Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2023103222-231

# *Clitoria Ternatea* Floral Mediated Synthesis, Characterization, Antioxidant, and Cytotoxicity Evaluation of Silver Nanoparticles

Hariharan Madhumitha[#a], Soundhararajan Ranjani[#a], Jenin Ravindranath Karunya[a], Srinivasan Hemalatha*[a]

[a]*School of Life Sciences, B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamilnadu, India*

#Authors with equal contribution

ARTICLE INFO

**Received:**
10 December 2022
**Revised:**
16 April 2023
**Accepted:**
19 April 2023

**Keywords:**
Metal Nanoparticles, Clitoria, Breast cancer, Molecular docking, Antioxidant, Anticancer agent

ABSTRACT

**Background:** Green silver nanoparticles offer a reliable and secure alternative to drugs and prevent cancer. The floral part of *Clitoria ternatea* is traditionally well known for its application as a medicine and food in various cultures around the world. Phytochemicals of these floral extracts are enriched with various properties. Floral extracts can be utilized as an alternative that can target the proteins and other molecules involved in the progression of cancer.

**Methods:** Silver nanoparticles (CT-AgNPs) were synthesized from the extracts of blue flowers of *Clitoria ternatea* (CT). The synthesized CT-AgNPs were characterized by various physicochemical methods that revealed the size, shape, and stability of the nanoparticles. Docking was performed between the phytocompounds of *Clitoria ternatea* and apoptotic proteins involved in breast cancer such as APAF-1, BCL-2, and BAX, to determine the ability of phytocompounds present in the floral extract to control breast cancer by binding to the targets.

**Results:** Based on the docking results, the binding energies ranged from -6.2 Kcal/mol to -7 Kcal/mol with Quercetin having the highest binding energies. Toxicity analysis of CT-AgNPs in *Artermia nauplii* and *Vigna radiata* seedlings confirmed that these nanoparticles were not toxic to both the model systems. Free radical scavenging activity assay revealed the antioxidant nature of CT-AgNPs was similar to that of standard ascorbic acid. *In vitro* cytotoxicity analysis using MCF-7 breast cancer cell lines revealed that CT-AgNPs were cytotoxic.

**Conclusion:** *In vitro* antioxidant and cytotoxicity analysis using MCF-7 breast cancer cell lines revealed that CT-AgNPs were potent antioxidant and cytotoxic, correlating with the results of *in silico* analysis and hence demonstrating the anticancer potential of *Clitoria ternatea* floral mediated nanoparticles



## INTRODUCTION

Breast cancer is one of the most commonly affecting cancers in the world and also the most common malignant cancer in women. The survival rate for breast cancer is high when diagnosed at early stages. However, diagnosis at later stages is incurable, which poses the need for better detection and enhanced treatment methods. Hence, the detection of breast cancer at early stages is crucial as it significantly increases the survival rates of patients.[1] Paclitaxel is one of the frequently used anticancer medicine owing to its apoptotic activity in cancer cells and also helps to improve the immune response

***Address for correspondence:**
Dr. S. Hemalatha,
Professor and Dean, School of Life Sciences, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai, Tamil Nadu, India 600048.
Tel: +4422759215
Email: hemalatha.sls@bsauniv.ac.in

against cancer cells. However, patients often develop resistance to paclitaxel. Therefore, the development of alternatives with better efficiency and reduced side effects is a need of the hour.[2]

Nanoparticles are utilized in various areas including drug delivery, diagnosis, imaging, and miniature medical devices, etc.[3] Nanoparticles have a high surface area to volume ratio and hence utilized as anti-cancer agents and for targeted cancer therapy.[4] Nanomedicine is used for breast cancer; however, it is not specifically designed to target breast cancer cells alone. Therefore, further research is necessary to understand and develop improved nanomedicines for breast cancer in order to improve the quality of treatment of breast cancer and to aid in its detection during onset.[5]

Nanoparticles are available in different nanoscale ranges and in different types, and one such type is metal-based nanoparticles, e.g., silver nanoparticles (AgNPs), gold nanoparticles, platinum, palladium, etc. AgNPs are reported to have anticancer, anti-fungal and anti-bacterial properties. Studies on anti-cancer therapy have proved that AgNPs have apoptosis-inducing and anti-proliferative properties. AgNPs are known to work against cancer by increasing the chemotherapeutic ability of other drugs against multi-drug-resistant cancer cells. This proves that these nanoparticles have greater potential as a combination drug.[6] There are reports supporting that nanoparticles could break the double-strand bonding of DNA, and thereby inducing apoptosis in cancer cells. This property facilitates the use of silver nanoparticles as a drug targeting the DNA of cancer cells.[7]

*Clitoria ternatea* is a plant that comes under the family of Fabaceae and is usually widespread throughout the tropical and lowland regions. The common name of *Clitoria ternatea* is the butterfly pea plant, which produces a flower of dark blue color. Traditionally, this plant has been used as a medicine and food in various cultures worldwide. This plant is reported to have antimicrobial, anti-inflammatory, antistress, antipyretic, and various other properties.[8] *Clitoria ternatea* contains various phytochemicals such as tannins, flavonoids, steroids, anthocyanin, resins, etc. These phytochemicals provide the plant with various properties and can be used as medicine to target proteins and other molecules involved in various diseases. However, further research is needed to validate and develop such products to cure various ailments.[9]

Various molecules were involved in cancer progression pathways and are often used to diagnose or as therapeutic targets. APAF-1 is one of the proteins involved in apoptosis and is often downregulated in cancer, due to which the process of apoptosis does not occur properly in cancerous cells.[10] BCL-2 is one of the anti-apoptotic proteins that is often upregulated in cancer. Drugs targeting the inactivation or inhibition of this protein could be potentially developed to aid in the treatment of cancer.[11] BAX is a pro-apoptotic protein and is usually downregulated in cancer.[12] In this study, apoptotic proteins involved in the breast cancer APAF-1 (Apoptotic protease activating factor 1), BCL-2 (B-cell lymphoma 2), and BAX (Bcl-2-associated X protein) were docked with phytocompounds of *Clitoria ternatea*. *In vitro* approaches including synthesis, characterization, and toxicity studies in animals and plants (*Artemia nauplii* and *Vigna radiata*) were performed using nanoparticles synthesized from floral extracts of *Clitoria ternatea (*CT-AgNPs*)*. Further, CT-AgNPs were explored for their antioxidant and anti-cancer properties in MCF-7 breast cancer cell lines.

## METHODS

### *In silico studies*

#### *Screening of ligands by using SwissADME*

Lipinski's rule of five was used to identify drug-likeness properties for the phytocompounds of *Clitoria ternatea*. Canonical smiles of the ligands were retrieved from PubChem. Ligands were analyzed in SwissADME which gives information about the drug-likeness properties of the ligands by adopting Lipinski's rule of 5.[13]

#### *Ligand preparation*

Different phytochemicals were chosen from *Clitoria ternatea* and their 3D structures were downloaded from PubChem and Chemspider. Octadecanoic acid, Quercetin, Hexadecanoic acid, 9,17-octadecadienal, D-Allose and Kaempferol monoglucoside were chosen for this study. The PubChem ID of Octadecanoic acid is 5091, the ID of Hexadecanoic acid is 960, the ID of 9,17-octadecadienal is 4936623 and that of D-Allose is 395203 and the Chemspider IDs of Quercetin and Kaempferol monoglucoside are 5280343 and 5282102, respectively. The breast cancer drug paclitaxel was used as a standard (ID 36314). These selected ligands were prepared for docking using PyRx via Open Babel by minimization.[14]

#### *Protein preparation*

Biomarkers found in breast cancer were identified and the chosen proteins were Apoptosis protease activating factor-1 (APAF-1), B cell lymphoma 2 (BCL-2) and BCL-2 associated X protein (BAX). These proteins were downloaded from RCS PDB in PDB format. The PDB ID of APAF-1 is 1CY5, that of BCL-2 is 4AQ3 and that of BAX is 4BDU. The

downloaded proteins were prepared with the help of Discovery Studio. Water and hetero atoms were deleted and polar hydrogen was added. The prepared protein was then saved for docking using PyRX. The protein was set as a macromolecule and suitable grid parameters ranging from 35 to155 were set.[15,16]

*Molecular docking using PyRx*

PyRx was used for molecular docking analysis. PyRx runs on the Vina wizard application by determining the values of binding energies between the proteins and ligands. The values were recorded and the interaction between the ligand and protein was visualized using Discovery studio visualizer tool.[16-18]

*In vitro studies*

*Preparation of floral extract from flowers of Clitoria ternatea*

10g of dried blue flowers was taken and boiled in 200ml of water for a few minutes. The mixture was blended and strained. The prepared extract was centrifuged at 3000rpm for 20 minutes and then filtered thrice with Whatman filter paper to remove particulate matter.[19]

*Synthesis of Clitoria ternatea floral mediated nanoparticles (CT-AgNPs)*

The floral extract was mixed with 1mM silver nitrate in a ratio of 1:4. The mixture was kept at room temperature for 24-48hours under dark conditions. After color change, the mixture was centrifuged thrice for 15minutes at 4000rpm. Synthesized CT-AgNPs were purified using ethanol with repeated centrifugation.[20-24]

*Characterisation of CT-AgNPs*

The characterization of CT-AgNPs was performed using UV Visible spectroscopy, FTIR spectroscopy, FESEM coupled with an EDAX analyzer, and DLS coupled with a zeta potential analyzer. Ultraviolet-Visible spectroscopy (UV) was used to measure the absorption and scattering of light passing through CT-AgNPs. The synthesis of nanoparticles can be identified by a color change and confirmed using UV-spectroscopy. Fourier transform infrared spectroscopy (FTIR) helps to understand the functional groups present in the floral extract of *Clitoria ternatea* and CT-AgNPs. The FTIR analysis of powdered dried flower of *Clitoria ternatea* and CT-AgNPs synthesized from the floral extracts were scanned between the wavelength ranging from 400 $cm^{-1}$ to 4000 $cm^{-1}$. Field emission scanning electron microscope (FESEM) uses electrons to produce an image of CT-AgNPs. This usually produces an image of nanoparticles microstructure and helps us to study the surface morphology, shape, and size of CT-AgNPs. Energy-dispersive X-ray spectroscopy (EDAX) aids in understanding the elemental constituents of CT-AgNPs. Dynamic light scattering (DLS) and Zeta potential give us information about the aggregation, diameter, Z-average, and polydispersity index of CT-AgNPs. Zeta potential analysis gives information about the charges on the surface of CT-AgNPs.[25,26]

*Toxicity screening of CT-AgNPs in Artemia nauplii and Vigna radiata*

*Artemia nauplii* and *Vigna radiata* seedlings were used to observe the toxicity of synthesized CT-AgNPs. Hatched *Artemia* were allowed to grow in a saltwater medium containing 10PPM, 5PPM and 1PPM of CT-AgNPs and mortality was observed after 24hours. Soaked *Vigna radiata* (green gram) seeds were placed in the seedling tray and were covered with soil. Water containing 1PPM, 5PPM and 10PPM of CT-AgNPs was sprayed for 7 days. Seed germination percentage was calculated for the control and treated seeds. At the end of the $7^{th}$ day, the grown plants were taken from the soil, and the root, shoot length, and fresh weight were calculated to study the toxicity of CT-AgNPs. The average length of the root and shoot was measured and then the values of the average were converted to one-fold to the values of the control.[27,28] All the experiments were performed in triplicates.

*DPPH assay for evaluating the radical scavenging activity of CT-AgNPs*

Free radical scavenging activity of CT-AgNPs was studied as described in the DPPH (α-Diphenyl-β-Picrylhydrazyl) assay protocol without any modification.[29] The standard ascorbic acid was used and compared with the CT-AgNPs at various concentrations ranging from 100, 75, 50, 25, 12.5, and 6.125µg/ml, respectively.

*Cytotoxicity studies using MTT in MCF-7 breast cancer cell lines*

MTT (3-(4,5-Dimethylthiazolyl-2)-2, 5-Diphenyltetrazolium Bromide) assay was performed to calculate the cytotoxicity and viability of cancer cells. In a 96 well plate, MCF-7 breast cancer cells were seeded at a density of $1\times10^4$ cells/well. The cells were treated with CT-AgNPs (100µg/ml, 80µg/ml, 60µg/ml, 40µg/ml, 20µg/ml and 10µg/ml) and incubated for 24 hours. After treatment, the cells were treated with 0.5mg/ml of MTT, and incubated for 3hours. MTT was removed and DMSO was added to dissolve the formazan crystals. After incubating the plate in dark for 15minutes, the absorbance was measured at 570nm (test wavelength) and 620nm (reference wavelength) using a multi-mode plate reader.[25]

## RESULTS AND DISCUSSION

*In silico docking studies on ligand-protein interaction*

Lipinski's rule of 5 clearly showed the drug-likeness properties of a selected ligands from *Clitoria ternatea*. Among the ligands, Kaempferol monoglucoside has two violations, whereas other ligands obey Lipinski's rule of 5 which makes it suitable for docking analysis (Table 1).

**Table 1**. Lipinski's rule of five

| | Molecular mass | Lipophilicity YLogP | No. of H-bond donors | No. of H-bond acceptors | Molar refractivity | Drug likeness |
|---|---|---|---|---|---|---|
| D-Allose | 180.16 | 0.24 | 5 | 6 | 35.74 | Yes; 0 violation |
| Hexadecanoic acid | 256.42 | 3.85 | 1 | 2 | 80.8 | Yes; 0 violation |
| Kaempferol monoglucoside | 448.38 | 0.53 | 7 | 11 | 108.13 | No; 2 violations |
| Octadecanoic acid | 284.48 | 4.3 | 1 | 2 | 90.41 | Yes; 0 violation |
| 9,17-octadecadienal | 264.45 | 4.41 | 0 | 1 | 87.89 | Yes; 0 violation |
| Quercetin | 302.02 | 1.63 | 5 | 7 | 78.03 | Yes; 0 violation |

Docking was performed between apoptotic proteins involved in breast cancer and the ligands (phytochemicals) found in *Clitoria ternatea*. The binding energies and the ligand interactions of the docking are shown in (Table 2)

**Table 2**. Binding energies between ligands and proteins

| Protein | D-Allose (DA) | Hexadecanoic acid (HD) | Octadecanoic acid (OC) | 9,17-octadecadienal (OD) | Quercetin (QR) | Paclitaxel |
|---|---|---|---|---|---|---|
| APAF-1- Apoptotic protease activating factor 1 | -4.6 | -3.8 | -3.1 | -3.4 | -6.2 | -6.7 |
| BCL-2- B-cell lymphoma 2 | -4.8 | -5.4 | -5.6 | -5.6 | -7 | -9.2 |
| BAX- Bcl-2-associated X protein | -4.8 | -4.1 | -3.7 | -4.5 | -6.9 | -7.8 |

(Figure 1 i-iii) Quercetin showed the highest binding energies for the three proteins with values ranging from -6.2Kcal/mol to -7Kcal/mol. Quercetin has binding energies of -6.9Kcal/mol for BAX, -7Kcal/mol for BCL-2 and -6.2Kcal/mol for APAF-1. The hydrogen bond involved between Quercetin and BCL2 was ASN 102 (asparagine) and between Quercetin and BAX it was PHE 1105 (phenylalanine) and ARG 168 (arginine). Paclitaxel has a binding energy of -6.7Kcal/mol for APAF-1, -9.2Kcal/mol for BCL-2 and -7.8Kcal/mol for BAX. The binding energy for quercetin and paclitaxel was very similar, which confirms the potential activity of the phytochemical quercetin against cancer.

*Floral mediated synthesis and physico-chemical characterisation of CT-AgNPs*

Synthesis of CT-AgNPs using floral extract was visually confirmed by prominent color change. Synthesized CT-AgNPs was further confirmed by measuring the absorbance of CT-AgNPs between the ranges of 200 nm-800 nm using a UV-visible spectrometer. SPR peak of CT-AgNPs was observed between 400 and 500 nm which confirmed the synthesis of CT-AgNPs (Figure 2a).

The FTIR analysis of dried flower extract showed a peak of 3292.86cm$^{-1}$ which specifies the presence of Si-OH where condensation OH stretching vibration occurs, a peak of 2921.63cm$^{-1}$ which specifies the presence of -C-H ($CH_2$) where stretching symmetric vibration occurs, a peak of 1629.55cm$^{-1}$ which specifies the presence of diketones and a peak of 1049.09cm$^{-1}$ which specifies the presence of polysaccharides (Fig. 2b). In the FTIR analysis of the CT- AgNPs, the peaks from the floral extract sample were shifted into two wide peaks. A wide peak, ranging from 2300cm$^{-1}$ - 4000cm$^{-1}$ with very small peaks at 3770.15cm$^{-1}$, 3651.55cm$^{-1}$, 3297.68cm$^{-1}$ and 2343.09cm$^{-1}$ corresponds to alcohols and carbon dioxide respectively and another peak ranging from 600cm$^{-1}$ - 1900cm$^{-1}$ with very small peaks ranging from 610cm$^{-1}$, 781.029cm$^{-1}$, 1067.41cm$^{-1}$, 1222.65cm$^{-1}$, 1373.07cm$^{-1}$, 1592.91cm$^{-1}$ and 1739.48cm$^{-1}$ corresponds to halo compounds, alkene, sulfoxide, vinyl ether, sulfone, nitro compounds and aldehyde groups, respectively. A peak present at 2114.56 cm$^{-1}$, indicates the presence of alkynes (C≡C) (Figure 2c).

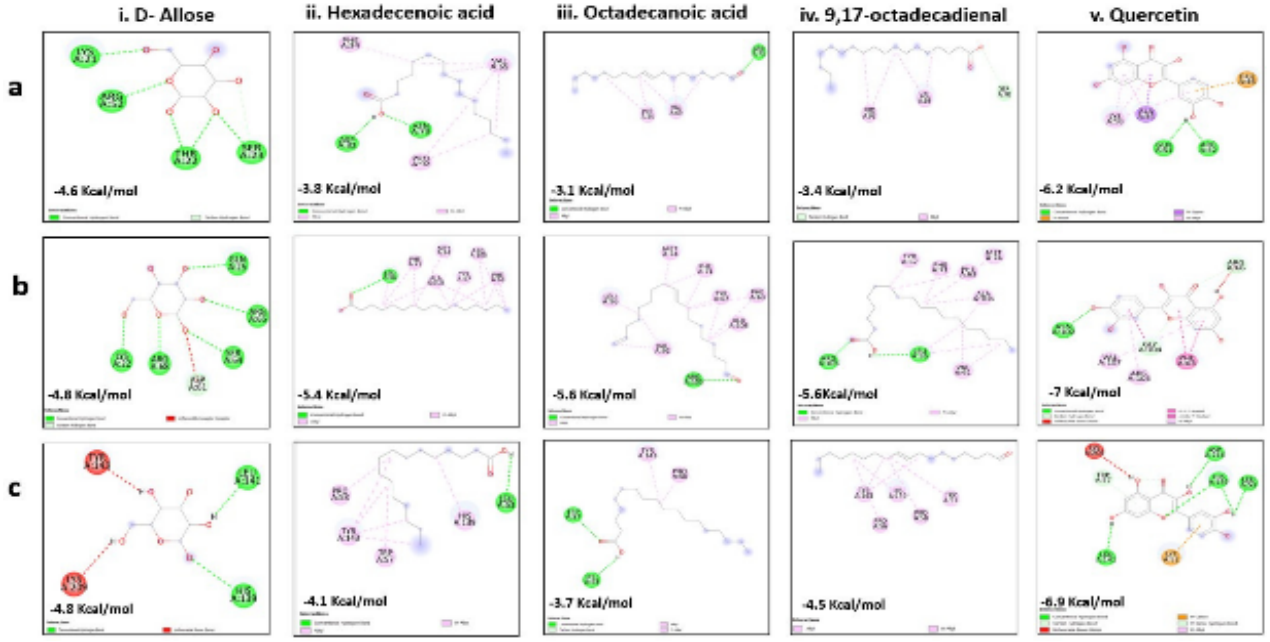


**Figure 1**. **a)** Binding interactions between ligands and APAF-1, **b)** Binding interactions between ligands and BCL-2, **c)** Binding interactions between ligands and BAX. Where i) D-Allose (DA), ii) Hexadecanoic acid (HD), iii) Octadecanoic acid (OC), iv) 9,17-octadecadienal (OD), v) Quercetin (QR)

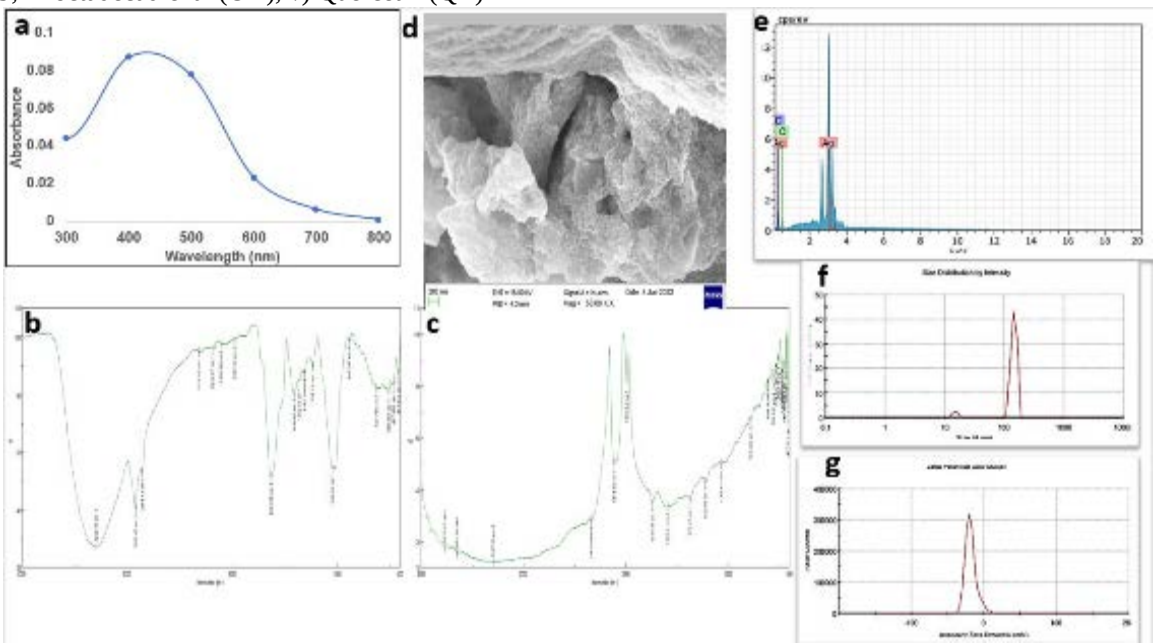


**Figure 2**. Biophysical characterization of CT-AgNPs: a) SPR peak of CT-AgNPs obtained using UV-Visible spectroscopy. b) Fourier transform infrared spectrum of powdered flower of *Clitoria ternatea*. c) Fourier transform infrared spectrum of CT-AgNPs. d) Field emission scanning electron microscopic image of CT-AgNPs at 100KX. e) Energy-dispersive X-ray spectrum of CT-AgNPs. f) Zeta sizer analysis depicting the size of CT-AgNPs. g) Zeta potential analysis of CT-AgNPs.

FESEM aids in understanding the morphology and structure of the synthesized CT-AgNPs. The CT-AgNPs were imaged at a magnification of 100KX with a 200-nanometer scale. The structure of CT-AgNPs was found to be polymorphic in nature (Figure 2d). Energy-dispersive X-ray spectroscopy was used to understand the composition of CT-AgNPs. The analysis showed that CT-AgNPs contain Ag, O, and C. A peak occurring at 3 keV indicates the presence of Ag in CT-AgNPs. The result implies the presence of Ag at a significantly higher percentage than other elements. A higher silver content would facilitate the function of CT-AgNPs as an anti-oxidant and cytotoxic agent (Figure 2e).[30]

In Dynamic light scattering, the average diameter of the AgNPs was around 145.6d.nm which corresponds to a respective peak between 100 – 1000nm, with PdI of 0.906 and intercept of 1.01. The polydispersity index was 0.926 (Figure 2f). The zeta potential was -18.4mV. A negative zeta potential indicates the charge of the CT-AgNPs was negative and was dispersed well in the medium (Figure 2g). The negative charge confirms the stability of the CT-AgNPs.

*Toxicity analysis of CT-AgNPs*

Toxicity study helps in determining the toxicity of CT-AgNPs by calculating the mortality rate of the shrimp (*Artemia nauplii)* (Figure 3 a). After 24 hours of treatment, mortality rate was 0, 3.4, 6.8, and 6.8% for control, 1 PPM, 5 PPM, and 10 PPM respectively. It was also noted that as the concentration of the CT-AgNPs increased, mortality rate also increased consecutively but the percentage of mortality was less than 7%. Hence, this proves that CT-AgNPs were not toxic for *Artemia nauplii*.

Toxicity of CT-AgNPs in *Vigna radiata* seedlings was carried out by observing the seed germination percentage and various other growth parameters (Figure 3 b). The seed germination was found to increase by 0.8, 0.86, and 1-fold in 1PPM, 5PPM, and 10PPM, respectively. It can be seen that the length of the root had increased by 1.04, 1.09, and 1.37-fold for 1PPM, 5PPM and 10PPM when compared to control root length, respectively. The shoot length was increased by 1.25, 1.6, and 1.89 in 1PPM, 5PPM, and 10PPM in comparison to the control shoot length, respectively. The weight of seedlings was also observed to increase by 1.18, 1.54, and 1.78-fold for 1PPM, 5PPM and 10PPM in comparison to the control. The root length, shoot length, weight, and seed germination percentage were observed to increase as the concentration of CT-AgNPs increased. The above data indicates the positive effect on *Vigna radiata* seedlings in the presence of CT-AgNPs, hence proving the non-toxic effect on plants and their growth. Hence, the toxicity study confirms that CT-AgNPs were not toxic to both the tested model systems.

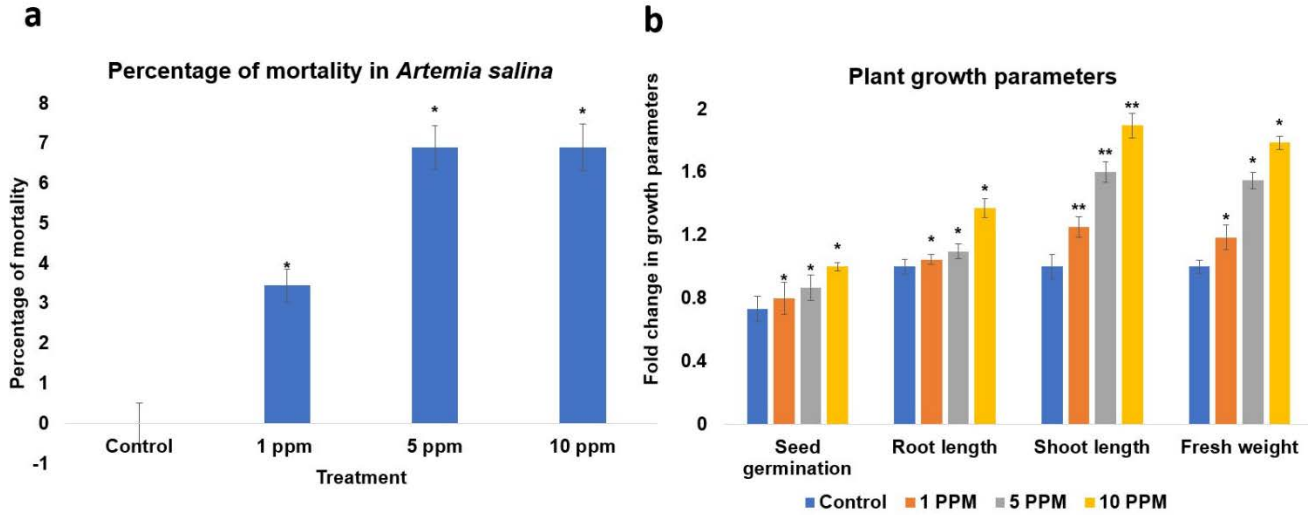


**Figure 3.** Toxicity studies of CT-AgNPs a) Mortality rate of *Artemia nauplii.* b) Fold change in growth parameters of *Vigna radiate* seedlings

*Detection of antioxidant activity of CT-AgNPs*

Based on the DPPH radical scavenging activity, it can be observed that the antioxidant properties of CT-AgNPs were similar to the activity of standard ascorbic acid. Ascorbic acid is a very popular antioxidant that can scavenge free radicals effectively. Ascorbic acid, also known as Vitamin C has various benefits to the human body and its deficiency produces various effects on our body. Higher levels of free radicals in our body are known to cause DNA damage which can potentially lead to cancer development. Antioxidants could reduce these

free radicals by a significant amount. Hence, this signifies the role of antioxidants in cancer pathway and its treatment.[31] Based on the free radical scavenging assay, CT-AgNP showed 73%, 66%, 54%, 44%, 37% and 32.6% for 100, 75, 50, 25, 12.5, and 6.125µg/ml, respectively. The antioxidant property of ascorbic acid was observed to be 76.5%, 69.49%, 58.26%, 47.38%, 40.93%, and 34.73% for 100, 75, 50, 25, 12.5, and 6.125µg/ml, respectively (Figure 4 a). Since the antioxidant property of CT-AgNP is similar to ascorbic acid, it can be seen that it has significant antioxidant properties. From the results, it was observed that the antioxidant level decreased as the concentration of CT-AgNPs decreased.[32] The antioxidants properties of the CT-AgNPs were due to the phytochemicals found in the floral extract of *Clitoria ternatea* which could have acted as a capping and reducing agent during the synthesis of nanoparticles. The phytochemicals of the flowers are known to possess antioxidants properties. There was a report on the extracts of *Clitoria ternatea*, which was found to have a free radical scavenging activity which proves the antioxidant properties of the phytochemicals present in the flower.[33] In another study, AgNPs synthesized from *Clitoria ternatea* proved to have significant antioxidant properties similar to that of extracts of *Clitoria ternatea*. Our results supported the previous studies proving the better antioxidant potential CT-AgNPs. Several flavonoids and various other phytochemicals including quercetin are reported to have antioxidant properties. Based on this study, CT-AgNPs can be utilized in cancer treatments owing to their better anticancer activity. These antioxidants may help to reduce the toxicity caused by chemotherapy and radiotherapy and also help to improve the efficacy of the drugs.[34]

*Cytotoxic effect of CT-AgNP in breast cancer cell lines*

MTT assay was performed to check the cytotoxic effect of CT-AgNP in MCF-7 cell lines. Upon CT-AgNPs treatment, the percentage of cell viability was 60%, 44%, 32%, 19%, 8%, and 6% at the concentration of 10, 20, 40, 60, 80, and 100µg/ml, respectively (Figure 4 b).

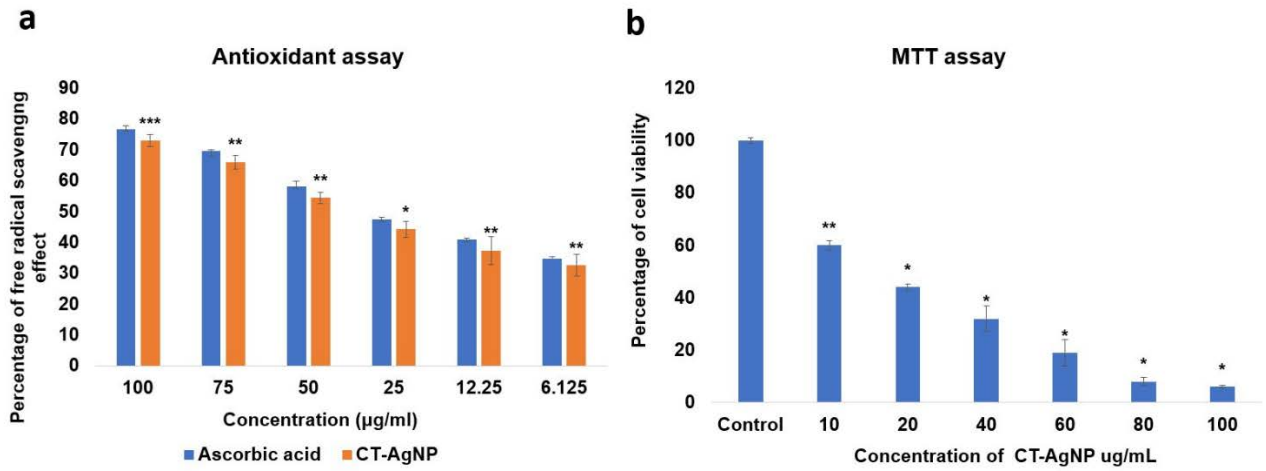


**Figure 4.** a) DPPH free radical scavenging activity of CT-AgNPs b) Cytotoxicity effect of CT-AgNPs in MCF-7 breast cancer cell lines

From the cytotoxic studies, it was observed that the viability of the cells decreased with an increase in the concentration of CT-AgNPs. Minimum inhibitory concentration (i.e.) IC50 of CT-AgNPs was calculated and the value was found to be 17µg/ml. CT-AgNPs had a significant inhibitory effect on the tested breast cancer cells. This result correlates with the docking results where the ligands identified in *Clitoria ternatea* were docked against the apoptotic proteins found in breast cancer. Quercetin had significant binding energies on all of the three proteins. The CT-AgNPs could have potentially induced the expression of these proteins which could inhibit the proliferation of breast cancer cells. The results of i*n silico* docking studies supported the *in vitro* cytotoxic effect of CT-AgNPs on MCF-7 cell lines. The AgNPs were reported to have good inhibition effects on cancer cells through triggering the apoptosis, and this was confirmed via DNA fragmentation. It was also observed that the AgNPs also produced an anti-angiogenic response by reducing ascites secretion and decreasing the density of cancer cells. The anti- angiogenesis response was found to be related to vascular endothelial growth factor.[35] There was a report showing that the green synthesis of AgNPs increases cellular uptake due to its unique phyico-chemical nature. Furthermore, AgNps treated cells showed a reduction in size and an

increase in apoptosis. It was hypothesized that AgNPs can easily enter the cancer cell and lead to the deactivation of cancer DNA, causing its death.[36] In another study, it was also observed that the biosynthesized AgNPs also aided in the downregulation of BCL-2 and improved the expression of BAX protein which increases the apoptosis in MCF-7 cells. AgNPs also helped to regulate the expression of other proteins such as caspases, p53, and cytochrome c. AgNPs were reported to cause genotoxicity in cancer cells by increasing ROX levels and lipid oxidation of cancer cells causing cell death.[37,38] The findings of our study correlate with other reports which confirmed the potential application of CT-AgNPs in cancer therapy due to the ability to produce apoptotic and anti-angiogenic response against cancer.

## CONCLUSION

CT-AgNPs were synthesized successfully and characterized. The binding energies from the docking study showed significant values against the proteins, BCL-2 and BAX. Plant phytochemicals are known to increase cell apoptosis by improving BCL-2/BAX ratios in cancer cells. The BCL-2 and family of proteins play an important role in cancer cells and therefore regulation of these could aid in the creation of anti-cancer drugs. Quercetin showed the highest binding energies out of all ligands against the proteins and the result correlates with the docking results of paclitaxel with the proteins. *In silico* findings proved the anti-cancer capability of CT-AgNPs. Toxicity tests in *Artemia nauplii* and *Vigna radiata* for CT-AgNPs were also performed showing that the CT-AgNPs were not toxic to plants and animal models. The DPPH radical scavenging assay also proved the significant scavenging activity of CT-AgNPs similar to that of ascorbic acid. The results from the MTT assay showed that CT-AgNPs had significant inhibitory effects against MCF-7 breast cancer cell with the IC50 of 17µg/ml. The P-value was calculated for all the experiments and was statistically significant. Hence, this study confirmed that the CT-AgNPs have greater potential as an anti-oxidant and anti-cancer agent but further research is needed to validate the findings of this study.

## FUNDING

Ministry of Science and Technology, Department of Science and Technology (KIRAN Division) (GoI), New Delhi. (Ref No. DST/WOS-B/2018/1583-HFN (G)).

## ACKNOWLEDGMENTS

Authors are thankful to B.S. Abdur Rahman Institute of Science and Technology, Chennai for providing research facilities in the School of Life Sciences. The authors also gratefully acknowledge the Ministry of Science and Technology, Department of Science and Technology (KIRAN Division) (GoI), New Delhi. (Ref No. DST/WOS-B/2018/1583-HFN (G)).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## DATA AVAILABILITY

Data will be available on request.

## ETHICAL CONSIDERATIONS

Not Applicable.

## REFERENCES

1. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer* (Dove Med Press). 2019;11:151-164. 2019. doi: 10.2147/BCTT.S176070
2. Kumar SA, Mohaideen NSMH, S H. Phytocompounds From Edible Oil Seeds Target Hub Genes To Control Breast Cancer. *Appl Biochem Biotechnol*. 2023 Feb;195(2):1231-1254. doi: 10.1007/s12010-022-04224-9.
3. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer*. 2017 Jan;17(1):20-37. doi: 10.1038/nrc.2016.108.
4. Jin C, Wang K, Oppong-Gyebi A, Hu J. Application of Nanotechnology in Cancer Diagnosis and Therapy - A Mini-Review. *Int J Med Sci*. 2020;17(18):2964-2973. 2020 Oct 18. doi: 10.7150/ijms.49801.
5. Wu D, Si M, Xue HY, Wong HL. Nanomedicine applications in the treatment of breast cancer: current state of the art. *Int J Nanomedicine*. 2017 16;12:5879-5892. doi: 10.2147/IJN.S123437.
6. Xu L, Wang YY, Huang J, Chen CY, Wang ZX, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics*. 2020;10(20):8996-9031. 2020. doi: 10.7150/thno.45413
7. Kovács D, Igaz N, Gopisetty MK, Kiricsi M. Cancer Therapy by Silver Nanoparticles: Fiction or Reality?. *Int J Mol Sci*. 2022;23(2):839. 2022. doi: 10.3390/ijms23020839
8. Oguis GK, Gilding EK, Jackson MA, Craik DJ. Butterfly Pea (Clitoria ternatea), a Cyclotide-Bearing Plant With Applications in Agriculture and Medicine. *Front Plant Sci*. 2019;10:645. 2019. doi: 10.3389/fpls.2019.00645.

9. Jeyaraj EJ, Lim YY, Choo WS. Extraction methods of butterfly pea (*Clitoria ternatea*) flower and biological activities of its phytochemicals. *J Food Sci Technol*. 2021;58(6):2054-2067. doi: 10.1007/s13197-020-04745-3.
10. Soengas MS, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, et al. Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 409, 207–211 (2001). doi: 10.1038/35051606.
11. Radha G, Raghavan SC. BCL2: A promising cancer therapeutic target. *Biochim Biophys Acta Rev Cancer*. 2017 Aug;1868(1):309-314. doi: 10.1016/j.bbcan.2017.06.004.
12. Liu Z, Ding Y, Ye N, Wild C, Chen H, Zhou J. Direct Activation of Bax Protein for Cancer Therapy. *Med Res Rev*. 2016 Mar;36(2):313-41. doi: 10.1002/med.21379.
13. Ravindranath KJ, Christian SD, Srinivasan H. Screening of Anti-carcinogenic Properties of Phytocompounds from Allium ascalonicum for Treating Breast Cancer Through In Silico and In Vitro Approaches. *Appl Biochem Biotechnol*. 2023 Feb;195(2):1136-1157. doi: 10.1007/s12010-022-04202-1.
14. Abu Samaan TM, Samec M, Liskova A, Kubatka P, Büsselberg D. Paclitaxel's Mechanistic and Clinical Effects on Breast Cancer. Biomolecules. 2019 Nov 27;9(12):789. doi: 10.3390/biom9120789.
15. Ravindranath KJ, Mohaideen NSMH, Srinivasan H. Phytocompounds of Onion Target Heat Shock Proteins (HSP70s) to Control Breast Cancer Malignancy. *Appl Biochem Biotechnol*. 2022 Oct;194(10):4836-4851. doi: 10.1007/s12010-022-04016-1.
16. Juzer T, Ranjani S, Hemalatha S. *Camellia sinensis* mediated synthesis and characterization of nanoparticles and applications to control Gram-negative ESBL producing antibiotic resistant bacterial pathogens. *Food Bioscience*. 2020. Volume 50, Part A, 102070. doi:10.1016/j.fbio.2022.102070.
17. Janarthanan S, Ranjani S, Hemalatha S. Myconanoparticles Break Antibiotic Resistance in Staphylococcus aureus and Acinetobacter baumannii. *Appl Biochem Biotechnol*. 2022;10.1007/s12010-022-04125-x. doi:10.1007/s12010-022-04125-x
18. Mendie EL, Hemalatha S. Molecular Docking of Phytochemicals Targeting GFRs as Therapeutic Sites for Cancer: an In Silico Study. *Applied Biochemistry and Biotechnology*. 2022. 194. 1-17. doi: 10.1007/s12010-021-03791-7.
19. Charlz Nithin J, Ranjani S, Hemalatha S. Mimusops elengi Flower-Mediated Green Silver Nanoparticles Control Staphylococcus aureus and Acinetobacter baumannii. *Appl Biochem Biotechnol*. 2022;194(7):3066-3081. doi:10.1007/s12010-022-03882-z.
20. Architha V, Ranjani S, Hemalatha S. Engineered green nanoparticles interact with *Nigrospora oryzae* isolated from infected leaves of *Arachis hypogaea*, *Journal of Basic Microbiology*, 2022. doi:10.1002/jobm.202100623.
21. Jenish A, Ranjani S, Hemalatha S. *Moringa oleifera* Nanoparticles Demonstrate Antifungal Activity against Plant Pathogenic Fungi. *Applied biochemistry and biotechnology*. 2022. 194(10), 4959–4970. doi:10.1007/s12010-022-04007-2.
22. Sai Nivetha S, Ranjani S, Hemalatha S. Synthesis and application of silver nanoparticles using Cissus quadrangularis. 2022. *Inorg. Nano-Met. Chem* 52:1, 82-89, doi: 10.1080/24701556.2020.1862219.
23. Ranjani S, Hemalatha S. Triphala decorated multipotent green nanoparticles and its applications. *Materials Letters*. 2022. doi: 10.1016/j.matlet.2021.131184.
24. Ranjani S, Faridha Begum I, Santhoshini J, Senthil Kumar N, Ruckmani K, Hemalatha S. Mimosa pudica floral nanoparticles: a potent antibiotic resistance breaker. 2021. *Inorg. Nano-Met. Chem*. 51:12, 1751-1758. doi: 10.1080/24701556.2020.1852429.
25. Ranjani S, Mohamed Sheik Meeran S, Prakash SP, Mohammad W, Kandasamy R, Hemalatha S. Multi potent aromatic nano colloid: synthesis, characterization and applications. 2020. *AMB Express*, 10(1), 168. doi:10.1186/s13568-020-01104-5.
26. Janarthanan S, Ranjani S, Hemalatha S. Myconanoparticles Break Antibiotic Resistance in Staphylococcus aureus and Acinetobacter baumannii. 2022. *Appl Biochem Biotechnol*. doi: 10.1007/s12010-022-04125-x.
27. Ranjani S, Shariq Ahmed M, MubarakAli D, Ramachandran C, Senthil Kumar N, Hemalatha S. Toxicity assessment of silver nanoparticles synthesized using endophytic fungi against nosacomial infection. 2021. *Inorg. Nano-Met. Chem*, 51:8, 1080-1085. doi: 10.1080/24701556.2020.1814332.
28. Ranjani S, Noorul Samsoon Maharifa H, Raihanathus Sahdhiyya A, Hemalatha S. Phytotoxicity assessment of synthesized green nanosuspension on germination and growth in Vigna radiate. 2021. *Inorganic and Nano-Metal Chemistry*. doi: 10.1080/24701556.2021.1993916
29. Ranjani S, Hemalatha S. Triphala decorated multipotent green nanoparticles and its applications, *Materials Letters*. 2022. doi: 10.1016/j.matlet.2021.131184.
30. Ranjani S, Shariq Ahmed M, Ruckmani K, Hemalatha S, Green Nanocolloids Control Multi Drug Resistant Pathogenic Bacteria. *Journal of Cluster Science*. 2019, doi:10.1007/s10876-019-01694-6.
31. Pehlivan, Fadime Eryılmaz. 'Vitamin C: An Antioxidant Agent'. Vitamin C, InTech, 2. 2017. *Crossref*, doi: 10.5772/intechopen.69660.
32. Jaafar NF, Ramli ME, Mohd Salleh R. Optimum Extraction Condition of Clitorea ternatea Flower on Antioxidant Activities, Total Phenolic, Total Flavonoid and Total Anthocyanin Contents. *Trop Life Sci Res*. 2020;31(2):1-17. doi: 10.21315/tlsr2020.31.2.1
33. Duangruedee K, Rasimate M, Parawee R, Green synthesis of silver nanoparticles using Clitoria ternatea flower: an efficient catalyst for removal of methyl orange. 2020. *International Journal of Environmental*

*Analytical Chemistry*. doi: 10.1080/03067319.2020.1793974.
34. Lamson DW, Brignall MS. Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. Altern Med Rev. 1999 Oct;4(5):304-29.
35. Gurunathan S, Kang MH, Kim JH. Combination Effect of Silver Nanoparticles and Histone Deacetylases Inhibitor in Human Alveolar Basal Epithelial Cells. *Molecules*. 2018;23(8):2046. doi: 10.3390/molecules23082046.
36. Alahmdi M, Khasim S, Sekar V, Panneerselvam C. Green Nanoarchitectonics of ZnO Nanoparticles from Clitoria ternatea Flower Extract for In Vitro Anticancer and Antibacterial Activity: Inhibits MCF-7 Cell Proliferation via Intrinsic Apoptotic Pathway, *Journal of Inorganic and Organometallic Polymers and Materials*. doi: 10.1007/s10904-022-02263-7.
37. Ismail A, Ahmed MM, Salem A. Biosynthesis of Silver Nanoparticles Using Mushroom Extracts: Induction of Apoptosis in HepG2 and MCF-7 Cells via Caspases Stimulation and Regulation of BAX and Bcl-2 Gene Expressions, *Pharm Biomed Sci*. 5(1)1-9, 2015.
38. Arpudhamary V, Priya S, Manzoor MAP, Mubarakali D, Hemalatha S. Apoptotic-inducing factor 1 (AIF1) plays a critical role in cembranoid mediated apoptosis to control cancer: Molecular docking and dynamics study. 2019. *Biocatalysis and Agricultural Biotechnology*. doi: 10.1016/j.bcab.2019.101343.

**How to Cite This Article**

**Madhumitha H, Ranjani S, Karunya JR, Hemalatha S. Clitoria Ternatea Floral Mediated Synthesis, Characterization, Antioxidant, and Cytotoxicity Evaluation of Silver Nanoparticles. Arch Breast Cancer. 2023; 10(3):222-31.**
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/655