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RESEARCH ARTICLE

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *Cassia Fistula* LEAF EXTRACT AND ITS APPLICATIONS

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ABSTRACT

Nanobiotechnology is an emerging field in biotechnology and it has vast applications in all fields. In this study silver nanoparticles has been synthesized by using Cassia fistula leaf extracts which was confirmed by using UV and FT-IR.The structure and size of synthesized nano materials was confirmed by using SEM and EDAX.The synthesized nanoparticles has effective results for antioxidant and antimicrobial studies. The anticancer effects of synthesized nanoparticles were analysed by using MCF-7 cell line and this paws a way to use silver nanoparticles as anticancer drug in future.

Keywords: Cassia fistula, silver nanoparticles, UV, FT-IR, SEM, EDAX, antioxidant, anticancer

INTRODUCTION

Nanotechnology is a science, engineering, and technology conducted at nanoscale, which is about 1 to 100 nanometres and is the study and application of extremely small things that can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanotechnology means scaling down to nanolevel starting from the bottom [21]. One nanometre is a billionth of a meter, or 10^{-9} of a meter. Remarkable advances are made in the field of nanotechnology for the benefit of life sciences, healthcare [1] and industrial biotechnology. Bionanotechnology generally refers to the study of how biological "machines" work and adapting these into existing nanotechnologies or creating new ones. Nanobiotechnology, on the other hand, refers to the ways that nanotechnology is used to create devices to study biological systems. In other words bionanotechnology is miniaturized biotechnology, whereas nanobiotechnology is a specific application of nanotechnology.

DNA nanotechnology is one important example of bionanotechnology[23]. The utilization of the inherent properties of nucleic acids like DNA to create useful materials is a promising area of modern research. Lipid nanotechnology is another major area of research in bionanotechnology, where physico-chemical properties of lipids such as their antifouling and self-assembly is used to build nanodevices with applications in medicine and engineering [11]. In nanotechnology particles are classified according to diameter. Coarse particles range between 2,500 and 10,000 nanometers. Fine particles are between 100 and 2,500 nanometers. Ultrafine particles, or nanoparticles, are sized between 1 and 100 nanometers in size. Nanoparticle research is an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields [8, 19]. They can self-assemble at water/oil interfaces and act as solid surfactants. Various types of liposome nanoparticles are currently used clinically as delivery systems for anticancer drugs and vaccines [20].

Plant description

Cassia fistula L. also known as the golden shower tree is a flowering plant belonging to the family Fabaceae. It is native to South Asia. This tree is the national tree of Thailand and its flower is its national flower. In Tamil, it is called as Konrai.

The golden shower tree is a medium-sized tree, growing to 10–20 m tall with fast growth. The leaves are deciduous. The flowers are produced in pendulous racemes. The fruit is a legume, with a pungent odour and containing several seeds. In ayurvedic medicine, the golden shower tree is known as Aragvadha, meaning "disease killer". The bark of the tree and fruits help in purification of the blood. Root is useful against cardiac disorders, biliousness, rheumatic condition and haemorrhages. The roots of *Cassia fistula* have the property to reduce blood sugar by about 30 %.

Cassia fistula

Green synthesis of silver nanoparticles

The synthesis of nanoparticles using plant and plant extracts was termed as 'green chemistry approach'. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts, particularly *Allamania nadiflora, Cardiospermum halicacabum* [14], *Murraya koenigii* (curry leaf) [10], *Euphorbia hirta, Aloe vera*, *Emblica officinalis* (Amla, Indian Gooseberry). Different plant constituents such as geraniol possess reducing property and reduce Ag^{+} to silver nanoparticles with a uniform size and shape.

MATERIAL AND METHODS

Sample collection

Cassia fistula leaves were collected from the campus of Madurai Kamaraj University, Madurai. Silver nitrate was obtained from Merck specialties Private Limited, Mumbai.

Preparation of leaf extract

20 g of leaves were washed with distilled water to remove the dust particles and then air dried. The dried *C.fistula* leaves were cut into small pieces and boiled with 100 ml of distilled water at 80° C for 1 h. After boiling, the brown coloured extract was separated by

filtration and it was used for the reduction of silver nitrate to silver nanoparticles.

Phytochemical screening

After obtaining the crude extract from plant material, phytochemical screening can be performed with the appropriate tests as shown in the table to get an idea regarding the type of phytochemical existing in the extract.

Table1: brief summary of phytochemical screening

Secondary metabolite	Name of test	Methodology
Alkaloid	Wagner's test	To 1 ml of extract few drops of Wagner's reagent was added.
Phenol	Lead acetate test	To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added.
	$FeCl_3$ test	To 1 ml of extract, 1 ml of 5% $FeCl_3$ solution was added.
Saponin	Frothing test	A small amount of extract was shaken vigorously in a test tube.
Reducing sugar	Fehling test	To 1 ml of extract Fehling's A & B solutions were added

Optimization parameters

Effect of concentration

Silver nitrate was taken in different concentrations (1.0 mM, 1.5 mM and 2 mM) for the synthesis of AgNP. The UV-Visible absorption spectrum for the above mentioned concentrations of silver nitrate were observed.

Effect of temperature

The leaves of *C.fistula* were boiled at different temperatures (60˚ C, 80˚ C, 90˚ C and 120˚ C) for 1 h. The UV-Visible absorption spectrum for the extract was observed.

Effect of ratio

The different ratio of plant extracts with silver nitrate like 1:5, 1:7, 1:9 were used for the reduction mechanism of silver ions to nanoparticles and it was investigated through UV-visible spectrophotometer.

Synthesis of silver nanoparticles

The synthesis of silver nanoparticles was done by mixing *C.fistula* leaf extract and $AgNO_3$ solution in the ratio of 1:9 and incubated in dark for 24 h. The AgNPs produced by *C.fistula leaf* extract was centrifuged at 6000 rpm for 20 min. The supernatant was discarded and the collected pellet was air dried.

Characterization of silver nanoparticles

UV-Visible spectral analysis

The bio reduction of pure silver ions to AgNPs was measured. This was carried out on a Systronic UV-Visible absorption spectrophotometer at a scanning speed of 200-1000 nm.

Fourier transform infra-red analysis

The dried sample (AgNPs) was subjected to FT-IR analysis. 2 mg of the sample was mixed with 200 mg of KBr and pressed into a pellet. It was placed into the sample holder and FT-IR spectra were recorded at a resolution of 4500-350 cm^{-1}.

Scanning Electron Microscopy

The pellet was subjected for SEM analysis. Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis.

Energy dispersive X-ray spectroscopy

Energy dispersive analysis X-ray (EDX) spectrometer takes advantage of the photon nature of the light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse X-ray. A semiconductor material is used to detect the X-ray along with processing electronics to analysis the spectrum.

Atomic force microscopy

A thin film of the sample was prepared on a glass slide by dropping 100 µl of the sample on the slide, and was allowed to dry for 5 min. The slides were then scanned with the AFM. The AFM characterization was carried out at ambient temperature in non-contact mode using silicon nitride tips with varying resonance frequencies at a linear scanning rate of 0.5 Hz.

Screening of antimicrobial activity

The antimicrobial effect of synthesized AgNPs, silver nitrate and crude plant extract were evaluated against human pathogens such as gram negative bacteria (*E. coli, Vibrio cholerae, Proteus mirabilus, Pseudomonas aeruginosa and Salmonella typhi*) and gram positive bacteria (*Staphylococcus aureus*) by agar disc diffusion method. Cultures were maintained at −20 ◦ C on glycerol stock. The strains were sub-cultured in nutrient broth for 24 h at 37˚ C[2,4]. Each strain was spread uniformly into the individual nutrient agar plates using sterile glass rod spreader. 1 mg/ml of AgNPs, 1 mg/ml of 2 mM silver nitrate, 1 ml of leaf extract, and 1 ml of distilled water along with standard antibiotic amoxillin 1 mg/ml discs were impregnated in the nutrient agar medium. After 24 h incubation at 37 ˚ C, the different level of zone of inhibition was measured.

Determination of total antioxidant by DPPH assay

The free radical scavenging activity of the synthesized silver nanoparticles was determined using DPPH (1, 1-diphenyl-2-picrylhydrazyl). DPPH solution (0.004% w/v) was prepared in 95% methanol. Sample was mixed with distilled water to prepare the stock solution (1 mg/ml). To 0.5 ml of the prepared DPPH solution silver nanoparticles in varying concentrations (20-60 µg/ml) were added. The mixture was left at room temperature for 30 min and the absorbance was measured at 517 nm. Then the scavenging ability was calculated using the following equation:

% DPPH radical scavenging =100 x (control OD- sample OD)/ Control OD

Anticancer activity

The anticancer activity of the synthesized AgNPs against MCF 7 human breast cancer cell line was determined by MTT assay.

Culturing of cells

The MCF 7 cells were seeded onto 13-mm glass coverslips in a 24-well plate at a density of 1x10 cells per well in 1 ml of complete medium for 24 h. After 24 h, medium was removed and the cells were washed with phosphate-buffered saline followed by viability staining.

***In-vitro* cell viability studies**

The MTT assay is a simple, nonradioactive colorimetric assay to measure cell viability. Metabolically active cells are able to convert this dye into a water-insoluble dark purple formazan by reductive cleavage of the tetrazolium ring. Formazan crystals, then, can be dissolved in an organic solvent such as dimethylsulphoxide (DMSO) and quantified by measuring the absorbance of the solution at 545 nm, and the resultant value is related to the number of living cells. To determine cell cytotoxicity/viability, the cells were plated at a density of 1x10 cells/well in a 96-well plate at 37˚ C in 5% CO_2 incubator. After 24 h of culture, the medium in the wells was replaced with the fresh medium containing nanoparticles in varying concentrations. After 24 h, 20 µl of MTT dye solution (5 mg/ml in phosphate buffer pH

7.4) was added to each well. After 4 h of incubation at 37˚ C and 5% CO_2, the medium was removed and formazan crystals were solubilised with 200 µl of DMSO and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader at 545 nm. The

spectrophotometer was calibrated to zero absorbance, using culture medium without cells. The relative cell viability (%) related to control wells containing cell culture medium without nanoparticles was calculated by the following formula:

% of cell viability = 100 x (Sample absorbance / Control Absorbance)

RESULTS AND DISCUSSION

Plant extract preparation

Plant extract was prepared at 90^0c and the brown colour extract was shown in Plate-1.

Plate 1: Plant extracts preparation

Phytochemical screening assay

The results revealed the presence of medicinally active compounds like phenols, alkaloids, saponins and reducing sugar Table 1. The alkaloids present are used as analgesic, antipyretic and anaesthetic [12]. Phenols play an important role in cancer prevention and treatment. They prevent apoptosis by arresting cell cycle [2]. They are also responsible for regulating carcinogen metabolism, inhibiting DNA binding and cell adhesion, migration, proliferation and blocking signalling pathways. The presence of saponins has been reported to resist microbial pathogens and have detergent like properties [22].

Table1: Phytochemical constitute in *Cassia fistula*

Phytochemical constituent	Observation	Result
Alkaloid	Formation of reddish brown precipitate	+
Phenols	Formation of bluish black colour	+
Saponin	Foam formation	+
Reducing sugar	Formation of brick red precipitate	+

Optimization parameters

Effect of concentration

The UV-Visible spectra of different concentration of $AgNO_3$ showed maximum absorption for 2 mM $AgNO_3$ as shown in Fig.1&2.

Effect of temperature

The plant extract taken at different temperatures were found to have same absorption maximum 400 nm as shown in Fig.3.

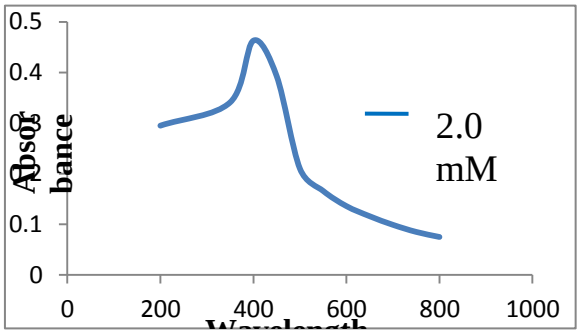


Fig 1: Effect of concentration-2.0mM

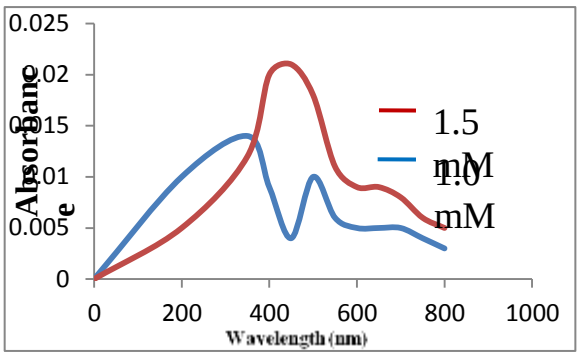


Fig.2: Effect of concentration-1.0&1.5mM

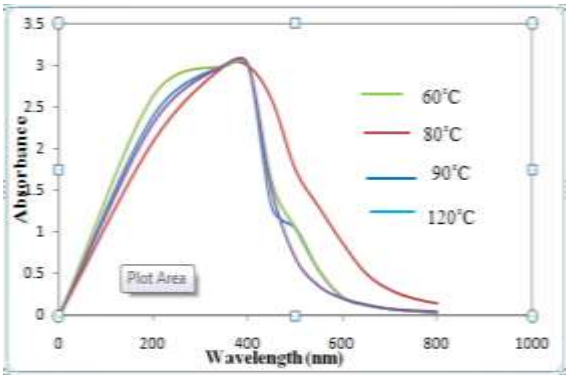


Fig.3: Effect of temperature

Effect of ratio

The reduction of silver ions to AgNPs had an absorption maximum at 400 nm for the ratio of 1:9 as shown in Fig.4.

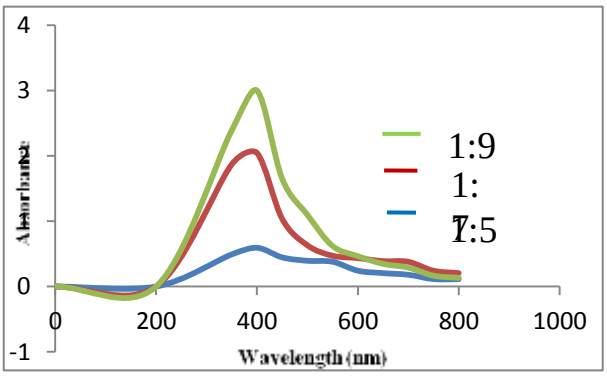


Fig. 4: Effect of ratio

Synthesis of silver nanoparticles

The green synthesis of AgNPs using 2 mM silver nitrate is shown in Plate 2. The yellow colour of the mixture of silver nitrate and *C.fistula* leaf extract changed to a black coloured mixture after 24 h of incubation which indicates the reduction of silver ions to silver nanoparticles.

Plate 2: Plant extract mixed with silver nitrate.

Characterization of silver nanoparticles

UV-Visible spectral analysis

The absorption spectrum of AgNPs formed in the reaction had a peak at 400 nm which confirms the presence of silver nanoparticles Fig. 5.

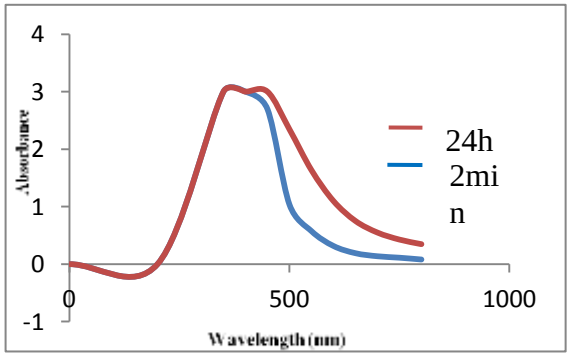


Fig.5:UV-Visible spectra of AgNPs at different incubation time

Fourier transform infra-red analysis

The spectrum was recorded in the wavelength region between 350 cm^{-1} to 4000 cm^{-1}. The FT-IR spectrum showed peak at 3437.26 cm^{-1} which indicates the presence of O-H stretching of alcohols and phenols Fig-6. The peaks at 2922.25 cm^{-1} and 2856.67 cm^{-1} indicate the presence of C-H stretching of alkanes. The peak observed at 1732.13 cm^{-1} represents the C=O stretch of aldehydes. The peak at 1529.80 cm^{-1} represents N-O asymmetric stretch of nitro compounds. The C-H bend of alkanes occurs at 1454.38 cm^{-1}. Further, the peaks at 1276.92 cm^{-1}, 1213.27 cm^{-1}, 1165.04 cm^{-1}, 1118.75 cm^{-1} and 1060.88 cm^{-1} correspond to C-N stretch of aliphatic amines. The C-Cl stretching occurs as a weak band at 740.69 cm^{-1}.

The Fig. 6 indicates the presence of many fundamental groups involved in conversion of silver ions to silver nanoparticles.

Scanning Electron Microscopy

The SEM image provided the morphology and size of the silver nanoparticles. SEM image showed individual silver nanoparticles as well as a number of aggregates Fig-7. The morphology of the silver nanoparticles was aggregated into larger irregular structure with no well defined morphology.

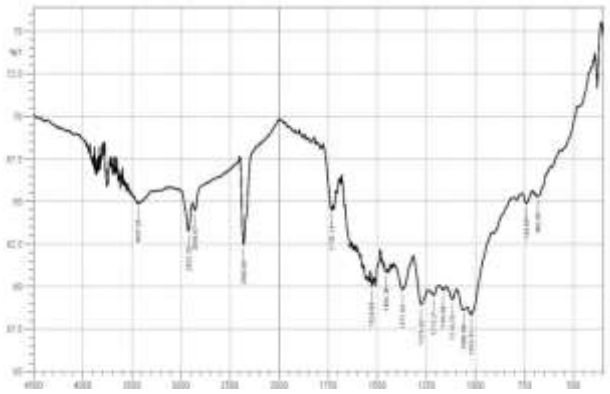


Fig. 6:FT-IR spectrum of AgNPs

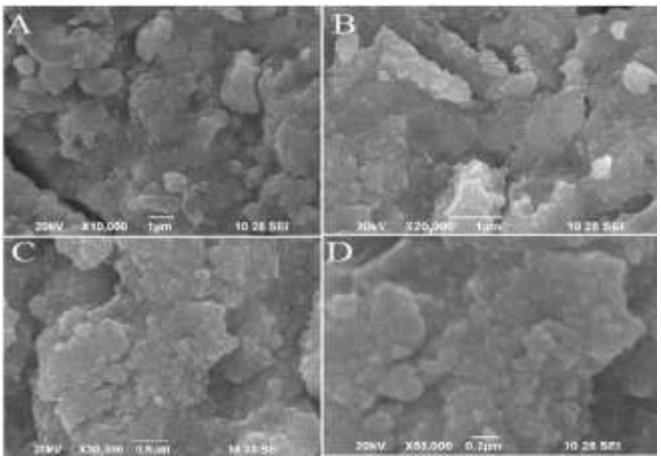
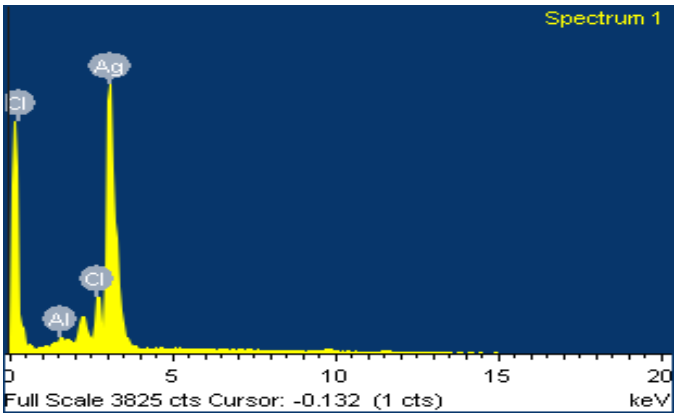


Fig 7:SEM image

Note: **A**-magnification-X10,000;**B**-magnification-X20,000;**C**-magnification- X30,000; **D**- magnification-X55,000.

Energy dispersive X-ray spectroscopy

The EDAX results confirm the presence of elemental silver in the sample. The optical absorption peak was observed at 3 keV, which is typical for the absorption of metallic AgNPs Fig-8. Strong signals were observed from Ag atoms, while weaker signals from Cl and Al atoms were also recorded. From the EDX signals, it was clear that AgNPs reduced by *C.fistula* extract have the weight percentage of elemental Ag as 92.88%.



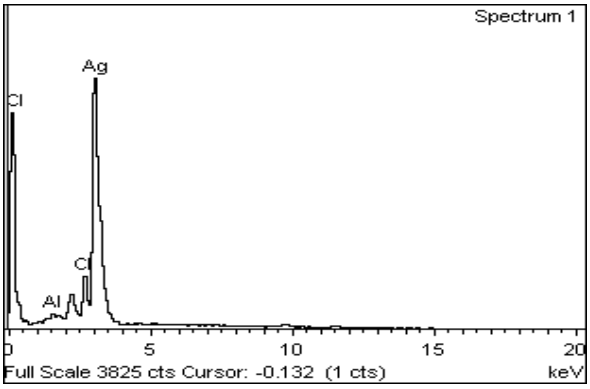


Fig.8:EDX spectra

Atomic Force Microscopy

Surface topology of the synthesized silver nanoparticles was studied by Atomic Force Microsopy (AFM) analysis Fig.9. The micrographs clearly indicate that the AgNPs possess the size range of 30-50 nm and are irregular is shape.

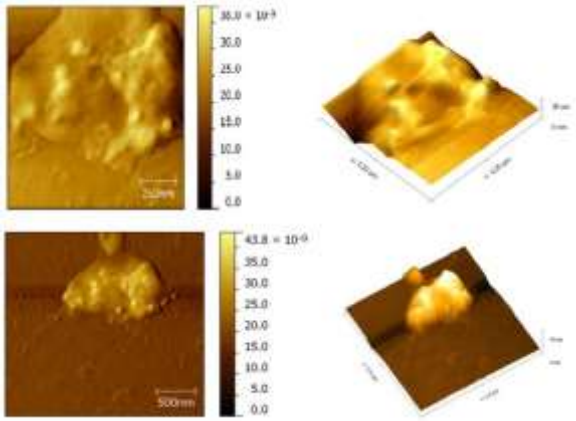


Fig.9:AFM image

Antibacterial activity

The antibacterial assay was performed against Gram positive and Gram negative bacterial pathogens using the synthesized AgNPs, plant extract, silver nitrate and antibiotic each of concentration 1 mg/ml. It was observed that there was no activity against plant extract and silver nitrate. The AgNPs showed inhibition against all the studied bacteria Plate3 & Fig 10. The organisms *E.coli, Pseudomonas aeroginosa* and *Vibrio cholerae* exhibit similar zone of inhibition. The most significant effect of AgNPs was against *Staphylococcus aureus a*and *Salmonella typhi.*

The AgNPs bind with cytoplasmic membrane and kills the bacterial cell. This is because of the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles [3,6,7]. However,[16,18] reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticles and was closely associated with the formation of pits in the cell wall of bacteria.

DPPH free radical scavenging activity

Oxygen Radical Absorbance Capacity (ORAC) value was measured to determine the free radical scavenging capacity of the plant extracts, while a fluorescent probe was used to evaluate the ROS scavenging ability. An SOD assay was used to find out if the plants' aqueous extracts can stimulate the production of the SOD. Plant extracts which were found to exhibit high antioxidant activity such as curry leaves, rooibos tea and fenugreek showed superior

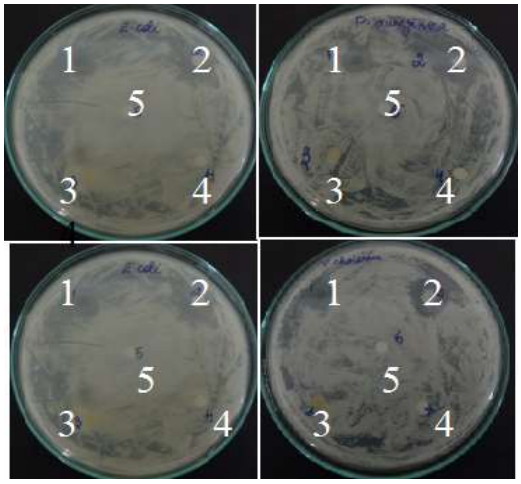


Plate 3: Antimicrobial activity

1. AgNP, 2. Antibiotic, 3. Extract, 4. Silver nitrate, 5.Water control

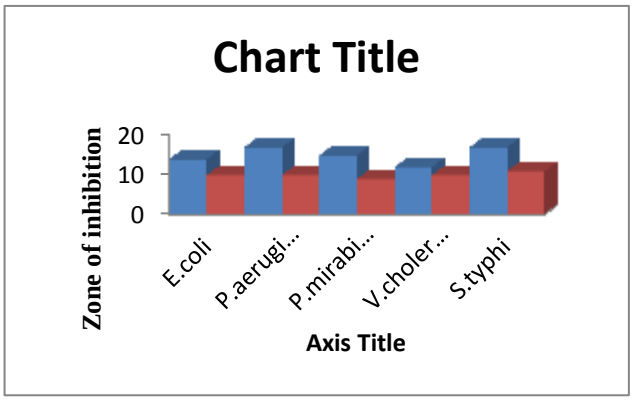


Fig.10: Antimicrobial activity

free radical scavenging ability in hyperglycemia-induced oxidative stress cell-line model. Plant extracts which exhibit low antioxidant activity such as Indian malabar, red silk cotton, cowitch, holy fruit tree and bitter gourd showed insignificant free radical scavenging ability. Methanolic extracts of *Cassia fistula* showed the highest amount of phenolic and flavonoid content and reducing capacity, whereas hexane extracts exhibited the lowest level of reducing capacity. [5,9]The DPPH free radical scavenging assay showed potent inhibitory capacity of AgNPs Fig.11. The percentage of inhibition of free radicals increased with increase in concentration of AgNPs.

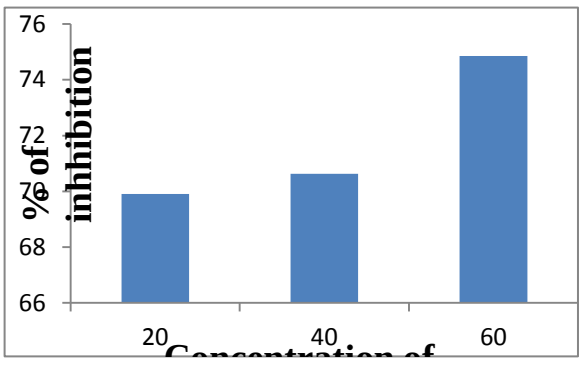
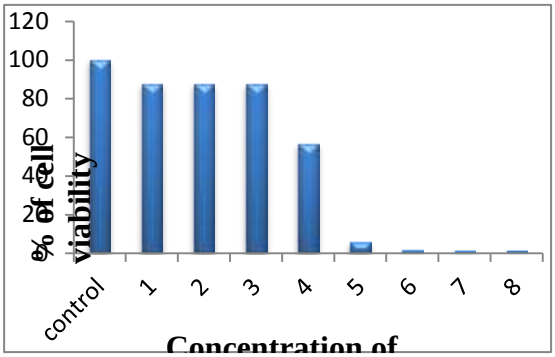


Fig.11: DPPH free radical scavenging activity

***In-vitro* anticancer activity**

The viability of MCF 7 cells was measured by MTT assay after culturing for 24 h. All nanoparticles affected the metabolic activity in concentration dependent manner when they were added in the concentration range of 1-8 µg/ml to the cells [13, 15]**.** Cytotoxicity of the nanoparticles increased in relation to increasing concentration, as shown in Fig-12. The cytotoxic effects of AgNPs are the result of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA.



Fig,12: MTT assay by MCF-7cell line

SUMMARY AND CONCLUSION

In this study, silver nanoparticles were synthesized using *C.fistula* leaf extract and were characterized by UV-visible, FT-IR, SEM, EDX and AFM. The antimicrobial activity was tested against six different human pathogens and it was found that AgNPs were more effective against gram positive bacteria. The anticancer activity was tested against MCF 7 human breast cancer cell line and it was found that the cytotoxicity increased with increase in concentration of AgNPs.

Thus the application of such eco-friendly silver nanoparticles in bactericidal, anticancer and other medical and electronic applications, make this method potentially exciting for the large scale synthesis of other nanomaterials and hence may be useful for relevant drug delivery and other biomedical applications.

ACKNOWLEDGEMENT

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REFERENCES

1. Ahmad, N., Sharma, S., Singh, V.N., Shamsi, S.F., Fatma, A. and Mehta, B.R. (2011) 'Biosynthesis of silver nanoparticles from *Desmodium triflorum*: a novel approach towards weed utilization', Biotechnol. Res. Int. American Journal of Nanoscience and Nanotechnology Vol.1, pp.57-64.
2. Alagesaboopathi C. and Sivakumar R. (2011) 'Antimicrobial properties of various extracts of *Andrographis neesiana* an endemic medicinal species from India', International Journal of Pharmatech Research Vol.3, pp.27-31.
3. Awal, M.A., Ahsan, S.M., Haque, E., Asghor, Q.H. and Ahmed, M.D. (2010) 'In-vitro Antibacterial Activity of Leaf and Root Extract of *Cassia Fistula*', Med. Col. J Vol.3, pp.10-13.
4. Cao, X.L., Cheng, C., Ma, Y.L. and Zhao, C.S. (2010) 'Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities', J. Mater. Sci. Mater. M Vol.21, pp.2861–2868.
5. Choo Suet Cheng Candy., Viduranga Waisundara. and Lee Yian Hoon.(2013) 'Evaluation and Characterization of Antioxidant Activity of Selected Herbs and Spices', Journal of natural remedies Vol.13.
6. Dibrov, P., Dzioba, J., Gosink, K.K. and Hase, C.C. (2002) 'Antimicrobial Agents', Chemother. Vol.46, pp .2668 - 2670.
7. Dragieva, I., Stoeva, S., Stoimenov, P., Pavlikianov, E. and Klabunde, K. (1999) 'Extracellular synthesis of silver nanoparticles using dried leaves', Nanostruct. Mater. Vol.12, pp.67-72.
8. Hewakuruppu, Y. L., Dombrovsky, L. A., Chen, C., Timchenko, V.,Jiang, X., Baek, S. and Taylor, R. A. (2013) 'Plasmonic "pump–probe"method to study semi-transparent nanofluids', Applied Optics, Vol.24,pp.6041–6050.
9. Irshad, Md., Zafaryab, Md., Man Singh. and Moshahid, M. (2012) 'Comparative Analysis of the Antioxidant Activity of *Cassia fistula* Extracts', International Journal of Medicinal Chemistry Vol.12.
10. Laura, C., Vivekanandhan, S., Misra, M. and Amar, K.M. (2011) 'Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size', Adv. Mat. Lett. Vol.2, pp.429-434.
11. Mashaghi, S., Jadidi, T., Koenderink, G. and Mashaghi, A. (2013) 'Lipid Nanotechnology', Int. J. Mol. Sci, Vol.14, pp.4242–4282.
12. Mukeshwar Pandey. and Mousumi Debnath. (2011) 'Phytomedicine: An ancient approach turning into future potential source of therapeutics', Journal of Pharmacognosy and phytotherapy Vol.3, pp.113-117.
13. Muthu Irulappan, S., Bharath Manikanth, S., Kalishwaralal, K., and Gurunthan S. (2010) 'Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumour model', Int. J. Nanomedicine Vol. 5, pp.753–762.
14. Niranjana, V.A., Narendhar, C. and Anbarasan, B. (2012) 'Synthesis and Evaluation of the Silver Nanoparticles for the Anticancer Activity using *Cardiospermum Halicacabum* Extract', International Journal of Drug Discovery and Herbal Research Vol.2, pp.504-508.
15. Pandya, J., Vishal, L., Tusharbindu, R., Rutu, R., Swati, D. and Amee J. (2012) 'Pharmacognostic and phytochemical evaluation of leaves of *Cassia fistula*', International journal of Pharmacy & life sciences.
16. Srivastava, R., Roseti, D. and Sharma, A.K. (2007) 'The evaluation of microbial diversity in a vegetable based cropping system under organic farming practices', Applied Soil Ecology Vol.36, pp.116 -123.
17. Sudhir Yadav., Hemraj Seer. and Rayasa Ramachandra Murthy. (2012) 'Cytotoxic, apoptotic and anti- anti-angiogenic efficacy of silver nanoparticles, synthesized using tannic acid as reducing agent under controlled temperature', Int. J. Phar. Res. Biosci. Vol.1, pp.160-177.
18. Szczepanowicz, K., Stefariska, J.,Socha, R.P. and Warszyriski, P. (2010) 'Preparation of silver nanoparticles via chemical reduction and their antimicrobial activity', Physiochem. Probl. Miner. Process Vol.45, pp.85 -98.
19. Taylor, R., Coulombe, S., Otanicar, T., Phelan, P., Gunawan, A., Wei, L., Rosengarten, G. and Prasher. (2013) 'Small particles, big impacts: A review of the diverse applications of nanofluids', Journal of Applied Physics Vol.113.
20 Vert, Michel., Doi, Yoshiharu., Hellwich, Karl-Heinz., Hess, Michael., Hodge, Philip., Kubisa, Przemyslaw., Rinaudo, Marguerite., and Schue, Francois. (2012) 'Terminology for biorelated polymers and application, Pure and Applied Chemistry Vol.84, pp.377–410.
21. Vicky, V.M., Rodney, S., Singh, A. and Hardik R.M. (2010), 'Introduction to metallic nanoparticles', J. Pharm. Bioallied Sci. Vol.2, pp.282–289.
22. Victor, O.N. and Chidi Obi. (2009) 'Phytochemical constituents of some selected medicinal plants', African Journal of Pure and Applied Chemistry Vol.3, pp.228-233.
23. Zadegan Reza, M. and Norton Michael, L. (2012) 'Structural DNA Nanotechnology: From Design to Applications', Int. J. Mol. Sci. Vol.13, pp.7149–7162.