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DESIGN AND SYNTHESIS OF BIOGENIC SILVER NANOPARTICLES FROM AQUEOUS LEAF EXTRACT OF *MIRABLIS JALAPA L.*, AND EXPLORING IT'S *INVITRO* ANTI-CANCER ACTIVITY

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ABSTRACT

In recent science Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1 – 100 nm in one dimension used significantly concerning medicinal chemistry, atomic physics, and all other known fields. *Mirablis Jalapa* L(Family: Nyctaginaceae) is versatile medicinal plants enriched with novel bioactive molecules and displays board-spectrum pharmacological actions including anticancer potential. The study planned to biosynthesize anticancer potent silver nanoparticles using aqueous leaf extract of *Mirablis Jalapa*. The synthesized nanoparticles were confirmed by colour transformation and UV-Vis spectrophotometry. The size and morphology of the silver nanoparticle were characterized by Scanning electron microscope (SEM). The stability of silver nanoparticles were detected by Fourier Transform Infra Red Spectroscopy (FT-IR). EDAX and Zeta potential were also detected for silver nanoparticles. Anticancer activity of silver nanoparticles tested against Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma(EAC) cells. The appearance of reddish brown colour and UV absorption 428 nm (OD=0.0695) confirmed the synthesized silver nanoparticles. The silver nanoparticles showed spherical structure and their size were ranging from 1- 100 nm under SEM observations. FT-IR spctra of silver nanoparticles showed the peaks for the functional groups, C=O,-C=C,C-H and OH which indicate the stability of synthesized silver nanoparticles. The synthesized of silver nanoparticles using aqueous extract of leaves of *Mirablis Jalapa* would be helpful for the preparation of potent cytotoxic agents against DLA and EAC cells to destroy cancer cells.

KEYWORDS: *Mirablis Jalapa*, Green synthesis, Silver nano particles, EDAX, Anti cancer activity.

INTRODUCTION

Nanotechnology deals with processes that take place on the nanometer scale, that is, from approximately 1 to 100 nm. Nanoparticles with controlled size are of fundamental and technological interest as they provide solutions to technological and environmental challenges in the areas of medicine, solar energy conversion, catalysis and water treatment. Thus, production and application of nanomaterials from 1 to 100 nanometers (nm) is an emerging field of research.[1] Nanoparticles are collection in aggregate of atoms in the range of 1-100 nm with unique structure and properties, which are widely used in an increase amount of applications. Silver nanoparticles (Ag-NPs) in particular, provide effective growth inhibition of various microorganisms in suspension and on solid medium. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner.[2] Green synthesis of nanoparticles reports to be clean, nontoxic, cost effective, and environmentally benign. Among the various biological methods available, the use of microbe-mediated synthesis has limited industrial use, as they require antiseptic conditions. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which was found to be reliable and eco friendly. The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields.

Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties they are gaining the interest of scientist for their novel methods of synthesis. Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science. Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for "green nanotechnology". Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants.

Plants provide a better platform for nanoparticles synthes Nis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. On the contrary, the use of plant extract for the nanoparticles synthesis is valuable due to the ease of scaleup, less biohazardous nature, and avoiding the hideous procedure of maintaining the cell lines.[3] Cancer is a life threatening disease and leads the cases of deaths around the world.[4] According to the WHO, the annual cancer cases are to rise from 14 million in 2012 to 22 million in the next two decades.[5] The development of potent and effective antineoplastic drugs is one of the most persuaded goals. Among the various approaches, the exploitation of natural products is one of the most successful methods to identify novel hits and leads.[6] Based on literature survey the plant *Mirablis Jalapa* has no comprehensive review available so far which explains the synthesis of biogenic silver nanoparticles and *in vitro* anti-cancer activity. So the study extends for the effective integration of information on chemical composition extracts, in-vitro anti-cancer activity studies of the synthesized silver nanoparticles as well as indigenous knowledge of traditional healers. *Mirablis Jalapa* is a shrub belonging to the family Nyctaginaceae. Previous study revealed that the presence of antioxidant, anti-inflammatory, anti-microbial but the biogenic synthesis of silver nanoparticles and in-vitro anti cancer study, of this plants is not get reported. In the present study, we report for the first time synthesis of silver nanoparticles, reducing the silver ions of silver nitrate by the aqueous extract of Mirablis *jalapa* leaves. Morphological characterizations are performed using SEM analysis. The optical absorption spectrum of silver nanoparticles was recorded using UV-Vis spectrophotometer. EDAX and Zeta Potential were carried out. *In Vitro* anticancer activity of the prepared silver nanoparticles were carried for using Dalton's lymphoma ascites cell (DLA) and Ehrlich ascites carcinoma cell (EAC) method.

MATERIALS AND METHODS

Silver nitrate used for the synthesis of silver nanaoparticles was produced from E. merck, (india) Limited, Mumbai, India. Mirablis jalapa used in this work were collected from Coimbatore and authentified from Botanical survey of India, Tamilnadu Agricultural university, coimbatore. Cultures dehydrated luria broth and nutrient agar media used for bacterial growth study were the products of E. Merck (india), Limited, Mumbai, India.

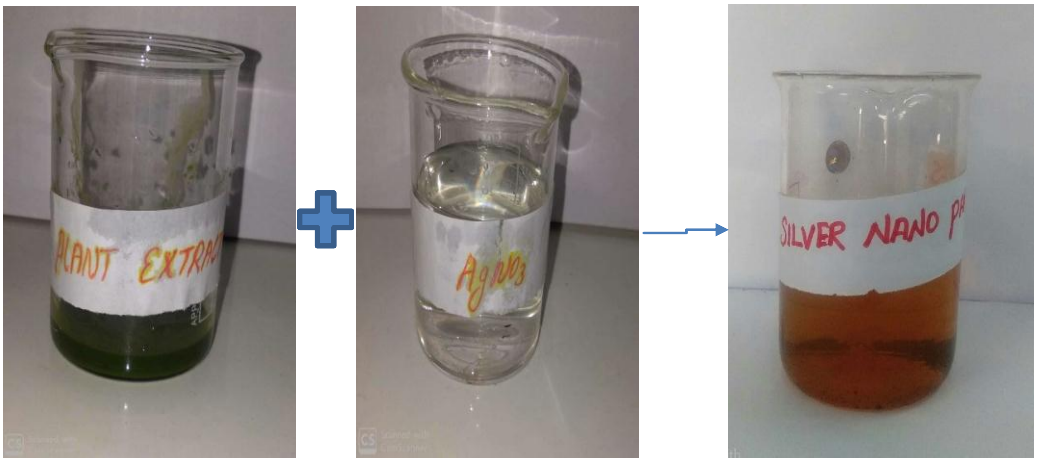
PREPARATION OF LEAF EXTRACT

Indian medicinal plant M.jalapa was selected from Coimbatore, India, on the basis of cost effectiveness, ease of availability and medicinal property. 20g of fresh leaves of *M.Jalapa*, wash thoroughly with double distilled water and were then cut into small pieces. These finely cut pieces were then mixed with 100ml double distilled water and this mixture was kept for boiling for a period of 5mins. After cooling it was filtered through whatman filter paper no.1.

SYNTHESIS OF SILVERNANO PARTICLES

10ml of aqueous extract of *M.Jalapa* leaves were added to 90ml of silver nitrate solution so as to make its final concentration to 10-3M the solution was allowed to react at room temperature. Periodic sampling after 30mins was carried out to monitor the formation of AgNPs. The qualitative evaluation of reducing potential of aqueous extract of *M.Jalapa*

The reducing of Ag+ was confirmed from the UV-Vis spectrum of solution. The nanoparticles were separated out from the mixture by ultracentrifugation.



CHARACTERIZATION OF SILVER NANO PARTICALES

ULTRA VIOLET SPECTROSCOPY

UV-Visible spectroscopy analysis was carried out on a Systronic UV Visible absorption spectrophotometer 117 with a resolution of ±1 nm between 200 and 1000 nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension (0.5 mL) were taken and analysed at room temperature. The progress of the reaction between metal ions and the leaf extract was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of silver ions and formation of silver nanoparticles occurred within an hour of reaction. Control was maintained by using AgNO3.

FT IR SPECTROSCOPY

The interaction between protein-silver nanoparticles were analyzed by Fourier transform infrared spectroscopy (FTIR) in the diffuse refluctance mode at a resolution of $4cm^{-1}$ in the KBr pellets and the spectra were recorded in the wavelength interval of 4000 to 400nm. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag+ ions and the capping of the bioreduced AgNPs synthesized by seaweed extract. For comparison, the seaweed filtrate was mixed with KBr powder and pelletized after drying properly and subjected to measurement.

SCANNING ELECTRON MICROSCOPY

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan patterns. Synthesized silver nanoparticles were subjected to Scanning Electron Microscope (JEOL model JSM-6390 LV) analysis. Thin films of the sample was prepared on the copper grid by dropping a small quantity of sample on the grid and then the films on the grid was allowed to dry under mercury lamp for 5 mind. Then it was fixed in to sample holder and placed in a vacuum chamber of the scanning electron microscope and observed under low vacuum and SEM images were recorded.

ZETA POTENTIAL

The ZP is related to the surface charge, a property that all materials possess or acquire, when suspended in a fluid. The sign and magnitude of ZP affects process control, quality control, and product specification. At the simplest level, it can help maintain a more consistent product and at a complex level, it can improve product quality and performance. Zeta potential is a scientific term for electrokinetic potential in colloidal dispersion. In the colloidal chemistry literature, it is usually denoted using the greek letter zeta, hence zeta potential. From a theoretical view point, the zeta potential is the electro potential in the interfacial double layer at the location of the slipping plane relative to a point in the bulk fluid away from the interface. In other words, zeta potential difference between the disposal medium and the stationary layer of fluid attached to the dispersion particle. Zeta potential is not measurable directly but it can be calculated using theoretical models and an experimentally determine electrophoretic mobility or dynamitic electrophoretic mobility.

ENERGY DISPERSIVE X-RAY (EDAX)

Analysis through energy dispensive X-Ray (EDX) spectrometers confirmed the presence of element silver signal of the silver nano particles. The vertical axis displays the number of X-Ray counts while the horizontal axis displays energy in kev. The EDAX spectrum observed a strong signal from the silver atoms in the nanoparticles at 3kev and weak signal are from the plant organic constituents. The presence of elemental Silver was confirmed through EDS. Energy dispersive analysis X-ray 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; the output of an ultra low noise pre-amplifier connected to the low noise is a statistical measure of the corresponding quantum energy. A semiconductor material is used to detect the X-rays together with processing electronics to analyses the spectrum.

RESULTS AND DISCUSSION

UV-VIS SPECTROPHOTOMETER ANALYSIS

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 428 nm in case of *Mirablis Jalapa*. The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles the insets to Figure: 1 represent the plots of absorbance at λmax (i.e., at 428 nm) versus time of reaction. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag^+ ions is complete within 4 Hrs. after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the Nanoparticle solution with time.

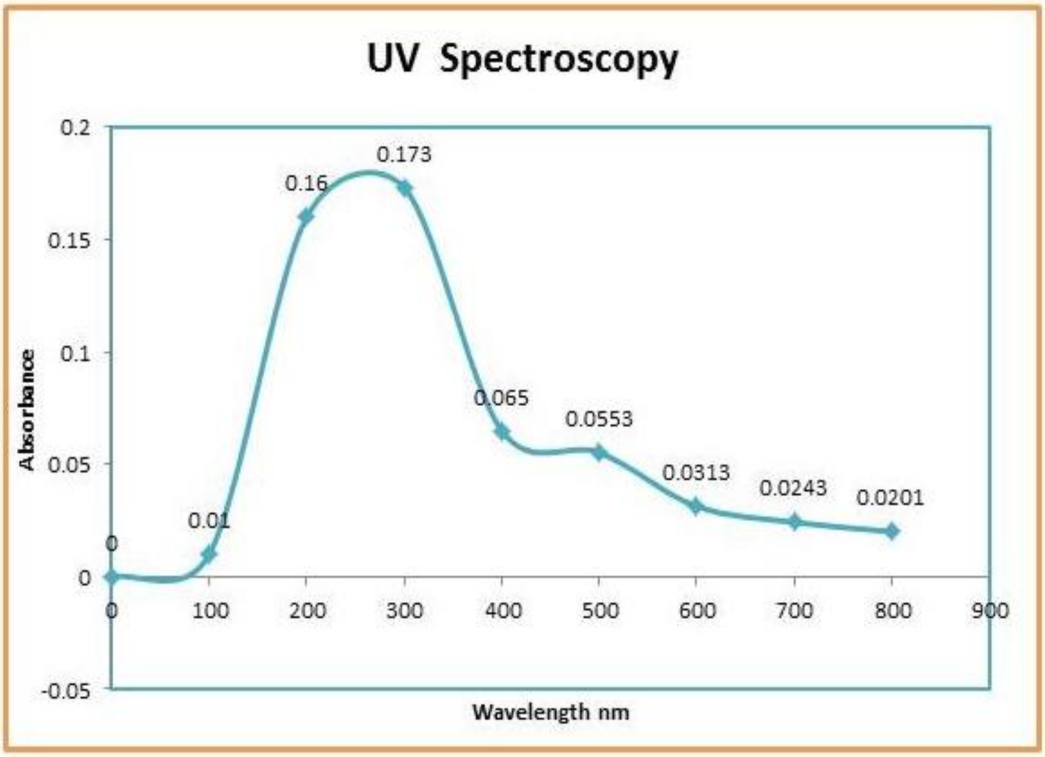


Fig. 1: UV-Vis absorbtion spectra of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

SCANNING ELECTRON MICROSCOPE (SEM)

The scanning electron microscopy (SEM) analysis was carried by a Carl Zeiss EVO-18 electron microscope. For SEM imaging to visualize the morphological size and shape of the silver nanoparticles, a sample of a nanoparticles solution was placed on a carbon strip attached to a SEM brass, extra solution was detached using blotting paper and then allowed to dry by putting it under a mercury lamp for 5 min. The elemental compositions of AgNPs were obtained using BRUKER EDS –(QUANTAX 200-XFLASH) SDD (Silicon Drift Detector) by variable pressure mode, at acceleration voltage of 20 KeV.

The Fig.2 showed spherical structure and their size were ranging from 1- 100 nm under SEM observations.

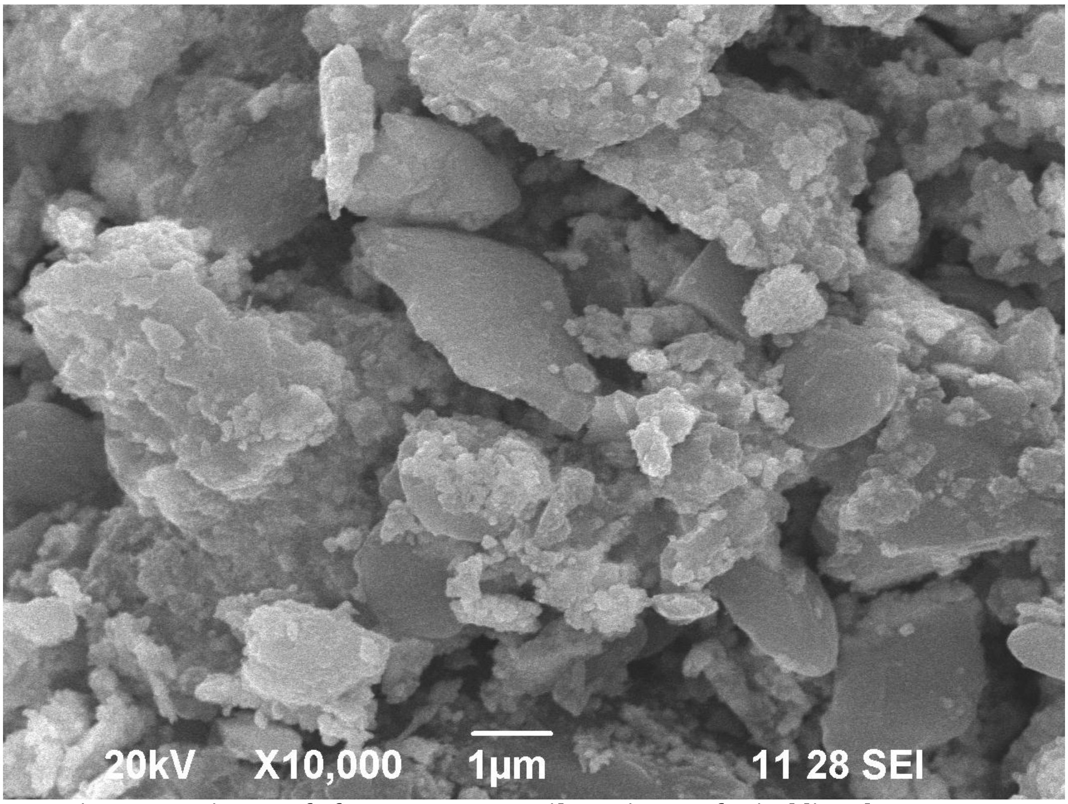


Fig. 2: SEM image of of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

ENERGY DISPERSIVE X-RAY (EDAX)

Analysis through energy dispensive X-Ray (EDX) spectrometers confirmed the presence of element silver signal of the silver nano particles. The vertical axis displays the number of X-Ray counts while the horizontal axis displays energy in kev. The EDX spectrum observed a strong signal from the silver atoms in the nanoparticles at 3kev and weak signal are from the plant organic constituents. The analysis revealed that the nanostructures formed were solely of silver. From EDX spectra it is clear that the silver nanoparticles reduced by *mirablis jalapa* have the weight percentage of silver as.

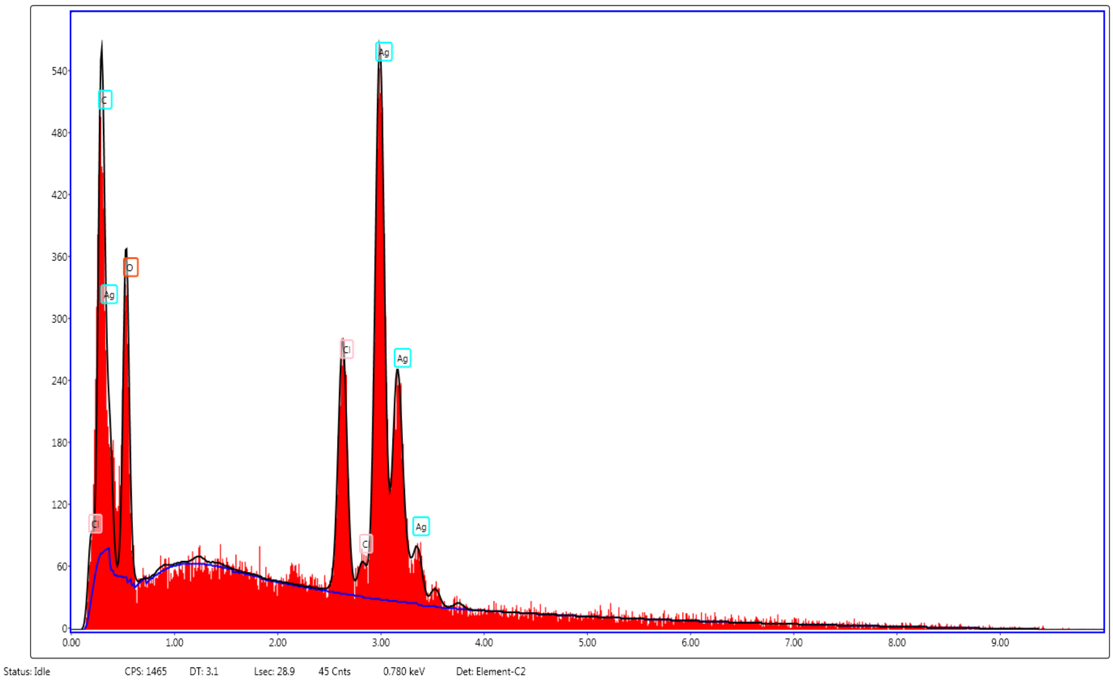


Fig. 3: EDAX image of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelength at a time. the FTIR image of o. sanctum medicated synthesized silver nanoparticles indicates presence of biomolecules involved in the reduction process.

FTIR spectrum silver nanoparticles shows the characteristic peaks in the region of 4000-500 cm-1. Mirablis jalapa AgNPs had the absorption at 3142 cm-1 is notably enhanced indicating -OH stretch of hydroxyl group. Peak at 1637 cm-1 represents C=O stretch of carbonyl group and 1402 cm-1 indicates C=C stretch of alkene group.

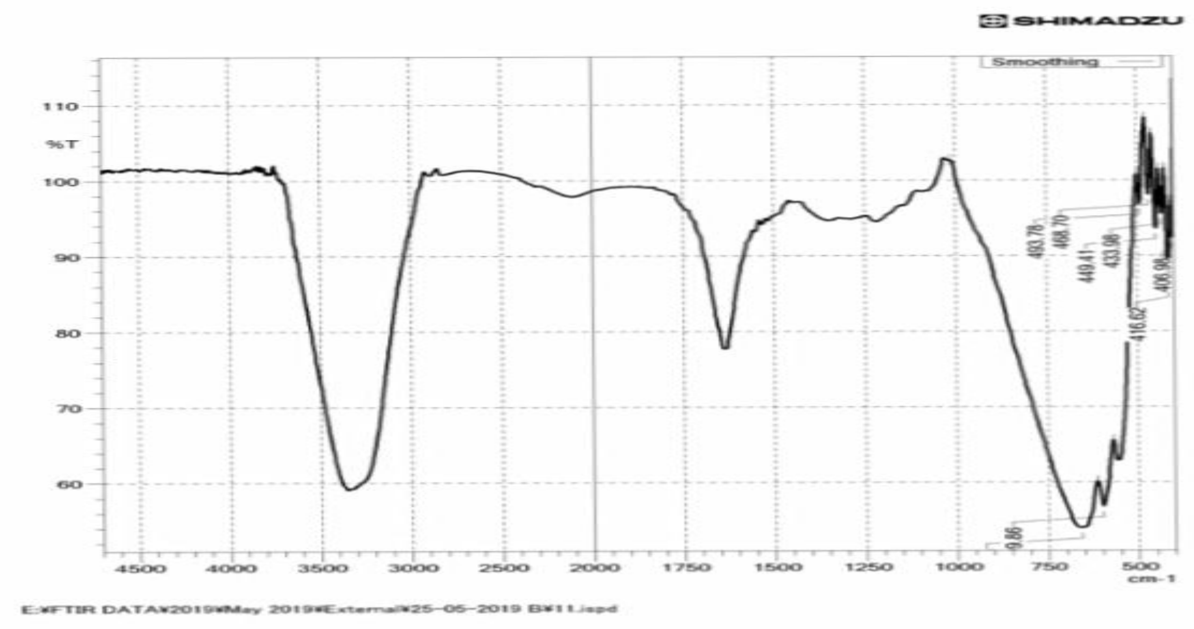


Fig. 4: FT-IR spectrum of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

ZETA POTENTIAL

Zeta potential is a scientific term for electrokinetic potential in colloidal dispersion. In the colloidal chemistry literature, it is usually denoted using the greek letter zeta (.), hence z - potential. Form a theoretical view point, the zeta potential is the electrical potential in the double layer (DL) at the location of the slipping plane relative to the point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer off fluid attached to the dispersed particle. The zeta potential is caused by the net electrical charge contained with in the region bounded by the slipping plane, and also depends on the location of the plane. Thus it is widely used for quantification of the magnitude of the charge. However, zeta potential is not equal to the different location. Such assumption of equality should be applied with caution. Zeta potential is often the only available path for characterization of double layer properties. The zeta potential is a key indicator of the stability of colloidal dispersion. The magnitude of the zeta potential indicates the degree electrostatic repulsion between adjacent.

MEASUREMENT

The potential is not measurable directly but it can be calculated using theoretical models and an experimentally determined electrophoretic mobility or dynamic electrophoretic phenomenon.

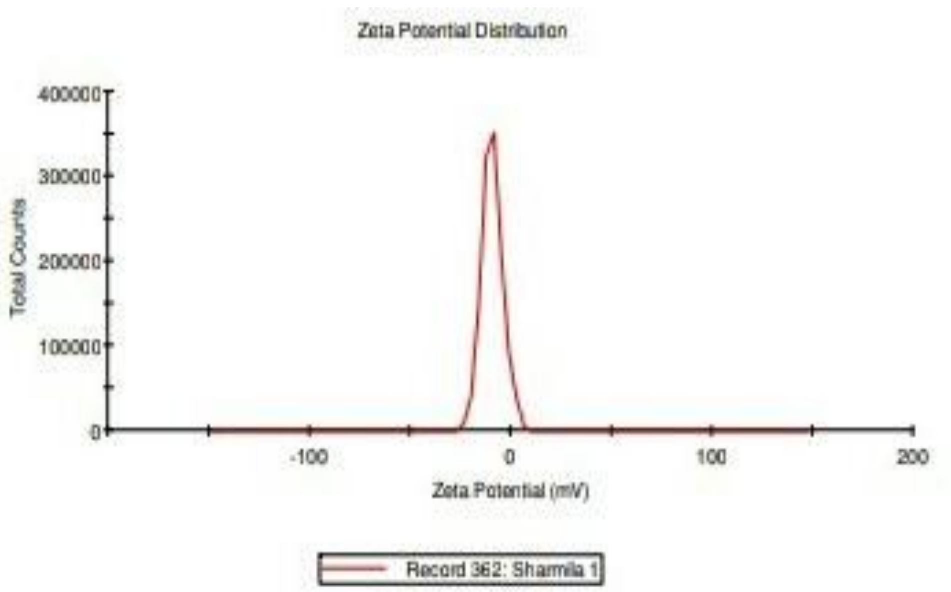


Fig. 5: Zeta potential of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

ELECTROKINETIC PHENOMENA

Electrophoresis is used for estimating zeta potential of particulates, whereas streaming potential of particulates, whereas streaming potential or current is used for porous bodies and flat surface. In practice, the zeta potential of dispersion is measured by applying an electric field across the dispersion. Particles with in the dispersion with a zeta potential will migrate toward the electrode of opposite charge with a velocity proportionally to the magnitude of the zeta potential. Diagram showing the ionic concentration and potential difference as a function of distance from the charged surface of a particles suspended in a dispersion medium.

ZETA POTENTIAL REPORT

Zeta potential of silver Nano particles of Mirablis jalapa.

The result from the study demonstrated the potential of using nanoparticle the range of - 24. 1, so that the silver nanoparticles exhibit superior cellular drug delivery.

***IN VITRO* ANTI – CANCER STUDIES**

The test compound was studied for short term *in vitro* cytotoxicity using Dalton's lymphoma ascites cell (DLA) and Ehrlich ascites carcinoma cell (EAC). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×106 cells in 0.1 ml) was added to tubes containing various concentration of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixture were intubated for 3 hour at 37 c. Further cell suspension was mixed with 0.1 ml 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cell do not take up the dye. The number of stained and unstained cells were counted separately.

$$\%\ cytotoxicity = \frac{No.\ of\ dead\ cells}{No.\ of\ live\ cells + No.\ of\ dead\ cells} \times 100$$

The whole plant of mirablis jalapa showed ANTI-CANCER activity. For a drug concentration of 200ug/ml, the plant leaves showed 100% (DLA) and of anti-cancer activity. The percentage of inhibition are showed in table. When the standard concentration increases, the percentage of cell inhibition also increases.

Table 1: *Invitro* anticancer activity of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

S.NO	DRUG CONCENTRATIONUG/ML	PERCENTAGE CELL DEATH(DLC)
1	200	100
2	100	100
3	50	90
4	20	78
5	10	65

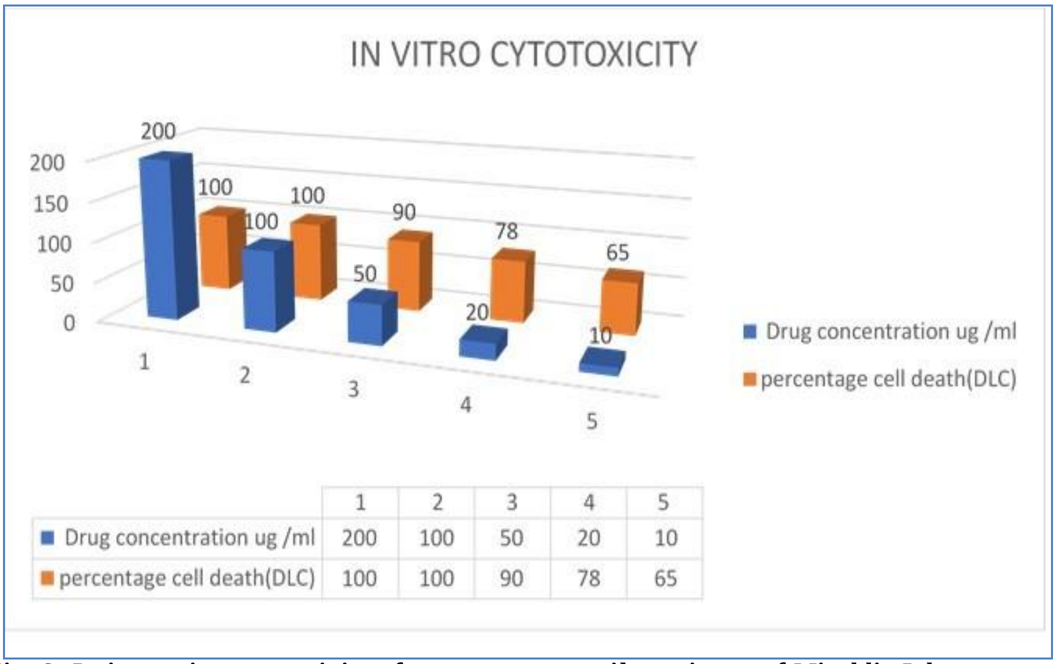


Fig. 6: *Invitro* anticancer activity of aqueous extract silver nitrate of *Mirablis Jalapa* extract.

CONCLUSION

In the present investigation, novel approach for biosynthesis of AgNPs from leaves extract of *Mirablis Jalapa* was given. The synthesized AgNPs were spherical in shape with size ranging around 20-30 nm as observed in SEM analysis. The FT-IR spectrum ascribed the biological molecules which perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. AgNPs began to form within 10min and higher formation yield at 70min after addition of leaf extract to silver nitrate as shown by the UV-vis spectrum at 428nm. It was found that the formation of AgNPs was increased with time. Zeta potential and EDAX of the prepared silvernanoparticles was carried out. Thus, our findings suggest the anticancer potential of biosynthesized AgNPs for DLA and EAC and could play an important role in the development of new therapeutic agent for the treatment of cancer. Moreover, this process could be easily scaled up for the industrial applications to increase the yield of the nanoparticles significantly, which undoubtedly would establish its commercial viability in medicine.

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