Anticancer Activity of Phytogenic Bimetallic Nanoparticles against MCF-7 Cells

Dr. Ch. S. Anuradha

Assistant Professor, Department of Chemistry, Dr. V. S. Krishna Government Degree College (A), VISAKHAPATNAM -530013, INDIA.

ABSTRACT

Now- a- days, green synthesis of metallic nanoparticles is a growing area of research because of their potential therapeutic applications for various diseases. In the present work, bimetallic silver nickel nanoparticles (BMNPs) are synthesized from silver nitrate ($AgNO_3$) and cobalt nitrate (Ni $(NO_3)_2$) precursor solutions using aqueous leaf extract of *Aerva lanata* as bioreducing, stabilizing and capping agent. The synthesized BMNPs were characterized using UV–Vis spectroscopy, FTIR, SEM, EDX, XRD and TEM analyses. The cytotoxic response was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. These biogenic Silver nickel BMNPs were found to be significantly toxic to MCF-7 cells (human breast cancer cells) via induction of apoptosis.

KEY WORDS

Bimetallic nanoparticles (BMNPs), *Aerva lanata*(AL), Phytomolecules and invitro cytotoxicity

1. INTRODUCTION

Breast cancer is the most common malignancy in mankind that causes major mortality worldwide every year. Over the past decenniums, treatments to this notable life-threatening cancer has become more exigent owing to the prevalence of multiple drug resistance, damaging side effects and the inadequacy of innovative measures [1]. Nanomedicine is a hopeful and exciting field that could potentially lead to improvements in cancer treatment procedures, offering a modern perspective on tumour identification, prevention and bioremediation [2]. The successful application of nanoparticles as an anticancer drug is due to their exclusive qualities like large surface area for volume, porosity, solubility, increased bioavailability and different structural properties. Ultimately this can improve the stability and permanence of the drugs; moreover it will offer many biomedical perceptions for clinical level applications [3]. Another interesting feature of nanoparticles, it can easily cross the cellular barriers and strongly interact with functional biomolecules [4]. Silver nanoparticles are among the most common and applicable nanostructures, according to their distinctive catalytic, therapeutic activities and stability as well as development of nanodevices and therapeutic preparation for diagnoses and treatment of cancer [5]. The treatment of a variety of cancers with silver NPs has been well documented [6]. Antitumor potentiality (cytotoxicity) of the silver NPs is expressed through oxidative stress as well as inflammation through production of reactive oxygen species that lead to DNA damage and mitochondrial membrane potential disorder, releasing cytochrome c and resulting in mitochondrial related apoptosis and necrosis to cell proliferation and carcinogenesis [7].

Aerva lanata is a medicinal plant that belongs to the family *Amaranthaceae*. It is wealthy source of secondary metabolites that have antibacterial [8], antifungal [9], antioxidant [10], cytotoxic [11], anti-HIV [12], anti tumour[13], anti diabetic[14,15] and anticancer[16] activities. Moreover the green synthesized nanoparticles are found to be more potent antitumour agents than the uncoated nanoparticles as the former are capped with bioactive phytomolecules [17].

2. EXPERIMENTAL

2.1. Preparation of *Aerva lanata* leaf extract: 100 g of fresh *Aerva lanata* leaves were taken and cleaned with running tap water to remove dust on surface of leaves followed by deionised water to eliminate other contaminants from leaves and dried up under shade for ten days. The dried leaves were powdered by using home blender. Now 200 mL deionised water was taken in 500 mL beaker to this 10g leaf powder was added. The contents in the beaker heated for 30 minutes at 50^oC with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth was filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4^o C. This was taken as leaf extract (figure (1)) for the experimental studies.

2.2. Synthesis of Ag-Ni bimetallic nanoparticles:

Equimolar (25 mM) concentrations of silver nitrate and nickel nitrate aqueous solutions were prepared separately in 100 mL volumetric flasks by dissolving 0.4246 g, 0.7267 g weight of $AgNO_3$ and $Ni(NO_3)_2$ in deionized water respectively. Synthesis of Ag-Ni BMNPs was done by taking 100 mL of $AgNO_3$ solution in a 500 mL beaker, to this 90 mL of leaf extract, 100 mL of $Ni(NO_3)_2$ solution were added by drop wise in simultaneous addition process. After this addition the beaker was placed on a magnetic stirrer for continuous agitation. This mixture was stirred at 70^oC for 70 minutes at pH 8 on magnetic stirrer. These synthesized BMNPs were separated out by doing centrifugation at 4000 rpm for 30 minutes. The obtained BMNPs were washed with deionized water two times to remove unwanted constituents and dried in oven at 85 ^oC for two hours. The resultant BMNPs particles were collected (**Figure: (2)**) and used for characterization.

Fig 1(a): *Aerva lanata* leaf extract

Fig 1(b): Ag-Ni BMNPS

2.3 Characterization:

The synthesized BMNPs are characterized by various instrumental techniques. UV-Visible analysis shows the formation of BMNPs by SPR band at band at around 437 nm (**Figure (3)**)and FTIR spectrum of Ag-Ni BMNPs exhibits major peak positions at 3213 cm^{-1}, 3416 cm^{-1} and 3381 cm^{-1}which indicate the N-H stretching vibrations of amines; O-H stretching of hydroxyl groups of alcohols and phenols. Intense peak at 1641 cm^{-1} is due to C=O stretching of amide group. Very small peak at 601 cm^{-1} indicates the presence of C-Cl group. From energy dispersive X-ray analysis (EDX), we can analyze all the elements present in the BMNPs which indicate the existence of Ag and Ni which confirms the formation of Ag-Ni bimetallic nanoparticles. This is also supported by the EDX study which gives quantitative data of silver and nickel compositions in BMNPs**.** By Field Emission Scanning electron microscopic (FESEM) images of Ag-Ni BMNPs (**Figure (4)**), it can be clearly noted that the synthesized Ag-Ni bimetallic nanoparticles are in the size range between 50 - 100 nm. Powder XRD analysis confirms that BMNPs have FCC crystal structure with average particle diameter of 24.5 nm.

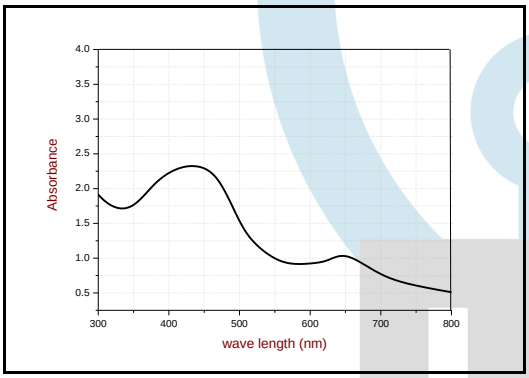


Fig 2(a): UV-Vis spectrum of Ag-Ni BMNPs

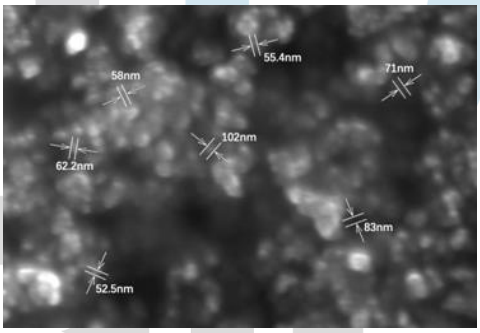


Fig 2(b): FESEM image of Ag-Ni BMNPs

2.4 In vitro Cytotoxic Activity by MTT assay

2.4.1 Materials and Methods

Apparatus and chemicals required

- Spectrophotometer
- Incubator
- Inverted microscope
- Centrifuge
- MCF cells (purchaged from NCCS, Pune, India)
- 96 well microplates
- Micropipette
- Doxorubicin
- MTT (1 mg/mL)
- DMSO

MTT assay is a quantitative colorimetric assay for evaluating cellular growth, cell proliferation and cell survival derived from the ability of living cells. The assay was conducted using (3-(4, 5- dimethyl thiazol-2yl) - 2, 5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells resulting a measurable purple product formazan. The amount of formazan formed is directly proportional to the viable cell count and is inversely proportional to the extent of cytotoxic activity. The effect of invitro cytotoxicity of Ag-Ni nanoparticles on breast cancer cell lines (MCF 7 cell line) is tested and recorded at 24 hours and 48 hours.

Concentration (mg/mL)	OD at 540 nm	Percent inhibition	IC50 (mg/mL)
2	1.36	8.11	**18.37**
4	1.30	12.16	
6	1.21	18.24	
8	1.17	20.95	
10	1.04	29.73	

2.4.2

Preparation of nanocompounds for the assay

Five hundred micro litre of stock (100 mg/ mL) nanoparticles were dissolved in 4.5 mL of DMSO for a concentration of 10 mg/ mL. Prior to the assay, the new working suspension was filtered through a 0.45 µm membrane filter. Five gradient concentrations (2 mg, 4 mg, 6 mg, 8 mg and 10 mg) of were used for this analysis. 500 µL of 48 h culture of MCF 7 cell lines at a concentration of 10^5 cells/ mL was applied to each well. Two control wells received only cell suspensions without nanoparticles, also the drug, doxorubicin used as positive control at same concentrations. The plate was placed in a humidified CO_2 incubator for 4 - 6 h at 37°C. Microscopically, the plate was tested for confluent cell monolayer, turbidity and toxicity.

2.4.3 Assay Process

After incubation, the medium from the well was carefully aspirated and then disposed. Each well was washed with Eagle's Minimum Essential Medium (EMEM) without Fetal Calf Serum (FCS). 200 µL of MTT solution (5mg MTT/ ml of PBS, pH 7.2) will be added to each and every well. In a CO_2 incubator with 5 percent CO_2, the plate was incubated for 6-7 h at 37°C. 1 mL of DMSO was applied to each well after incubation, combined with a pipette and left at room temperature for 45 seconds. In the wells, purple formazan was developed [22]. In order to compare full cell viability in cytotoxicity and antitumor activity assessments, cell control and solvent controls were used in each assay. The suspension was moved to a cuvette of the spectrophotometer and the optical density (OD) was calculated as blank at 540 nm using DMSO. With the following formula, cell the %viability was determined.

Cell viability % = Mean OD of wells receiving each plant extract dilution / Mean OD of control wells x 100.

The determination of IC50, the compound concentration needed to inhibit 50 percent cell growth, was calculated by plotting a log graph (extract concentration) vs. percent cell inhibition. A line drawn on the Y axis from the 50% value meets the curve and interpolates to the X axis. The value of the X axis gives the log value (concentration of the compound). The IC50 value is given by the anti-log of that value.

3. RESULTS AND DISCUSSION

The synthesized Ag-Ni BMNPs were investigated for their cell viability assay and cytotoxic activity against human breast cancer cell line (MCF-7) were assessed by applying standard MTT assay and doxorubicin was used as a standard drug. The compounds were treated with MCF-7 cell line at five different concentrations (2mg, 4mg, 6mg, 8mg and 10mg). The cytotoxic activities of Ag-Ni nanocompound and doxorubicin drug against MCF-7 Cell Line at different concentrations are depicted in Table 3.1 and Table 3.2. The Linear graphs of percent inhibition of Ag-Ni nano compound and doxorubicin are shown in Fig 3.1 and fig 3.2. The results clearly demonstrate that the Ag-Ni nano compound exhibits a maximum of 29.73 percentage inhibition at 10 mg concentration and a IC50 of 18.37 mg/mL. Figure 3.3 shows the morphological analysis of materials treated with MCF-7 cells.

The results clearly demonstrates that all the synthesized compound Ag-Ni (for 24 and 48hrs) have shown moderate to significant cytotoxic activity with values ranging from 2mg to 10mg respectively. So, it is confirmed that all the novel synthesized derivatives exhibit cytotoxic activity and it is also noted that cytotoxic activity increases as the concentration of the BMNPs in the solution increases. Based on these results, compound Ag-Ni exhibited remarkable cytotoxic activity comparable to standard drug, doxorubicin.

Table 3.1: Cytotoxicity of Ag-Ni nanoparticles against MCF-7 Cell Line at Different Concentrations

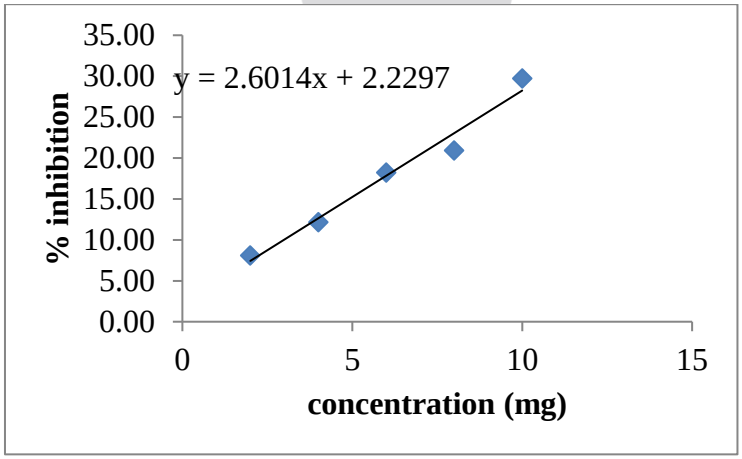
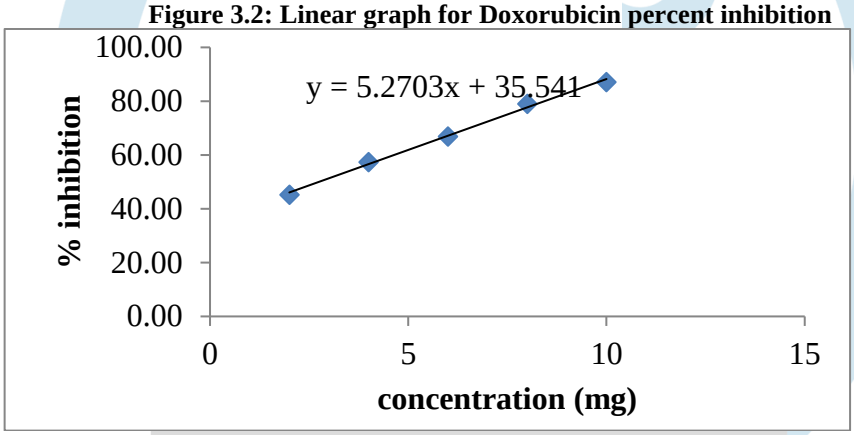


Fig 3.1: Linear graph for Ag-Ni nanoparticles percent inhibition

Table 3.2: Cytotoxicity of Doxorubicin against MCF-7 Cell Line at Different Concentrations

Concentration (mg/ml)	OD at 540nm	Percent inhibition	IC 50 (mg/ml)
2	0.81	45.27	2.74
4	0.63	57.43	
6	0.49	66.89	
8	0.31	79.05	
10	0.19	87.16	

Figure 3.2: Linear graph for Doxorubicin percent inhibition



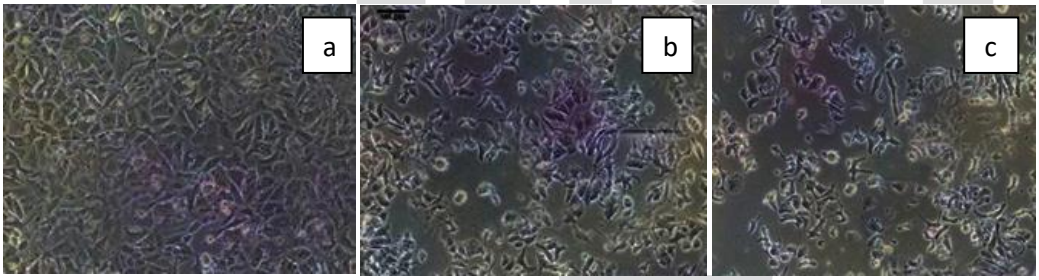


Figure 3.3: Morphological analysis of nano compounds treated MCF-7 cells.
a- control; b- AgNi treated at 10 mg/mL concentration; c- Doxirubicin treated at 10 mg/mL concentration

4. CONCLUSIONS

In this present study phytochemicals of *Aerva lanata* leaf extract are involved in the bioreduction, formation and stabilization of nanoparticles, the future studies might move towards the optimization of the reaction parameters for generation of high amount of biomolecules to stabilize and cap the formed nanoparticles.In conclusion, this study implies that green synthesized Ag-Ni nanoparticles may be potent for treatment of MCF-7 cells of human breast cancer. A linear correlation was observed between the number of the tumor cells and the dose-dependent cytotoxic effects the synthesized nanoparticles. From the results, it is concluded that these nanoparticles can be used for the development of new preparations for the therapy of tumors. The future research may be directed for the genetic manipulation of plants to increase the metal tolerance and cytotoxicity which may be useful in cancer therapy.

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