O-H stretching, bands at ~ 1620 corresponds to; where as small band at ~1100 is corresponds to alcoholic C-O bond, metallic silver bond is seen at ~450 cm-1. Fig 2 where A-water, B Ethanol, C methanol & D acetone solvents respectively.

3.4) Field Emission Scanning Electron Microscopy (Fe-SEM) & Energy Dispersive X-Ray spectroscopy (EDS) Analysis. Thin film of the as obtained sample was prepared on cleaned glass plate using drop casting technique. This film is dried under Infra-Red lamp at room temperature. As depicted in electron micrographs, sample consist of clusters of ultrafine nanoparticles of size ~40-75 nm. Fig 4 To confirm the composition of the sample, EDS analysis is done. As seen from the spectra

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depicted in chart1, Sample prominently consist of Ag along with S, P, O and Si.

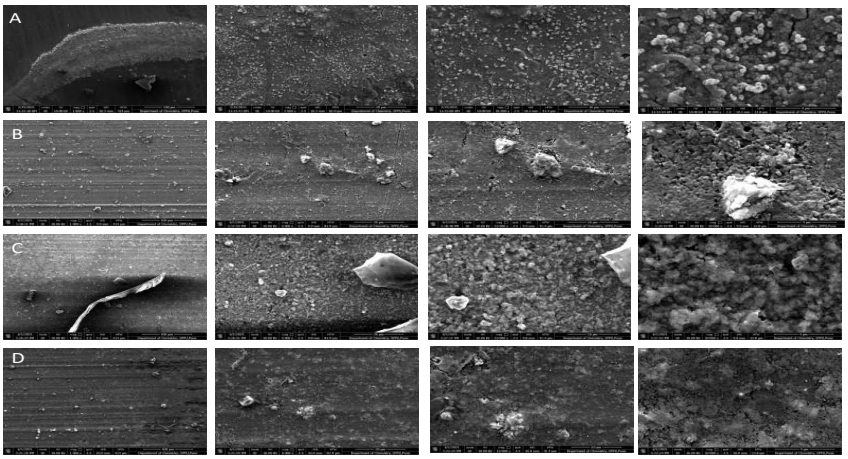


**Fig 3. Field Emission Scanning Electron Microscopy (Fe-SEM)**

**Table 1: Elemental composition of as synthesized sample**

| Element | Atomic No | A | B | C | D |
|---|---|---|---|---|---|
| Carbon | 6 | 23.81 | 20.64 | 19.07 | 22.53 |
| Aluminum | 13 | 10.18 | ND | ND | ND |
| Silver | 47 | 47.10 | 45.49 | 44.70 | 45.60 |
| Sulfur | 16 | 5.20 | ND | ND | ND |
| Phosphorus | 15 | 1.50 | ND | ND | ND |
| Silicon | 14 | 1.23 | ND | ND | ND |
| Oxygen | 8 | 10.98 | 9.91 | 36.24 | 31.88 |

**Table 2: Effects of *Buteamonosperma* AgNPs solutions against MCF7 (human breast cancer cell line) by MTT assay.**

| Sr. No. | Sample | Concentration (µg/ml) | OD | % Inhibition |
|---|---|---|---|---|
| 1 | Control | | 1.275 | |
| 2 | Std. 5 FU | 10 | 0.965 | 75.68 |
| | | 40 | 0.736 | 57.72 |
| | | 80 | 1.123 | 96.00 |
| | | | | |
| 3 | A | 10 | 0.658 | 51.00 |
| | | 40 | 0.756 | 59.29 |
| | | 80 | 0.822 | 64.47 |
| | | | | |
| 4 | B | 10 | 1.003 | 78.66 |
| | | 40 | 1.118 | 87.68 |
| | | 80 | 1.273 | 99.03 |
| | | | | |
| 5 | C | 10 | 1.223 | 74.90 |
| | | 40 | 1.257 | 76.70 |
| | | 80 | 1.273 | 88.94 |
| | | | | |
| 6 | D | 10 | | 95.92 |

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Fig. 3: At the Concentration 80µg/ml, sample Code A, B, C, D samples showed good percent inhibition MCF7cell line as compared to standard drug.

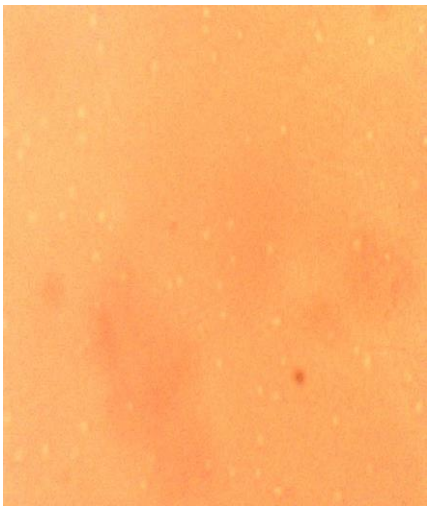

**Fig. 54: Control**

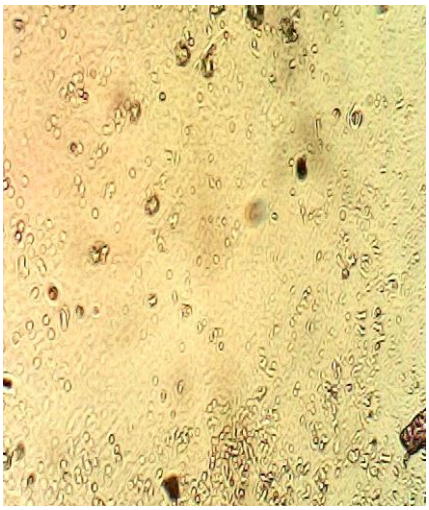

**Fig. 55: 5-FU**

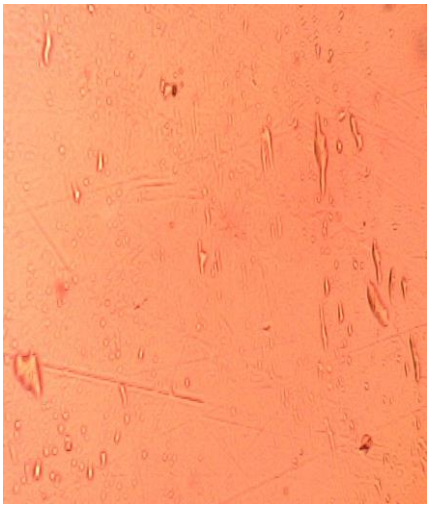

**Fig. 56: Aqueous AgNPs (A**

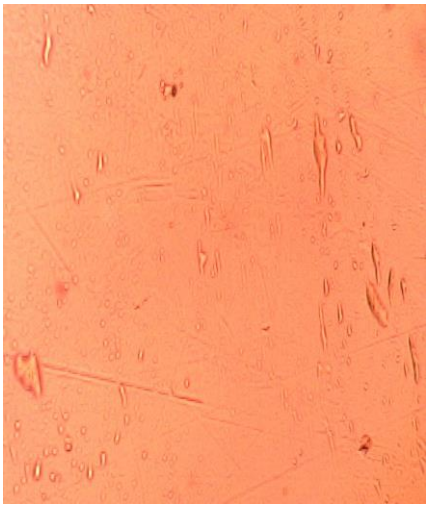

**Fig. 57: Ethanol AgNPs (B)**

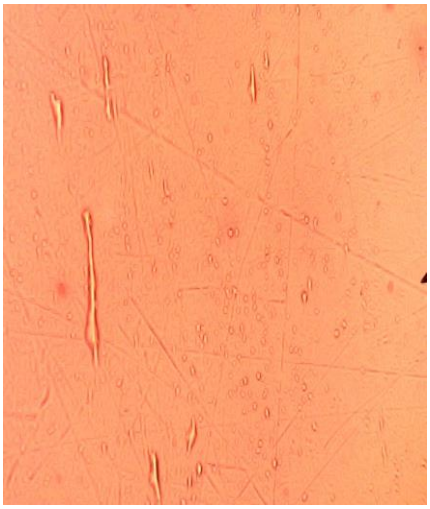

**Fig. 58: Methanol AgNPs (C)**

**Fig. 59 Acetone AgNPs (D)**

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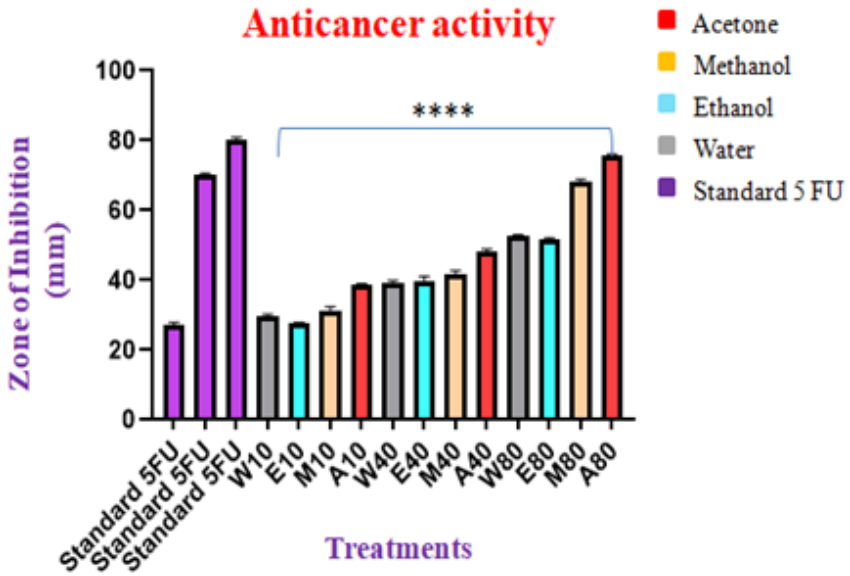


**Fig. 52:Anti-cancer Activity of *Butea monosperma*AgNP's against MCF7 cell line**

Concentration 80µg/ml AgNPs samples & Standard 5FU inhibition was observed in above study. Maximum inhibition was observed in Acetone AgNPs against MCF7cell line using mmt assay which was around 75.89% as compared to standard which is 80.75 %.

## IV. CONCLUSION

In this study, different temperatures were used for silver nanoparticle biosynthesized by using *Buteamonosperma* extract. The greatest Plasmon resonance was acquired at 60 °C previously, improved the procedure parameters for incorporating AgNPs by utilizing aqueous extracts of *Buteamonosperma*. UV–Vis spectroscopy and FT-IRanalysis confirmed the formation of the silver nanoparticles. The size ofAgNPs was confirmed by TEM analysis, which illustrates that AgNPswere spherical in shape with size ranging from 5 to 40 nm. Synthesized AgNPs appeared polydispersed and well scattered. The synthesized silver nanoparticles and *Buteamonosperma* extracts were compared and showed promising cytotoxicity activity against MCF-7 cell line. From the study, it can be concluded that the silver nanoparticles synthesized using plant, possess high cytotoxicity activity against cell lines which suggests the potential therapeutic use of these nanoparticles. At the Concentration 80µg/ml, Sample Code A, B, C, D samples showed good percent inhibition MCF7cell line as compared to standard drug.

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