



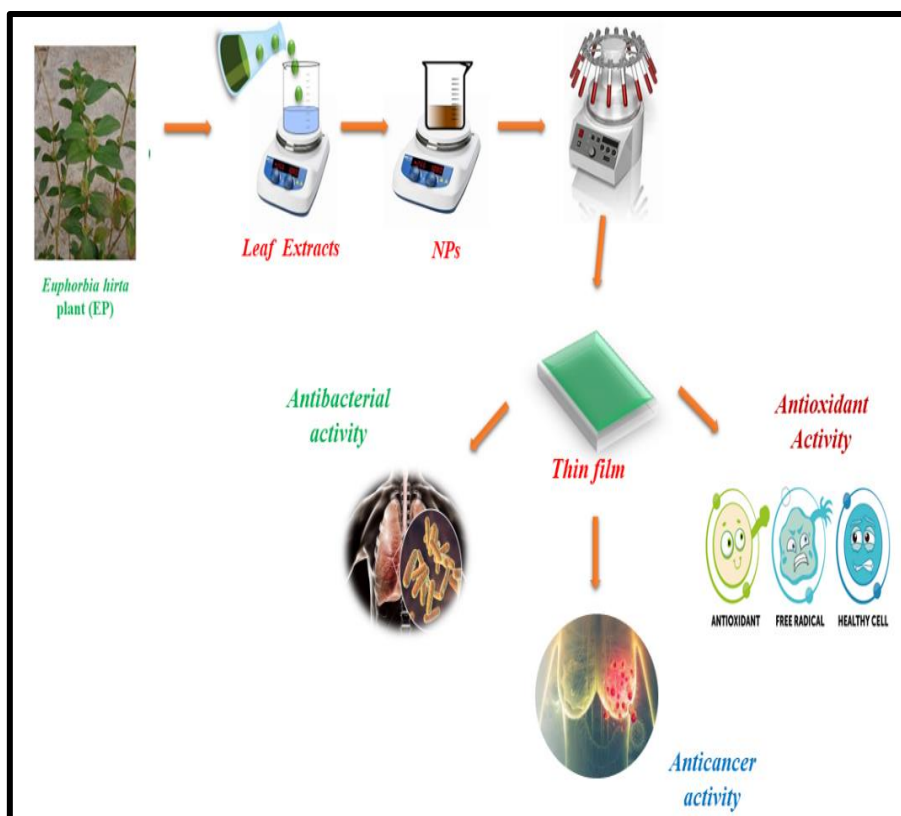
## Bioactive Nanocomposite Film Based on Polyethylene Glycol/ Polyvinyl Alcohol Containing Biogenic Silver Nanoparticles

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### Graphical Abstract



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[doi: 10.33472/AFJBS.6.1.2024.338-351](https://doi.org/10.33472/AFJBS.6.1.2024.338-351)**ABSTRACT:**

The incorporation of biogenic silver nanoparticles (AgNPs) into a film matrix of polyvinyl alcohol (PVA) and polyethylene glycol (PEG) offers a promising opportunity for diverse biomedical applications. In this research, we successfully synthesized biogenic AgNPs using plant extracts through an environmentally friendly synthesis method, and then integrated them into a PVA/PEG film. The resulting film was thoroughly assessed for its antibacterial, antioxidant, and cytotoxic effects on MCF-7 human breast cancer cells. The biogenic AgNPs synthesized were analyzed using various techniques including UV-Visible spectroscopy, scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR). The PVA/PEG film incorporated with AgNPs exhibited significantly improved antibacterial activity against both Gram-positive and Gram-negative bacteria compared to the pristine PVA/PEG film. This was demonstrated through agar diffusion assay. Furthermore, the antioxidant activity of the film was evaluated using DPPH radical scavenging assay, demonstrating notable radical scavenging potential. This multifunctional film has great promise for a variety of biological applications, including wound dressings, implant coatings, and anticancer treatments. More study is needed to better understand its mechanisms of action and optimise its efficacy for therapeutic use.

**Keywords:** Biogenic silver nanoparticles, Antibacterial activity, Antioxidant activity, Cytotoxicity

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**1. Introduction**

In the field of biomedical research, the search for innovative materials capable of addressing multifaceted challenges such as bacterial infections, oxidative stress, and cancer has always been pressing [1-3]. Among the emerging candidates, biogenic silver nanoparticles (AgNPs) stand out for their remarkable properties, which include powerful antibacterial activity, antioxidant capacity, and cytotoxic effects against cancer cells. Using these properties, researchers have begun to explore novel strategies for harnessing the therapeutic potential of AgNPs in combination with versatile polymeric matrices [4-6].

In this regard, a promising direction for multipurpose biological applications is the incorporation of biogenic silver nanoparticles into a polyvinyl alcohol (PVA)/polyethylene glycol (PEG) film. PVA, which is well-known for its mechanical stability and biocompatibility,

acts as a strong scaffold to guarantee the continuous release of AgNPs while preserving structural integrity. PEG enhances hydrophilicity and resistance to protein fouling, which are important properties for interacting with biological environments, and it complements PVA [7-11].

Silver nanoparticles have attracted significant interest because of their natural antimicrobial properties, which stem from their capacity to interfere with bacterial cellular processes. The eco-friendly and cost-effective synthesis of AgNPs through biogenic methods involves using plant extracts or microorganisms as natural sources. By incorporating these nanoparticles into a PVA/PEG film, their stability and controlled release properties are improved, thereby enhancing their therapeutic capabilities and ensuring compatibility with biological systems [12-16].

In recent years, there has been a growing interest in exploring natural sources for the development of antimicrobial agents due to the emergence of antibiotic resistance and the limitations of synthetic antimicrobials. *Euphorbia hirta*, commonly known as "asthma weed" or "gatas-gatas," is a herbaceous plant found in many parts of the world, particularly in tropical and subtropical regions. It has been traditionally used in various folk medicine systems for its medicinal properties, including its antimicrobial activity. The utilization of plant extracts in pharmaceutical and cosmetic formulations has gained significant attention due to their potential therapeutic benefits and relatively lower toxicity compared to synthetic counterparts. *Euphorbia hirta* is recognized for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities [17-23].

This work discusses the production, characterisation, and biological applications of biogenic silver nanoparticles embedded in a PVA/PEG film. We investigate the processes behind this composite material's antibacterial, antioxidant, and cytotoxic activities against human breast cancer cells, emphasising its potential for treating infections, reducing oxidative stress, and slowing cancer growth. Furthermore, we explore the synergistic interactions between AgNPs and the polymeric matrix, explaining how their combined properties improve therapeutic efficacy while maintaining biocompatibility.

## **2. Materials and Methods**

### **Collection of Plant Materials and Preparation of Leaf Extracts**

Three Indian medicinal plant (*Euphorbia hirta*) were selected for the synthesis of silver nanoparticles due to its ease of availability and medicinal properties. Fresh and healthy leaves were cut and washed in distilled water to remove the adhering dust particles and allowed to dry under the sunlight. The dried leaves of each plant were then grinded using sterile mortar and

pestle. About 5g of each plant types were weighed and added to 50ml distilled water. Then the mixture was heated at 50°C for 1 hour. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) for further use [24-25].

### **Green Synthesis of Silver Nanoparticles**

Synthesis of Silver nanoparticle was carried out according to the method described by Mankadet *al* with slight modifications. 5ml of each plant extract was added to 20ml of 1mM aqueous AgNO<sub>3</sub> solution at room temperature. Then each solution was stirred in a magnetic stirrer until the colour of the solution changes to dark brown in colour which indicating the formation of AgNP<sub>s</sub>. The resulting nanoparticles were collected by centrifugation at 10000rpm for 15 minutes. After centrifugation the resulting pellet was washed with distilled water to obtain pure AgNP<sub>s</sub> and was dried in hot air oven at 50°C for 24 hours[26].

### **Preparation of PVA-PVG Agnp Film**

Synthesis of PVA-PVG AgNP<sub>s</sub> films were carried out by solvent casting method. Initially Poly Vinyl Alcohol (PVA) solution was prepared by adding 5ml PVA into 100ml distilled water and heated at 50°C for 3 hours until the complete dissociation of PVA. The PVA solution was then cooled down to room temperature. After cooling 7.5g of Poly Ethylene Glycol (PEG) was added to the above solution. After that the solution mixture was stirred for about 2 hours under constant stirring. Then the AgNP<sub>s</sub> synthesized from each three plants were added separately to each PVA-PVG mixture, and the resulting solution were casted into Petri plates and dried in hot air oven at 50°C for 24 hours [2-6].

### **In Vitro Antibacterial Activity**

Antibacterial studies of (P-VA/EG)-AgNP<sub>s</sub> has been tested against two different bacterial strains like, by well diffusion method. The drugs gentamycin (antibacterial) was used as the standard [27].

### **In Vitro Antioxidant Activity**

Utilizing four different techniques, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide dismutase (SOD), nitric oxide (NO), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assay, the antioxidant activity of (P-VA/EG)-AgNP<sub>s</sub> is investigated. Ascorbic acid was used as a standard drug[28-29].

### **In Vitro Anticancer Studies by MTT Assay**

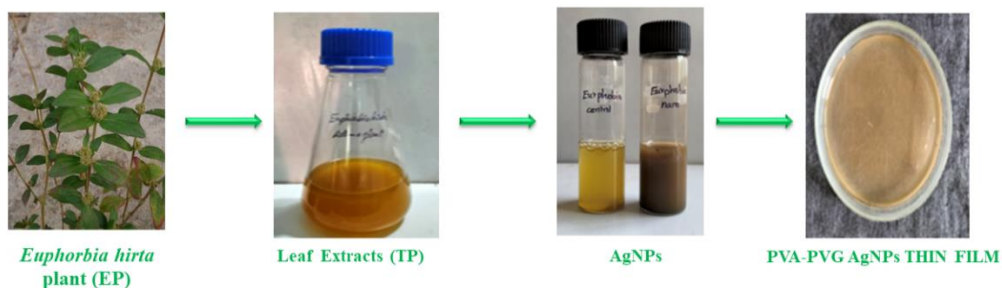
In vitro anticancer activity of (P-VA/EG)-AgNP<sub>s</sub> in vitro is assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. The anticancer activity of

the synthesized Nanoparticles was investigated using cancer cells such as MCF-7 (breast cancer) [30-31].

### 3. Result and Discussion

#### Visual observation

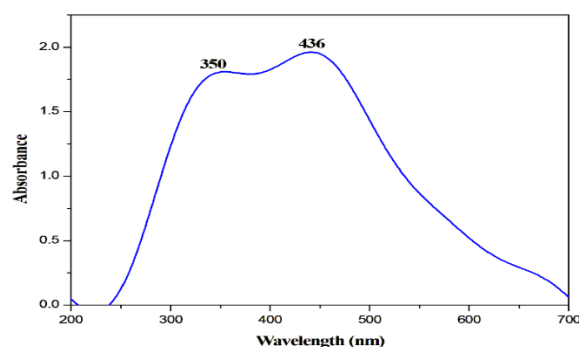
The Ag-NPs solution has been found to have a dark brown color based on the results of earlier studies. Before being exposed to a silver nitrate solution, the *Euphorbia hirtaleaf* extract was first shown to have a dark green hue (Fig. 1). Nevertheless, the extract's hue changed to a dark brown tint after the reaction (Fig. 1). This hue shift indicates that a chemical event occurred that produced silver nanoparticles (Ag-NPs). It is thought that the extract's active molecules are responsible for this process by reducing the silver ions. Surface plasmon resonance is a phenomena that causes this hue and is a size-dependent characteristic of nanoparticles.



**Figure.1. Preparation of PVA-PEG AgNPs films**

#### UV Analysis of PVA-PEG AgNPs films

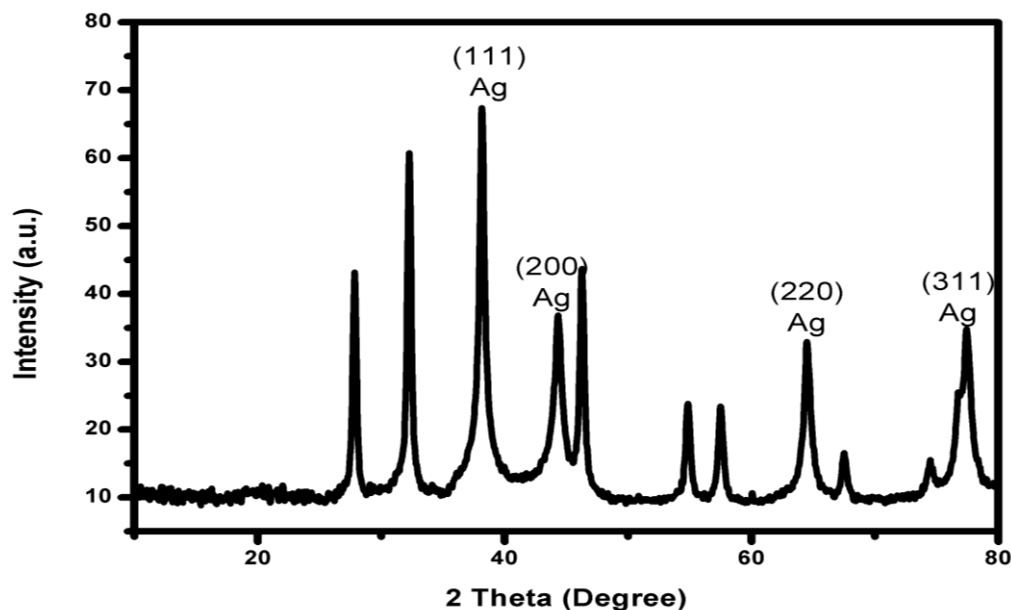
The extraction of *Euphorbia hirtaleaf* extract was used to successfully synthesize silver nanoparticles (AgNPs) as evidenced by the notable shift in color to dark brown in the colloidal solution. In addition, a large surface plasma resonance (SPR) band between 390 and 475 nm was detected in the colloidal solution using UV-vis absorption spectra analysis (fig. 2), suggesting the existence of spherical AgNPs



**Fig. 2. The UV-visible spectrum of PVA/PEG Films Containing AgNPs**

### X-Ray Diffraction (XRD) Spectroscopy

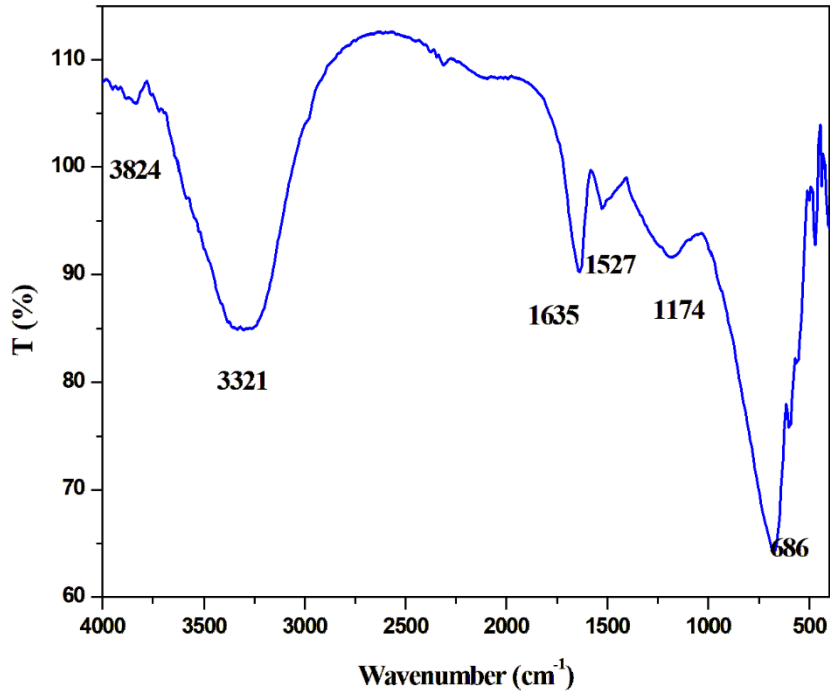
Fig:3 shows the X-ray diffraction spectra which depicts the presence of *Euphorbia hirta* silver nanocomposite diffraction peak were  $2\theta$  values of  $32^\circ$ ,  $37^\circ$ ,  $46^\circ$ ,  $55^\circ$ ,  $57^\circ$ ,  $76^\circ$  corresponding to the crystalline planes of (110), (200), (211), (220), (221), (311), respectively and it is compared with silver nanoparticle XRD spectrum [19]. These diffraction peaks says the tetragonal structure of Isorhamnetin, Calotropone, Gofruside, Amyrin and also the silver nanoparticles presence.



**Fig. 3. X-ray Diffraction patterns of the prepared PVA/PEG Films Containing AgNPs**

**Fourier Transform-Infrared Spectra**

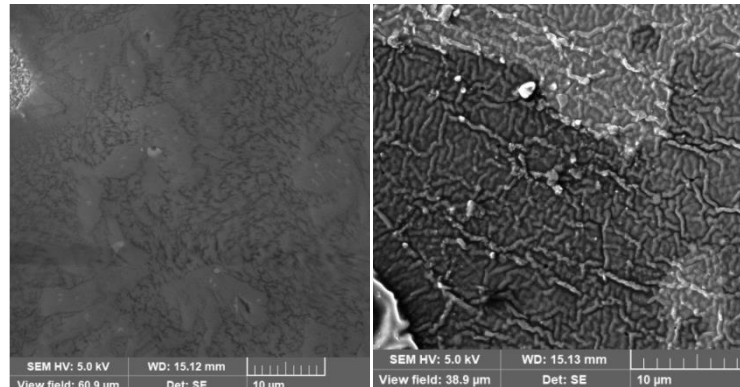
FTIR spectroscopic analysis was conducted on the as-prepared thin film sample to characterize its organic compound content, specifically focusing on the plant *Euphorbia hirta*. The FTIR spectra revealed distinctive peaks, including N-H Amide or amine group asymmetric stretching at  $3302.13\text{ cm}^{-1}$ , C=C bending of Alkene, indicating the presence of aromatic compounds at  $1635.64\text{ cm}^{-1}$ . Additionally, the absorption peak at  $1527.62\text{ cm}^{-1}$  suggested the presence of a polyphenol skeletal aromatic ring. Aromatic C-H bending and Alkene group elements were evident at the peaks around  $686.66\text{ cm}^{-1}$ . The FTIR analysis provides valuable insights into the molecular composition and functional groups present in the thin film derived from *C. gigantea*.



**Fig.4. FTIR spectra of PVA/PEG Films Containing AgNPs**

#### **Field Emission Scanning Electron Microscopy (FESEM)**

Field emission scanning electron microscopic analysis for *C.gigantea*-AgNPs thin film was carried out to analyze the morphology which shows the rod like structure with the width of 15.8nm in the view field of both 26.8 and 6.99 micrometer (Fig.5a).

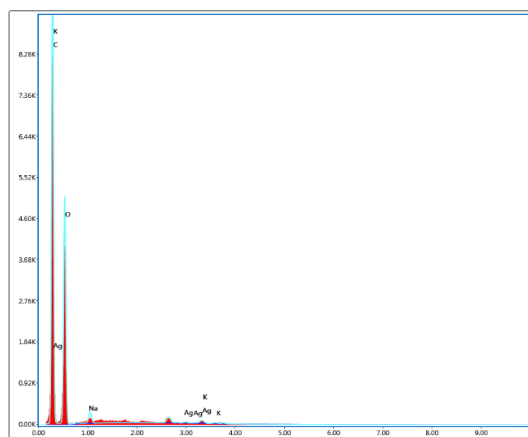


**Fig.5. (a-b) Scanning Electron Microscopy (SEM) with PVA/PEG Films Containing AgNPs**

#### **Energy dispersive X-RAY spectroscopy (EDS)**

Electron dispersive X-ray spectroscopic analysis was used to determine the composition of AgNPs and *C.gigantea* leaf extract in the prepared thin film. The spectra show the presence of 82.25% silver (Ag), 7.21% carbon (C), 5.82% oxygen (O), 3.41% nitrogen (N), 1.07%

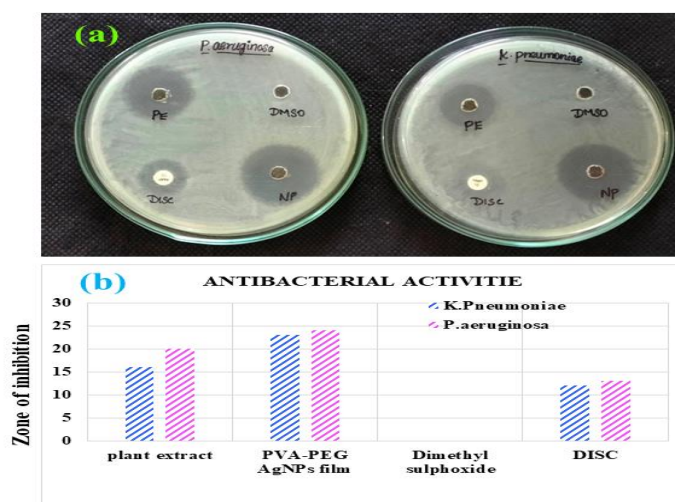
potassium (N) and 0.24% magnesium (Mg) in the sample, indicating the high level of purity of the silver nanoparticles. (Fig.6)



**Fig.6. The Spectrum of Energy-Dispersive X-rays (EDX).**

**Antibacterial activities**

Agar well diffusion method was used to screen the antibacterial activities of the extracts as displayed. 70µl of 24hours old culture of *K.pneumoniae* and *P.aeruginosa* was spread on Mueller hinton agar plate (39g of MHA was dissolved in 1000 ml of distilled water and sterilized under autoclave at 121°C for 15 minutes), using sterile cotton swab and wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 20µl of the samples were added to respective wells. The plates were incubated at 37°C for 24 h. Antibacterial activity was detected by measuring the zone of inhibition appeared after the incubation period. DMSO at a concentration of 10% was employed as a negative control and antibiotic disc of ampicillin AMP-10mcg was used as positive control and it is shown in Figure7.



**Fig. 7. Screening of antibacterial activity of PVA-PEG AgNPs against *K.pneumoniae* and *P.aeruginosa* and Histogram showing the Zone of inhibition**

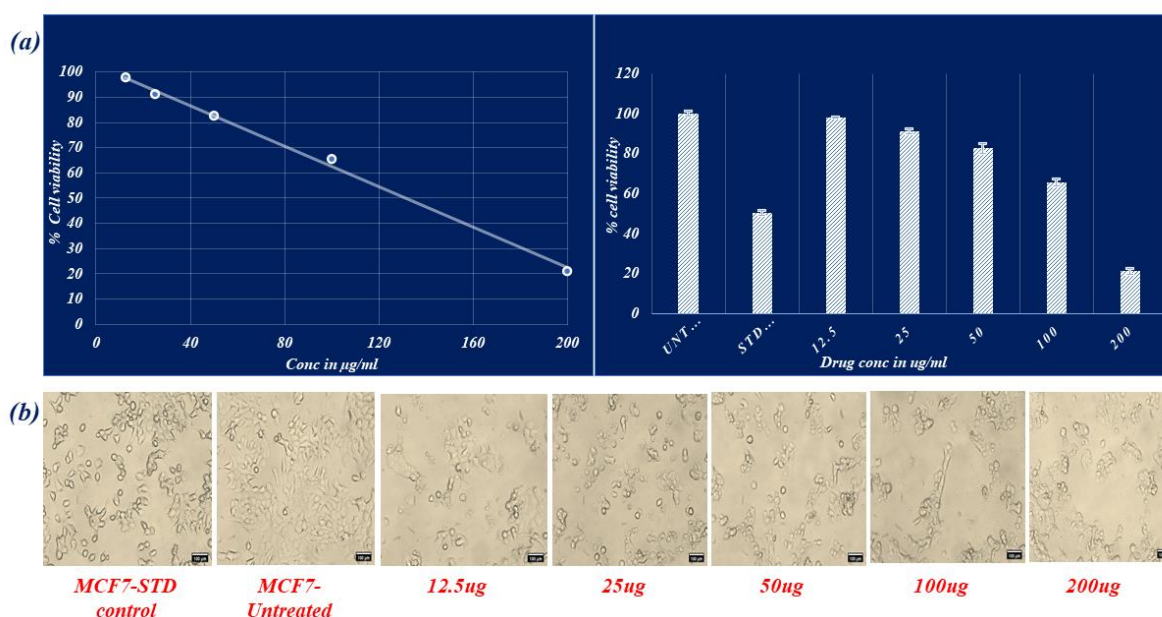


## Anticancer Activities

Anticancer studies of PVA-PEG AgNPs against cancer cell lines Human Breast cancer (MCF7) by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay were shown in the Fig. 8. The obtained results were related with cisplatin as standard. For cancer cell lines, we found our PVA-PEG AgNPs has significant anticancer activity. 5, 12.5, 25, 50, 100, 200  $\mu\text{g}/\text{mL}$  of PVA-PEG AgNPs is needed to abolish the MCF7 cancer cell. This will be more permeable through cell membranes eventually behave as carriers of antitumor agents.

## Morphological Changes

Following a 24-hour treatment with PVA-PEG AgNPs at several concentrations to cause cytotoxicity in MCF7 cells, the cells were inspected under a microscope. The bulk of the cells perished of apoptosis, according to the cytological changes, with the exception of the MCF7 cells. A large number of the treated cells had early apoptotic morphological features, including different PVA-PEG AgNP concentrations (Fig.8).



**Fig.8. (a) Histogram showing the cell viability percentage of PVA-PEG AgNPs against MCF-7 cells**

**Treated With Different Concentrations for 24 H and (B) Morphological Assessment of PVA-PEG Agnps against MCF-7 Cells Lines at Different Concentrations**

## DPPH Assay

In this experiment, a peak at 517 nm was seen due to the rich purple color of the DPPH radical. With the addition of Pt complex, the peak intensity of the DPPH solution gradually dropped, and the color of the DPPH vanished, showing that the synthesised PVA-PEG AgNPs

film has a strong radical scavenging ability. Figure 9(a) shows the percentage inhibition of AgNPs and PVA-PEG AgNPs films by the DPPH radical.

### SOD assay

The radicals known as ( $O_2^-$ ) may bind to biomolecules (DNA/protein) and cause immediate damage to biological systems. The superoxide radical quenching activities of the PVA-PEG AgNPs film increased as concentrations increased (Figure 9(b)).

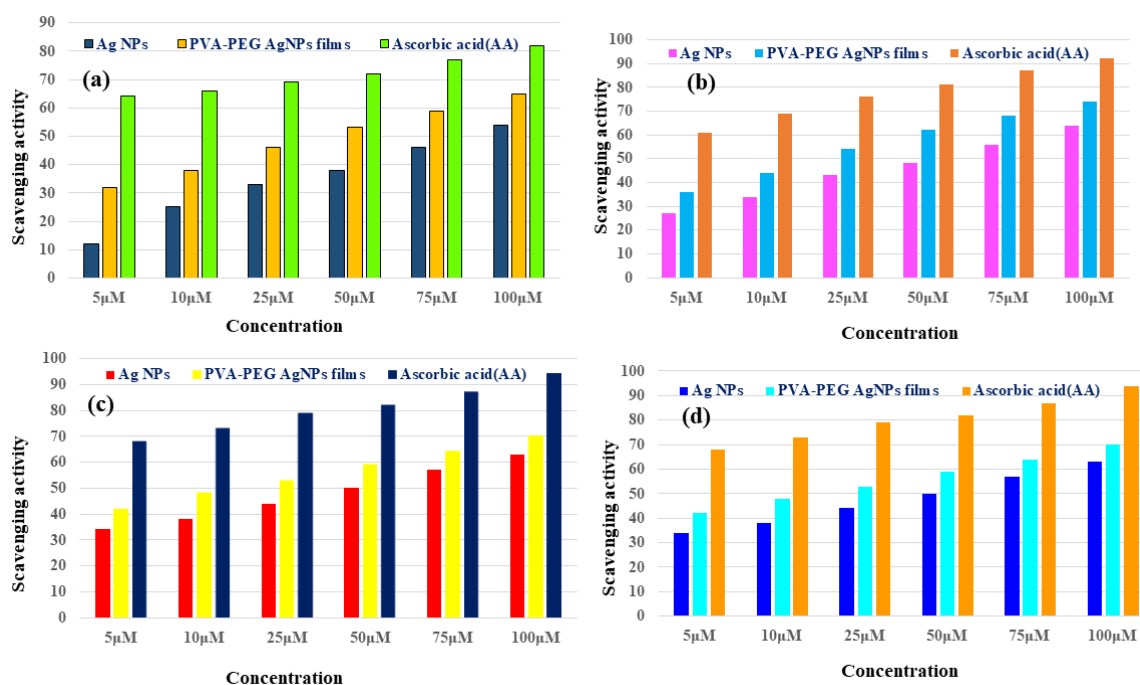
### NO assay

In addition to being an essential component of several biological systems, nitric oxide is a dynamic, pleiotropic mediator of a broad variety of physiological processes. The NO activity of a PVA-PEG AgNPs film is depicted in Figure 9(c).

### $H_2O_2$ assay

$H_2O_2$  can be switched to  $H_2O$  by absorbing protons ( $H^+$ ) or electrons ( $e^-$ ). The Pt complex acts as an  $H_2O_2$  radical scavenger in this experiment by donating azomethine protons to decrease the hydrogen peroxide to  $H_2O_2$ . Figure 9(d) depicts the  $H_2O_2$  scavenging of a PVA-PEG AgNPs film.

The percentage inhibitions of AgNPs, PVA-PEG AgNPs and Ascorbic acid with DPPH, SOD, NO and  $H_2O_2$  radical scavengers are given in Figure 9(a–d). These results suggest that PVA-PEG AgNPs film has good radical scavenging activity than ligand



**Fig.9. In vitro antioxidant activities of AgNPs, PVA-PEG AgNPs and Ascorbic acid.**

(a) DPPH; (b) SOD; (c) NO and (d)  $H_2O_2$ .

#### 4. Conclusions

The green synthesis process emerges as a highly efficient method for the production of silver nanoparticles, characterized by its environmentally friendly nature, straightforward procedure, cost-effectiveness, and overall efficacy. This study focuses on the synthesis of PVA-PEG AgNPs films using an extract derived from *Calotropis gigantea* leaves. The PVA-PEG AgNPs was thoroughly characterized through FT-IR, UV-Vis, SEM, XRD, and EDX spectral studies. Furthermore, the *in vitro* anticancer activity of PVA-PEG AgNPs against MCF-7 cancer cell lines was assessed using the MTT assay, with the observed results compared to the standard drug cisplatin. Remarkably, PVA-PEG AgNPs exhibited superior anticancer potency against a spectrum of cancer cell lines compared to other complexes and the free ligand. These findings suggest that the synthesized PVA-PEG AgNPs may represent a promising class of novel anticancer agents.

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