

Comparative cytotoxic analysis of plant synthesized metallic nanoparticles and their extracts against breast cancer cells

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Abstract

Background: Breast cancer remains one of the leading causes of mortality among women worldwide. Several therapeutic strategies including surgery, hormonal therapy, radiotherapy and chemotherapy have been used to fight against cancer. However, these therapies have several drawbacks such as non-specificity, drug resistance, less efficiency and high doses. These disadvantages lead researchers to find therapies which offer more specificity and sensitivity. Among these, plant-based metallic nanoparticles have emerged as a promising approach due to their improved bioactivity and reduced side effects. The present study aimed to evaluate and compare the anticancer potential of aqueous extracts of selected medicinal plants and their corresponding silver (Ag) NPs against the human breast cancer cells.

Methods: Aqueous extracts of the plants were prepared, and their AgNPs were synthesized. Cytotoxic effects were assessed using the MTT assay over a concentration range of 0.5–150 µg/ml for both extracts and NPs. Microscopic examination revealed pronounced morphological changes in NP-treated cells compared to those treated with plant extracts only and untreated controls.

Results: The MTT assay demonstrated significantly higher cytotoxicity of NPs formulations relative to their respective extracts. The LC₅₀ values of fenugreek-AgNPs, *N. sativa*-AgNPs, *C. colocynthis*-AgNPs, *A. indica*-AgNPs, *Z. officinale*-AgNPs, and *A. sativum*-AgNPs were 3.6, 11.2, 4.97, 18, 24.4, and 31.43 µg/ml, respectively. Fenugreek-AgNPs showed the strongest anticancer activity at the lowest concentration.

Conclusion: These findings suggest that plant-based NPs exhibit significant anticancer efficacy at lower doses compared to crude plant extracts and may serve as promising candidates for breast cancer therapy.

Key Words: Cytotoxicity, Plant, Synthesis, Metallic nanoparticles

INTRODUCTION

In Europe, cancer is the main cause of death and despondence. Breast cancer is the most frequently diagnosed cancer in woman, with an incidence rate of 1 out of every 8 women. In breast cancer, a group of cells starts uncontrolled proliferation in breast and can also invade in proximate tissues or metastasize to distant areas of body [1]. Inherited genetic changes including mutations in BRCA1 and BRCA2 genes and family history of cancer account for 5–10% of the breast cancer cases. [2].

Breast cancer prevalence is linked to many factors like menstruation (early age at menarche and late age at menopause), nutrition and food (alcohol intake), reproductive factor (late age at the first child birth, fewer children and nulliparity), anthropometry (high weight, weight gain while adulthood, and distribution of body fats) and exogenous intake of hormones (hormone replacement therapy and use of oral contraceptives). Targeted therapies are the most applied therapies for cancer treatments as these therapies have advantages including less dose requirements, increased efficiency, decreased side effects, and avoiding non-specific targets [3]. Metallic Nano particles (NPs) are widely used in drug delivery approaches where they induce changes in the pharmacokinetic properties of drug to enhance their efficiency and lessen the side effects [4]. Gold and silver NPs have adjustable optical properties which can be personalized to specific wavelengths as per size, shape and composition. For instance, to

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evaluate their in vivo activity, soybean agglutinin has been evaluated in conjugation with silver NPs for breast cancer cell lines (MCF-7 and MDA-MB-237) and in non-cancerous cell line (MCF 10A). Their results showed that soybean agglutinin-AgNPs could kill cancer cells more effectively [5]. A study indicated anti-proliferative and proapoptotic effects of capecitabine-AgNPs against breast cancer cells (MCF-7). The crew synthesized NPs of different sizes and then performed MTT assay and Annexin V for anti-proliferating and proapoptotic effects. The results indicated that NPs of 10nm size were least toxic to MCF-7 cells. In 2019, Jessica Swanner and coworkers reported that AgNPs showed promising effects in the treatment of triple-negative breasts. Their study also evaluated that systemically directed silver NPs have reduced the growing triple negative breast cancer tumors in mice [6].

Silver nanoparticles derived from green plants have proved to be remarkable approach in cancer research [7]. Thymoquinone (TQ), the main constituent of *Nigella sativa* enhances the antitumor activity of drugs and decreases their side effects. TQ also controls the signaling pathways that are involved in cancer progression [8]. In a recent study, researchers indicated that fenugreek extracts showed cytotoxicity at concentrations 100µg/ml, 200µg/ml and 300µg/ml against breast cancer cell line (MCF-7), T-lymphoma, Thyroid papillary cancer and B-cell lymphomas. Citrullus colocynthis belongs to Cucurbitaceae family. Glycosides, alkaloids and flavonoids are the known compounds present in this fruit [9]. Due to the broad range of biological activities, *C. colocynthis* as anti-neoplastic, anti-allergic, anti-diabetic and anti-rheumatic agent [10].

Azadirachta indica (Neem) has high levels of antioxidants [11,12]. Neem has various constituents like nimbidin, nimbin, nimbolide, and limonoids. These constituents have positive roles in disease management by modulations in their genetic pathways [13]. *Zingiber officinale*, Ginger and its compounds have flavonoid, paradol, sesquiterpenes and phenolic compounds. It also possesses several activities including anti-apoptotic, anti-clotting, anti-diabetic, analgesic properties and anti-tumor activities. Ginger also possesses pharmacological activities due to its active phytochemicals such as 6-shogaol, 6-gingerol and 6-zingerone. Shogaol and gingerol are important due to anti-inflammatory and anti-oxidant properties [14]. Amagase reported that *Allium sativum*, garlic is involved to prevent a number of cancers including lung cancer, breast cancer, stomach cancer, colon cancer, bladder cancer and prostate cancer. Garlic, also known as medicinal plant, not only boosts immune system but also plays an important role in prevention of fungal and bacterial infections [15].

The aim of this study was to determine the cytotoxic effect of six different plant synthesized metallic nanoparticles including *Trigonella foenum-gracecum* (fenugreek)-AgNPs, *N. sativa*-AgNPs, *C. colocynthis*-AgNPs, *A. indica*-AgNPs, *Z. officinale*-AuNPs and *A. sativum*-AuNPs against breast cancer cells and comparing their cytotoxicity with aqueous plant extracts at the same dosage to determine the more efficient group. The investigation also focuses to determine the most efficient plant-synthesized metallic nanoparticle.

METHODS

Cell Culture

All practical work was performed under aseptic conditions in biosafety cabinet level 2. DMEM media (Gibco) was used for cell culturing experiments. The complete media was prepared by adding 10% FBS and 1% of 100U/ml penicillin/streptomycin (Gibco). The revival media differed only in having 15% FBS. The breast cancer cells (MCF-7) were grown in humidified incubator at 37°C, supplied with 5% CO₂.

Plant Extracts and Nanoparticles Preparation

Aqueous plant extracts of *N. sativa*, *Trigonella foenum-gracecum*, *C. colocynthis*, *A. indica*, *Z. officinale* and *A. sativum* were prepared and were referred to Centre for High Energy Physics, University of the Punjab for the synthesis and characterization of their metallic nanoparticles. Nanoparticles were sterilized using syringe filter, while the concentrations are given in Table 1.

Cytotoxicity Assay

Percentage viability of MCF-7 cells treated with nanoparticles was assessed by performing MTT assay. A total of 10,000 MCF-7 cells were plated in 200µl of media in each well of 96-well plate and incubated the plate at 37 °C with 5% CO₂ overnight. After 24 hours of incubation, old media was removed and the cells were exposed to nanoparticles formulation. The dose ranges selected for *T. foenum-gracecum*-AgNPs, *N. sativa*-AgNPs, *C. colocynthis*-AgNPs, *A. indica*-AgNPs, *Z. officinale*-AuNPs and *A. sativum*-AuNPs were 0.5µg/ml to 18µg/ml, 0.5µg/ml to 18µg/ml, 0.5µg/ml to 20µg/ml, 0.5µg/ml to 40µg/ml, 5µg/ml to 150µg/ml and 5µg/ml to 150µg/ml respectively. After the applied treatment and waiting period of 72 hours, old media containing nanoparticles was removed and 100µl of the MTT dilution was added in each well. Afterwards, purple-colored formazan crystals were dissolved in 150µl DMSO for 1 hour. The absorbance was taken in ELISA reader plate at 570nm and LC50 was calculated by GraphPad Prism v.10.

Table 1: Nanoparticles concentration.

Nanoparticles	Concentration
<i>N. sativa</i> (black seed)-AgNPs	98 µg/ml
<i>T. foenum-graecum</i> -AgNPs	102 µg/ml
<i>C. colocynthis</i> -AgNPs	513.61 µg/ml
<i>A. indica</i> -AgNPs	105.21 µg/ml
<i>Z. officinale</i> -AuNPs	234.47 µg/ml
<i>A. sativum</i> -AuNPs	562.74 µg/ml

RESULTS

Effect of *N. sativa* on MCF-7 cell line

The cells treated with *N. sativa* extracts and their nanoparticles were regularly observed under Olympus Fluorescent microscope to see the behavior of cells. Nanoparticles treated cells lost the original morphology from 8µg/ml to onward while the pure plant extract treated cells still showed the original morphology even at high doses (Figure 1). The cytotoxicity analysis revealed that the cells treated with nanoparticles showed clear cell death compared to the cells treated with the extracts only. The cells in case of plant extract treatment were alive even after 72-hour treatment incubation. LC50 value was calculated using GraphPad prism. The LC50 for *N. sativa* nanoparticles was calculated to be 11.224µg/ml while the LC50 value for *N. sativa* extract was not achieved till the maximum dose.

Effect of Fenugreek on MCF-7 cell line

The cells treated with fenugreek extract and nanoparticles were regularly observed to see the morphological changes in cells under treatment. The cells treated with nanoparticles lost their morphology at some of the higher doses after 48 hours, but the cells treated with plant extracts showed intact morphology. The next day, nanoparticles treated cells lost the original morphology even at lowest doses while the plant extract treated cells were still showing the original morphology. The cytotoxicity analysis revealed that the cells treated with nanoparticles showed clear cell death compared to the cells treated with nanoparticles. The cells in case of plant extract treatment were alive even after 72-hour treatment incubation. MTT assay was performed and LC50 value was calculated using GraphPad prism and it was found to be 3.614µg/ml while the LC50 value in case of fenugreek extract was not achieved till the maximum dose (Figure 2).

Effect of *C. colocynthis* on MCF-7 cell line

The cells treated with nanoparticles lost their morphology at some of the higher doses after 48 hours, but the cells treated with plant extracts showed intact morphology. The next day, nanoparticles treated cells lost the original morphology even at lowest doses, but the cells treated with extracts still showed the original morphology at the highest dose. MTT assay was performed and LC50 value was calculated using GraphPad prism. The LC50 for *C. colocynthis* nanoparticles was calculated to be 4.977µg/ml (Figure 3).

Effect of *A. indica* on MCF-7 cell line

The cells treated with nanoparticles lost the original morphology even at lowest doses, but the plant extract treated cells were still showed the original morphology at the highest dose. MTT assay was performed after 72-hour incubation. The LC50 for *A. indica* nanoparticles was calculated to be 18µg/ml as shown in Figure 4.

Effect of *Z. officinale* on MCF-7 cell line

The nanoparticles treated cells lost the original morphology even at lowest doses, but the plant extract treated cells still showed the original morphology at the highest dose (Figure 5). MTT assay was performed and LC50 value was calculated using GraphPad prism. The LC50 for *Z. officinale* nanoparticles was calculated to be 24.40µg/ml.

Effect of *A. sativum* on MCF-7 cell line

The cells treated with nanoparticles lost their morphology at some of the higher doses after 48 hours, but the cells treated with plant extracts showed intact morphology. The next day, nanoparticles treated cells lost the original morphology even at lowest doses, but the plant extract treated cells were still showing the original morphology at the highest dose. MTT assay was performed after 72 hours of treatment and LC50 value was calculated using GraphPad prism. LC50 for *A. sativum* nanoparticles was calculated to be 31.43µg/ml. The LC50 in case of *A. sativum* plant extract was not achieved (Figure 6). Overall, the cell viability in case of plant-based nanoparticles decreased as the dose of nanoparticles increased. Neither of the plant extracts gave any clear cell death at the selected dose ranges. The comparison of their cytotoxicity showed that the nanoparticles derived from plants are far more efficient in killing cancer cells than their respective plant extracts.

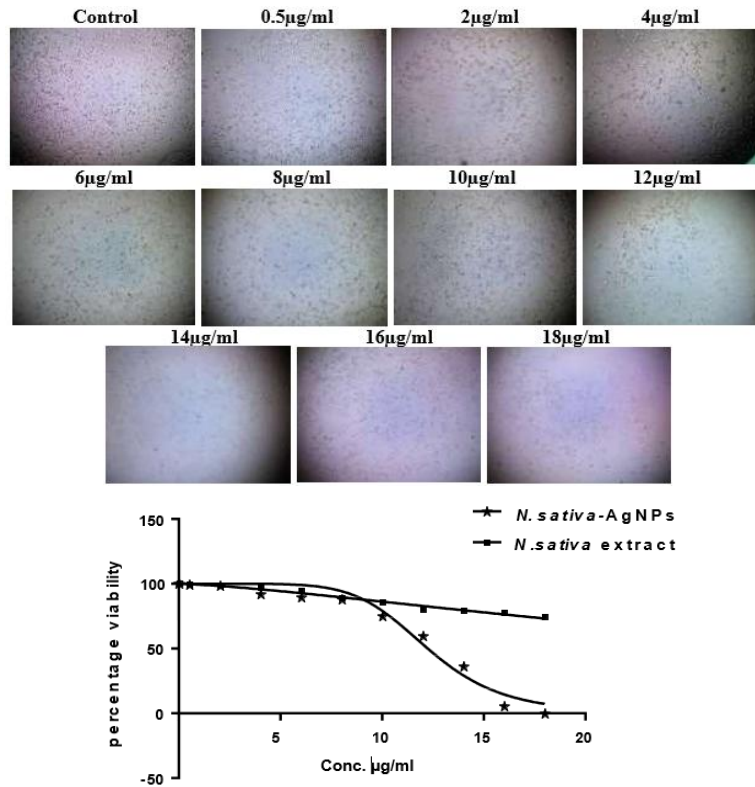


Figure 1: Treatment with different concentrations of *N. sativa*-AgNPs. MCF-7 cell line; 72hour post treatment with different concentrations of *N. sativa*-AgNPs (0.5µg/ml-18µg/ml) (Magnification=4X). Graphical comparative analysis of cytotoxicity by *N. sativa*-AgNP and its extract.

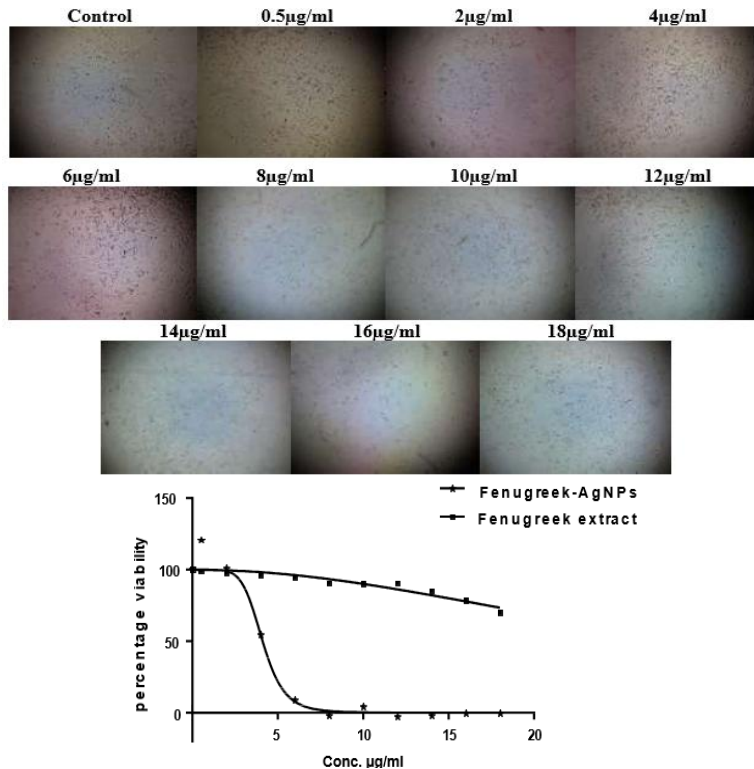


Figure 2: Treatment with different concentrations of Fenugreek-AgNPs. MCF-7 cell line 72-hour post treatment with different concentrations of Fenugreek-AgNPs (0.5µg/ml-18µg/ml) (Magnification=4X). Graphical comparative analysis of cytotoxicity by Fenugreek-AgNP and its extract.

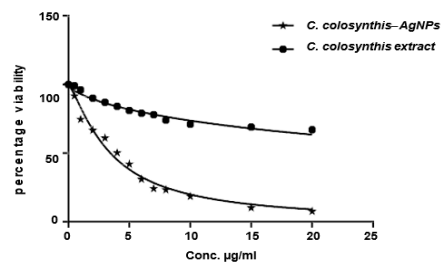
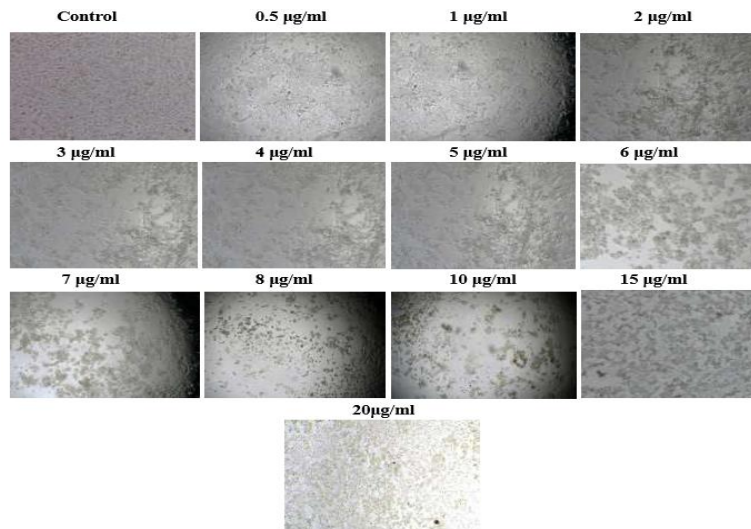


Figure 3: Treatment with different concentrations of *C. colocynthis*-AgNP. MCF-7 cell line 72 hour post treatment with different concentrations of *C. colocynthis*-AgNP (0.5µg/ml-20µg/ml) (Magnification=4X, Resolution=200 um). Graphical comparative analysis of cytotoxicity by *C. colocynthis*-AgNP and its extract.

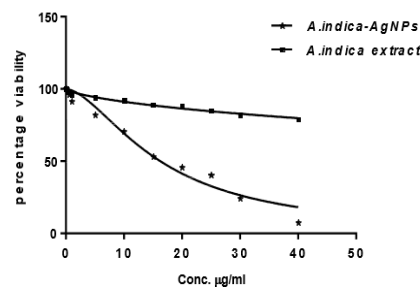
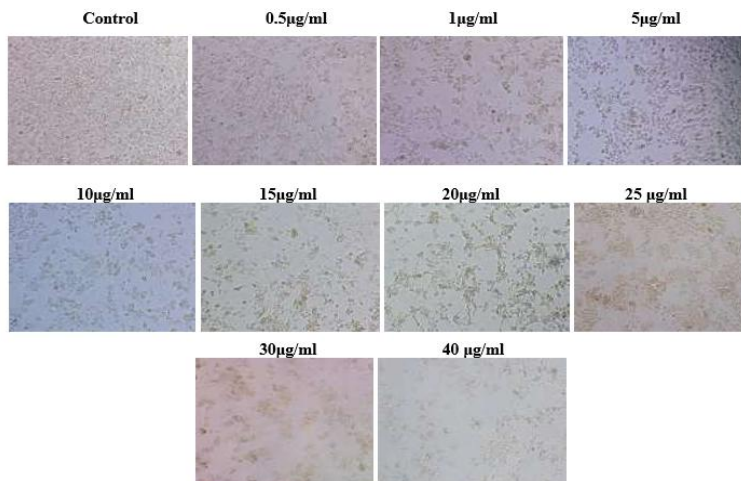


Figure 4: Treatment with different concentrations of *A. indica*-AgNP. MCF-7 cell line 72hour post treatment with different concentrations of *A. indica*-AgNP (0.5µg/ml-40µg/ml) (Magnification=4X, Resolution=200 um). Graphical comparative analysis of cytotoxicity by *A. indica*-AgNP and its extract.

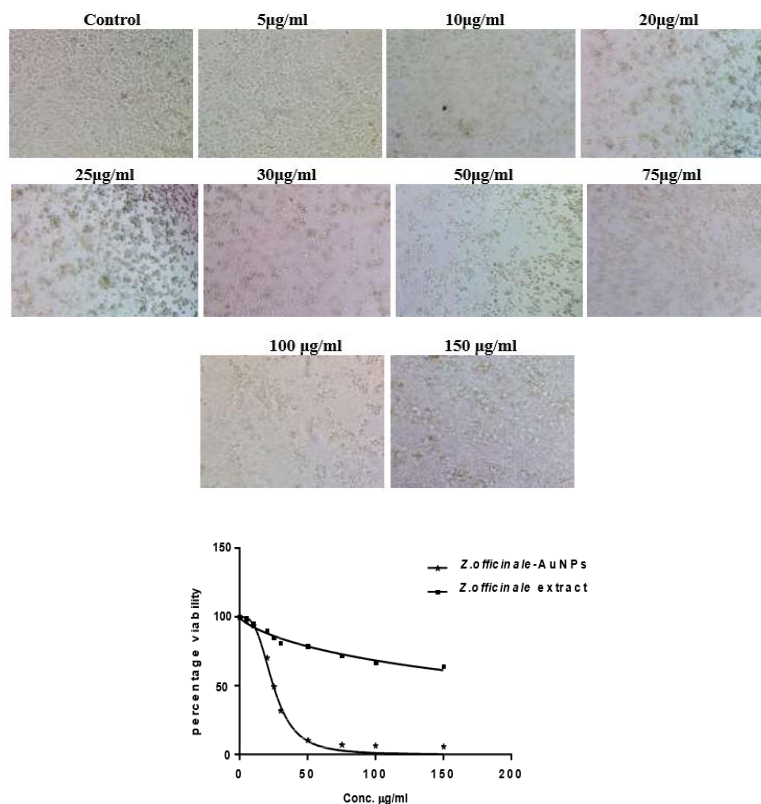


Figure 5: Treatment with different concentrations of *Z. officinale*-AuNPs. MCF-7 cell line 72-hour post treatment with different concentrations of *Z. officinale*-AuNPs (5µg/ml-150µg/ml) (Magnification=4X, Resolution=200 µm). Graphical comparative analysis of cytotoxicity by *Z. officinale*-AuNP and its extract.

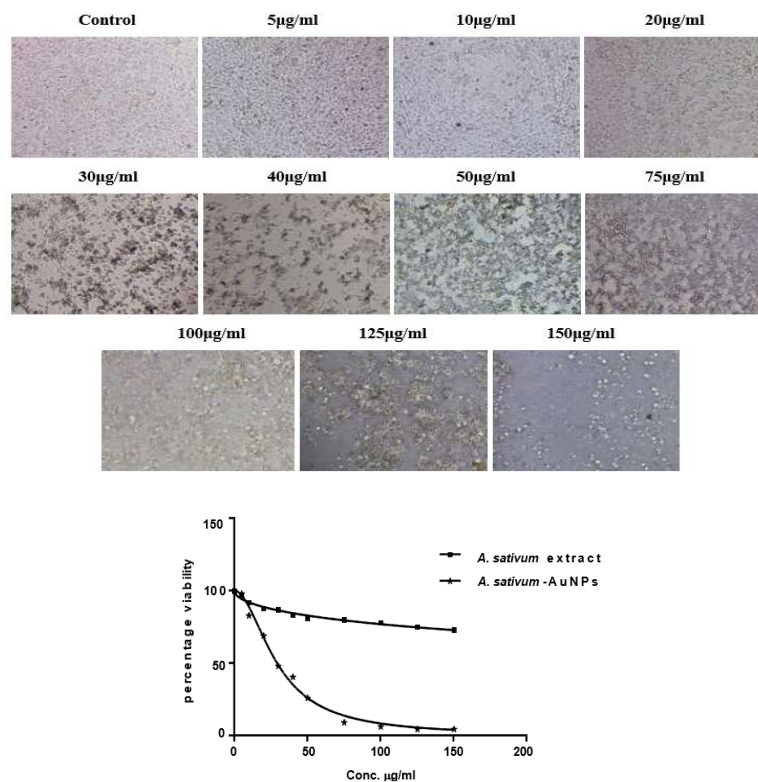


Figure 6: Treatment with different concentrations of *A. sativum*-AuNPs. MCF-7 cell line 72-hour post treatment with different concentrations of *A. sativum*-AuNP (5µg/ml-150µg/ml) (Magnification=4X, Resolution=200 µm). Graphical comparative analysis of cytotoxicity by *A. sativum*-AuNP and its extract.

Comparison of cytotoxicity of Plant synthesized metallic nanoparticles

All samples were observed for 72 hours and then MTT assay was performed. All the samples were found cytotoxic against MCF-7 cell line showing their potential to be used in therapeutics in future. In case of Fenugreek-AgNPs, cells were under stress after only 24 hours of incubation. The results of MTT assay showed greatest cytotoxic potential of

Fenugreek-AgNPs as indicated by LC50 value among all provided samples upon MCF-7 cells. The most effective nanoparticles for cytotoxic purpose were Fenugreek-AgNPs and *C. colocynthis* which gave LC50 at 3.614µg/ml and 4.977µg/ml respectively. The cells treated with LC50 for different plant nanoparticles are shown in Figure 7.

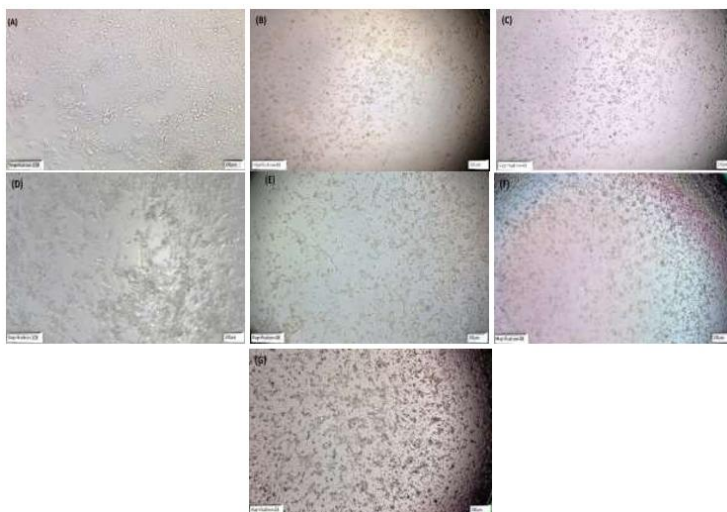


Figure 7: Comparison of LC50 images of all nanoparticles. Morphology of MCF-7 A) Control showing normal morphology of MCF-7 cells, B) LC50 dose of *N. sativa*-AgNPs, C) LC50 dose of Fenugreek-AgNPs, D) LC50 dose of *C. colocynthis*-AgNPs, (E)LC50 dose of *A. indic*-AgNPs. (F) LC50 dose of *Z. officinale*- AuNPs, E) LC50 dose of *A. sativum*-AuNPs.

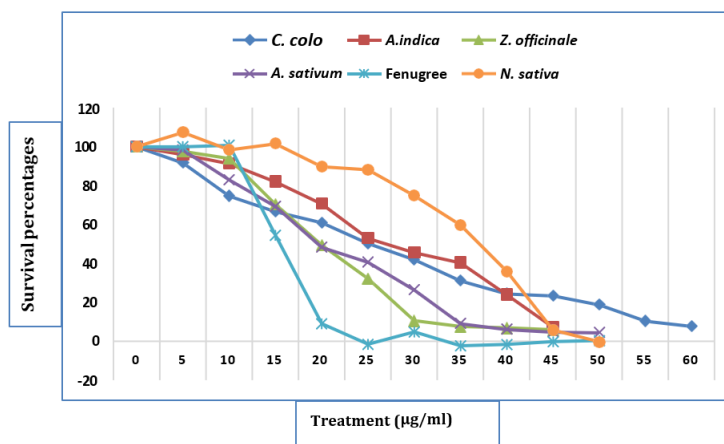


Figure 8: Graphical Comparison of cytotoxicity of all plant synthesized metallic nanoparticles.

Table 2: LC50 values of different Nanoparticles as calculated by GraphPad Prism.

Nanoparticles	LC50 value
<i>T. rigonella foenum-graecum</i> -AgNPs	3.614 µg/ml
<i>C. colocynthis</i> -AgNPs	4.977 µg/ml
<i>N. sativa</i> (black seed)-AgNPs	11.224 /ml
<i>A. indica</i> (Neem)-AgNPs	18 µg/ml
<i>Z. officinale</i> (Ginger)-AuNPs	24.40 µg/ml
<i>A. sativum</i> (Garlic)-AuNPs	31.43 µg/ml

DISCUSSION

Breast cancer is becoming the leading cancer type in women, and it emerges as the second among most leading death causing cancer in women [16]. There have been several treatments used to cure this lethal cancer, but no permanent cure has been found yet, same as in the case of many other types of cancer [17]. It requires more reliable treatments than traditional ones which may offer severe side effects. There is a need to develop such therapies that offer advantages like less doses, more efficiency, decreased side effects, and avoiding non-specific goals [18].

Nanoparticles are widely used in drug delivery approaches as NPs protect the drug, increase the efficiency of drug and target the exact location via systemic release [19]. Nanocarriers like liposomes, polymeric NPs, dendrimers, nanomicelles, nanogels, exomes and many other are used in targeted delivery of drugs [20]. Nanoparticles derived from green plants have been proved a remarkable approach in cancer research. The plants including *Artemisia vulgaris*, *Ocimum gratissimum*, *Nigella sativa*, *J. glauca*, *Corchorus capsularis*, *Morus alba*, *Ficus benghalensis*, *Coffea arabica*, *Andrographis echioides*, *Solanum tuberosum*, *Chlorella vulgaris* and *Alcea rosea* have been used to prepare nanoparticles for their biological values and many of them have been applied for cancer research [21].

The present study aimed at determining the cytotoxic effect of different plant extracts and plant-based metallic nanoparticles and assessing the most efficient plant synthesized nanoparticles. The cytotoxicity of samples was analyzed by performing MTT assay. MCF-7 breast cancer cells were selected for their convenient doubling time, and the behavior of the cells is easy to monitor. MCF-7 cells were taken from School of Biological Sciences cell culture bank, University of the Punjab. The growing conditions for MCF-7 cells were kept standard as for any cell culture lab. The cells were deforested and placed in humidified incubator at 37°C with 5% CO₂. The cells were healthy and their shape and structure were intact as seen by the light microscope.

The aqueous extracts of medicinal plants were prepared, and their metallic nanoparticles were designed by Center of Solid-State Physics. The cells were subjected to plant extracts and nanoparticles treatment. The dose range for treatment was random for the initial attempts of treatment. After repeated experimentations with higher dose range to narrow down the range, we get defined dose range of plant extracts and their nanoparticles. The cells were treated with plant extracts and nanoparticles at the same dose range and compared the behavior of cells on a single platform. The cells in each well were regularly observed under

fluorescent microscope (Olympus) at different magnifications.

The findings clearly displayed that nanoparticles synthesized from medicinal plants demonstrated significantly higher cytotoxicity as compared to crude plant extracts. Morphological changes, reduced cell viability, and lower LC₅₀ together confirmed the better anticancer effectiveness of the nanoparticle formulations.

Among tested samples, fenugreek nanoparticles have shown the robust cytotoxic effect with the lowest LC₅₀ value (3.614 µg/ml), followed by *C. colocynthis* nanoparticles (4.977 µg/ml) and *N. sativa* nanoparticles (11.224 µg/ml). In contrast, the crude extracts did not achieve LC₅₀ within tested concentration scale, indicating relatively weak anticancer activity. These findings indicate that nanoparticle synthesis markedly improves the bioactivity present in medicinal plants.

Microscopic interpretation further favored the cytotoxicity results. Cells treated with nanoparticles increasingly lost their normal morphology, detached from the surface and presented characteristics associated with the apoptosis and cell damage. Interestingly, these changes were observed even at lower nanoparticle concentrations and became more obvious after prolonged incubation. On other hand, cells treated with crude extracts largely retained their normal morphology even after 72 hours of exposure. This indicated limited cytotoxic activity under the tested conditions. Improved anticancer activity of plant derived nanoparticles can be assigned to several factors, including improved solubility, increased surface area, augmented penetration into cancer cells, and more effective delivery of bioactive compounds. Lower LC₅₀ values monitored for nanoparticles compared to crude extracts clearly support this hypothesis.

The deviation in cytotoxic ability among different plant nanoparticles may be correlated to differences in their phytochemical makeup. Although *A. zadirachta indica*, *Z. officinale* and *A. sativum* nanoparticles also displayed cytotoxic effects against MCF-7 cells, their LC₅₀ values were reasonably higher, suggesting relatively lower potency. Nevertheless, all nanoparticle formulations showed noticeably better anticancer effects than their corresponding crude extracts, pointing out the importance of nano formulation in improving therapeutic efficacy.

Overall, the study demonstrated that plant-mediated nanoparticles have promising anticancer ability against breast cancer cells and may assist as effective alternatives to conventional therapies. However, additional studies are needed to investigate the exact molecular mechanisms triggering nanoparticle-induced cytotoxicity.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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