Invitro Anti–Breast Cancer Evaluation of *Ipomoea aquatica* Silver microparticles by using MTT assay on MCF-7 cell line

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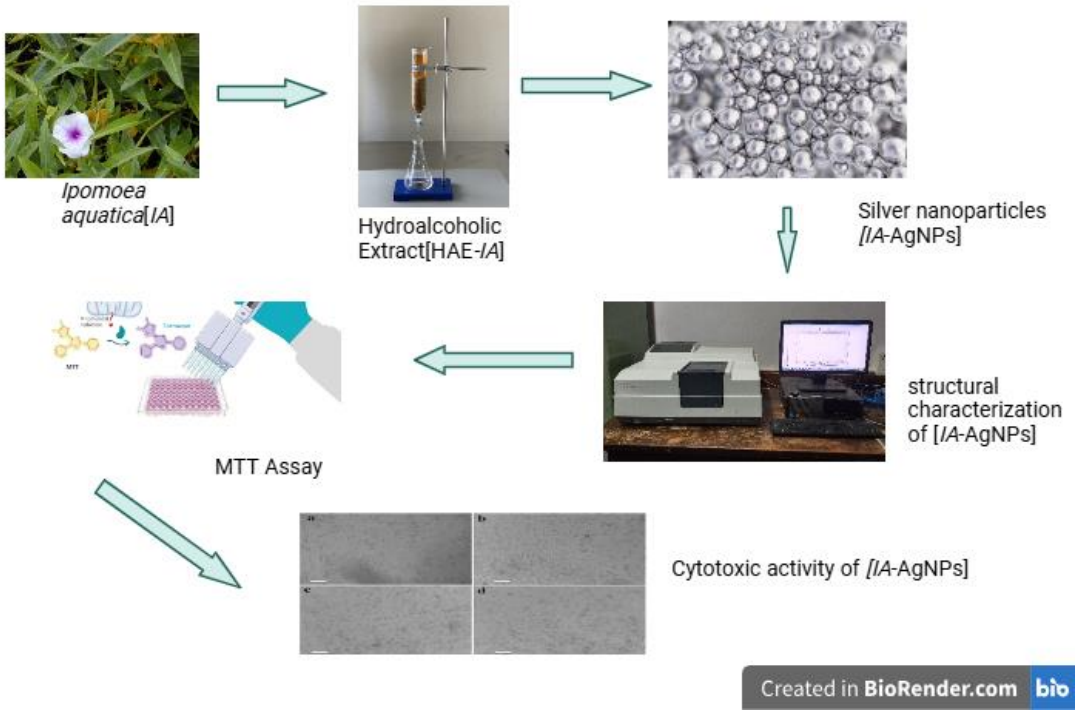
Abstract

Herbs are often used as raw materials in traditional medicine for a variety of nano formulations. **Methodology:** The cells were Seeded cells in 96 well plate. The plate was Incubated at 37^0C for 24 hrs. The MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) reagent was added to each well and it was incubated again. Then DMSO was added to each well Absorbance was read at-620nm (ELISA multiwall plate reader). **Results:** The experimental results demonstrate that the extract inhibited cell proliferation in a dose dependent manner. The IC_{50} values of samples against cancer cells were calculated and it is found to be 6,17, and 32 µg/ml for standard drug [Doxorubicin], IA-Ag MPs, IA plant extract respectively. It can be noticed from the results that the observed IC_{50} values of the samples was low and significantly inhibited the proliferation of selected human breast cancer cells **Conclusion:** The study indicated by comparing the standard drug (DOX- Doxorubicin) with IA-Ag MPs and IA plant extract exhibits cytotoxic activity thus might be developed as a new type of anti –cancer agent. And the microparticles demonstrated dose-dependent cytotoxicity, with higher concentrations leading to a greater reduction in cell viability. This indicates their ability to effectively target and reduce the viability of breast cancer cells.

Keywords: Silver microparticles [Ag MPs], MTT assay, cytotoxic activity, MCF-7 cell lines, Ipomoea aquatica [IA]



1. Introduction

Microparticles are colloidal solid particles with a size range of 100–1000nm, are used in medicine for carrying antibodies, drugs, imaging agents, and other substances to specific parts of the body. They have been studied for their detection, diagnosis, and gene delivery into tumours due to their unique physiochemical properties [1-3]. Biologically-mediated synthesis of microparticles has emerged as a viable option, with high yield production of microparticles using various biological systems. *Ipomoea aquatica* silver microparticles (IA-Ag MPs) have antimicrobial activity due to Ag+ ions, which inhibit microbes' electron transport chain, damage DNA and RNA, and inhibit cell division. The International Agency for Research on Cancer (IARC) estimates that globally, 1 in 5 people develop cancer during their lifetime, and 1 in 8 men and 1 in 11 women die from the disease. Breast cancer represents 1 in 4 cancers diagnosed among women globally. Colorectal, lung, cervical, and thyroid cancers are also common among women (MamtaSaxena et al., 2013; Marvibaigi et al., 2016) Breast cancer is a common cancer in women worldwide, with over 1.5 million diagnosed annually. Early detection of the disease can lead to a good prognosis and a high survival rate. As per the report on the plant, Convolvulaceae plants would have resin glycosides like aquaterins, convolvulins and jalapins (Ono, 2017). Aquaterins (I to XIX) present in the I. aquatica plant had evaluated for aquaterin II induced G0/ G1 arrest regulated by related proteins CDK4/6, cyclin D/E and p21 used mitochondria-mediated apoptosis featured by MMP decrease, ROS accumulation, caspase cascade activation and Bax/Bcl-2 alteration (Bo-Yi Fan et al., 2015). Anticancer activity of Ipomoea aquatica, a common leafy vegetable in prostate cancer and reduction of cisplatin-induced hepatotoxicity in vivo were reported. From the above literature information, it is clear that I. aquatica plant extract has potential as anti-tumor active while its effect has not been explored with breast cancer cell lines. Resin glycosides, as members of glycolipids have shown antitumor activity. The activities include mammalian cytotoxicity of tricolorin, antimetastaticacivity of cairicoside, HepG2 inhibition by aquaterin-II (Chen et al., 2017; Fan BY et al., 2015; Pereda-Miranda et al., 2010; Rencurosi et al., 2004).

The purpose of this research is to produce *Ipomoea aquatica*- mediated AgMPs (IA-AgMPs) ensuring the green synthesis method and to assess the anti-breast cancer potential using MTT assay on MCF-7 cell lines.

2. Material and Methods

ELISA multi well plate reader, Nikson bright field inverted light microscope, Double beam UV/VIS Spectroscopy (Shimadzu UV-1800), Thermo Scientific Nicolet Summit FTIR Spectrometer, Centrifuge, Hot Plate (Cintex), Magnetic Stirrer, silver nitrate, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], DMSO (Dimethyl sulfoxide, Hot air oven, Ethanol,), Methanol, Millipore water, Distilled water.

2.1 Preparation Hydroalcholic extract of *Ipomoea aquatica* (IA)

The Ipomoea aquatica (IA) plants were collected and washed thoroughly with distilled water three times. Following the washing, the plants were dried under sunlight and then finely ground into a powder. This powder underwent hydroalcoholic extraction using a cold percolation method with a mixture of water and 95 % ethanol (1:1 v/v). The resulting extract was concentrated using a rotary evaporator to obtain a concentrated extract. Analysis of the aqueous alcoholic extract of the whole plant of I. aquatica revealed the presence of various compounds, including flavonoids, saponins, tannins, alkaloids, glycosides, phenols, terpenoids, and coumarins. Further analysis of the leaves of I. aquatica identified triterpenes, flavonoids, saponins, alkaloids, tannins, glycosides, and additional flavonoids [4].

2.2 Synthesis of Metal Nano Particles

The concentration of the vegetal extract used for the synthesis of microparticles was 2 mg/ml. Upon the addition of 5 mM AgNO3, the color of the extract rapidly changed to brown within 30 min, with no further observable alteration after 24 h (Fig. 1). The observed colour change can be attributed to the presence of active phytochemicals within the I. aquatica extract. These phytochemicals act as reducing agents, facilitating the transformation of silver ions into AgMPs through the excitation of surface plasmon resonance. The bioreduction of Ag + ions from the $AgNO_3$ solution into silver microparticles using phyto chemicals from I. aquatica was confirmed through UV–Visible spectroscopy. AgMPs were synthesized by slowly adding 50 mL of IA extract dropwise into 50 mL of a freshly prepared aqueous silver nitrate (AgNO3) solution with a concentration of 1 mM in a glass beaker. The mixture was stirred continuously using a magnetic stirrer at room temperature (24^0C). After suspension, the solution underwent centri- fugation at 1500 rpm for 1 h. The resulting solid particles were collected and subsequently dried in a beaker on a hot plate at 80 ˚C for 2 h. Following this, the dried powder was finely ground using a mortar and pestle to achieve the desired AgNMPs. These AgMPs were then transferred into an Eppendorf tube and stored in a refrigerator for further analysis [4,5]

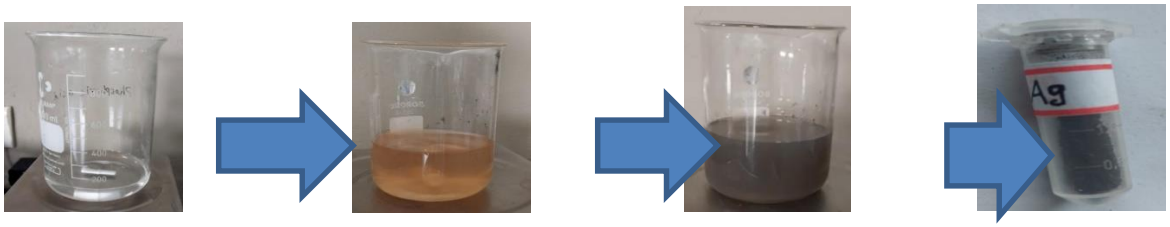


Figure 01: Silver microparticles (IA-Ag MPs) formation was confirmed by color changed to brownish black from reddish brown

2.3 UV-visible spectrophotometric analysis

A Double beam UV-Visible spectrophotometer (Model: UV-1800, Shimadzu) was used to identify the primary formation of microparticles. The synthesized metal microparticles were diluted with methanol and taken a quartz cell for the recording the absorbance spectrum at the wavelength of 200nm to 800nm against the methanol as blank [5]

2.4 Fourier transform infrared analysis

Fourier transform infrared spectroscopy makes the information about the functional groups determination present in the surface of metal microparticles in the form of stretching and bending frequencies of the molecules. The functional groups of microparticles were recorded from 4000 cm^{-1} to 500cm^{-1} by Altenuated Total Reflectance Method [to recognize the organic, inorganic, biomolecule residues and nanoparticle formation] with a FT-IR Spectrophotometer [5]

2.5 ZETA SIZER

The synthesized silver microparticles were characterized using a Zeta Sizer (Malvern Instrument) to determine the particle size distribution and zeta potential. The analysis was performed at room temperature with the sample dispersed in methanol. Measurements were taken to evaluate the average particle size and polydispersity index (PDI). The results were recorded and analyzed to assess the stability and dispersion quality of the synthesized microparticles.

2.6 MTT assay

The selected cells that were grown on cover slips (1×105 cells/cover slip) were incubated with complex at different concentration, and they were then fixed in an ethanol: acetic acid solution (3:1, v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were micro graphed. The morphological changes of the cells were analysed using Nikon (Japan) bright field inverted light microscopy at 10× magnification

The inhibitory concentration (IC50) value was evaluated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The cells were grown (1×104 cells/well) in a 96-well plate for 48 h in to 80% confluence. The medium was replaced with fresh medium containing serially diluted sample, and the cells were further incubated for 24h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37oC for 4 h. After removal of the supernatant, 50µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula [11-16].

$$\%\ \boldsymbol{OF\ VIABILITY} = \frac{OD\ VALUE\ OF\ EXPERIMENTAL\ SAMPLE}{OD\ VALUE\ OF\ EXPERIMENTAL\ CONTROL} \times 100$$

3. Result and Discussion

3.1 UV-visible spectrophotometric analysis

Synthesized microparticles were subjected to UV–visible spectrophotometer analysis which confirms the formation of particles in the initial stage. The results through UV-VIS spectrum of obtained for IA-Ag NPs, and the peak obtained for metal microparticles varies in the range of 200-800nm which is identical to the characteristics of UV spectral analysis for metal microparticles. Surface Plasmon Resonance (SPR) patterns are used as indicative tool for metal microparticles. The exact position of Surface Plasmon Resonance (SPR) band may shift depending on individual particle properties including size, shape, dielectric constant and capping agents. For the metal nanoparticle Silver from silver nitrate at 300nm and 321nm-peak obtained at absorbance of 0.050 and 0.313 indicates the presence of metal microparticles and their format.

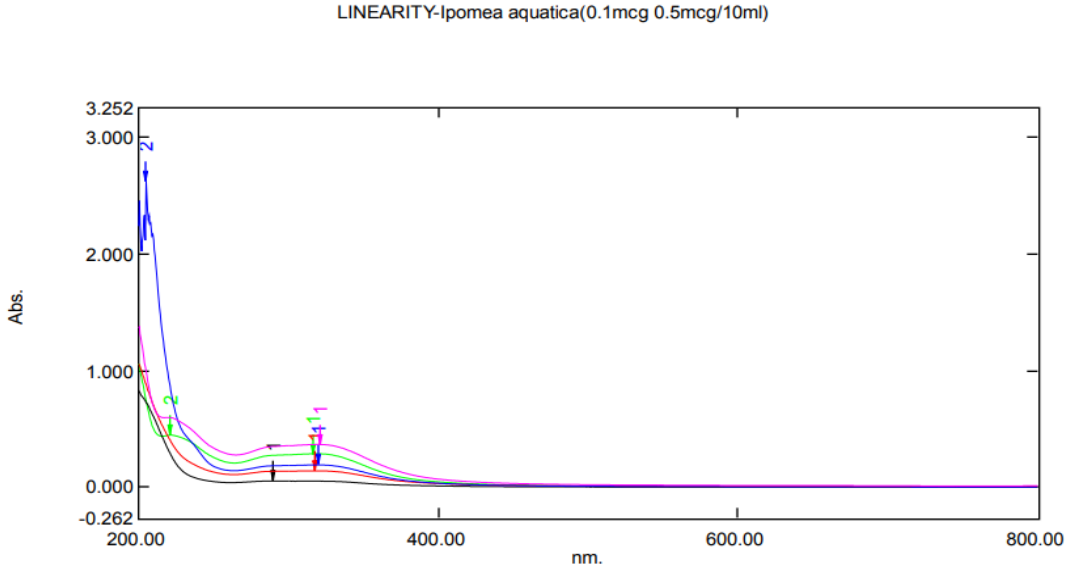


Figure 02: UV overlay spectrum for silver microparticles

3.2 Fourier transform infrared (FT-IR) analysis

FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of the metal ions and which could act as capping agent of the reduced metal microparticles thereby helping stabilization of microparticles. FTIR spectra of *Ipomoea aquatica* extract and synthesized metal microparticles and wavelength absorption bands are presented in table [01]. The phytochemical analysis of *Ipomoea aquatica* reveals the presence of flavonoids, alkaloids, steroids, saponins and proteins. *Ipomoea aquatica* extract, the peaks are observed at 3292cm^{-1},2875cm^{-1},1592cm^{-1}, 1381cm^{-1}, 1259cm^{-1}, 1046cm^{-1}. Various bands

for silver microparticles from silver nitrate -obtained in the frequency range of 3335.86cm^{-1},2918.71cm^{-1},1719.34 cm^{-1},1599.64 cm^{-1},1514.46 cm^{-1}.

The bands at 3335 cm^{-1} corresponding to O-H stretching vibrations and vibration stretch at 1599 cm^{-1} is because of C=C stretch in aromatic ring is confirmation for the presence of aromatic group. The peak at 1366 cm^{-1} corresponds to N=O stretch of nitro compounds, 2918 cm^{-1} corresponded to CH, CH_2 Aliphatic hydrocarbon and the weaker band at 1272 cm^{-1} corresponding to C-N stretch was observed. The C-O stretching vibrations of IR spectrum observed at 1071cm^{-1}. Based on the result obtained, it can see the predominant functional groups present in all microparticles are phenolic O-H, aliphatic hydrocarbons of CH, CH_2 and amine groups. Hence it can conclude that the presence of phenolic functional groups is responsible for formation and stabilization of microparticles

Table 01: characterization by FTIR spectroscopy

S.NO	Metal microparticles	Frequency range cm^{-1}	Functional group
1	IA-Ag MPs	3335.86 2918.71 1719.34 1599.64 1514.46	Hydroxyl group Methyl group Carbonyl group Phenol ring Phenol ring

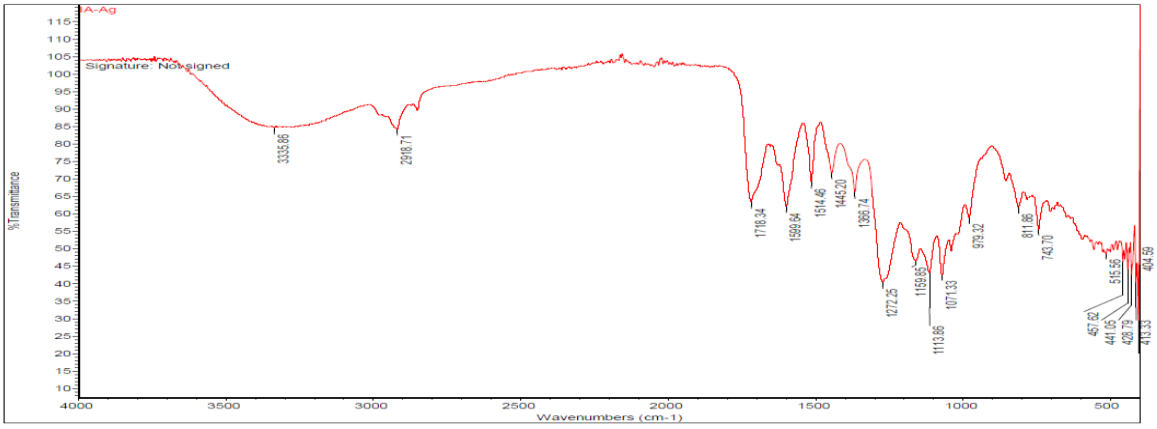


Figure 03: FTIR spectrum for silver microparticles

3.3 ZETA SIZER

The synthesized silver microparticles were characterized using a Zeta Sizer to determine their particle size distribution and zeta potential. The analysis was conducted at

room temperature after dispersing the sample in distilled water. The average particle size obtained from the Zeta Sizer was found to be 5626 nm, indicating that the synthesized particles fall within the microparticle range rather than the nanoparticle range. The polydispersity index (PDI) was recorded as 0.481, which indicates a moderate level of size distribution homogeneity. This value suggests that the microparticles exhibit some variation in size, which is typical in green synthesis methods.

The zeta potential value was measured as -19.7 mV, indicating moderate stability of the microparticles in the colloidal suspension. A negative zeta potential suggests that the particles carry a negative surface charge, providing electrostatic repulsion that helps maintain dispersion stability and prevents aggregation.

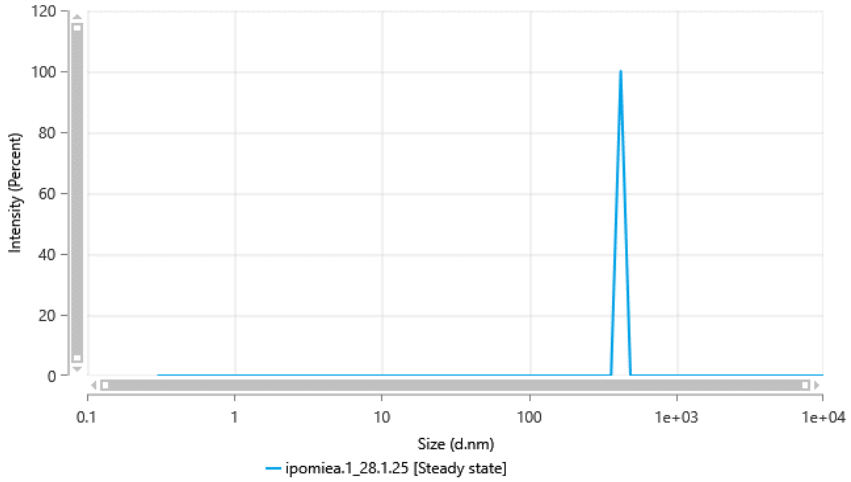


Figure 07: size distrubition by intensity

Table 03: Determination of particle size

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	5626	-	-	5626	5626
Polydispersity Index (PI)	1	-	-	1	1
Peak 1 Mean by Intensity ordered by area (nm)	420.2	-	-	420.2	420.2
Peak 1 Area by Intensity ordered by area (%)	100	-	-	100	100

3.4 MTT assay:

We analyses the effect of the extract on the cell response of the Human breast cancer cells by using the MTT assay. the *in vitro* cytotoxicity activity of sample (upto 50µg/ml concentrations) against selected breast cancer cells. The experimental results demonstrate that the extract inhibited cell proliferation in a dose dependent manner. From the IC_{50} values of samples against cancer cells were calculated and it is found to be 17, and 32µg/ml Ag NPs, and Extract respectively. It can be noticed from the results that the observed IC_{50} values of the samples is low and significantly inhibits the proliferation of selected Human breast cancer cells.

However, in the presence of samples the cells show the improved cell shrinkage, membrane blebbing and forms floating cells in a dose-dependent manner. It is well accepted that cytological investigations elucidate the antiproliferative effect routed through membrane blebbing, membrane instability and distressing the cytoskeleton of the cells by the sample.

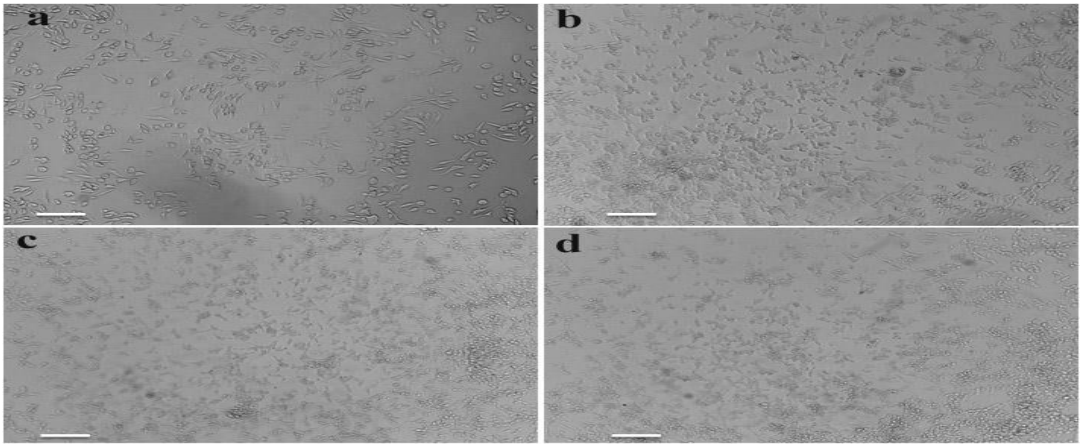


Figure 05: [a] effect of control sample in MCF-7 cell lines
[b] effect of 10 µg/ml of IA-Ag NPs on MCF-7 cells
[c] effect of 25 µg/ml of IA-Ag NPs on MCF-7 cells
[d] effect of 50 µg/ml of IA-Ag NPs on MCF-7 cells

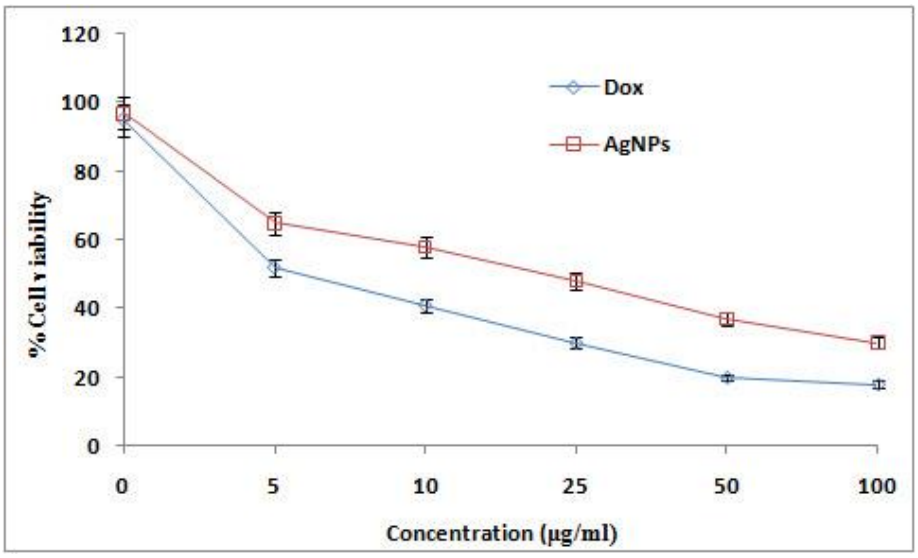


Figure 06: % cell viability of IA- Ag MPs compare with the standard drug Doxorubicin

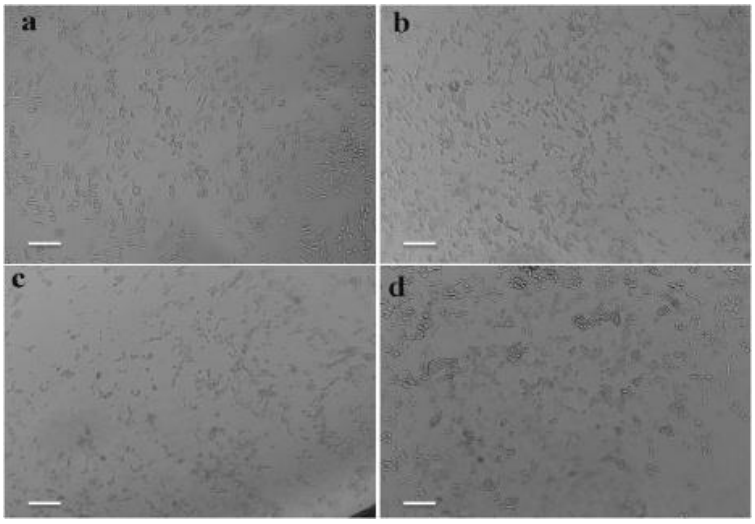


Figure 07: [a] effect of control sample in MCF-7 cell lines
[b] effect of 10 µg/ml of IA plant extract on MCF-7 cells
[c] effect of 25 µg/ml of IA plant extract on MCF-7 cells
[d] effect of 50 µg/ml of IA plant extract on MCf-7 cells

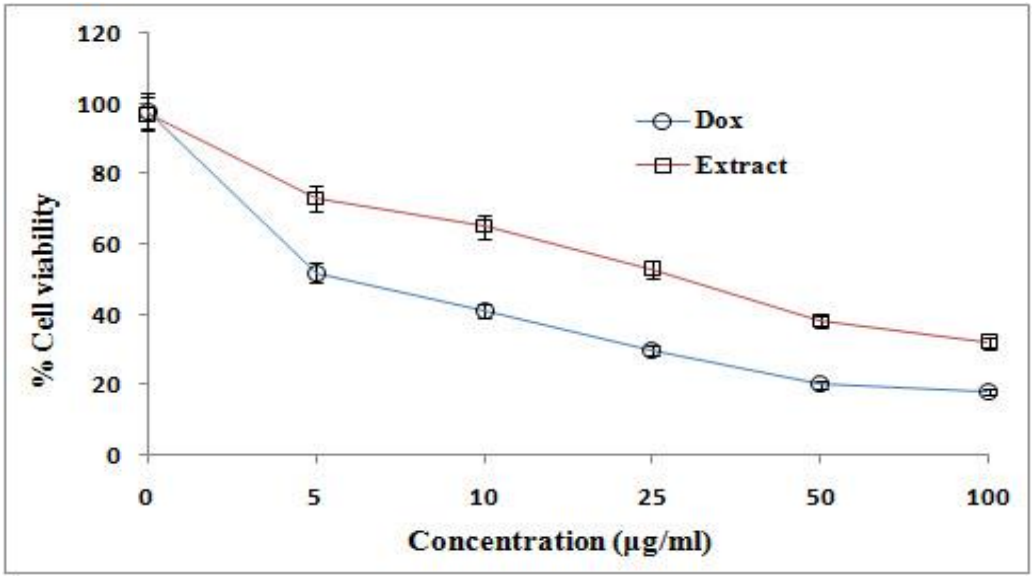


Figure 08: % cell viability of IA plant extract compared with the standard drug Doxorubicin

IC_{50} values:

Table 02: Cytotoxic activity of sample (µg/ml)

Sample code	(Inhibitory Concentration/ IC_{50}) (MCF-7)
1. Ag NPs	17 ± 0.5
3. Extract	32 ± 0.4
4. Dox (STD)	6.5 ± 0.2

4. CONCLUSION:

The study characterized IA-Ag MPs from silver nitrate using UV-Vis, FTIR, and ZETA SIZER analysis. The results showed that the metal microparticles were characterized by bands on visible wavelengths of 200nm-800nm. FT-IR spectra confirmed the presence of carboxyl, amine, and phenol compounds in the reaction mixture, reducing metal ions to zero valent microparticles. The IA-Ag MPs were found to be more powerful against selected breast cancer cells than the crude plant extract. The study revealed the potency of Ipomoea aquatica IA-Ag MPs as an anti-cytotoxic agent, suggesting their potential applications in biomedical research. The synthesized metal microparticles were highly thermally stable and supported cytotoxic properties.

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