Eco-friendly Green Synthesis of Silver Nanoparticles (AgNPs) via Aqueous Extraction from Annona Squamosa Leaves and Its Genetic Effects on the MCF-7 Cell Line

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ABSTRACT:

Nanotechnology treatments are among the numerous unconventional cancer medicines that have emerged in recent years. Even with its positive results, additional research and study are required. This work included the green synthesis of silver nanoparticles (AgNPs) utilizing an aqueous extract of Annona Squamosa L. The aqueous extract was analyzed qualitatively and quantitatively for phytochemical content. The morphological features, optical characteristics, and surface properties of synthesized AgNPs were examined using transmission electron microscopy (TEM), ultraviolet-visible (UV-Vis) absorption spectroscopy, transform infrared spectroscopy (FTIR) and Dynamic Light Scattering (DLS). The analysis revealed phenols (8.70 mg GAE/g), and flavonoids (7.50 mg/g). The plant extract demonstrated antioxidant activity, attributed to its high flavonoid and phenolic content, suggesting its potential source demonstrated that the bio-synthesized AgNPs exhibited a remarkable level of homogeneity concerning their surface morphology. The mean diameter of the particles was around ~16.7 nm to ~37.0 nm. A. squamosa extract and AgNPs showed low toxicity and effective treatment trial for cancer with a significant decrease in cell viability with a percentage (58.2%) and (79.57%) respectively. In conclusion, the present work introduces a straightforward and economically viable approach for synthesizing AgNPs using environmentally friendly processes, intending to use them as a promising treatment for breast cancer of MCF-7 cell line

Keywords: Green synthesis; Annona squamosa leaves; Silver Nanoparticles; Cytotoxicity; Gene expression.

INTRODUCTION:

Cancer is a complex disease marked by uncontrolled cell growth and spread, leading to tumor formation and tissue invasion. It remains a major global health threat, with rising incidence and mortality rates worldwide, emphasizing the need for effective prevention, early detection, and treatment strategies (Deepti, *et al*., 2024).

Breast cancer, develops in the mammary gland, and ranks as the most prevalent cancer among women. Furthermore, breast cancer represents approximately 15.2% of all cancer cases In the United States, approximately 281,550 new cases of invasive breast cancer are projected to be diagnosed in 2021 (Rui, *et al.*, 2024). while in Egypt, 56.8% of BC patients present at stage 3, and 62% undergo mastectomy Sallam, et al., (2024).. In the resent years, Modern treatment methods for cancer have witnessed significant advancements, with a notable focus on nanotechnology-based approaches like silver nanoparticles (AgNPs). AgNPs exhibits potent antimicrobial and anticancer properties, making it a promising alternative to conventional therapies like chemotherapy, which often lead to severe side effects, including toxicity to healthy tissues and drug resistance (Maher, *et al*., 2023). Recent advancements in breast cancer research highlight green AgNPs, synthesized through eco-friendly methods, as a promising therapeutic agent due to its unique properties and biocompatibility Ullah, *et al*., (2020). AgNPs offer advantages such as targeted deliverance, biocompatibility, and minimal off-target effects Jose, *et al*., (2021). A sustainable and eco-friendly approach to synthesizing AgNPs involves green plant extraction, where plant biomolecules act as reducing agents, producing stable nanoparticles without hazardous chemicals Shaik, *et al.*, (2018). The phytochemicals in Annona Squamosa (A. squamosa) leaves, such as alkaloids, flavonoids, and tannins, act as natural agents with reducing and stabilizing properties, enabling the production of stable AgNPs without harmful chemicals Malik, *et al*., (2022). The biosynthesized AgNPs exhibit potent anticancer properties, specifically against breast cancer cells, via inducing apoptosis, inhibiting cell proliferation, and disrupting

tumor angiogenesis Ullah, *et al*., (2024). A. squamosa leaves are rich in phenols and flavonoids, which help stabilize AgNPs and increase their biological activity, including their antibacterial and anticancer qualities Shehata, *et al*., (2021) & Mokhtar, *et al*., (2022). Nevertheless, the antioxidant capacity of these extracts not only promotes the creation of nanoparticles but also enhances their therapeutic effectiveness by scavenging free radicals and reducing oxidative strain in biological systems Ruddaraju, *et al*., (2019).

In this regards, the present study introduces the novel green synthesis of AgNPs using an aqueous extract from A. Squamosa leaves. This is a ground-breaking technique that offers a low-toxicity, safe, selective, and effective therapeutic alternative for biological interventions and cancer treatment.

MATERIALS AND METHODS

Materials:

All chemicals ($AgNO_3$, dimethyl sulfoxide (DMSO), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), $ALCL_3$, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) ... were prucged from Sigma–Aldrich, USA, Ltd.,) and all the solvents were purchase from El-Gomhouria Company for Chemicals, which located in Cairo, Egypt and whole chemicals were of high quality and purification.

Methods:

Collection of Plant Leaves and Extraction Prosses

The leaves of the *A. squamosa* Plant were collected from Al-Ghataty Farms located in Sadat City, Menoufia Governorate, Egypt [Coordinates: 30.3811°N 30.5266°E]. The fresh leaves were cleaned by rinsing first with running tap water, followed by distilled water. Then air-dried in a dark environment for 72 hours and subsequently ground to powder using an electric blender. A portion of the powdered leaves was mixed with 1000 ml of distilled water, stirred continuously at 400 rmb , and heated to a temperature of 60–70°C for two hours. The crud extract was filtered using Whatman No. 41 filter paper and stored at 4°C for further use, following the described methodology (Vivek, *et al*., 2012*)* with some modifications.

Qualitative techniques for the determination of phytochemicals

phytochemicals compounds such as phenols were detected according to (Silva, et al., 2017). Flavonoids were detected according to (Saxena *et al*., 2013), Saponins were detected according to (Banu and Cathrine 2015), Alkaloids were detected according to (Banu and Cathrine 2015), Tannins were detected according (Silva, et al., 2017).

Determination of Total Flavonoids Compounds

from A. squamosa powder were extracted by 25 ml of petroleum ether, followed by 95% ethanol to 50 ml. Then 5 ml of extract was mixed Then 5 ml of 0.1 M $AlCl_3$, and the absorbance was measured at 445 nm using UV spectroscopy, Quercetin was used as stander soulution (Chun-Hong., 2009).

Determination of Total Phenolic Compounds

For total phenolic quantification, 200 µL of 70% methanolic extract (1 mg/mL) was diluted to 3 mL with water, mixed with 0.5 mL Folin-Ciocalteu detector, and reacted for 3 min. 2 mL of 20% sodium carbonate was added. The mixture was kept in the dark for 60 min, and absorbance was measured at 650 nm. Results were passed as mg gallic acid equivalents per gram of dry weight. Emmanuel, et al., (2018).

Assessment of Antioxidant Activity (DPPH)

The antioxidant activity was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. according to (Russo, et al., 2023).

HPLC analysis

The qualitative analysis compounds of A. squamosa sample was determined by HPLC Himesh, et al., (2011). The thermo eventual 3000 system was complete with pump, automatic sample injector, and Dell-compatible computer with Cromelion 7 software for the interpretation of data. It was fitted with a DAD-3000 diode array detector using a Thermo-hypersil reversed-phase C18 column (2.5 × 30 cm) at 25°C. The mobile phase is composed of 0.05% trifluoroacetic acid/acetonitrile, (, solvent A) and distilled water,(solvent B). UV imbibition spectra were registered for both standards and samples between 230-400 nm. Samples, standard solutions, and the mobile phase were degassed and distilled through a 0.45 µm diaphragm filter before use.

Green Synthesis of AgNPs using Annona leaves extract

This process done according to Vivek et al., 2012.

Characterization AgNPs

UV-Visible Spectroscopy

The UV-Vis absorption spectra have been recorded using TG80 UV-Vis spectrophotometer (, PG instruments Inc., UK), inside a spectral ranging from 200 to 900 nm, with an growing of 5 nm (Akinwumi, et al., 2020).

Fourier Transforms Infrared Spectroscopy (FT-IR)

(FT-IR) spectroscopy was performed to determine the chemical bonding characteristics between atoms. Measurements were conducted using a Vertex 70 spectrometer (Bruker Optics, Germany) within the range of 4000 cm^{-1} to 400 cm^{-1}, with a spectral resolution of 2 cm^{-1} and a scanning speed of 2 mm/s, employing the potassium bromide (KBr) disk method Zhang, & Wang., (2023).

Transmission electron microscope (TEM)

The AgNPs morphology, surface structure, and particle size were analyzed using a TEM. The analysis was performed with a JEOL 2100 LB6 TEM at the National Research Center in Cairo, Egypt, operating at a voltage of 160 kV. The powder sample was dispersed in distilled water through ultrasonication (UP 100 H) to ensure proper dispersion. Subsequently, two drops of the dispersed solution were placed onto carbon-coated copper grids and allowed to dry for 10 minutes before imaging Zhang, & Wang.,(2023).

Dynamic Light Scattering (DLS) Measurements:

DLS size and zeta potential measurements were performed on a nano-zeta sizer (Malvern InstrumentZS-Nano, UK, λ=532 nm) at a temperature of 25 °C. Powdered samples were dispersed in ethanol before measurement. The surface charge measurements were carried out after suspending the investigated powders double distilled water at pH 7.0 (Rahdar, et al., 2019).

Anticancer Activity

The breast cancer MCF-7 cell line samples were divided into three groups and the treatment was as follows: First Group (G1): Control was treated with DMSO. Second Group (G2): The cell line was treated with A. squamosa extract with a concentration of 74 µg/ml. Third Group (G3): The cell line was treated with AgNPs with concentration of 74 µg/ml

After treatment, the cell line was cultured for three days, followed by an MTT assay to determine the viability of the cell line (cytotoxicity) and molecular evaluation.

Cytotoxicity Study

The inspection process is a precise, sensitive, and quantitative colorimetric technique used to assess cell viability. This assay relies on the ability of mitochondrial lactate dehydrogenase (LDH) enzymes in living cells to convert the water-soluble substrate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), at a concentration of 5 mg/ml, into insoluble dark blue formazan crystals. To dissolve the insoluble purple formazan, a solubilizing agent, typically (DMSO), is added, producing a colored solution. The absorbance of this solution was measured using a spectrophotometer, typically at a wavelength ranging from 500 to 600 nm (Kumar, et al., 2018). and (Chen *et al*., 2009).

Molecular Evaluations

To evaluate the impact of A. squamosa extract and AgNPs on MCF-7 cell line, a set of genes (*P53* and *Bax*) and their primer sets were measured. The treated and non-treated carcinomas cell line was collected. Afterwards, RNA extraction and qRT-PCR were carried out to measure the expression levels of the genes of interest.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from both control and treated cells using the RNeasy Mini Kit (Qiagen) following the manufacturer's guidelines. cDNA synthesis was performed in a reaction volume of 20 µL using the Sure Cycler 8800 thermocycler (Agilent Technologies). The reaction conditions included an initial enzyme activation step at 42°C for 1 hour, followed by enzyme inactivation at 95°C for 5 minutes

Real-time RT-qPCR

RT-qPCR was procedure exercise the SYBR Green PCR Master Mix (Fermentas, USA). Each reaction consisted of a 25 µL mixture, which contained 1.5 µL of 10 pmol L^{-1} forward primer, 1.5 µL of 10 pmol L^{-1} reverse primer, 1 µL of template cDNA (50 ng), 12.5 µL of 2×SYBR Green and 3.5 µL of nuclease free water. The used primers sequences were mentioned in Table (1). Each sample was run in triplicate. The response was done via Rotor-Gene 6000 (QIAGEN, ABI arrangement, USA) (Ratika, et al., 2024).

Statistical Analysis

All the experience was proceeded in triplicates, and the results are presented as means and standard deviation. One-way ANOVA was proceeded at ($P<0.05$) (Santangelo, et al., 2018). and gene expression data subjected to statistical analysis through the CoStat software program to detect the significance between the samples. (COStat version 6.400 copyright © 2022-2008 COHORT SOFTWARE (798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA).

RESULTS AND DISCUSSION

Annona is a valuable medicinal plant with potent anticancer properties attributed to its diverse bioactive compounds. These compounds work through various mechanisms, including apoptosis induction, inhibition of cell proliferation, and antioxidant activity, making Annona a promising candidate for cancer treatment. Shehata, *et al* (2021). According to earlier studies, the Annona plant has several applications as an anti-bacterial, anti-fungal, and anti-cancer agent due to the phytochemicals it contains, including antioxidants Gavamukulya, et al., (2017).

The aqueous extracted of Annona plant leaves, it was utilized to identify the active compounds in the extract through qualitative followed by quantitative analysis.

Qualitative Evaluation of Phytochemicals

The photochemical composition of aqueous A. squamosa leaves in aqueous, extracted were displayed in Table (2). Phenols, flavonoids, saponins, alkaloids, and tannins were identified using chemical composition analysis. These results complemented those of other sources, including Nguyen *et al*., (2020) & Kumar, et al., (2021).

They reported that these photochemical are important in human health because they display different biological bounce like anticancer, antifungal, antibacterial activities.

Evaluation of Total Phenolic Content (TPC) and total flavonoids content (TFC)

(TPC) in the aqueous extract of A. squamosa leaves was determined and expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight. The TPC was found to be 8.70 ± 0.68 mg GAE/g dry weight (Table 3). The phenolic compounds contribute significantly to the antioxidant possession of plant extracts due to their ability to donate hydrogen atoms or electrons, thereby deaden free radicals (Maria, 2024 and Bishambar, *et al*., 2024).

Similar studies on Annona species have reported comparable phenolic content values. For instance, an aquatic extract of Annona Muricata leaves demonstrated a TPC of 9.52 ± 0.47 mg GAE/g Carvalho, et al., (2022). which aligns closely with our findings. The divergence in TPC values across studies can be imputed to differences in plant species, extraction methods, and environmental factors affecting secondary metabolite production Kadir, D. (2021).

On the other hand, TFC was quantified as milligrams of quercetin tantamount per gram of dryish weight, with a value of 7.50 ± 0.39 mg QE/g dried weight (Table 3). Flavonoids are known to exhibit strong antioxidants and anti-inflammatory activities because of

Their ability to celebrate metal ions and scavenge reactive oxygen species (ROS).

Crascí, et al., (2018). Similar study were observed in Annona reticulata, where the aqueous leaf extract exhibited a TFC of 7.82 ± 0.55 mg QE/g dry weight Sonali, Bhagat. (2021). The slight variations may arise from differences in dissolvent polarity, extraction time, and geographic location of the plant source.

The phenolic and flavonoids compounds play a synergistic role in determining the overall antioxidant capability of plant extracts. Higher TPC and TFC values generally correlate with enhanced free radical scavenging action Zhang, et al., (2011). This correlation proposition that Annona leaves can serve as a potential source of natural antioxidants for pharmaceutical and nutraceutical applications. The TPC value demonstrates their antioxidant and potential anticancer properties that include apoptosis induction, inhibition of angiogenesis, and disruption of cancer cell signaling pathways Rami, et al., (2023). Comparatively, A. squamosa leaf extracts exhibit similar or slightly higher TPC than other species within the genus. A study by Nguyen, et al., (2020). reported a TPC range of 6.5–10.4 mg GAE/g DW in hydrous extracts of *Annona muricata*, highlighting species-specific differences and the impact of extraction conditions Abhay, et al., (2023).

Our findings reinforce the curative potential of *A. squamosa* leaves, warranting further investigation through bioassays to

validate its efficacy against specific cancer cell lines.

DPPH Radical Scavenging Activity

DPPH is a constant free extremist, which modulates color from violet to yellow upon reduction either by the hydrogen or electron donation process Table (4) and Fig. (1). When the free extremist DPPH encounters an unpaired electron, the most significant absorption occurs at 517 nm, resulting in a purple streak. A scavenger antioxidant for free radicals reacts with DPPH to generate DPPH, which exhibits reduced absorbance compared to DPPH due to decreased hydrogen content. This radical form induces decolorization, manifesting as a yellow tint, as the quantity of accumulated electrons rises. Reveal that the mean antioxidant action of A. squamosa exhibited at 517 nm, indicating the percentage of antioxidant presence 83.78% in distilled water. Based on numerous previous studies, it has been established that the solution of A. squamosa leaves extract contains a significant quantity of DPPH radical scavenging activity (MAHAWAR, et al., 2019; Vikas *et al*., 2017; Awada *et al*., 2023).

The DPPH assay results reveal the strong antioxidant potential of the aqueous A. squamosa extract, with a scavenging percentage of 83.78%. This activity, though somewhat depressing than the standard antioxidant ascorbic acid (91.43%), underscores the efficacy of Annona-derived bioactive Vehicles in neutralizing free radicals. This antioxidant activity observed in Annona species has been previously imputed to the presence of phenolic compounds, flavonoids, and alkaloids, which act as hydrogen donors to stabilize free radicals (Mujumdar *et al*., 2016).

Similar studies have reported comparable antioxidant activities in aqueous Annona extracts. For instance, Chavan *et al*., (2017) demonstrated DPPH scavenging activity of 85.2% in A. squamosa leaf extracts, while Pereira *et al*., (2019) reported 82.3% activity for Annona muricata leaves extracts. The results of this study align closely with these findings, further validating the possibility of aqueous A. squamosa extracts as a source of natural antioxidants.

HPLC analysis

The HPLC analysis was carried out on the aqueous A. squamosa leaves extract. The detailed tabulations of HPLC permission of the extracts are given in (Table 5) and (Fig. 2). From the analysis, five flavonoids have been detected in aqueous A. squamosa leaves extract were rutin with retention Time time (RT) = 2.520, diosmin (RT= 2.923), kampferol (RT= 10.627), quercitin (RT= 15.173), while In addition seven phenols component were detected from aqueous A. squamosa leaves extract are quinic (RT= 2.98), ellagic (RT= 3.190), cinnamic (RT= 3.593), chlorogenic (RT= 4.400), resorcinol (RT= 8.477), pyrochatechol (RT= 9.187) and phenantherine (RT= 16.007). These findings are consistent with those reported by V., Vanitha., et al., (2010), who found phenolic components in the watery extract of A. squamosa resorcinol and pyrochatechol, ellagic are the main phenolic compound followed by ellagic and phenantherine Manjunath, et al., (2020).

The presence of rutin, quercetin, and chlorogenic acid underscores the strong antioxidant potential of the aqueous A. squamosa extract. Rutin and quercetin have been widely studied for their capacity to deaden reactive oxygen species (ROS), thus mitigating oxidative stress is a critical factor in aging and various diseases Soha, et al., (2022). Chlorogenic acid, a vigorous antioxidant, is also associated with metabolic regulation and protection against cardiovascular diseases (Mathew *et al*., 2021). The disclosure of phenanthrene at a higher RT suggests the presence of more complex phenolic structures, which could contribute to bioactivity but require further characterization. In addition, studies by Chavan *et al*., (2017) and Pereira *et al*. ,(2019) declared that there are similar results reported the prevalence of such phenolic component in aqueous extracts of other Annona species, affirming the findings in this survey. These obtained results underscore not only the rich phytochemical profile of A. squamosa leaves, but also the synergistic effects of these compounds and their biological activity in vivo.

Green Synthesis of Nanosilver using Annona Leaves Extract

This process was done according to Vivek et al., 2012 with some modification.

Characterization of AgNPs

UV-Visible Spectroscopy

The UV-Visible spectral analysis of AgNPs was commonly used to assure their formation. The characteristic superficies plasmon rebound (SPR) band for AgNPs appears in the range of 400–450 nm. In the sitting study, the UV-Visible spectrum displayed a strong

absorption peak at 442 nm (Fig.3), indicating the successful synthesis of AgNPs. This peak corresponds to the localized SPR of AgNPs, which agrees with earlier studies on biologically synthesized AgNPs exercise plant extracts.

This result is harmonious with formerly announce studies where the biosynthesized AgNPs display absorption peaks in the range of 420–450 nm, reflecting particle formation and stability (Bilguun, et al., (2024); A., Ramesh., et al., (2024). The development of silver AgNPs was tracked by observing a color change and utilizing UV-Vis spectroscopy. The reaction admixture began to turn yellowish-brown within 10 minutes and transitioned to reddish-brown after one hour. This color shift indicated the formation of silver nanoparticles, as the silver ions (Ag+) were reduced to silver nanoparticles (Ag°) Vimala, et al., (2021).

Transmission Electron Microscopy (TEM)

The morphology and measuring of the synthesized AgNPs were examined using TEM. As shown in (Fig. 4), the particles exhibited a mostly spherical shape with varying sizes continue from 16.7 nm to 37.0 nm, with an intermediate size of around 20–30 nm. The images also reveal minimal aggregation, suggesting that the nanoparticles are well- scattered. The particle dimension observed aligns with previous reports on green synthesis using Annona squamosa leaf Extracted. For example, Fatma, et al., (2022). reported AgNPs with sizes between 10–50 nm using a similar biosynthesis route.

FTIR analysis

FTIR analysis was conducted to identify the feasible groups involved in the stabilization and reduction of AgNPs. The spectra of the nanoparticles (Fig.5) show prominent peaks corresponding to functional groups such as O-H (hydroxyl), C=O (carbonyl), and C=C (alkene) stretching vibrations.

The peaks at 3400–3200 cm^{-1} represent O-H stretching, indicating the involvement of phenolic groups from *Annona squamosa* leaf extract in reducing silver ions (Ag^{+}) to Ag^{0}. Other peaks at 1600 cm^{-1} and 1400 cm^{-1} suggest the presence of carbonyl and aromatic groups, which could stabilize the nanoparticles by capping them. These observations are consistent with earlier FTIR studies of green-synthesized AgNPs Siva., et al., (2024).

Zeta Potential Measurement

The zeta potential permission was performed to evaluate the stability of the synthesized nanoparticles. The zeta prospect value was measured to be -27.9 mV (Fig. 6). This passive zeta potential signalizes good colloidal settlement of the nanoparticles, as values greater than ±25 mV are typically considered stable due to electrostatic inconsistency between particles.

The result demonstrates the effective capping of AgNPs by biomolecules present in the Annona squamosa leaf extract, which prevents aggregation. Similar findings have been reported in studies on green synthesis of nanoparticles, where zeta potentials ranged from -20 to -35 mV, confirming stability (Santhosh., et al., (2020). A zeta potential value of -27.9 mV for nano-silver suggests that the particles have a negative surface charge. This negative charge arises from the turnout of negatively charged ions or groups on the surface of the silver nanoparticles.

Annona leaves Nanosilver and its extract Anticancer activity

MTT Cytotoxicity

MTT assay for 3 groups was done to assess cell viability and proliferation, G1 group of non-treated MCF-7 cell line showed no cell death while G2 group that treated with Annona extract only at a concentration of (50µg/ml) Raja, et al., (2016); Naik & Sellappan,. (2020); Vivek, et al., (2012). showed significant decrease in cell viability with percentage (58.2%). However, the most efficient decrease was recorded at G3 that was treated with both extract and nanosilver with the same concentration with percentage (79.57%) when compared with G1 group. The MTT inspection has been a valuable tool for evaluating the cytotoxic effects of plant excerpts and nano-silver on MCF-7 breast cancer cells. Numerous studies have expounded the prospect of these agents to induce cell death and inhibit cancer cell growth. Many plant extracts, including those from green tea, curcumin, resveratrol, and garlic, have shown significant cytotoxicity against MCF-7 cells in MTT assays. The cytotoxic effects of plant extracts are often attributed to their ability to induce apoptosis, inhibit cell propagation, and target specific molecular pathways involved in cancer cell survival Septaningsih, *et al*., (2024). Nano-silver has been reported to exhibit dose-dependent cytotoxicity versus MCF-7 cells in MTT assays Vivek, *et al*., (2012). The technicality of action of nano-silver is complex

and involves multiple pathways, including oxidative stress, DNA damage, and mitochondrial dysfunction Supavadee, *et al*., (2024); Ikram, *et al*., (2024).

P53 and BAX Gene Expression

The obtained results in **Fig (7&8)** revealed the potent enhancement of Annona extract and its nanosilver for induction of apoptosis process via expression of P53 and bax genes. Using COSTAT statistical method there are significant increase from G1 to G3 for P53 and bax gene expression. These genes play crucial roles in apoptosis, a programmed cell death mechanism that is essential for cancer prevention. P53 is often referred to as the " trustee of the genome," p53 is a tumor suppressor protein that is activated in response to cellular stress. When activated, p53 can prompt apoptosis by upregulating pro-apoptotic genes like bax. While bax is a pro-apoptotic protein that promotes the emission of cytochrome c from the mitochondria, leading to the activation of caspases and cell death Majid, *et al*., (2021); Dan, *et al*., (2023); Fatma, *et al*., (2022).

CONCLUSION:

The present study introduces Annona squamosa as a potent source of important bioactive ingredients for several biological processes such as antioxidant, anti-inflammatory and anticancer treatments. Moreover, the study recommends the aqueous extraction of Annona leaves and the effective and successful green synthesis of silver nanoparticles from Annona leaves as a novel method that offers safe, selective, low toxicity and effective treatment trial for cancer and biological treatments.

REFERENCES

Al-Nemari, R., Al-Senaidy, A., Semlali, A., Ismael, M., Badjah-Hadj-Ahmed, A., Bacha, A., 2020. GC-MS profiling and assessment of antioxidant, antibacterial, and anticancer properties of extracts of Annona squamosa L. leaves. *BMC Complementary Medicine and Therapies, 20.* https://doi.org/10.1186/s12906-020-03029-9.

Askal, H., Saleh, G., Backheet, E., 1992. A selective spectrophotometric method for determination of quercetin in the presence of other flavonoid. *Talanta, 39 3, 259-63* . https://doi.org/10.1016/0039-9140(92)80030-H.

Awada, N., Ayoub, A., Jaber, A., Ibrahim, F., El Ghotmi, N., Cheble, E., 2023. Evaluation of the Anticancer, Anti-Inflammatory, and Antioxidant Properties of Various Extracts of Annona squamosa L. *Pharmaceutical Sciences, 29(3), 384-394.* doi: 10.34172/PS.2023.5

Azim, H.A., Elghazawy, H., Ghazy, R.M., Abdelaziz, A.H., Abdelsalam, M., Elzorkany, A., Kassem, L., 2023. Clinicopathologic features of breast cancer in Egypt—contemporary profile and future needs: *a systematic review and meta-analysis. JCO Global Oncology, 9, e2200387.* https://doi.org/10.1200/GO.22.00387

Dayal, B., Kulkarni, A., Lea, M., Kaur, G., Karani, W., & Singh, J. (2024). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. https://doi.org/10.14233/ajomc.2023.ajomc-p28926

Buchman, J., Hudson-Smith, N., Landy, K., Haynes, C., 2019. Understanding Nanoparticle Toxicity Mechanisms To Inform Redesign Strategies To Reduce Environmental Impact.. *Accounts of chemical research*. https://doi.org/10.1021/acs.accounts.9b00053.

Carvalho, N., Monteiro, O., Da Rocha, C., Longato, G., Smith, R., Da Silva, J., Maia, J., 2022. Phytochemical Analysis of the Fruit Pulp Extracts from Annona crassiflora Mart. and Evaluation of Their Antioxidant and Antiproliferative Activities. *Foods*, 11. https://doi.org/10.3390/foods11142079.

Chavan, J.J., Gaikwad, D.K., Kshirsagar, P.R., 2017. Phytochemical and HPLC analysis of Annona squamosa leaf extracts. Journal of Plant Sciences, 7(3), 34-39.

Chun-Hong, S., 2009. The Extracting Techniques and Analysis Methods for Flavonoids in Plants. *Journal of Analytical Science*.

Crascí, L., Lauro, M., Puglisi, G., Panico, A. 2018. Natural antioxidant polyphenols on inflammation management: Anti-glycation activity vs metalloproteinases inhibition. *Critical Reviews in Food Science and Nutrition*, 58, 893 - 904. https://doi.org/10.1080/10408398.2016.1229657.

Enkhbat, B., Erdenebaatar, T., Badamtsetseg, M., Baasanjav, Z., Bayarsaikhan, E., 2024. Technological Study on The Synthesis of Silver Nanoparticles Using Plant Extracts Via Biosynthesis Methods. https://doi:10.14233/ajomc.2023.ajomc-p28926

Faraone, I., Russo, D., Labanca, F., Lela, L., Ponticelli, M., Sinisgalli, C., Milella, L., 2023. Antioxidant Activity Methods. Methods For Preclinical Evaluation of Bioactive Natural Products, 1-69.

Ganesan, K., Wang, Y., Gao, F., Liu, Q., Zhang, C., Li, P., Zhang, J., Chen, J., 2021. Targeting Engineered Nanoparticles for Breast Cancer

Therapy. *Pharmaceutics*,13.https://doi.org/10.3390/pharmaceutics13111829.

Gavamukulya, Y., Wamunyokoli, F., El-Shemy, H., 2017. Annona muricata: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects.. *Asian Pacific journal of tropical medicine*, 10 9, 835-848 . https://doi.org/10.1016/j.apjtm.2017.08.009.

Gentle, A.A., Omogbolahan, S.Q., Godwin, J.O., 2020. Characterization of biosynthesized silver nanoparticles using UV-visible and FTIR Spectroscopy. Afr J Environ Nat Sci Res, 3(5), 21-26.

Giaquinto, A.N., Sung, H., Newman, L.A., Freedman, R.A., Smith, R.A., Star, J., Siegel, R.L., 2024. Breast cancer statistics 2024. CA: A Cancer Journal for Clinicians, 74(6), 477-495.

Gul, A., Shaheen, F., Rafique, R., Bal, J., Waseem, S., Park, T., 2020. Grass-mediated biogenic synthesis of silver nanoparticles and their drug delivery evaluation: A biocompatible anti-cancer therapy. *Chemical Engineering Journal*, 127202.https://doi.org/10.1016/J.CEJ.2020.127202.

Himesh, S., Sarvesh, S., Sharan, P. S., Mishra, K., Singhai, A.K., 2011. Preliminary phytochemical screening and HPLC analysis of flavonoid from methanolic extract of leaves of Annona squamosa. *International Research Journal of Pharmacy*, *2*(5), 242-246.https://doi.org/10.2174/18715303176661711 14114321.

Huang, D., Wang, J., Zhou, S., Zhang, T., Cai, J., Liu, Y., 2023. Ag nanoparticles green-mediated by Scrophularia striata aqueous extract induce apoptosis via P53 and signal transducer and activator of transcription 3 signaling pathways in gastric cancer cells. *Inorganic Chemistry Communications, 155, 110942.*

Ibraheim, S.G.E., Shedeed, W.K., Abdelaziz, N.S. 2024. Breast Cancer Preventive Program among Women According to Sustainable Development Strategy 2030. *International Egyptian Journal of Nursing Sciences and Research*, *4*(2), 376-393. 10.21608/ejnsr.2024.258957.1345

Ullah, I., Khalil, A. T., Zia, A., Hassan, I., & Shinwari, Z. K. (2024). Insight into the molecular mechanism, cytotoxic, and anticancer activities of phyto-reduced silver nanoparticles in MCF-7 breast cancer cell lines. Microscopy Research and Technique, 87(7), 1627-1639.

Jahan, S., Karim, M., Chowdhury, E. 2021. Nanoparticles Targeting Receptors on Breast Cancer for Efficient *Delivery of Chemotherapeutics. Biomedicines, 9.* https://doi.org/10.3390/biomedicines9020114.

Jose, V., Raphel, L., Aiswariya, K., Mathew, P., 2021. Green synthesis of silver nanoparticles using Annona squamosa L. seed extract: characterization, photocatalytic and biological activity assay. *Bioprocess and Biosystems Engineering*, 44, 1819 - 1829. https://doi.org/10.1007/s00449-021-02562-2.

Kadir, D. 2021. Statistical evaluation of main extraction parameters in twenty plant extracts for obtaining their optimum total phenolic content and its relation to antioxidant and antibacterial activities. *Food Science & Nutrition*, 9, 3491 - 3499. https://doi.org/10.1002/fsn3.2288.

Kamble, D.D., Jadhav, P.P., 2024. Conventional and Modern Techniques to Treat Cancer. Asian Journal of Pharmaceutical Research, 14(3)., doi: 10.52711/2231-5691.2024.00047

Kiranmayi, G. 2021. Synthesis of silver nanoparticles using ethanolic extract of Annona squamosa fresh leaves and investigation of antioxidant, anti-arthritic, and thrombolytic activities. *International Journal of Green Pharmacy*, 15. https://doi.org/10.22377/IJGP.V15I2.3096.

Kumar, M., Changan, S., Tomar, M., Prajapati, U., Saurabh, V., Hasan, M., Sasi, M., Maheshwari, C., Singh, S., Dhumal, S.R., Thakur, M., Punia, S., Satankar, V., Amarowicz, R., Mekhemar, M. 2021. Custard Apple (Annona squamosa L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Biological Activities. *Biomolecules*, 11. https://doi.org/10.3390/biom11050614.

Kumar, P., Nagarajan, A., Uchil, P., 2018. Analysis of Cell Viability by the MTT Assay.. *Cold Spring Harbor protocols*, 2018 6, pdb.prot095505. https://doi.org/10.1101/pdb.prot095505.

Mahawar, V., Patidar, K., Joshi, N. 2019. Development and evaluation of herbal antiaging cream formulation containing Annona squamosa leaf extract. *DEVELOPMENT, 12(2).*

Maher, S., Kalil, H., Liu, G., Sossey-Alaoui, K., Bayachou, M. 2023. Alginate-based hydrogel platform embedding silver nanoparticles and cisplatin: characterization of the synergistic effect on a breast cancer cell line. *Frontiers in Molecular Biosciences*, 10. https://doi.org/10.3389/fmolb.2023.1242838.

Majid, S., Jabir., Yasmin, M, Saleh., Ghassan, M., Sulaiman., Nahi, Y., Yaseen., Usama, I., Sahib., Yaser, Hassan, Dewir., Mona, S., Alwahibi., Dina, A., Soliman. 2021. 5. Green Synthesis of Silver Nanoparticles Using Annona muricata Extract as an Inducer of Apoptosis in Cancer

Cells and Inhibitor for NLRP3 Inflammasome via Enhanced Autophagy.. Nanomaterials, doi: 10.3390/NANO11020384

Malik, M., Iqbal, M., Malik, M., Raza, M., Shahid, W., Choi, J., Pham, P. 2022. Biosynthesis and Characterizations of Silver Nanoparticles from Annona squamosa Leaf and Fruit Extracts for Size-Dependent Biomedical Applications. *Nanomaterials*, 12. https://doi.org/10.3390/nano12040616.

Manjunath, J., Shetty., K.R, Vasudeva., T, Sakthivel., G.J, Suresh., H.C, Krishna., K, Vishnuvardhana., Anil, Kumar, S. 2020. 2. Polyphenolic profiles in edible Annona spp. using high-performance liquid chromatography (HPLC-MS/MS). Environment Conservation Journal, doi: 10.36953/ECJ.2020.21325

Mathew, S., Abraham, T., Joseph, M. 2021. Chlorogenic acid and its derivatives: Role in antioxidant activity and therapeutic potential. Journal of Agricultural and Food Chemistry, 69(11), 3227-3240.

Mishra, A.P., Nigam, M., Nambooze, J., Salau, V.F., Olofinsan, K.A., Iriti, M., Matsabisa, M.G., 2023. GC-MS Analysis, Antioxidant, and Antidiabetic Properties of Methanol Extract of Annona muricata L. Leaves-An In vitro and In silico Study. Current Organic Chemistry, 27(17), 1531-1541.

Mishra, A.P., Nigam, M., Nambooze, J., Salau, V.F., Olofinsan, K.A., Iriti, M., Matsabisa, M.G., 2023. GC-MS Analysis, Antioxidant, and Antidiabetic Properties of Methanol Extract of Annona muricata L. Leaves-An In vitro and In silico Study. *Current Organic Chemistry*, *27*(17), 1531-1541.

Mokhtar, F.A., Selim, N.M., Elhawary, S.S., Abd El Hadi, S.R., Hetta, M.H., Albalawi, M.A., Ibrahim, R.M., 2022. Green biosynthesis of silver nanoparticles using annona glabra and annona squamosa extracts with antimicrobial, anticancer, apoptosis potentials, assisted by in silico modeling, and metabolic profiling. Pharmaceuticals, *15*(11), 1354.

Mokhtar, F., Selim, N., Elhawary, S., Hadi, S., Hetta, M., Albalawi, M., Shati, A., Alfaifi, M., Elbehairi, S., Fahmy, L., Ibrahim, R. 2022. Green Biosynthesis of Silver Nanoparticles Using Annona glabra and Annona squamosa Extracts with Antimicrobial, Anticancer, Apoptosis Potentials, Assisted by In Silico Modeling, and Metabolic Profiling. *Pharmaceuticals*, 15. https://doi.org/10.3390/ph15111354.

Montalvo-Quirós, S., Aragoneses-Cazorla, G., García-Alcalde, L., Vallet-Regí, M., González, B., Luque-Garcia, J. 2019. Cancer cell targeting and therapeutic delivery of silver nanoparticles by mesoporous silica nanocarriers: insights into the action mechanisms using quantitative proteomics.. *Nanoscale*, 11 10, 4531-4545 . https://doi.org/10.1039/c8nr07667g.

Mujumdar, A., Patel, S., Desai, K. 2016. Antioxidant potential of Annona squamosa leaf extract: An in-vitro evaluation. Journal of Plant Sciences, 7(3), 45-51.

Mujumdar, A., Patel, S., Desai, K. 2016. Antioxidant potential of Annona squamosa leaf extract: An in-vitro evaluation. Journal of Plant Sciences, 7(3), 45-51.

Naik, A., Sellappan, K. 2020. In vitro evaluation of Annona muricata L. (Soursop) leaf methanol extracts on inhibition of tumorigenicity and metastasis of breast cancer cells. *Biomarkers*, 25, 701 - 710. https://doi.org/10.1080/1354750X.2020.1836025.

Nga, E.N., Nchinda, G., Mahi, L.L., Figuei, P.B., Mvondo, J.M., Sagnia, B., Adiogo, D. 2019. Phytochemical screening and evaluation of antioxidant power of hydro-ethanolic and aqueous leaves extracts of Annona muricata Linn (soursop). *Health Sciences and Disease, 20(1).* *https://doi.org/10.5281/hsd.v20i1.1328*

Nguyen, M., Nguyen, V., Le, V., Trieu, L., Lam, T., Bui, L., Nhan, L., Danh, V. 2020. Assessment of preliminary phytochemical screening, polyphenol content, flavonoid content, and antioxidant activity of custard apple leaves (Annona squamosa Linn.). *IOP Conference Series: Materials Science and Engineering*, 736. https://doi.org/10.1088/1757-899X/736/6/062012.

Nugraha, A., Damayanti, Y., Wangchuk, P., Keller, P. 2019. Anti-Infective and Anti-Cancer Properties of the Annona Species: Their Ethnomedicinal Uses, Alkaloid Diversity, and Pharmacological Activities. *Molecules*, 24. https://doi.org/10.3390/molecules24234419.

Pereira, A.S., Monteiro, R.C., Silva, L.T. 2019. Assessment of phenolic profiles in Annona muricata extracts. Natural Product Research, 33(12), 1714-1720.

Rahdar, A., Amini, N., Askari, F., Susan, M., 2019. Dynamic light scattering and zeta potential measurements: *effective techniques to characterize therapeutic nanoparticles. .* https://doi.org/10.22034/JNA.2019.575819.1108.

Raj, S., Singh, H., Trivedi, R., Soni, V. 2020. Biogenic synthesis of AgNPs employing Terminalia arjuna leaf extract and its efficacy towards catalytic degradation of organic dyes. *Scientific Reports*, 10. https://doi.org/10.1038/s41598-020-66851-8.

Raja, Rajan. p., Senthil, Kumar, R. 2016. 3. Assessment of antineoplastic potential of

annona reticulata linn. on human cancer cell lines. Asian Journal of Pharmaceutical and Clinical Research,

Ramesh, A., Elanchezhiyan, J., Karthickeyan, D., Mani, M., Kaviyarasu, K., Uthrakumar, R., Alam, M.W., 2024. Biogenic Syntheses of AgNPs With Leaf Extract Abution indicum and Its Application for Antimicrobial and Photocatalytic Activity. *Applied Organometallic Chemistry, e7758.*

Rami, N., Kulkarni, B., Chibber, S., Jhala, D., Parmar, N., Trivedi, K. 2023. In vitro antioxidant and anticancer potential of Annona squamosa L. Extracts against breast cancer. *International Journal of ExperimentalResearchandReview*. https://doi.org/10.52756/ijerr.2023.v30.024.

Rahmasari, R., Raekiansyah, M., Aliyah, S. H., Yodi, P., Baihaqy, F., Irhamsyah, M., ... & Sauriasari, R. (2024). Development and validation of cost-effective SYBR Green-based RT-qPCR and its evaluation in a sample pooling strategy for detecting SARS-CoV-2 infection in the Indonesian setting. Scientific reports, 14(1), 1817.

Rivera, M., Vazquez-Duhalt, R., Castro-Longoria, E., Juárez-Moreno, K. 2024. Synergistic anticancer effects and reduced genotoxicity of silver nanoparticles and tamoxifen in breast cancer cells.. *Journal of biochemical and molecular toxicology*, 38 10, e23823 . https://doi.org/10.1002/jbt.23823.

Maria, Inês, Rouxinol. 2024. 1. Isolation and Identification of phenolic compounds. doi: 10.5772/intechopen.1005224

Ruddaraju, L., Pallela, P., Pammi, S., Padavala, V., Kolapalli, V. 2019. Synergetic antibacterial and anticarcinogenic effects of Annona squamosa leaf extract mediated silver nano particles. *Materials Science in Semiconductor Processing*. https://doi.org/10.1016/J.MSSP.2019.05.007.

Rui, Sha., Xiang-meng, Kong., X., Li., Yan, Wang. 2024. 1. Global burden of breast cancer and attributable risk factors in 204 countries and territories, from 1990 to 2021: results from the Global Burden of Disease Study 2021. Biomarker research, doi: 10.1186/s40364-024-00631-8

Santhosh, S. B., Natarajan, D., Deepak, P., Gayathri, B., Kaviarasan, L., Naresh, P., ... & Chandrasekar, M. J. N. (2020). Metabolic enzyme inhibitory and larvicidal activity of biosynthesized and heat stabilized silver nanoparticles using Annona muricata leaf extract. BioNanoScience, 10, 267-278.

Sallam, I., Amira, G., Down, S., Downey, S., Maraqa, L., Naguib, S., Amin, H. 2024. Enhancing Breast Cancer Education in Egypt: A Quality Improvement Initiative from the First Advanced Oncoplastic Skills Course by the Egyptian Group of Breast Surgeons. *European Journal of Surgical Oncology*, *50*.

Santangelo, C., Vari, R., Scazzocchio, B., De Sanctis, P., Giovannini, C., D'Archivio, M., Masella, R. 2018. Anti-inflammatory activity of extra virgin olive oil polyphenols: which role in the prevention and treatment of immune-mediated inflammatory diseases?. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 18(1), 36-50.* https://doi.org/10.2174/1871530317666171114114321.

Septaningsih, D., Suparto, I., Achmadi, S., Heryanto, R., Rafi, M. 2024. Untargeted metabolomics using UHPLC-Q-Orbitrap HRMS for identifying cytotoxic compounds on MCF-7 breast cancer cells from Annona muricata Linn leaf extracts as potential anticancer agents.. *Phytochemical analysis : PCA*. https://doi.org/10.1002/pca.3373.

Shaik, M., Khan, M., Kuniyil, M., Al-Warthan, A., Alkhathlan, H., Siddiqui, M., Shaik, J., Ahamed, A., Mahmood, A., Khan, M., Adil, S. 2018. Plant-Extract-Assisted Green Synthesis of Silver Nanoparticles Using Origanum vulgare L. Extract and Their Microbicidal Activities. *Sustainability*, 10, 913. https://doi.org/10.3390/SU10040913.

Shehata, M., Abu-Serie, M., El-Aziz, N., El-Sohaimy, S. 2021. Nutritional, phytochemical, and in vitro anticancer potential of sugar apple (Annona squamosa) fruits. *Scientific Reports*, 11. https://doi.org/10.1038/s41598-021-85772-8.

Soha, Dehsorkhi., M., Deepali, Shirsath, 2022. 4. A Review on: Plant Based Antioxidant. International Journal For Multidisciplinary Research, doi: 10.36948/ijfmr.2022.v04i06.1221

Sonali, Bhagat. 2021. 1. Phytochemical Screening, Determination of Total Phenol Content, Total Flavonoid Content and Quantitative Estimation of Rutin and Quercetin Using RP-HPLC in the Fruits of Capparis decidua (Forsk.) Edgew. doi: 10.18782/2582-2845.8666

Strasser, R., Kam, S.M., Regalado, S.M. 2016. Rural health care access and policy in developing countries. *Annual review of public health*, *37*(1), 395-412.

Supavadee, Boontha., Benjaporn, Buranrat., Kamchai, Saepang., Prapapan, Temkitthawon., Tasana, Pitaksuteepong. 2024. 1. Cytotoxic, Cell Apoptosis, Colony Formation and Anti-Migratory Activity of Three Herbal Plant Extracts in MCF-7 Breast Cancer Cells. doi: 10.12982/nlsc.2025.004

Taheri-Ledari, R., Zhang, W., Radmanesh, M., Mirmohammadi, S., Maleki, A., Cathcart, N., Kitaev, V. 2020. Multi-Stimuli Nanocomposite Therapeutic: Docetaxel Targeted Delivery and Synergies in Treatment of Human Breast Cancer Tumor.. *Small*, e2002733 . https://doi.org/10.1002/smll.202002733.

Takeuchi, T., Kitamura, Y., Sobue, T., Utada, M., Ozasa, K., Sugawara, Y., Tsuji, I., Hori, M., Sawada, N., Tsugane, S., Koyanagi, Y., Ito, H., Wang, C., Tamakoshi, A., Wada, K., Nagata, C., Shimazu, T., Mizoue, T., Matsuo, K., Naito, M., Tanaka, K., & Inoue, M. 2021. Impact of reproductive factors on breast cancer incidence: Pooled analysis of nine cohort studies in Japan. *Cancer Medicine*, 10, 2153 - 2163. https://doi.org/10.1002/cam4.3752.

Ullah, I., Khalil, A., Ali, M., Iqbal, J., Ali, W., Alarifi, S., Shinwari, Z. 2020. Green-Synthesized Silver Nanoparticles Induced Apoptotic Cell Death in MCF-7 Breast Cancer Cells by Generating Reactive Oxygen Species and Activating Caspase 3 and 9 Enzyme Activities. *Oxidative Medicine and Cellular Longevity*, 2020. https://doi.org/10.1155/2020/1215395.

SIVA, V., MANIKANDAN, S., PALPPERUMAL, S., VAISHALY, G., JEYAKUMARAN, N., & ALAGU, A. Biosynthesis of Silver Nanoparticles with Aqueous Leaf Extract of Commiphora caudata and their Antibacterial Activity against Human Pathogens.

V., Vanitha., K.J., Umadevi., K., Vijayalakshmi. 2010. 6. In vitro assessment of alcoholic leaf extracts of annona squamosa and aegle marmelos. Vikas, B., Akhil, B.S., Remani, P., Sujathan, K. 2017. Free radical scavenging properties of Annona squamosa. *Asian Pacific journal of cancer prevention: APJCP*, *18*(10), 2725.

Jose, V., Raphel, L., Aiswariya, K. S., & Mathew, P. (2021). Green synthesis of silver nanoparticles using Annona squamosa L. seed extract: characterization, photocatalytic and biological activity assay. Bioprocess and Biosystems Engineering, 44, 1819-1829.

Vivek, R., Thangam, R., Muthuchelian, K., Gunasekaran, P., Kaveri, K., Kannan, S. 2012. Green biosynthesis of silver nanoparticles from Annona squamosa leaf extract and its in vitro cytotoxic effect on MCF-7 cells. *Process Biochemistry*, 47, 2405-2410. https://doi.org/10.1016/J.PROCBIO.2012.09.025.

Wang, H., Agarwal, P., Zhao, S., Yu, J., Lu, X., He, X. 2016. Combined cancer therapy with hyaluronan-decorated fullerene-silica multifunctional nanoparticles to target cancer stem-like cells.. *Biomaterials*, 97, 62-73 . https://doi.org/10.1016/j.biomaterials.2016.04.030.

Youssef, S., El-Moneim, A., Elsayed, H. 2024. The Relationship between Reproductive Factors and Risk of Breast Cancer among Pre–Menopausal Women. *Helwan International Journal for Nursing Research and Practice*, *3*(5), 468-486.

Zhang, K., Liu, X., Ravi, S., Ramachandran, A., Ibrahim, I., Nassir, A., Yao, J. 2019. Synthesis of silver nanoparticles (AgNPs) from leaf extract of Salvia miltiorrhiza and its anticancer potential in human prostate cancer LNCaP cell lines. *Artificial Cells, Nanomedicine, and Biotechnology*, 47, 2846 - 2854. https://doi.org/10.1080/21691401.2019.1638792.

Zhang, L., Ravipati, A., Koyyalamudi, S., Jeong, S., Reddy, N., Smith, P., Bartlett, J., Shanmugam, K., Münch, G., Wu, M. 2011. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds.. *Journal of agricultural and food chemistry*, 59 23, 12361-7 . https://doi.org/10.1021/jf203146e

Zhang, S., Wang, C., 2023. Precise Analysis of Nanoparticle Size Distribution in TEM Image. *Methods and Protocols*, 6. https://doi.org/10.3390/mps6040063.

Table 1: Specific primers sequence used in qRT-PCR

Primer	Direction	Sequences 5′-3′
P53	F	TAACAGTTCCTGCATGGGCGGC
	R	AGGACAGGCACAAACACGCACC
Bax	F	CCCTTTTGCTTCAGGGTTTCATCCA
	R	CTTGAGACACTCGCTCAGCTTCTTG
GAPDH	F	GTCTCCTCTGACTTCAACAGCG
	R	ACCACCCTGTTGCTGTAGCCAA

Table 2: Preliminary phytochemical screening of aqueous extracts of *A squamosa* leaves

Compounds	Aqueous
Phenols	+
Flavonoids	+
Saponins	+
Alkaloids	+
Tannins	+

Table 3: Total Phenolic Content (TPC) &Total Flavonoids Content(TFC) in Aqueous Extract of Annona squamosa Leaves

Compounds	amount (medium)
Phenols (mg GAEequivalent /g dry weight)	8.70± 0.68
Flavonoids (mg of quercetin equivalent /g dry weight)	7.50 ± 0.39

Table 4: DPPH radical scavenging activity of samples.

Sample	Abs. Avg	Scav.%
Control	0.581	-
Ascorbic acid	0.111	91.43
Water extract	0.150	83.78

Table 5: Analysis of Aqueous extract of *ANNONA Squamosa* leaves

NO	Peak Name	Retention Time (min)
	Flavonoids compounds	
1	Rutin	2.520
2	Diosmin	2.923
3	Kampferol	10.627
4	Quercitin	15.173
5	hisperiden	6.52
	Phenolic compounds	
5	quinic	2.983
6	Ellagic	3.190
7	cinnamic	3.593
8	chlorogenic	4.400
9	resorcinol	8.477
10	pyrochatechol	9.187
11	phenantherine	16.007

Table 6. Effect of aqueous extraction *Annona squamosa* leaves and green nano silver on the MCF-7 Breast cancer cells using MTT assay.

Groups	Dose	Mean absorbance	Viability %	Cytotoxicty %
Control (G1)	----	0.306	99.5	0.5
Extract (G2)	50 ul/ml	0.126666667	41.3	58.2
Nano-Extract (G3)	50 ul/ml	0.061	19.9	79.6

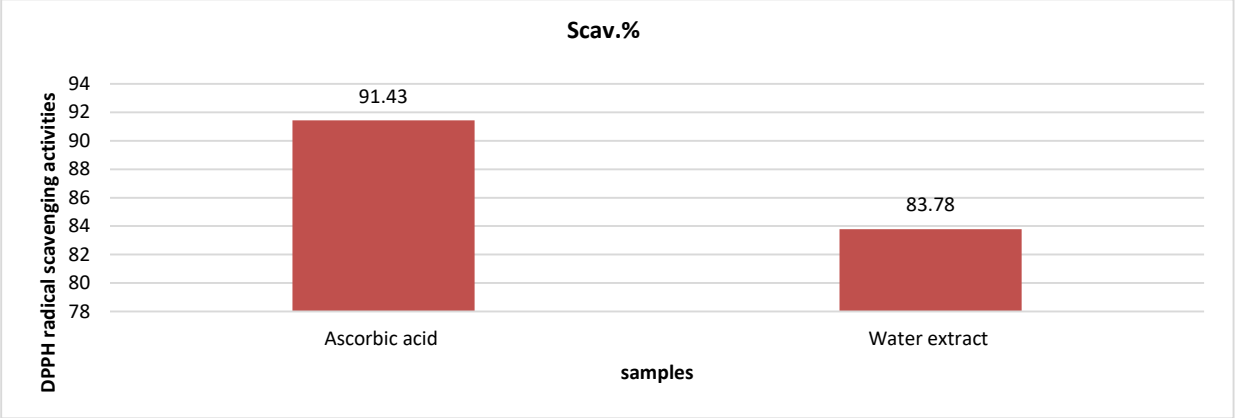

Figure 1: DPPH radical scavenging activity (%) of ascorbic acid and aqueous Annona extract.

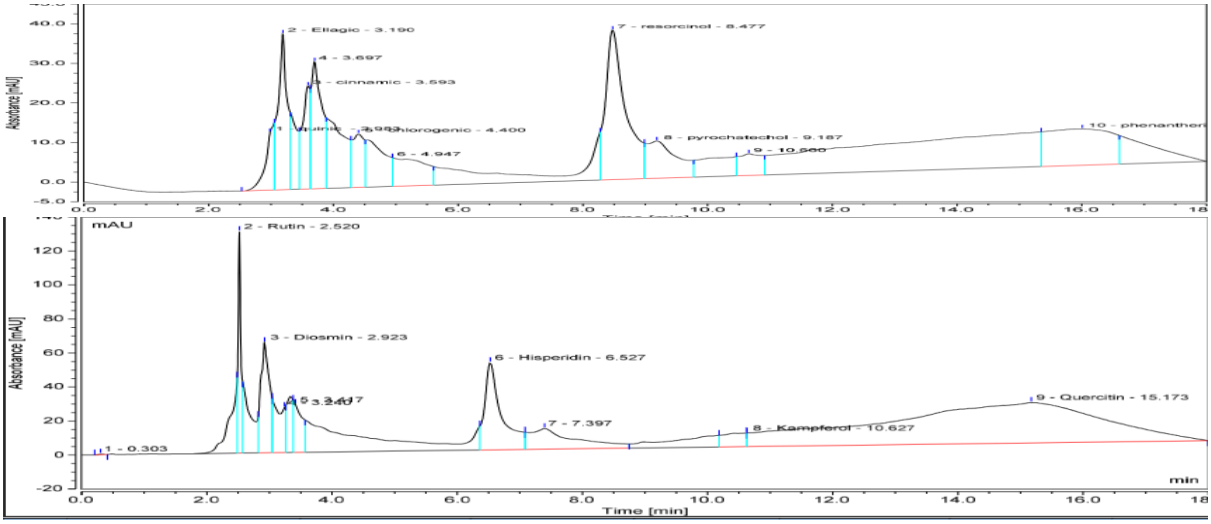

Figure 2: Identification of Aqueous extract of Annona Squamosa leaves

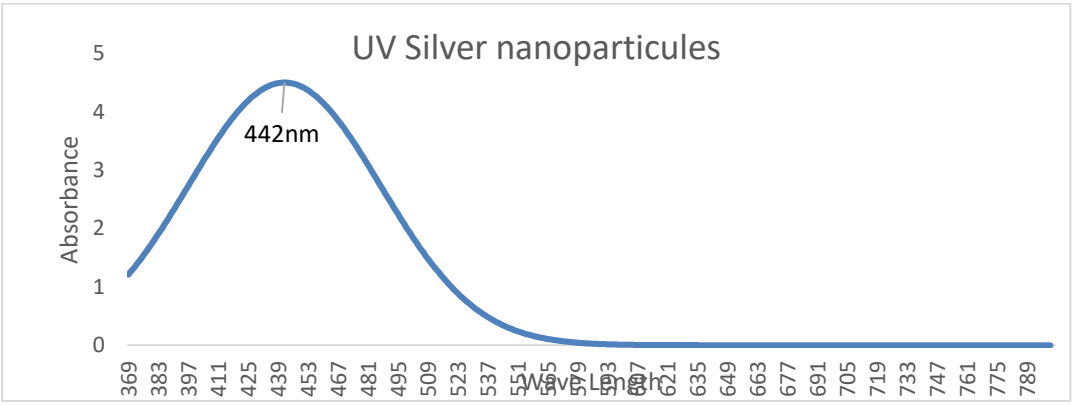

Figure 3: UV-Visible Spectroscopy Characterization of silver nanoparticles green synthesis by aqueous Annona Squamous leaves extract.

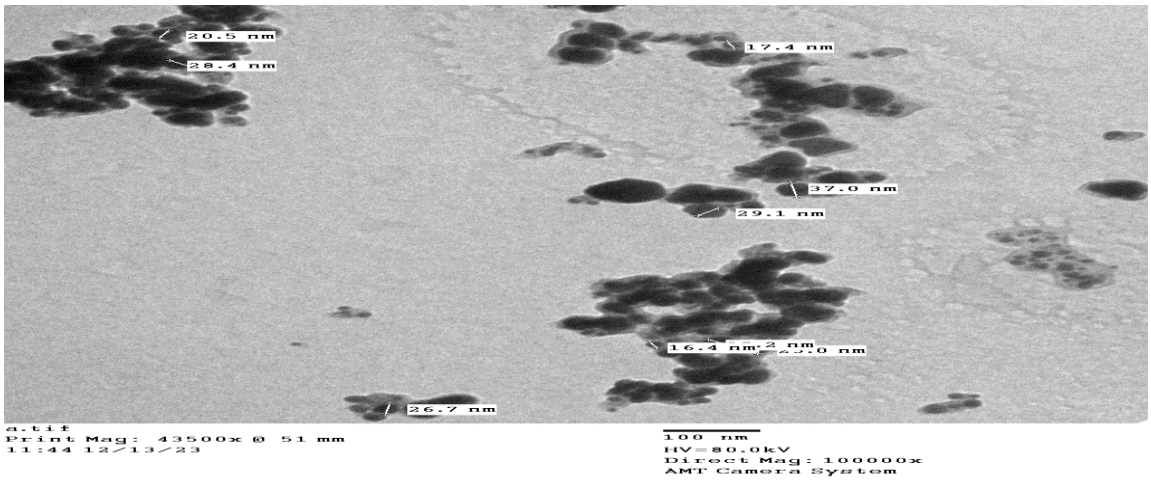

Figure 4:TEM analysis of silver nanoparticles green synthesis by aqueous *Annona squamous* leaves extract.

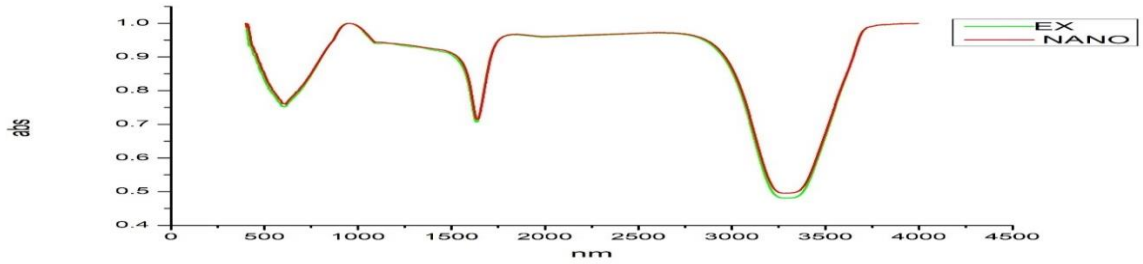

Figure 5: FTIR Spectroscopy Characterization of silver nanoparticles green synthesis by aqueous Annona Squamous leaves extract.

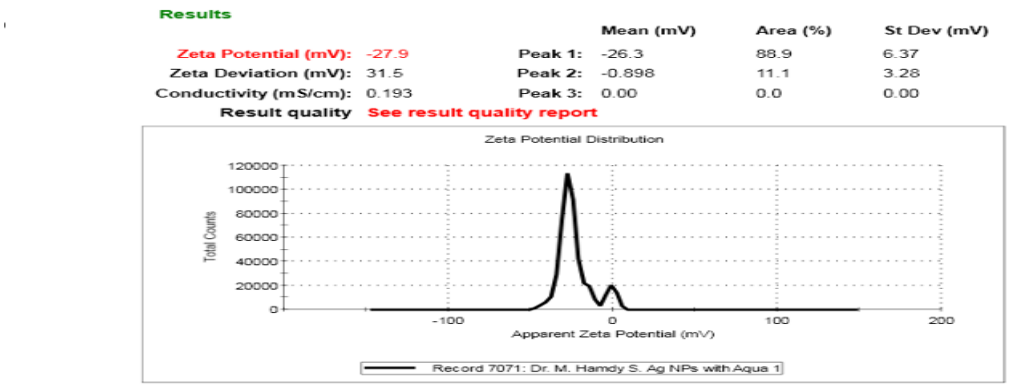

Figure 6: Zeta Potential Measurementof silver nanoparticles green synthesis by aqueous Annona Squamous leaves extract.

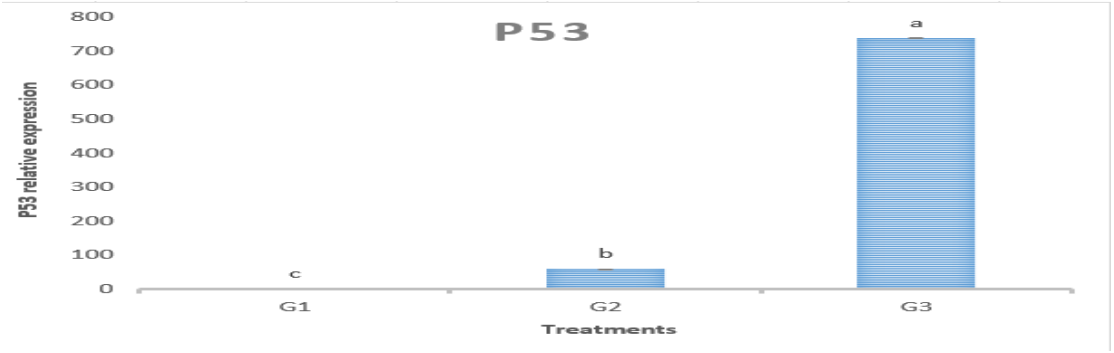

Figure 7: Significance level variance increased from (a) to (c) when compared with G1 (Costat statistical analysis).

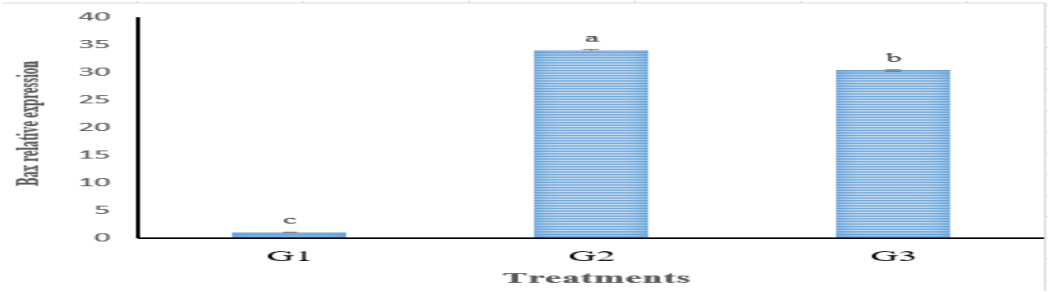

Figure 8: Significance level variance increased from (a) to (c) when compared with G1 (Costat statistical analysis).

تخليق جزيئات الفضة النانوية صديقة للبيئة عن طريق الاستخلاص المائي من أوراق نبات القشطة ودراسة تأثيراتها الجينية على خلايا MCF-7

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الملخص بالعربي

في السنوات الأخيرة، ظهرت العديد من العلاجات غير التقليدية للسرطان، وأبرزها علاجات النانو تكنولوجي. وعلى الرغم من نتائجها الواعدة إلا أنها بحاجة إلى المزيد من الدراسات والتحقيقات. ومن ناحية أخرى، يتقدم البحث في العلاجات البديلة من المصادر الطبيعية، وخاصة تلك القائمة على النباتات. وتركز الدراسة المقدمة على استخدام نبات Annona Squamosa، الذي يتميز بغنى استثنائي بالعديد من المكونات البيولوجية العلاجية. وتستخرج الدراسة هذه المكونات بشكل فريد من الأوراق وتستخدمها في إنتاج جسيمات نانوية فضية عن طريق التخليق الأخضر، مما يدل على ضمان في علاج أنواع مختلفة من السرطان، وخاصة سرطان الثدي. وقد تلاعبت الدراسة بالاستخراج والتحديد النوعي والكمي لمكونات Annona الضوئية المهمة مثل الفينولات (8.70 مجم GAE / جم)، والفلافونويدات (7.50 مجم / جم)، ومضادات الأكسدة. علاوة على ذلك، قدمت الدراسة تخليقًا أخضرًا مبتكرًا للنانو فضة مع قياسات توصيفها (التحليل الطيفي للأشعة فوق البنفسجية، والتحليل الطيفي للأشعة تحت الحمراء باستخدام تحويلات فوريه، والمجهر الإلكتروني النافذ (TEM) والتشتت الضوئي الديناميكي (DLS)) ومراقبة تأثيراتها السامة والجزيئية (تعبيرات جين P53 وBax) على خلايا سرطان الثدي. وأكدت الدراسة الأهمية الكبيرة للنانو نفضة من خلال التخليق الأخضر كعلاج واعد لسرطان الثدي.

الكلمات الاسترشادية : التخليق الأخضر، أوراق نبات القشطة، جسيمات النانو فضة، السمية الخلوية، التعبير الجيني.