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**SYNTHESIS OF SILVER NANOPARTICLES USING FLOWER OF  
*NELUMBO NUCIFERA* ITS ANTIOXIDANT ACTIVITY, ANTI CANCER  
ACTIVITY ON HUMAN BREAST CANCER CELL LINE (MDA-MB-231)**

**PRIYADHARSHINI PS<sup>\*1</sup>, SANDHIYA G<sup>1</sup> AND R. VIJAYAN<sup>2</sup>**

**1:** UG student, Department of Biotechnology, Muthayammal Engineering College, Rasipuram

**2:** Assistant Professor, Department of Civil Engineering, Muthayammal Engineering College,  
Rasipuram

**\*Corresponding Author: Ms. Priyadharshini P S: E Mail: [aravindvijav90@yahoo.com](mailto:aravindvijav90@yahoo.com)**

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**ABSTRACT**

In the present study, synthesis of silver nanoparticles (AgNPs) using flower of *Nelumbo nucifera* were tested for antioxidant activity and against human breast cancer cell line (MDA-MB-231). The synthesized AgNPs were characterized using UV-visible spectroscopy, X-ray powder diffraction (XRD), fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM) and EDAX analysis. The synthesized silver nanoparticles were checked with the colour variation and it was confirmed by UV-vis spectral analysis. The morphology of the synthesized nanoparticles were analysed using SEM. The XRD was done to find out the crystalline structure of the compound. FTIR measurements are carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver NPs synthesized using flower of *Nelumbo nucifera*. The anti oxidant analysis showed better percentage inhibition for the synthesized silver nanoparticles for the DPPH (65.74µg/ml), Hydrogen peroxide (51.48 µg/ml) and for metal chetaling (60.93 µg/ml) at a concentration 100µg/ml. The synthesized flower-AgNPs of *Nelumbo nucifera* was tested against the MDA-MB-231 cell line to find out the cell viability. The synthesized silver nanoparticles using flower of *Nelumbo nucifera* inhibited the proliferation of human breast cancer cell line with an IC<sub>50</sub> value of (11.25 µg/ml). From this study, concluded that synthesized AgNPs using flower of *Nelumbo nucifera* have potential anti-oxidant and anticancer activity.

**Keywords:** flower of *Nelumbo nucifera*, silver nanoparticles, MTT assay, MDA-MB-231 cell line, ethanol extract

## INTRODUCTION

Nanotechnology is important in the field of life science. In biological field, nanotechnology provides rapid research in the field of genetics. Silver nanoparticles are mainly used for its distinctive properties in bio sensing, catalysis, photonics, chemical sensing and pharmaceuticals [1]. In physical and chemical methods, the synthesis of nanoparticles requires temperature, pressure and also energy is consumed. In Biological method, synthesis of silver nanoparticles using plant is more advantageous because of its low-energy consumption and environment friendly [2]. Silver nanoparticles used in wide range of application such as anti-inflammatory [3], antimicrobial, antiviral [4] and anti-diabetic [5] and also involved in the prevention of diabetic wound healing (ointments) [6].

Cancer is a proliferating disease combining of physical, environmental, metabolic, chemical and genetic factors [7]. In US country, Breast cancer is the second leading cause of death in women. An estimated 39,620 breast cancer death and 232,340 new cases are expected among women in 2013 [8]. In 2019, breast cancer has been diagnosed among 268,600 people in US women and 41,760 people were died. The mortality rate for breast cancer is projected to

decrease from 16.1 in 2015 to 14.7 in 2030. Breast cancer cell targeting drug development without affecting normal cells is a challenging task in the field of drug discovery for cancer [9]. Cytotoxic agents are expensive and induce severe side effects [10, 11]. Currently, a variety of cytotoxic agents have been used in the treatment of breast cancer, such as doxorubicin, cisplatin, and bleomycin. Although usage of drugs provides beneficial effect, but the efficacy and demerits are uncertain [12]. In olden centuries, medicinal plants are used as the effective anticancer agents [13]. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer [14]. *Nelumbo nucifera* is a large aquatic herb, belongs to family Nymphaeaceae, distributed throughout the India and Sri Lanka, northern Indochina and East Asia [15]. The plant possesses treat cuts, rheumatism, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, and bilious fever, ataxy of abdominal viscera, sores, measles, fevers, cold and high blood pressure. The whole plant is used to cure bronchitis and the powdered root in milk was given to children for stomach-ache and as a vermifuge. Lantana oil is used to treat a skin

infection, itches, and as an antiseptic for wounds [16]. The chemical constituents shows that the presence of tannin, alkaloids, catechin, steroids, saponins, phenol, anthraquinone, protein, terpenoids, flavonoids, glycosides, different reducing sugars and essential oils [17, 18]. So, it is necessary to find novel therapeutic agents against cancer, which are biocompatible and cost effective. Therefore, this study was designed to synthesize AgNPs using flower of *Nelumbo nucifera* and to evaluate potential antioxidant activity and the general mechanism of synthesized AgNPs in human breast cancer cells (MDA-MB-231 cells) separately.

## MATERIAL AND METHODS

### Collection of plant material

The flower of *Nelumbo nucifera* was collected in the month of March from the mullipatti, pudukkottai, Tamil Nadu, India. The plant was identified and flower of *Nelumbo nucifera* were authenticated and confirmed from Dr. Amlanandan, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu for identifying the plants. The voucher specimen number SGP001 (V.S.001).

### Chemical reagents and instruments required:

**Chemicals:** Silver nitrate, DMEM medium, Fetal Bovine Serum (FBS) and

antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson (India).

**Instruments:** Lambda 35, Perkin Elmer Spectrophotometer, Malvern zetasizer version 2.2., XPERT-PRO Machine and TEM, JEOL-JEM 2100

### Ethanolic extract of plant preparation:

The flower of *Nelumbo nucifera* plant were collected and washed with fresh water. After, it was dried and made them into fine powder. In a dry beaker, absolute amount of powder and few amount of ethanol were added. These solution were shifted into another beaker, stirred with glass rod and closed them with watch glass. Boiling this solution for 20-30 minutes; the colour changes occurs from slight green into dark green in colour and cooled at room temperature for 1 hour. The extract was filtered using Whatman No.41 filter paper in a clean beaker. Finally, these stock solutions were transferred into a brown bottle and then stored in as cooled place for further study [19].

### Phytochemical Screening of flower of *Nelumbo nucifera*

The preliminary qualitative analysis is performed to find the presence of various primary metabolites in *Nelumbo nucifera* extract and the quantification of phytochemicals such as flavonoids, tannins, saponins, alkaloids, phenols and terpenoids was analysed using the standard procedure [19].

#### **Optimization and synthesis of silver nanoparticles:**

1mm silver nitrate was prepared in a 50 ml standard flask. 2.5 ml of the ethanolic extract of flower extracts was mixed with 50 ml of 1mm of silver nitrate solution without any contamination with continuous and constant stirring which react at an ambient condition and Ag get reduced in to Ag<sup>+</sup> ion. The color change was observed for the reaction mixture from transparent white to dark brown indicates the formation of silver nitrate. The presence of reduction of Ag<sup>+</sup> ion was confirmed overtime by the UV-Spectral analysis [20].

#### **Characterization techniques:**

Characterization of synthesized nanoparticles were carried out to learn the characteristic wavelength and functional group bound to silver nanoparticles by UV-Vis spectra and FTIR and its size, crystalline nature and elemental composition using SEM, XRD [21, 22].

#### **UV-visible analysis:**

The optical properties of silver nanoparticles were characterized using UV-Vis spectrophotometer. Silver nitrate was added to the ethanolic extract of flower of *Nelumbo nucifera*. The color change was observed for the reaction mixture indicates the formation of silver nitrate. UV was taken after 24 hours of addition. The absorbance was recorded between 350-500nm

#### **FTIR analysis**

Fourier Transform Infrared Spectroscopy is otherwise called as FTIR Analysis or FTIR Spectroscopy. The test sample can be scanned by infrared light and chemical properties like organic, polymeric and inorganic materials were observed by this method. Fourier Transform Spectrometer absorbs infrared spectra within the range of 400-4500cm<sup>-1</sup>. At a particular frequency, multiple functional groups may be absorbed and it gives rise to different characteristic absorptions.

#### **XRD:**

X-ray diffraction (XRD) analysis is used to study the nanomaterials (with structural features in the range of 1-100 nm). The structure of nanomaterials has been probed by XRD method. The position of values of product (crystallinity or amorphous nature) can be identified by this technique. With

respect to d-spacing values; the fingerprint regions of relative intensity are found in XRD analysis.

### SEM and EDAX Analysis

Samples were mounted on 12 mm aluminium specimen stubs with double-sided carbon tape, coated with gold palladium, and examined with a FEI Quanta 250 FEG SEM operating at 10 kV. The composition and elements of the green synthesized silver nanoparticles was investigated using EDX.

### ANTIOXIDANT ACTIVITY

#### DPPH Assay method

The antioxidant activity of synthesized silver nanoparticle was examined by stable DPPH free radical activity. Ethanolic solution of DPPH (0.05 mM) (500 $\mu$ l) was added to 1000 $\mu$ l of synthesized silver nanoparticle with the different concentrations (20-100 $\mu$ g/ml). The freshly prepared DPPH solution was kept in the dark at 4°C. Then 96% (2.7 ml) of ethanol was added in the mixture and shake vigorously. The mixture was kept to stand for 5 minutes at 540nm, absorbance was measured spectrophotometrically. Absorbance was set to zero by using ethanol. A blank sample contains the same amount of ethanol and DPPH was prepared. They all performed in triplicate. The radical activity of the tested samples, expressed as percentage of inhibition were calculated [23].

Percent (%) inhibition of DPPH activity =  $[(A-B)/A] \times 100$ .

Where A and B – absorbance values of blank and sample, respectively

#### Hydrogen peroxide method

The synthesized silver nanoparticles was dissolved in phosphate buffer (0.1M, pH 7.4) at various concentration (20-100  $\mu$ g/ml) and 1000 $\mu$ l of hydrogen peroxide solution was added. The reference compound was Ascorbic acid. At 230nm, the absorbance of the hydrogen peroxide was measured after 10 minutes. By using the Molar absorptivity percentage of Inhibition was determined [23].

#### Metal ion chelating method

The ferrous ion chelating potential of samples was measured according to the method of Yamaguchi *et al.*, Take 1ml of  $\text{FeSO}_4$  solution was mixed with synthesized silver nanoparticle of different concentration (20-100  $\mu$ g/ml). 1ml of Tris HCl buffer was added (pH 7.4) and 2,2' – bipyridyl solution, hydroxyl amine, HCl and ethanol respectively. 5ml of distilled water was added to final volume in reaction mixture, incubated for 10 minutes, shaken well and kept in room temperature. At 522nm absorbance was measured, for calculating metal chelating activity =  $[(A_0 - A_1)/A_0] \times 100$ , Where  $A_0$  is

absorbance of the control and A1 the absorbance presence in the sample [24].

### **Anticancer activity of synthesized silver nano particle using *N. Nucifera* on breast cancer MDA-MB-231 cell line**

#### **Source of chemical and reagents**

Dulbecco's Modified Eagle's Medium, streptomycin, penicillin-G, L-glutamine, phosphate buffered saline, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, 2',7'-diacetyl dichloro fluorescein, sodium dodecyl sulfate, trypan blue, trypsin-EDTA, ethylene diamine tetra acetic acid, acridine orange, ethidium bromide, rhodamine-123, triton X-100, ethanol, dimethyl sulfoxide (DMSO), and bovine serum albumin were purchased from Sigma Aldrich Chemicals Pvt. Ltd (India). All other chemicals used were of analytical grade, purchased from Hi media Laboratories Pvt. Ltd., India.

#### **MTT assay**

The cytotoxicity of synthesized silver nanoparticles on MDA-MB-231 cells was determined by the method of Mosmann, (1983) [25].

#### **Procedure**

Cell viability assay, MDA-MB-231 viable cells were harvested and counted using haemocytometer diluted in DMEM medium to a density of  $1 \times 10^4$  cells/ml was seeded in

96 well plates for each well and incubated for 24 h to allow attachment. After MDA-MB-231 cells treated with control and the containing different concentrations of synthesized silver nanoparticles 50 to 300 µg/ml were applied to each well. MDA-MB-231 cells were incubated at 37°C in a humidified 95% air and 5% CO<sub>2</sub> incubator for 24 h. After incubation, the drug-containing cells wash with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubated for another 4 h at 37°C. The purple precipitated formazan formed was dissolved in 100 µl of concentrated DMSO and the cell viability was absorbance and measured 540nm using a multi-well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC 50) values were calculated and the optimum doses were analysed at different time period (Nanda *et al.*, 2009; Suresh *et al.*, 2018).

The IC 50 values were determined from the sample dose responsive curve where inhibition of 50% cytotoxicity compared to vehicle control cells. All experiments were performed at least three times in triplicate.

### **RESULTS AND DISCUSSION:**

#### **QUALITATIVE ANALYSIS OF PHYTO-COMPOUNDS FROM THE *NELUMBO NUCIFERA* FLOWER**

Phytochemical constituents are found in the flower extract of *N. nucifera* as mentioned in the (Table 1). Various assays are used to identify phytochemical compounds such as phenol, terpenoids, xanthoprotein, Flavonoids, cardiac glycosides, saponins, leucoanthocyanin, tannins, carbohydrate, alkaloids, anthocyanins, steroids, protein, glycosides, coumarins and antraquinone are strongly present in the flower extract. The previous study reported that the petals of *N. nucifera* contained phytochemical compounds such as flavonoids, tannins, alkaloids, saponin and cardiac glycosides [26]. The aqueous extract of flower of *N. nucifera* revealed the presence of Carbohydrate, flavanoids, quinones, cardiac glycosides, terpenoids, phenol, coumarines, sterols, phyto sterols [27].

#### QUANTITATIVE INVESTIGATION OF *NELUMBO NUCIFERA* FLOWER EXTRACT

Phytochemical screening and quantitative analysis of *Nelumbo nucifera* flower extract were reported in various amounts. The phytochemical constituents such as Tannins (1.997mg/g), Saponins (0.198mg/g), Alkaloids (0.019mg/g), Terpenoids (0.660mg/g), Flavonoids (1.459mg/g) and Phenols (0.660mg/g) are present in the *Nelumbo nucifera* flower extract (Table. 2).

Gnana joyce and Estherlydia, 2014 [27] reported that the total polyphenol content (TPC) was found to be high in acetone extracts (130.88 mg GAE/100 ml).

#### VISUAL COLOR CHANGE AND UV-VIS SPECTROSCOPY:

In this experiment, addition of ethanol extract of plant sample of *Nelumbo nucifera* flower extract in to the glass vial containing AgNO<sub>3</sub> led to the change in color from colorless to reddish brown. Bio-reduction of silver nitrate into silver nanoparticles in the presence of flower of *N. nucifera* extract was confirmed from UV-Vis spectral measurements. Silver nanoparticles have free electrons which have the probability to give rise to a plasma resonance absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave. The UV-Vis absorption results of silver nanoparticles as shown in Figure 1. As shown in figure, the broad spectrum of silver nanoparticles with absorption maximum at 440.90 nm was seen due to the surface Plasmon resonance. The presence of phytocompounds terpenoids, flavonoids, steroids, amides and methoxy groups in the extracts of flower of *N. nucifera* may play an important role in reduction and stability of silver nanoparticles.

## FUNCTIONAL GROUP DETERMINATION USING FT-IR SPECTROSCOPY:

The functional groups of *N.Nucifera* responsible for the bio-reduction of  $\text{AgNO}_3$  into Ag nanoparticles can be explained from FT-IR analysis. The IR spectra of *N. nucifera* extract and Ag nanoparticles stabilized by the extract are shown in **Figure 2**. FT-IR shows the major absorption peaks around 3452, 2081, 1688, 1394, 1112 and 659  $\text{cm}^{-1}$ . The broad absorption band observed around 3452.30  $\text{cm}^{-1}$  is the characteristic band of hydrogen-bonded NH groups.

The absorption band positioned at 2081.97  $\text{cm}^{-1}$  could be assigned to the C-H stretching vibration of methyl, methylene, and methoxy groups [28]. The appearance of peak at around 1688.19  $\text{cm}^{-1}$  indicates the C=O stretching of amide I bands of peptide linkage [29]. The peaks at 1394.43  $\text{cm}^{-1}$  are the stretching vibration of N-H groups and the bending vibration of C-N groups, amide II and III bands, in the proteins [30]. The broad absorption positioned at 1112.49  $\text{cm}^{-1}$  revealed the presence of aliphatic amides [31]. From the FT-IR spectra, it is concluded that the proteins are responsible for stabilizing the silver nanoparticles synthesized by the flower extract of *N. nucifera*. The nanoparticles are bound to the functional organic groups (carboxyl and amine) from the *Nelumbo*

*nucifera* plant extracts, and these functional groups may act as template, reducing and capping agents of silver nanoparticles.

## EDX ANALYSIS

Energy dispersive X-ray (EDX) spectrometer analysis confirmed the elemental signal of silver nanoparticles. The Y-axis (vertical) represents the number of X-ray counts while X-axis (horizontal) shows the energy in KeV [32]. EDX spectrum recorded for the silver nanoparticles was shown in the below **Figure 3**. From EDX spectra it is found that silver nanoparticles are reduced by *N. nucifera* have the silver weight percentage as 64.42%.

## X-RAY DIFFRACTION (XRD)

The crystalline nature of silver nanoparticles was confirmed using X-ray crystallography. X-ray diffraction pattern of *Nelumbo nucifera* flower extract mediated synthesis of silver nanoparticles shows the peaks correspond to the Bragg's reflections of (111), (200), (220), (311) and (222) planes, which confirms the face-centered cubic (FCC) crystalline structure of silver (**Figure 4**). XRD analysis showed intense peaks at  $2\theta$  values of  $38.08^\circ$ ,  $45.57^\circ$ ,  $66.02^\circ$ ,  $78.23^\circ$  corresponding to Bragg's reflection based on the fcc structure of silver nanoparticles. This clearly indicates that the silver nanoparticles formed by the reduction of  $\text{Ag}^+$  ions by the *Nelumbo*

*nucifera* plant extracts are crystalline in nature [33].

### SEM

The SEM image is employed to predict the size and morphology of resultant silver nanoparticles using sample. The size(diameter) of the nanoparticles lie between region in case of flower –AgNps are shown in the below **Figure 5**. The size of the particle range was 63.47-70.72 nm in diameter and size was about 200nm. whereas the shapes were spherical and cubic. This may be due to availability of different quantity and nature of capping agents present in the different flower extracts. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis. Literature data by Bharathi *et al.*, 2019 [34]; Das *et al.*, 2008 [35]; Kumar *et al.*, 2018 [36] showed that silver nanoparticles of various nanosizes and morphologies (spherical, hexagonal, polygonal, etc.) have been synthesized using various stem bark extracts such as *Diospyros montana*, *Butea monosperma*, *Holarrhena antidysenterica*, etc.

### Antioxidant Activity By DPPH Assay Method

The result shows that the AgNps-flower exhibit antioxidant activities at high concentration when compared with standard ascorbic acid (**Table 3**). The synthesized

silver nanoparticles has 65.74% antioxidant activity at concentration of 100µg/ml, while the ascorbic acid has 89.52% at the same concentration. Previous study reported by Kharat and Mendhulkar, 2016 [32] studied the antioxidant activity of synthesized nanoparticles using DPPH assay and observed the antioxidant potentials of photosynthesized nanoparticles. They suggested that photosynthesized NPs can be used as a potential free radical scavenger. Priya *et al.* 2016 [33] studied in vitro antioxidant activity of biosynthesized nanoparticles from *P. pinnata* extract and found significant free radical scavenging potential. The results strongly recommend the application of AgNPs as useful natural antioxidants for health preservation against different oxidative stress associated with degenerative diseases.

### Antioxidant activity by Hydrogen peroxide method

In living systems, uninhibited accumulation of hydrogen peroxide leads to the development of oxygen free radicals like peroxide and hydroxyl radicals which causes huge damage to cell membranes. The hydrogen peroxide scavenging activity of AgNPs was quantified spectrophotometrically using ascorbic acid as a standard. The concentrations at 100 lg/mL inhibition were found to be 51.48 and 74.25 % for the AgNPs and ascorbic acid, respectively.

In the presence of hydrogen peroxide, the dispersed AgNPs can induce reactive oxygen species like hydroxyl radicals. Hydrogen peroxide inside a cell at a low dose can accelerate the dissolution of AgNPs and produce much stronger oxidative stress [36]. Our results are in good accordance with an earlier report on the H<sub>2</sub>O<sub>2</sub> scavenging effect of leaf extract of *Abutilon indicum* [37].

#### **Antioxidant activity of synthesized silver nanoparticles using *N. nucifera* by Metal chelating method**

As excess free irons have been implicated in the induction and formation of free radicals in biological systems, we tested our medicinal plant extracts in a metal chelating assay. The synthesized silver nanoparticles has 60.93% compared with standard drug ascorbic acid (81.66%) at a concentration of 100µg/ml (Table 5). Our results showed that synthesized silver nanoparticle using *N. nucifera* flower inhibited the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine [38].

#### **Anti-Cancer Activity of Synthesized Silver Nanoparticle Using Flower *Nelumbo Nucifera***

The Cytotoxicity study was carried out for the synthesized silver nanoparticles using flower of *N. nucifera* for its cytotoxicity against

MDA-MB-231 breast cancer cell lines at different concentrations 2.5-15 µg/ml by MTT assay was summarized in Table 6. The table's shows that the silver nanoparticles as found more cytotoxic on MDA-MB-231 cancer cell lines respectively. From this cell viability assay, the synthesized silver nanoparticles has more capacity to kill MDA-MB-231 cells. Photomicrograph (10x) represents morphological changes in MDA-MB-231 cells such as shrinkage, detachment, membrane blebbing and distorted shape induced by sample (2.5, 5, 7.5, 10 and 15 µg/ml for 24 h) as compared with control. Control showed normal intact cell morphology and their images were captured by light microscope. There is no drastic change in control treated cells when the time is increased, whereas the synthesized silver nanoparticles treated cells causes variation in cell viability when the time is increased at 10 and 15 µg/ml (Figure 6).

The previous study reported by Cetintas *et al.*, 2020 [39] showed the synthesis of silver nanoparticles synthesized using *Eucalyptus camaldulensis* leaf extract and its anticancer activity against MDA-MB-231 cancer cell lines was reported. The anticancer activity was investigated using MTT assay and the activity of the AgNPs was compared with the extract. The fabricated AgNPs displayed a

very high activity against all the cancer cells and higher activity the plant extract. The AgNPs showed good anticancer activity and its best activity came against MDAMB-231 cancer cell line. Nindawat and Agarwal, 2019 [40] and Ajith *et al.*, 2019 [41] reported that the silver nanoparticles synthesized using medicinal plants showed excellent inhibition against cancer cells due to the presence of the secondary metabolites. A green approach was

employed for synthesis of silver nanoparticles using *Fumaria parviflora* leaves extract for its anticancer activity evaluation against human breast cancer (MDA-MB-468) cell lines by MTT assay. The fabricated AgNPs showed a considerable activity which increased with the concentration of the product and it also displayed high selectivity (Sattari *et al.*, 2020) [42].

**Table: 1 Qualitative analysis of phytochemicals from the ethanolic extract of *Nelumbo nucifera* flower extract**

Test No	Test for	Observation	Result
1	Terpenoid	Reddish brown	+++
2	Flavonoids	Yellow colour	+++
3	Saponin	Formation of Emulsion	+++
4	Tannin	Green precipitate	+++
5	Alkaloids	Yellow colour Precipitate	+++
6	Steroids	Reddish brown ring	+++
7	Glycosides	Violet into blue into Green colour	+++
8	Phlobatanins	Red precipitate	+++
9	Proteins	White precipitate	+++
10	Coumarin	Yellow precipitate	+++
11	Emodin	Red colour	+++
12	Anthroquinone	Pink, Violet, Red Colour	+++
13	Anthocyanin	Pinkish red to bluish Violet colour	+++
14	Carbohydrate	Reddish violet ring Formation	+++
15	Laucoanthocyanin	Organic layer into Red	+++
16	Cardiac Glycosides	Formation of violet or Browning	+++
17	Xanthoprotein	Blue black colour	+++
18	Phenols	Reddish orange Colour	+++

A – Absence, + - Trace, ++ - Moderate, +++ - Strong

**Table 2: Quantitative Analysis Of *Nelumbo Nucifera* Flower Extract**

S. No.	Phytochemical Constituents	<i>Nelumbo nucifera</i> flower extract (mg/g)
1	Saponins	0.198
2	Alkaloids	0.019
3	Flavonoids	1.459
4	Phenol	0.734
5	Terpenoids	0.660
6	Tannins	1.997

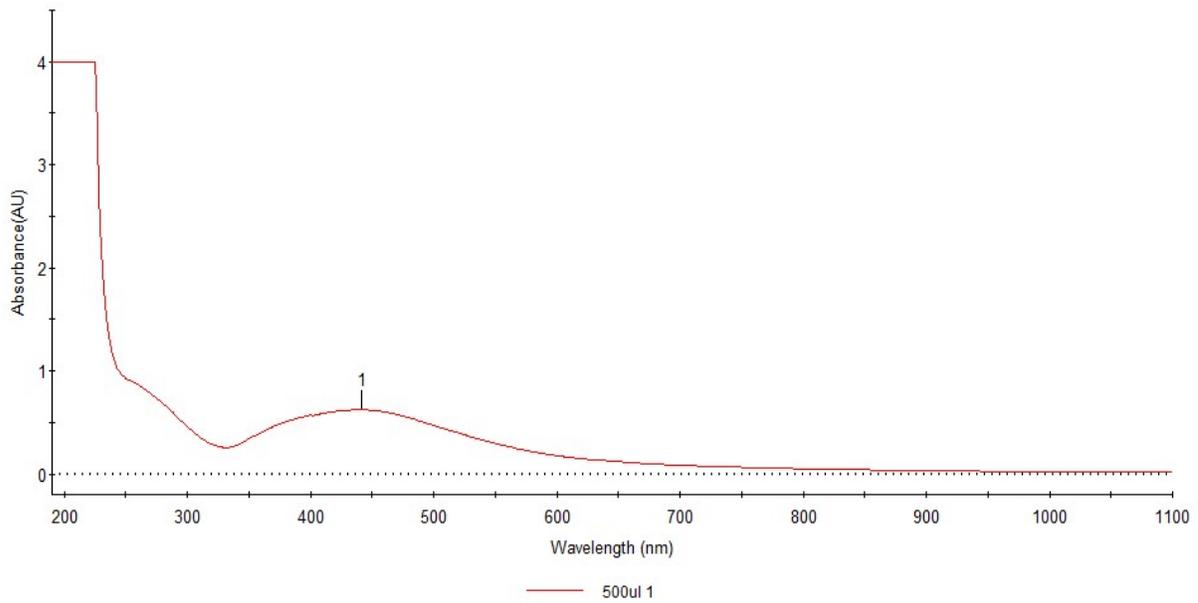


Figure 1: UV Spectra for Synthesized Silver Nanoparticles Using *Nelumbo Nucifera* Flower

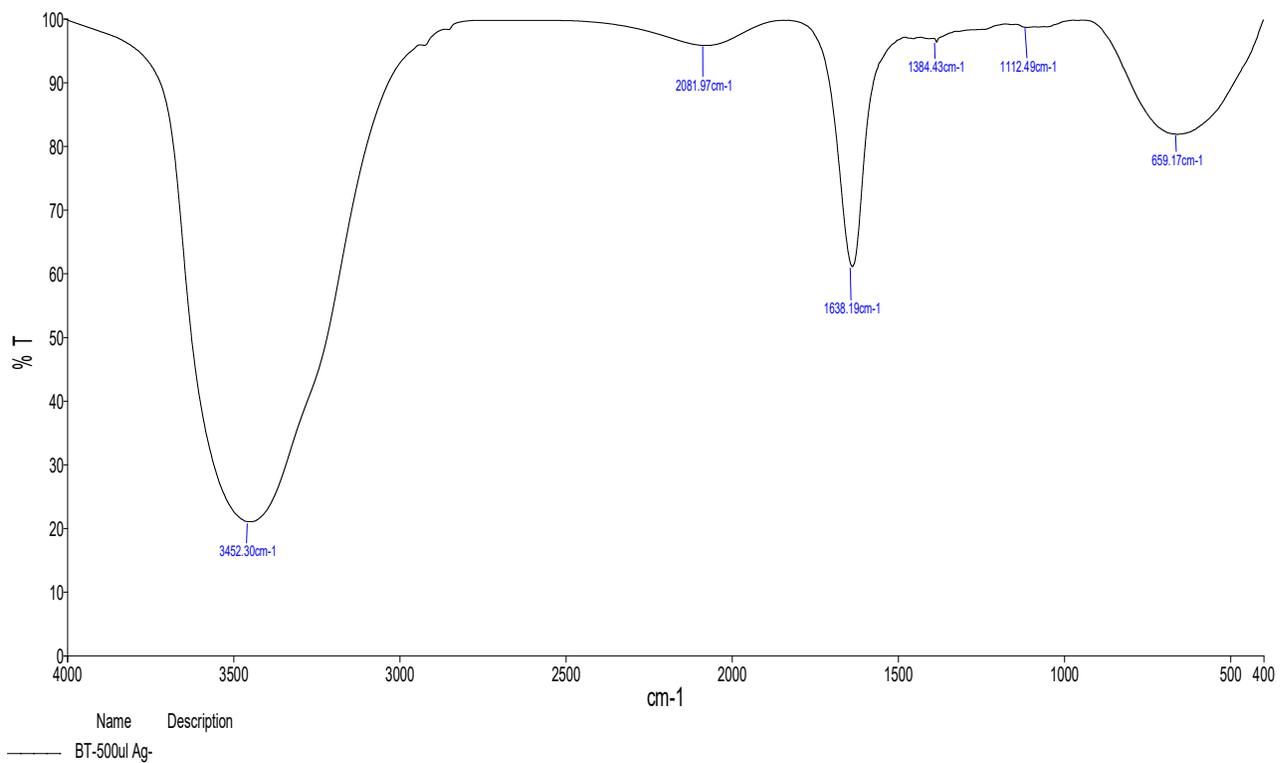
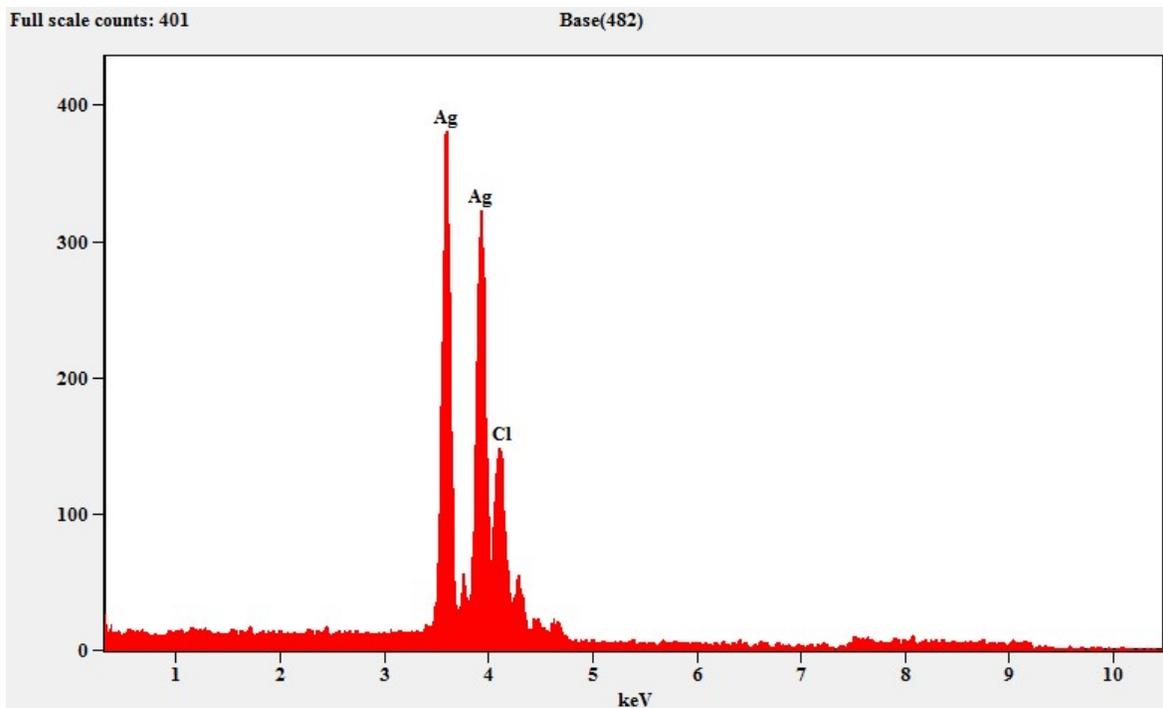
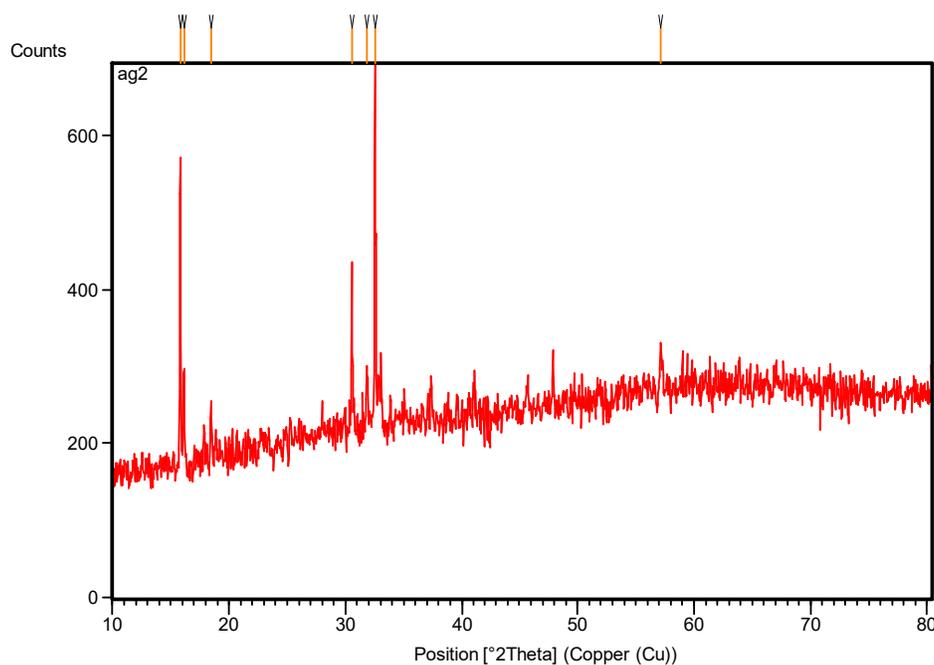


Figure 2: FTIR Spectra For Synthesized Silver Nanoparticles Using *Nelumbo Nucifera* Flower

Figure 3: EDAX Spectra For Synthesized Silver Nanoparticles Using *Nelumbo Nucifera* Flower

Quantitative Results for: Base (482)			
Element Line	Weight %	Weight % Error	Atom %
AgL	52.48	± 0.60	25.15
AgM	32.63	± 1.75	17.36
Cl K	14.89	± 0.24	57.49
Total	100.00		100.00

Figure 4: Xrd Spectra For Synthesized Silver Nanoparticles Using *Nelumbo Nucifera* Flower

Pos. [ $^{\circ}$ Th.]	Height [cts]	FWHM Left [ $^{\circ}$ Th.]	d-spacing [ $\text{\AA}$ ]	Rel. Int. [%]
38.2486	59.54	0.1238	1.23465	38.46
45.5784	92.37	0.1468	1.43576	42.00
66.0213	143.24	0.1801	1.35584	87.32
78.2365	128.33	0.1806	2.37542	76.57

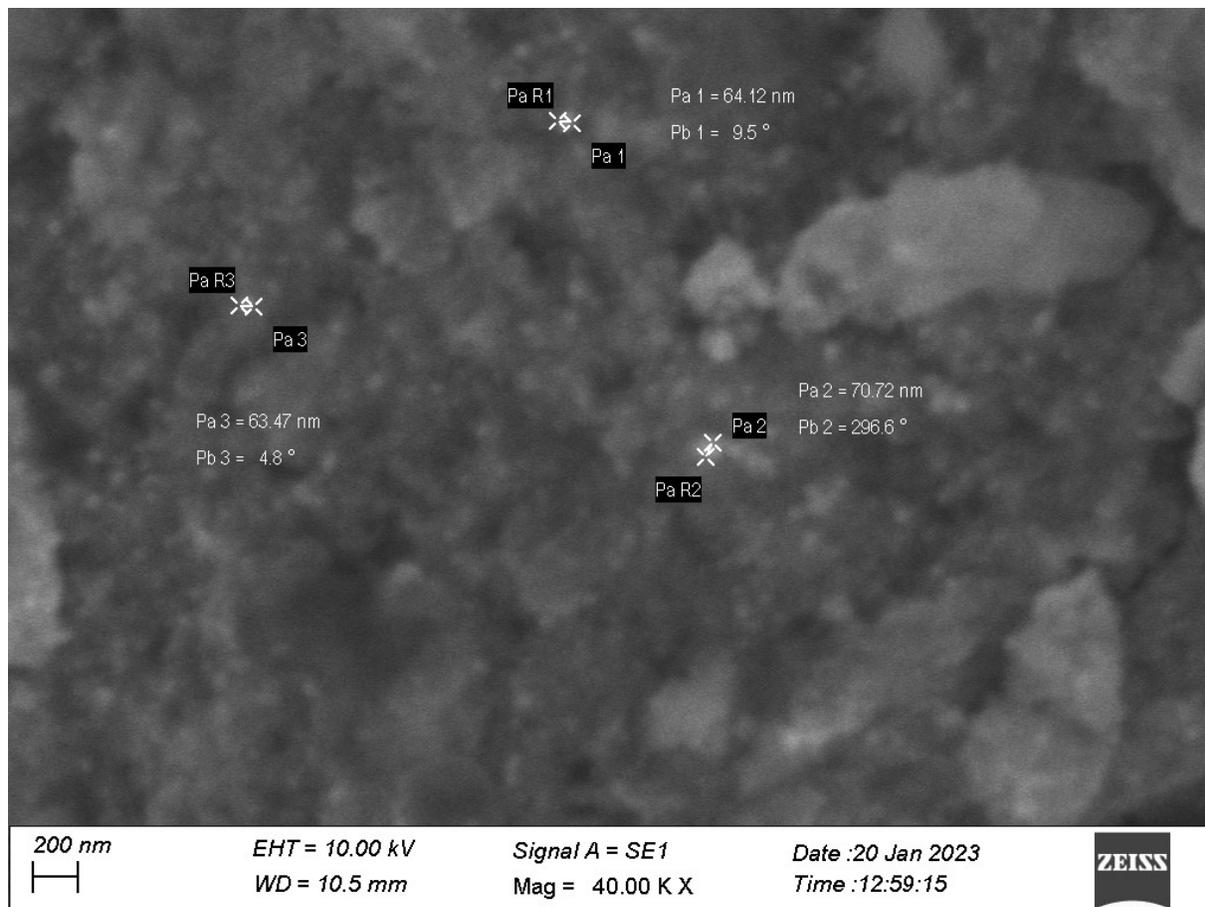


Figure 5: SEM Spectra For Synthesized Silver Nanoparticles Using *Nelumbo Nucifera* Flower

Table 3: Antioxidant activity of synthesized silver nanoparticle by DPPH assay method

S. No	Concentration ( $\mu\text{g/ml}$ )	Percentage of Inhibition %	
		Silver Nanoparticle	Ascorbic Acid
1.	20	49.07	66.66
2.	40	52.77	74.07
3.	60	56.48	80.55
4.	80	60.18	82.40
5.	100	65.74	89.52

Table 4: Antioxidant activity of synthesized silver nanoparticle by Hydrogen peroxide method

S. No.	Concentration ( $\mu\text{g/ml}$ )	Percentage of Inhibition %	
		Silver Nanoparticle	Ascorbic Acid
1.	20	35.64	56.43
2.	40	39.60	61.38
3.	60	43.56	67.32
4.	80	48.51	72.27
5.	100	51.48	74.25

Table 5: Antioxidant activity of synthesized silver nanoparticle by Metal chelating method

S. No.	Concentration (µg/ml)	Percentage of Inhibition %	
		Silver Nanoparticle	Ascorbic Acid
1.	20	46.09	63.28
2.	40	50	67.18
3.	60	54.68	69.53
4.	80	59.37	71.87
5.	100	60.93	81.66

Table 6: Cell Viability Of Synthesized Zinc Nanoparticles Using Medicinal Plants On Mda-Mb-231 Cell Line By Mtt Assay

S. No.	Sample concentration (µg/ml)	Synthesized silver nanoparticles				
		Cell Viability(%) R1	Cell Viability(%) R2	Cell Viability(%) R3	Average	SD
1.	Control	100	100	100	100	0
2.	2.5 µg/ml	93.60245	89.87864	85.65608	89.71239	3.975793
3.	5 µg/ml	83.11047	77.54148	74.46688	78.37294	4.381371
4.	7.5 µg/ml	67.63856	63.44396	55.18456	62.08903	6.336593
5.	10 µg/ml	55.98826	47.65725	42.96355	48.86969	6.596459
6.	15 µg/ml	42.75274	38.23714	36.40153	39.13047	3.268485

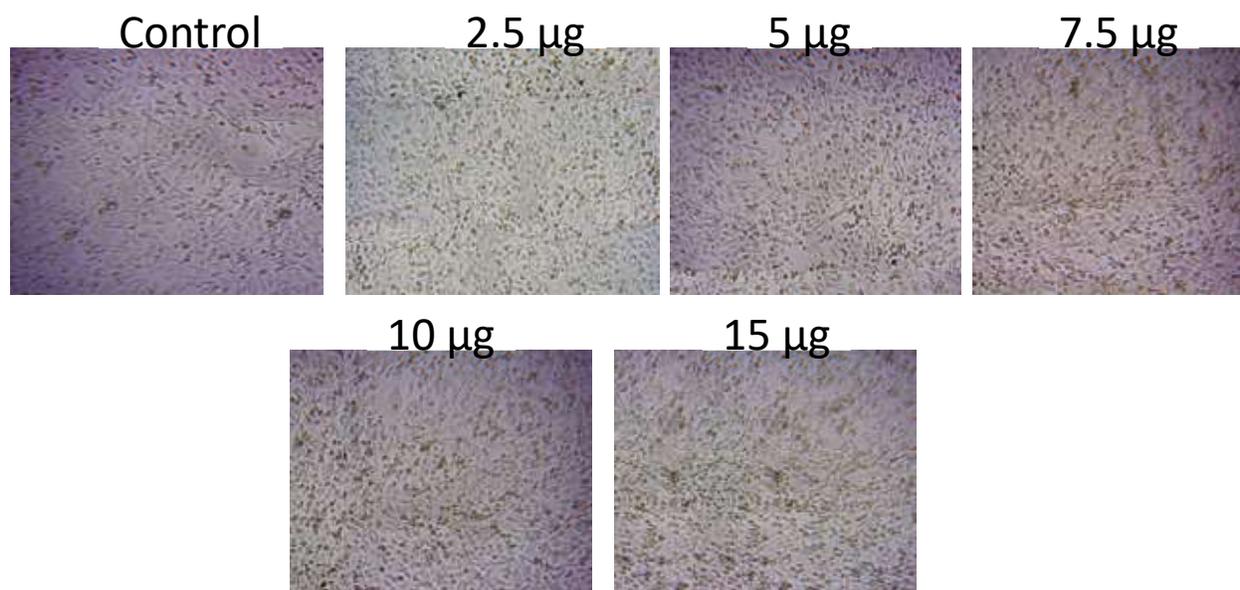


Figure 6: Morphological Changes In Control And Synthesized Silver Nanoparticles Treated Breast Cancer MDA-MB-231 Cells For 24 H

**CONCLUSION:**

In the present study, a simple and economic approach has been attempted to obtain a green eco-friendly synthesis of silver nanoparticles

which was obtained from bio-reduction of *Nelumbo nucifera* flower extracts with AgNO<sub>3</sub> solution. Silver nanoparticles synthesized by the green chemistry approach

reported in the present study may have potent applications in human breast cancer. Synthesized AgNPs from the plant extracts are characterized specifically using UV-Visible spectroscopy, EDAX analysis and SEM, whereas protocol to produce uniform sized nanoparticles has to be standardized for specific applications. The anti oxidant analysis showed better percentage inhibition for the synthesized silver nanoparticles in the DPPH (65.74%), Hydrogen peroxide (51.48%) and metal chetaling (60.93%) at a concentration 100µg/ml. The study of anti-cancer activity was carried out for synthesized silver nanoparticle from the flower of *N. nucifera*. This AgNps-flower were screened for its cytotoxicity against MDA-MB-231 Breast cancer cell line. Different concentration of samples was tested for Cell viability against cancer cell line. IC<sub>50</sub> value was found out to be 11.25 µg/ml. In conclusion, the result revealed that synthesized silver nano particles from *N.nucifera* flower extract has good potential for anti-oxidant and anti-cancer activities

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#### AUTHOR CONTRIBUTION

All authors contribute equally to this manuscript.

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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