PLANT NANOMATERIALS JOURNAL

Review articles

Green-Synthesized Silver Nanoparticles as Cytotoxic Agents in Triple-Negative Breast Cancer: A Review of ROS-Mediated Apoptosis Mechanisms

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ABSTRACT

Triple-negative breast cancer (TNBC) represents one of the most aggressive and therapeutically challenging subtypes of breast cancer due to the absence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression. The limited targeted therapy options and high recurrence rates have driven the search for novel anticancer agents. Green-synthesized silver nanoparticles (AgNPs) derived from plant extracts have emerged as promising cytotoxic agents owing to their unique physicochemical properties, environmental compatibility, and enhanced biocompatibility. This literature review aims to analyze the cytotoxic potential of plant-mediated green-synthesized AgNPs against TNBC cells and to elucidate the reactive oxygen species (ROS)-mediated apoptosis mechanisms based on current research findings. Literature searches were conducted across Google Scholar, PubMed, and ScienceDirect databases using relevant keywords, and selected articles were analyzed descriptively. The results demonstrate that green-synthesized AgNPs, characterized by sizes ranging from 10 to 100 nm, spherical morphology, and negative zeta potential, exhibit significant cytotoxic activity against TNBC cell lines, particularly MDA-MB-231, through dose-dependent and time-dependent mechanisms. The primary mechanism of action involves intracellular ROS overproduction that induces oxidative stress, mitochondrial dysfunction, upregulation of pro-apoptotic proteins such as Bax and p53, downregulation of anti-apoptotic Bcl-2, activation of caspase cascades, and DNA damage, ultimately leading to programmed cell death via the intrinsic apoptotic pathway. Plant-derived phytochemical capping agents further contribute synergistically to these effects. These findings establish a strong scientific foundation for the development of plant-based nano-chemotherapeutic agents as selective, safer, and more effective alternatives for TNBC treatment.

Keywords: Silver nanoparticles, green synthesis, triple-negative breast cancer, reactive oxygen species, ROS-mediated apoptosis.

INTRODUCTION

Breast cancer has become one of the biggest threats to global health, especially in women, to the point that it is one of the leading causes of cancer deaths in women worldwide. Data from the *Global Cancer Observatory* (Globocan) shows that global breast cancer cases reach 2.3 million cases or about 11.7% of total cancer cases, with the highest incidence found in Australia and New Zealand, at 94.2 per 100,000 population. In Indonesia, the prevalence of breast cancer is still high, recording 66,271 new cases or 16.2% of the total cancer cases, with an incidence rate of 44.3 per 100,000 women and a mortality rate of 18.6 per 100,000 women [1]. Among the various subtypes of breast cancer, *Triple-Negative Breast Cancer* (TNBC) which is the rarest subtype of molecular breast cancer is encountered. This type of cancer accounts for about 15-20% of all types. TNBC is a subtype of breast cancer that lacks ER, PR, and HER2 receptors, thus limiting the effectiveness of hormone-based therapies and targeted therapies. This condition causes treatment options to be limited, with a high recurrence rate and a suboptimal therapeutic response [2].

The development of science and technology, especially in the field of nanotechnology, has opened up new opportunities in cancer treatment. Metal nanoparticles, especially silver nanoparticles (AgNPs), are one of the materials that are widely researched because they have unique physicochemical properties such as nanosize, high surface area, and strong interaction with biological systems [3]. Various studies show that AgNPs have a wide range of biological activities, including as antimicrobial and anticancer agents [3]. In the context of cancer therapy, AgNPs are reported to be able to induce cytotoxic effects on different types of cancer cells through complex and *multifactorial mechanisms* [4].

However, conventional methods of synthesis of AgNPs generally involve harmful chemicals that have the potential to cause additional toxic impacts and pollute the environment [5]. This encourages the development of a green *synthesis approach* that utilizes plant extracts as a reducing and stabilizing agent. This method is considered more environmentally friendly, economical, and has the potential to increase the biocompatibility of nanoparticles [6]. In addition, the content of bioactive compounds in plants such as flavonoids, phenolics, and terpenoids can contribute to increasing the biological activity of AgNPs, including as cytotoxic agents against cancer cells [3].

Nanoparticles (NPs) are typically defined as particles with sizes ranging from 1 to 100 nm. At this scale, nanoparticles have different physical and chemical characteristics than conventional materials, thus exhibiting distinctive and unique properties [7]. The development of nanotechnology has encouraged the use of silver nanoparticles (AgNPs) as cancer therapy agents due to their unique physicochemical properties and biological activity. Various studies show that AgNPs have anticancer effects through multifactorial mechanisms, including oxidative stress, DNA damage, mitochondrial disorders, and the induction of apoptosis [7]. AgNPs are known to play a role in inducing apoptosis and necrosis in cancer cells through damage to the cell ultrastructure, increased oxidative stress (ROS), damage to genetic material, protein inactivation, and regulation of intracellular signaling pathways [8].

AgNP-induced apoptosis in cancer cells does not proceed exclusively through the ROS-mediated intrinsic pathway, but may also involve other mechanisms. Several studies have reported that AgNPs can activate the extrinsic apoptotic pathway through death-receptor interactions such as Fas/FasL and TNF-related apoptosis-inducing ligand (TRAIL), induce endoplasmic reticulum (ER) stress, and affect autophagy and cell-cycle progression as additional mechanisms restricting cancer cell proliferation [37.] In addition to these mechanisms, AgNP-induced cytotoxicity may also involve mitochondrial dysfunction, DNA damage, and alterations in intracellular signaling pathways associated with cell survival and proliferation. AgNP exposure has been reported to affect mitochondrial and ER homeostasis in breast cancer cells, while oxidative and cellular stress may contribute to DNA damage and subsequent activation of apoptosis[48].

These findings indicate that the cytotoxic effects of AgNPs are multifactorial and involve interconnected cellular responses rather than a single apoptotic pathway. Therefore, although ROS-mediated apoptosis is the primary focus of this review, other mechanisms should also be considered to provide a more comprehensive understanding of AgNP-induced cytotoxicity in TNBC cells.

Although these pathways also contribute to the overall cytotoxic effects of AgNPs, this literature review specifically focuses on the ROS-mediated apoptosis mechanism in TNBC cells, as this pathway is the most extensively reported and most relevant one for plant-based green-synthesized AgNPs[57].

The main mechanism of anticancer activity of AgNPs is known to be closely related to increased production of Reactive Oxygen Species (ROS) within cells [5]. Excessive ROS increases can lead to oxidative stress that impacts the damage of important biomolecules such as DNA, proteins, and lipids. This condition then triggers mitochondrial dysfunction, activation of pro-apoptotic signaling pathways, and ultimately induces apoptosis (programmed cell death) [9]. The mechanism of ROS-mediated apoptosis is of particular

interest in cancer therapy because cancer cells, including TNBC, generally have higher levels of basal oxidative stress and are therefore more susceptible to increased ROS than normal cells [9].

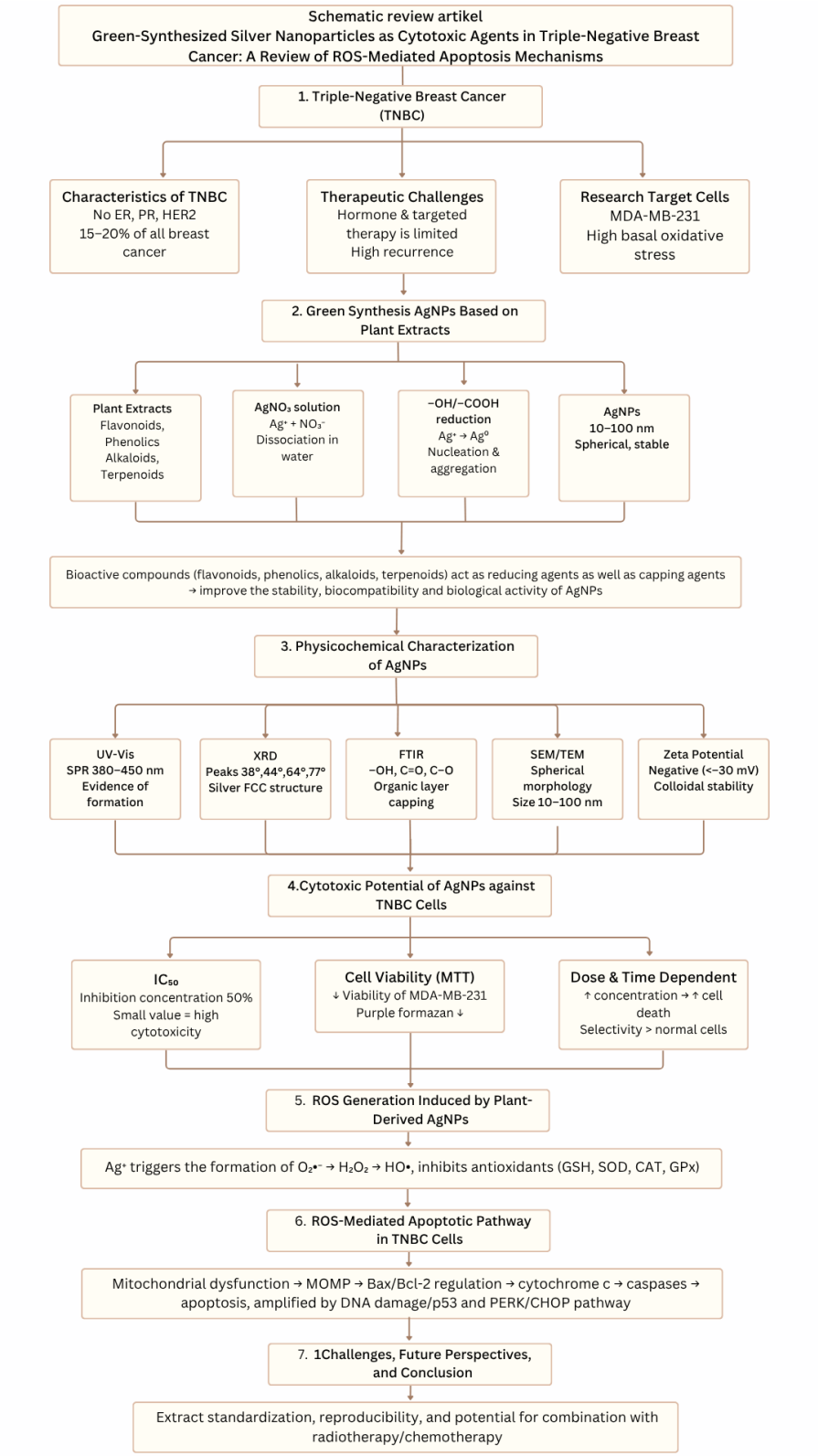
ROS plays an important role in activating apoptotic pathways, especially through intrinsic pathways involving mitochondria. This mechanism is characterized by increased expression of pro-apoptotic proteins such as Bax, decreased anti-apoptotic protein Bcl-2, as well as activation of p53 as a major regulator of apoptosis. In triple-negative breast cancer (TNBC) studies, green synthesized AgNPs were reported to increase the expression of Bax and p53 and decrease the expression of Bcl-2, indicating the activation of the apoptosis pathway in MDA-MB-231 cells [3]. In addition, the study also showed an increase in the expression of PERK and CHOP, which indicates the involvement of the endoplasmic reticulum (ER) stress mechanism in the apoptosis process of cancer cells. Thus, changes in the regulation of apoptosis proteins and activation of the PERK–CHOP pathway are thought to contribute to the cytotoxic effects of AgNPs on TNBC.

Although the potential of green synthesized AgNPs as anticancer agents has been widely reported, there are still significant scientific gaps in the current literature regarding the specific molecular mechanisms underlying their selectivity and toxicity to TNBC cell lines. Most previous studies have tended to focus on optimization of synthesis parameters and macroscopic cytotoxicity assays (such as IC values of 50), without comprehensively mapping the intracellular signaling pathways involved. In addition, the variability of the types of bioreducers used in green synthesis results in diverse physicochemical characteristics of AgNPs, which directly affect the efficiency of intracellular ROS formation.

A number of studies have also reported the potential of green synthesized AgNPs in inhibiting proliferation and inducing cell death in various types of cancer, including breast cancer. However, the results of these studies are still scattered and show variations in terms of plant material sources, synthesis methods, nanoparticle characteristics, and the mechanisms of action involved. In addition, studies that specifically integrate the role of ROS-mediated apoptosis mechanisms in TNBC are still limited and have not been comprehensively synthesized.

Based on this, a literature review is needed to thoroughly examine the potential of *silver nanoparticles* resulting from green synthesis as cytotoxic agents against *triple-negative breast cancer cells*. This study aims to analyze the cytotoxic potential of silver nanoparticles resulting from green synthesis against triple-negative breast cancer cells and examine the mechanism of apoptosis mediated by reactive oxygen species (ROS) based on the findings of the latest research.

Through the integration of data from various literatures, this study is expected to make a strong theoretical contribution to the advancement of science in the field of nanomedicine, especially in laying a strong scientific basis for the design and standardization of nano-chemotherapy-based materials that are safer, selective, and more effective in the future.



Schematic 1. Conceptual overview of this review, illustrating the pathway from green synthesis of plant-derived silver nanoparticles (AgNPs) through physicochemical characterization, cytotoxic activity against triple-negative breast cancer (TNBC) cells, reactive oxygen species (ROS) generation, and the resulting ROS-mediated apoptotic pathway, concluding with current challenges and future perspectives.

1. Plant-Mediated Green Synthesis of Silver Nanoparticles

Various studies show that the ability of plants to synthesize silver nanoparticles is influenced by the content of their secondary metabolites. Phytochemical compounds such as flavonoids, phenolics, alkaloids, terpenoids, proteins, and polysaccharides have active functional groups that are able to play a role in the process of reducing Ag^+ ions into neutral silver atoms (Ag^0). The content of these secondary metabolites is different in each type of plant so that it can affect the size, shape, stability, and biological activity of the nanoparticles produced. This has led many researchers to start exploring different types of medicinal plants as well as local plants as a source of natural ingredients in the synthesis of AgNPs [10].

Green synthesis process usually begins when a solution of silver nitrate ($AgNO_3$) is mixed with plant extracts. Plant extracts contain a variety of secondary metabolite compounds, such as polyphenols, terpenoids, polysaccharides, alkaloids, and other bioactive compounds that play a role in the process of reducing $AgNO_3$ to silver (Ag). The reduction mechanism begins when $AgNO_3$ dissociates in water into Ag^+ and NO_3^- ions. Active functional groups, especially hydroxyl (–OH) and carboxyl (–COOH) groups found in phytochemical compounds, are acidic so they are able to release H^+ ions and produce negative charges. The negative charge in the functional group then interacts electrostatically with the Ag^+ ion. This interaction causes the Ag^+ ion to be reduced to a neutral silver atom (Ag^0). On the other hand, NO_3^- ions will bind to H^+ ions released by phenol groups as well as organic acids to form HNO_3 that remains soluble in the water phase. The Ag^0 atoms that are formed then undergo a process of nucleation and aggregation to produce silver nanoparticles (AgNPs) [11].

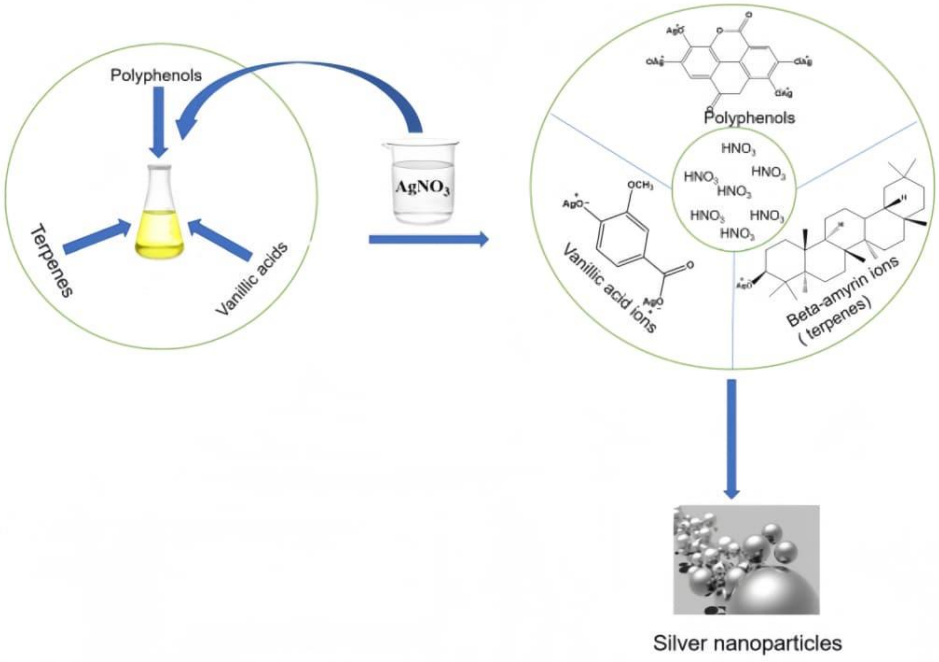


Figure 1. Mechanism of AgNO3 reduction into AgNPs [11].

Flavonoids are one of the compounds most often reported to play a role in the biosynthesis of silver nanoparticles [6]. This is because flavonoids have hydroxyl groups that are able to donate electrons effectively, thereby accelerating the process of metal ion reduction[12]. In addition to flavonoids, phenolic compounds are also known to have high antioxidant activity so they are very helpful in electron transfer during the synthesis process [6]. Meanwhile, alkaloids and terpenoids contribute to the formation and stabilization of nanoparticles through the interaction of their active functional groups with the surface

of AgNPs [13]. The existence of these various compounds makes plant extracts very effective for use as natural ingredients in the synthesis of nanoparticles.

The literature also shows that green synthesized silver nanoparticles have various advantages over conventionally synthesized nanoparticles. This method is relatively less expensive because it utilizes natural ingredients that are easy to obtain and do not require complicated equipment. In addition, green synthesis does not produce harmful chemical wastes so it is safer for the environment. The resulting nanoparticles also tend to have better stability and biocompatibility due to the presence of natural compounds that coat their surfaces. Therefore, green synthesized AgNPs have great potential to be developed in various fields, especially in the health and biomedical fields [14]. In the biomedical field, silver nanoparticles are widely researched because they have a wide range of biological activities, such as antibacterial, antioxidant, anti-inflammatory, antifungal, and anticancer [15]. Several studies have shown that AgNPs are able to inhibit the growth of various types of pathogenic bacteria by damaging cell membranes and disrupting the metabolism of microorganisms. In addition, AgNPs are also widely developed as cytotoxic agents against cancer cells [3].

Table 1. Summary of Various Plant Extracts in the Green Synthesis of Silver Nanoparticles (AgNPs)

Plants	Main Bioactive Compounds	Role in AgNP Synthesis	Biological Activity	Reference
Curcuma longa	Curcuminoids, flavonoids, phenolic compounds	Reducing and capping agents	High antibacterial activity	[63]
Ocimum sanctum	Eugenol, flavonoids, alkaloids	Nanoparticle stabilizers	Antioxidant and cytotoxic activities	[64]
Phyllanthus emblica	Vitamin C, tannins, phenolic compounds	Bioreductants of Ag^{+} ions	Antimicrobial activity	[65]
Andrographis paniculata	Andrographolide	Natural reducing agent	Oral antibacterial activity	[66]
Calotropis procera	Flavonoids, terpenoids	Reduction of Ag^{+} to Ag^{0}	Antimicrobial activity	[67]
Echinops kebericho	Flavonoids, phenolic compounds	Reducing and stabilizing agents	Antibacterial activity	[68]

2. Characterization of Plant-Derived AgNPs

Characterization of green synthesized silver nanoparticles (AgNPs) is an important stage to ensure the successful formation of nanoparticles and to know their physical and chemical properties. These characteristics include particle size, morphological shape, particle distribution, stability, crystal structure, and functional groups of organic compounds involved in the synthesis process. In plant-based synthesis, plant extracts act as a reducing agent as well as a capping agent because they contain

secondary metabolite compounds such as flavonoids, phenolics, alkaloids, and terpenoids [16]. Green synthesis was developed as an environmentally friendly method because it utilizes the bioreduction capabilities of plant extracts without the use of toxic chemicals [17].

2.1 Characteristics of AgNPs of Plant Extracts

The characteristics addressed in this section specifically encompass particle size and shape (morphology) as the two fundamental parameters that are evaluated first, which are then complemented by other physicochemical parameters such as functional groups, crystal structure, and colloidal stability discussed in the following subsections. Characterization of AgNPs is generally carried out using UV-Visible Spectroscopy (UV-Vis), Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) [18].

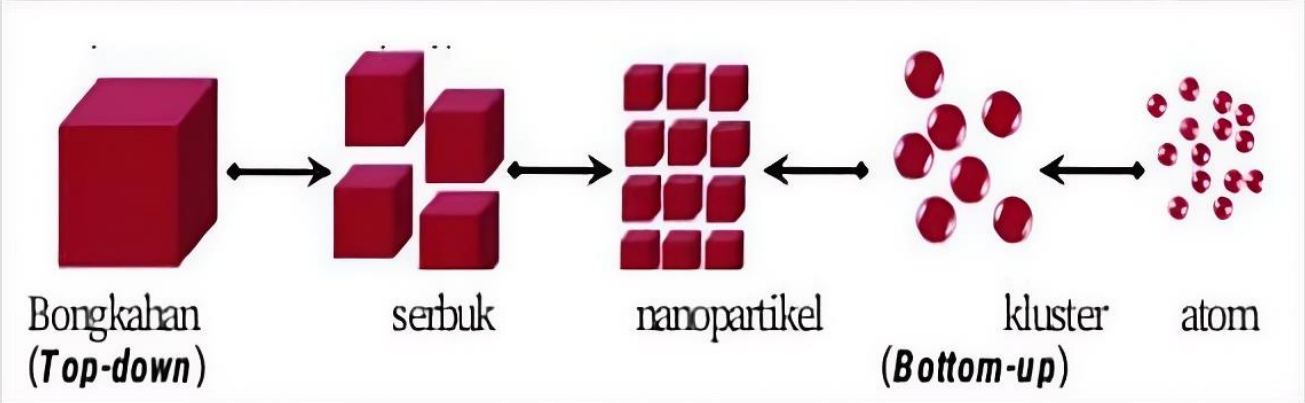


Figure 2. Method of synthesis of silver nanoparticles [19].

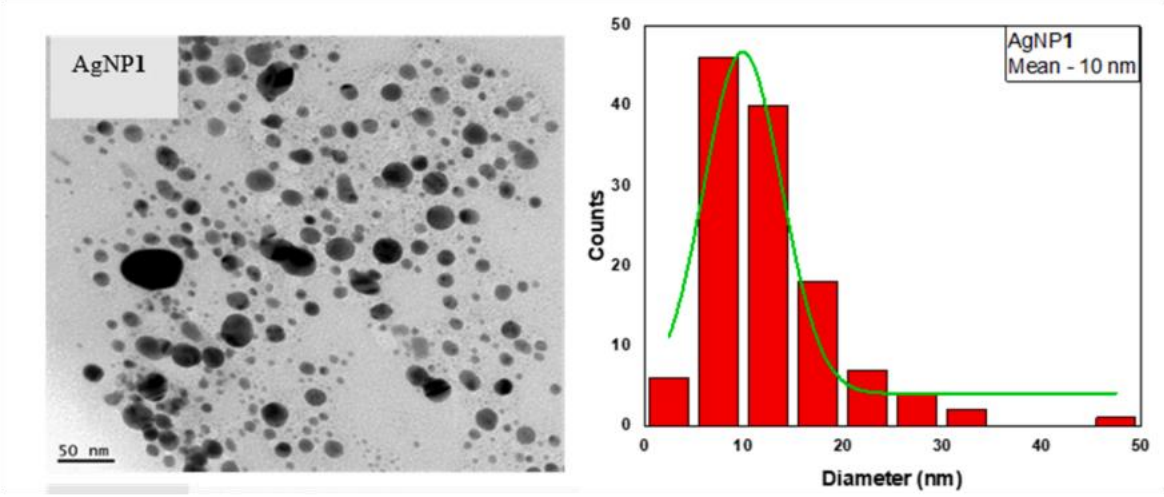


Figure 3. Illustration of the morphology and structure of AgNPs based on TEM characterization, showing the spherical shape and particle-size distribution at the nanoscale [69].

Size and morphology are the most basic parameters that are first evaluated in the characterization of AgNPs resulting from *green synthesis*. The size of the nanoparticles is a key characteristic that determines the biological activity of AgNPs. The smaller the size of the nanoparticles, the larger the relative surface area so that the interaction with the cell membrane increases [20]. Small-sized nanoparticles also enter target cells more easily and trigger the formation of reactive oxygen species (ROS) [21].

Silver nanoparticles from Indonesian plants are generally in the range of 10–100 nm, with the range of 10–30 nm being the most therapeutically relevant to cancer cells [16].

The shape of the nanoparticles also affects the biological effectiveness of AgNPs. Green synthesized AgNPs are generally spherical, although under certain synthesis conditions they can form irregular nanoparticles or undergo aggregation [22]. The spherical/spherical shape is considered more stable because it has a more even surface distribution so that the interaction with the cell membrane becomes more effective [23]. In addition, spherical nanoparticles are more easily dispersed and have a lower aggregation tendency than irregular shapes [22].

In the development of cancer therapies, the shape of nanoparticles is important because it affects the process of cellular internalization. Homogeneous-shaped nanoparticles more easily enter cancer cells and increase cytotoxic effectiveness [24]. Various studies have shown that small-sized spherical AgNPs have better cellular internalization and are able to increase the formation of intracellular reactive oxygen species (ROS) so that their cytotoxic effectiveness against TNBC cells is higher [25].

UV-Visible Spectroscopy (UV-Vis) is performed to prove the successful formation of AgNPs. The appearance of typical absorbance peaks in the wavelength range of 380–450 nm is the first optical evidence of the formation of silver nanoparticles, and this phenomenon is known as Surface Plasmon Resonance (SPR) [26].

Once the morphology is known, the next step is to use X-Ray Diffraction (XRD) to ensure that the particles formed are indeed silver in the desired crystalline phase. The diffraction pattern of XRD AgNPs from green synthesis consistently showed characteristic peaks at 2θ angles of approximately 38°, 44°, 64°, and 77°, corresponding to the crystal fields (111), (200), (220), and (311) of the face-centered cubic structure (FCC) of silver [26].

FTIR (Fourier Transform Infrared Spectroscopy) analysis plays an important role in identifying organic compounds from plant extracts involved in the reduction and capping processes during the synthesis of AgNPs. The spectrum of FTIR AgNPs resulting from green synthesis generally features several distinctive absorbance bands. Wide bands in the range of 3200–3500 cm^{-1} indicate O–H longitudinal vibrations originating from hydroxyl polyphenol groups or alcohols. The bands at about 1620–1650 cm^{-1} correspond to the C=O vibrations of the carbonyl group, which are commonly found in flavonoids and organic acids. Meanwhile, bands in the range of 1050–1200 cm^{-1} indicate C–O vibrations from ethers or alcohols, and weak bands at 1530–1550 cm^{-1} indicate N–H vibrations from amide groups that may be derived from proteins or peptides in plant extracts [27]. The presence of these functional groups on the FTIR spectrum of AgNPs that differ from the spectrum of plant extracts proves that these biomolecules are not merely present as contaminants, but are indeed actively bound to the surface of AgNPs and form a functional capping layer [28].

Furthermore, the composition of the element AgNPs is proven using *the Energy Dispersive X-ray Spectroscopy* (EDX/EDS) technique, generally carried out in an integrated manner with SEM or TEM, and produces a spectrum with a peak characteristic of the element Ag at an energy of about 3 keV, which is direct evidence of the presence of silver in the sample [29].

Characterization using Transmission Electron Microscopy (TEM) is carried out by firing a high-intensity electron beam at a very thin sample to produce high-resolution nanoparticle images. This technique allows for detailed observation of the size, shape, morphology, and distribution of nanoparticles. In TEM, a denser part of the sample inhibits electron transmission so that it appears darker in the resulting image [30].

Meanwhile, Scanning Electron Microscopy (SEM) works with the principle of scanning the surface of a sample using a high-energy electron beam to describe the surface morphology of the particle. Electrons reflected or emitted back from the sample surface will be captured by the detector and processed into a high-resolution surface topographic image [30]. Through SEM characterization, the surface shape, particle distribution, and aggregation rate of AgNPs can be observed more clearly. TEM and SEM techniques are widely used in the characterization of AgNPs because they are able to provide accurate information on the morphology of nanoparticles [31].

The general characteristics of green synthesized AgNPs from several studies in Indonesia can be seen in the following table:

Table 2. Comparison of the Physicochemical Characteristics of AgNPs from Various Plant Extracts

Plants	**UV-Vis**	**XRD**	**SEM**	**TEM**	**Shape**	**Potential Zeta**	**Cytotoxic Activity**	**Reference**
Mangrove Rhizophora stylosa	420 – 438 nm	38 – 77 nm	-	10 – 60 nm	Spherical	-	-	[18]
plant extract-mediated AgNPs	426 nm	16.4 nm		12-25 nm	Spherical	−25.6 ± 1.9 mV	-	[29]
Zinnia elegans L	462 nm	18 – 36 nm		25 nm	Spherical		High	(32)
Euphorbia fusiformis	412 - 439 nm	9,05 – 17, 9	3,55 – 16,42 nm	3,55 – 16,42 nm	Spherical	-19.5 ± 0.6 mV	High	(33)
Melaleuca alternifolia	402 nm	25,47 nm	-	10 nm	Cubic	-21 mV	High	(34)

2.2 Colloidal and Zeta Stability Potential

The colloidal suspension stability of AgNPs was evaluated quantitatively using potential zeta measurements via Doppler laser electrophoresis technique. Particles are considered colloidal stable if the zeta potential value is above 30 mV, a large potential zeta is needed to prevent silver particles from aggregating due to the presence of an attractive force so that it can be used to describe the stability of nanoparticles. The positive and negative values of the potential zeta value indicate the

type of charge on the surface of the nanoparticle. A negative value at the potential zeta indicates the formation of silver nanoparticles because silver ions that were originally positively charged lose their charge and form silver particles (Ag0) due to [35].

2.3 The Relationship of Physicochemical Characteristics with Bioactivity to TNBC

The structure-activity relationship of AgNPs to triple-negative breast cancer (TNBC) cells includes three interrelated dimensions. First, in terms of size, small nanoparticles exhibit higher cytotoxic activity because they have a larger surface area to volume ratio, thereby increasing the release of Ag^+ ions and the production of reactive oxygen species (ROS). Small-sized particles are also more easily internalized into the cell and can directly disrupt DNA stability [25]. In addition, the size of the nanoparticles affects the mechanism of cellular internalization, where very small particles tend to diffuse or enter cells more easily than larger particles [24].

Second, the surface charge of the nanoparticles also determines the interaction of AgNPs with the cancer cell membrane. Green synthesized AgNPs generally have a negative surface charge due to the presence of capping biomolecules from plant extracts, but are still able to interact with TNBC cell membranes and undergo effective internalization through endocytic or phagocytic pathways [25].

Third, the composition of phytochemical capping agents makes a synergistic contribution to the bioactivity of AgNPs. Characterization of these capping agents is generally performed by comparing the FTIR spectra of the AgNPs with those of the raw plant extract; a shift or change in the intensity of the absorbance bands of specific functional groups (e.g., hydroxyl and carbonyl groups, see Section 2.1) in the AgNP spectrum indicates that the phytochemical compounds are actively bound to the nanoparticle surface and form a functional capping layer, rather than merely being unbound extract residue. AgNPs based on *Curcuma longa* are known to contain bioactive compounds such as curcuminoids that have anticancer activity and are able to increase the cytotoxic effects of nanoparticles. The combination of phytochemical activity and silver nuclei can increase ROS production, activate apoptosis pathways, as well as increase the expression of caspase-3 in cancer cells [36]. This synergistic effect causes the value of green synthesis AgNPs to be lower than conventional silver nanoparticles at equivalent concentrations, the greater the contribution of phytochemical capping to cytotoxicity, the smaller the AgNP concentration required to achieve an equivalent cytotoxic effect. These three factors do not work independently, but rather form an integrated bioactivity profile that forms the basis for the development of green synthetic AgNPs as candidates for selective cytotoxic agents against TNBC.

3. Cytotoxic Potential of Plant-Synthesized AgNPs Against TNBC Cells

3.1 Synthetic activity of AgNPs against Cells

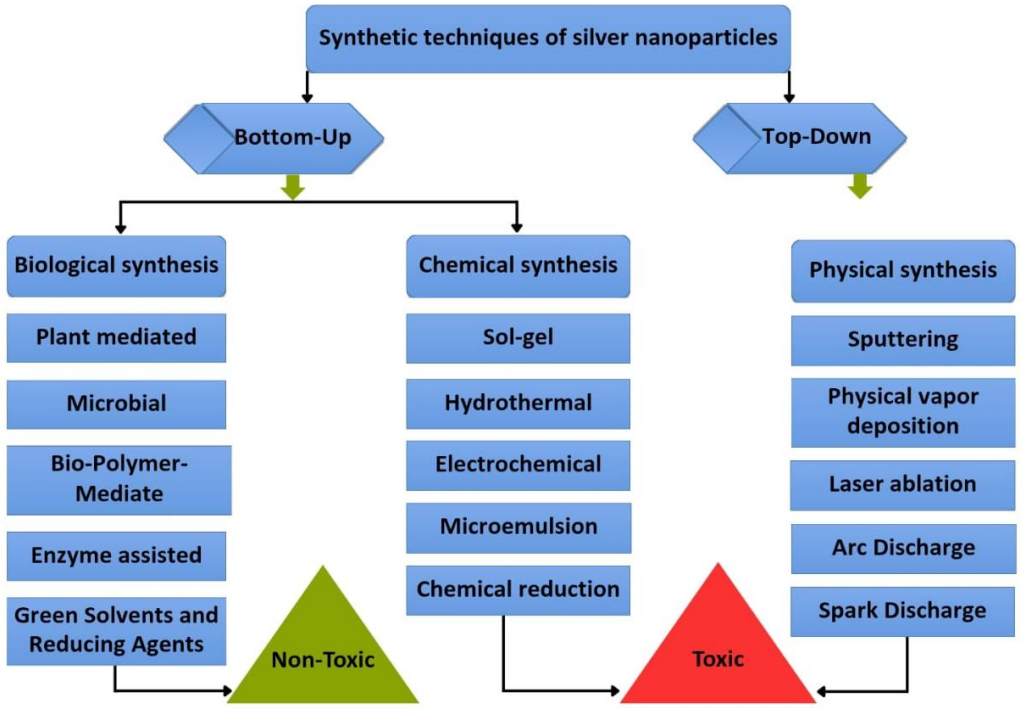


Figure 4. Schematic diagram for different synthetic techniques of AgNPs

Literature review shows that silver nanoparticles (AgNPs) from plant-based green synthesis have cytotoxic capabilities against triple-negative breast cancer cells (TNBC)[7]. These cytotoxic activities are shown through decreased cancer cell proliferation, changes in cell morphology, and increased cell death rates after administration of AgNPs. In AgNPs synthesized using Rubus ellipticus extract, TNBC cells undergo a smaller shape, cytoplasmic shrinkage, membrane damage, and decreased cell density [40].

The cytotoxic effect of AgNPs is related to the small size of the nanoparticles so that their surface area becomes larger and facilitates interaction with the cancer cell membrane. These interactions cause nanoparticles to enter the cell more easily and trigger oxidative stress through increased production of reactive oxygen species (ROS). Excessive ROS accumulation then leads to damage to cellular components such as lipids, proteins, DNA, and mitochondria and eventually triggers apoptosis [40].

3.2 IC_{50} (Half Maximal Inhibitory Concentration)

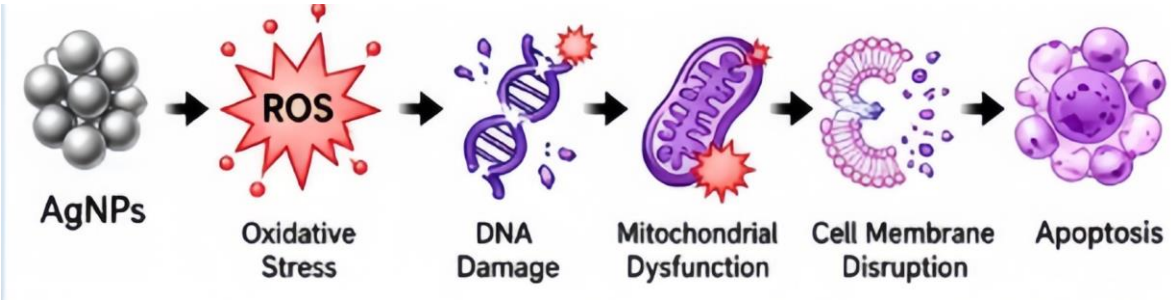


Figure 5. Mechanism of AgNP- Induced Reduction in TNBC Cell Viability[38]

The cytotoxic activity of AgNPs is generally analyzed using the IC_{50} (half maximal inhibitory concentration) parameter, which is the concentration of nanoparticles that are able to inhibit 50%

of cancer cell growth. The IC_{50} value is used as an indicator of the anticancer effectiveness of a compound. The smaller the IC_{50} value, the higher the cytotoxic ability of the nanoparticles against TNBC cells because the inhibiting effect has already occurred at low concentrations. The IC_{50} value is usually obtained through regression analysis between the concentration of AgNPs and the percentage of cell inhibition. The relationship graph shows that an increase in the concentration of nanoparticles will increase the percentage of inhibition against cancer cells [38].

3.3 Cell Viability

Cell viability is a parameter used to determine the ability of cancer cells to survive after treatment of AgNPs. Cell viability measurements are generally performed using the MTT assay method [6]. This method is based on the ability of living cell mitochondrial enzymes to reduce MTT compounds into purple formazan crystals. The higher the number of living cells, the more intense the purple color will form. In contrast, a decrease in color intensity indicates a reduced number of living cells due to the toxic effects of nanoparticles. The results of the study showed that the administration of AgNPs caused a significant decrease in the viability of TNBC cells compared to the control group. The decrease in viability occurs due to increased ROS which causes oxidative stress, DNA damage, impaired mitochondrial function, and ultimately triggers apoptosis in cancer cells [39].

3.4 Dose-Dependent Effect

The cytotoxic activity of AgNPs is dose-dependent, that is, the higher the concentration of nanoparticles given, the higher the cancer cell death rate. At low concentrations, AgNPs generally only inhibit the proliferation and metabolic activity of cells. However, at higher concentrations, AgNPs can cause cell membrane damage, ion imbalances, organelle function disorders, and apoptosis[7]. Green-synthesized AgNPs from Rubus ellipticus extract were reported to have an IC_{50} value of 30.12 µg/mL against MDA-MB-231 cells after a defined incubation period, comparable to the effectiveness of the standard chemotherapeutic drug 5-fluorouracil [40]. In another study on synthetic AgNPs against TNBC cells, the mean IC_{50} value was reported to be approximately 18.75 µg/mL, with cytotoxic effects increasing at two-fold (37.5 µg/mL) and four-fold (75 µg/mL) that IC_{50} value [25]. These figures confirm that the cytotoxic effectiveness of AgNPs depends heavily on the type and source of the nanoparticles, although the general pattern of increasing cytotoxic effect with increasing dose was consistently observed across the reviewed studies.

In addition to concentration, the length of exposure also affects the level of toxicity of nanoparticles to TNBC cells. The longer the cells are exposed to AgNPs, the greater the ROS accumulation and cell damage that occurs. Therefore, the relationship between concentration and exposure time is an important factor in determining the cytotoxic effectiveness of AgNPs against cancer cells [7].

3.5 Selectivity against Cancer Cells

AgNPs derived from plant-based green synthesis are reported to have a fairly good degree of selectivity against cancer cells compared to normal cells [6]. This selectivity is important in the development of cancer therapies because a good anticancer agent is expected to be able to kill cancer cells without damaging too many surrounding healthy cells. The content of secondary metabolites such as flavonoids, phenolics, and terpenoids from plant extracts is thought to increase the cytotoxic effectiveness of AgNPs against TNBC cells. In addition, cancer cells generally have higher metabolic rates and ROS production than normal cells so they are more sensitive to increased oxidative stress due to exposure to nanoparticles[7]. Therefore, plant-based AgNPs have great potential to be developed as alternative therapeutic agents in triple-negative breast cancer [40].

4. ROS Generation Induced by Plant-Derived AgNPs

Plant-derived silver nanoparticles (AgNPs) are known to be able to induce the formation of reactive oxygen species (ROS) which play an important role in the mechanism of cancer cell death. Mechanistically, after plant-based AgNPs are internalized by cancer cells through the process of endocytosis, the acidic intracellular environment of cancer cells will trigger the dissolution of those nanoparticles to release silver ions (Ag+). The interaction between the nucleus of the nanoparticles and these released Ag+ ions with the cellular component is the main trigger for a massive spike in free radical emissions. Based on the study [41], ROS is a reactive molecule that is formed as a by-product of cellular metabolism and consists of several main types, such as superoxide anion (O2•−), hydrogen peroxide (H2O2), and hydroxyl radical (OH•). The formation of ROS occurs through the gradual reduction process of oxygen molecules. The oxygen molecule receives one electron to form a superoxide, which then undergoes conversion to hydrogen peroxide and hydroxyl radicals through a series of redox reactions. The reaction of ROS formation can be described as follows:

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$

$$H_2O_2 + e^- \rightarrow HO^{\bullet} + OH^-$$

$$HO^{\bullet} + e^- + 2H^+ \rightarrow 2H2O$$

The formation of ROS can also occur through the Haber–Weiss & Fenton reaction

$$Fe_3^+ + O_2^{\bullet -} \rightarrow Fe_2^+ + O_2$$

$$Fe_2^+ + H_2O_2 \rightarrow Fe_3^+ + HO^{\bullet} + OH^-$$

Net reaction:

$$O_2^{\bullet -} + H_2O_2 \rightarrow HO^{\bullet} + OH^- + O_2$$

Based on the study, ROS is produced from various intracellular sources such as mitochondria, *NADPH oxidase (NOX), xanthine oxidase*, and endoplasmic reticulum. Mitochondria are known to be the main source of ROS formation due to electron leakage in the *electron transport chain (ETC)*, especially in complexes I and III. The leaking electrons will react with oxygen molecules and produce superoxides which further trigger the formation of other ROS. In addition, the NOX enzyme also plays a role in the production of ROS through the transfer of electrons from NADPH to oxygen resulting in superoxide and hydrogen peroxide. Excessive ROS accumulation can cause redox imbalances and trigger oxidative stress that leads to lipid, protein, DNA, and mitochondrial dysfunction [41].

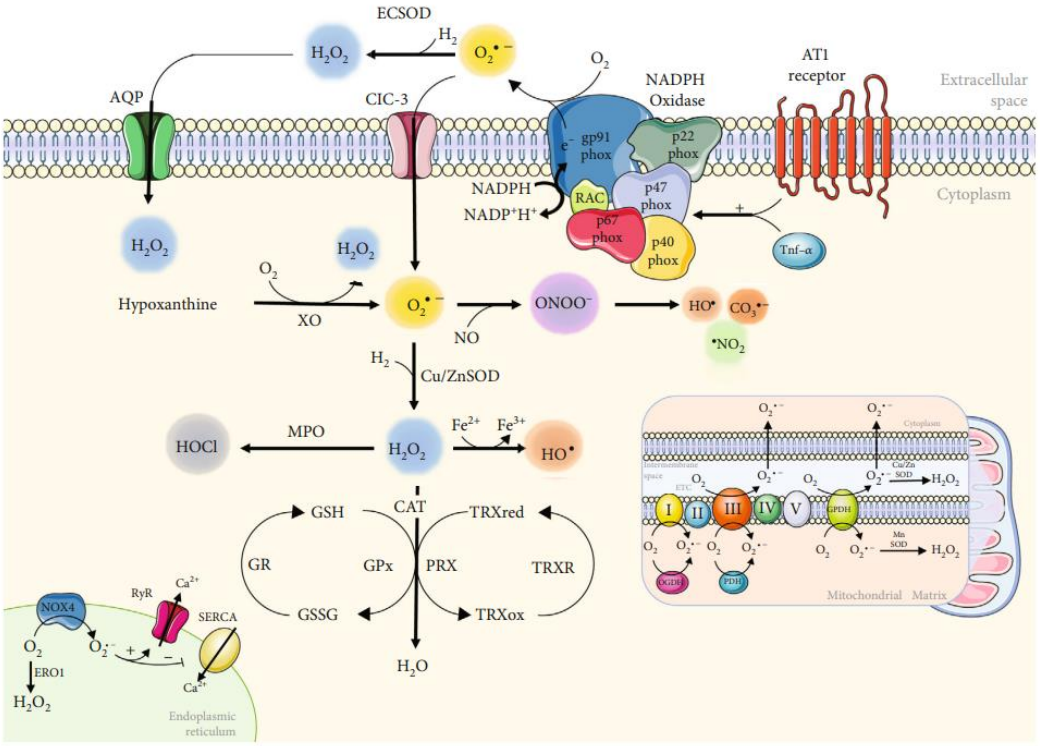


Figure 6. Mechanisms of formation of reactive oxygen species (ROS) from various intracellular sources and cellular antioxidant systems [41].

In relation to nanoparticle cytotoxicants, *exposure to plant-derived AgNPs* is specifically capable of triggering severe mitochondrial dysfunction. Based on the literature from [42]. plant extract-based silver nanoparticles that enter the cytoplasm will release Ag+ ions which then interact with electron transport chain (ETC) proteins in mitochondria. The adhesion of these metal ions interferes with the normal flow of electrons, causing intracellular electron leakage to increase many times over, and resulting in a sharp spike in superoxide production. This condition is supported by a study from [43]. which confirms that the cytotoxic effects of nanosilver mediated by plant extracts act directly through ROS-dependent oxidative stress enhancement pathways to impair the intracellular homeostasis of cancer cells.

Several studies report that oxidative stress artificially induced by plant-based AgNPs plays an important role in the development and death of cancer cells through an increase in the level of reactive oxygen species (ROS). According to [18], oxidative stress occurs when ROS production exceeds the capacity of the cell's antioxidant system to maintain a redox balance. This condition causes excessive ROS accumulation that triggers the breakdown of various cellular biomolecules, including lipids, proteins, and DNA [44].

[45] explains that increased ROS can also interfere with mitochondrial function through decreased mitochondrial membrane potential as well as trigger the release of cytochrome c and the activation of caspase which plays a role in the apoptosis pathway (Figure 5.2). This damaging effect is amplified by the release of Ag+ ions that chemically bind strongly to thiol (-SH) groups in the cellular antioxidant defense system such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) which function to neutralize ROS [45]. As a result, the binding by these Ag+ ions depreciates and drastically

decreases the capacity of the cell's antioxidant system, making oxidative stress levels and cell damage increasingly uncontrollable [18].

In cancer cells, including triple-negative breast cancer (TNBC), elevated ROS levels triggered by *plant-derived AgNPs* treatment are known to push cells past oxidative stress tolerance thresholds, activate an intrinsic apoptosis cascade, and ultimately trigger programmed cell death [44 43].

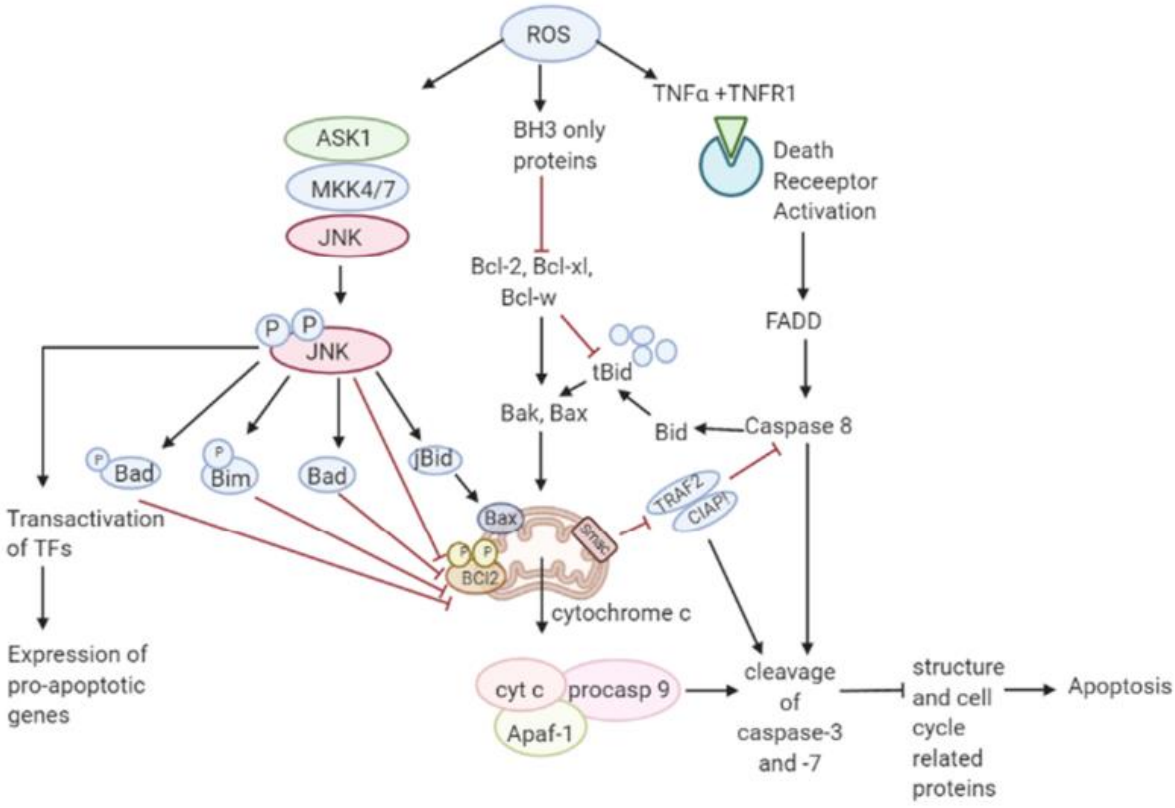


Figure 7. Molecular mechanisms of oxidative stress and ROS accumulation in inducing intrinsic apoptosis pathways of cancer cells [44].

5. ROS-Mediated Apoptotic Pathway in TNBC Cells

Based on the results of various studies, *silver nanoparticles* (AgNPs) from green synthesis showed significant cytotoxic activity against *triple-negative breast cancer* cells (TNBC), mainly through the mechanism of apoptosis mediated by an increase in *reactive oxygen species* (ROS). An increase in intracellular ROS is one of the main mechanisms that plays a role in triggering oxidative stress in cancer cells [9]. Under physiological conditions, ROS plays a role in a variety of biological processes, including proliferation and cellular signaling. However, excessive ROS accumulation can lead to disruption of cellular homeostasis and activate intrinsic apoptosis pathways. In TNBC cells, specifically the MDA-MB-231 cell line, green synthesized AgNPs are reported to be able to increase ROS levels beyond the capacity of the cell's antioxidant system, thereby initiating a series of molecular changes that end in programmed cell death.

AgNPs from green synthesis are known to exhibit cytotoxic activity to TNBC cells through increased ROS production which contributes to the induction of apoptosis. In MDA-MB-231 cells,

AgNPs can enter the cell through the process of endocytosis and cause an increase in intracellular ROS accompanied by inhibition of cell proliferation [46]. In addition, TNBC cells are known to have relatively higher levels of basal oxidative stress than normal cells due to high metabolic activity and rapid cell proliferation rates. This condition causes TNBC cells to be at a higher threshold of redox tolerance than normal cells [47]. Therefore, an increase in ROS due to exposure to AgNPs can exceed the capacity of cellular antioxidant systems, disrupt the balance of homeostasis, and initiate intrinsic apoptosis pathways.

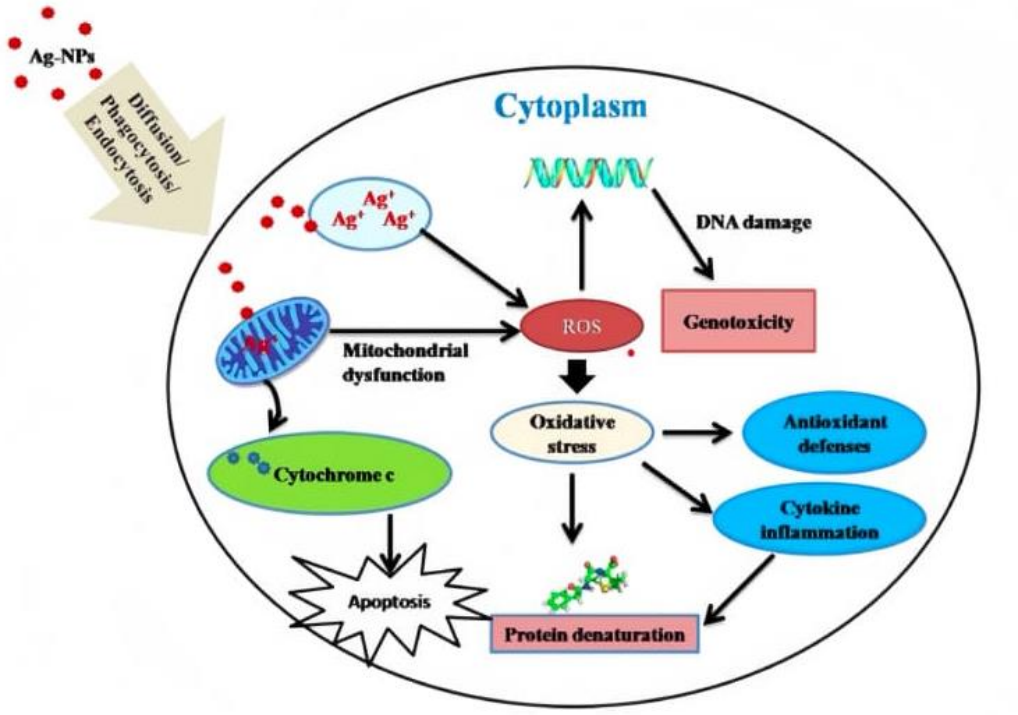


Figure 8. Mechanism of ROS-Mediated Apoptosis by AgNPs [70]

The increase in ROS that occurs subsequently affects the function of mitochondria, which play an important role as the center of energy metabolism as well as the main source of intracellular ROS formation. Exposure to AgNPs is known to cause impaired mitochondrial function characterized by depolarization of the mitochondrial membrane and increased production of mitochondrial ROS [48]. The increase in ROS results in oxidative stress that interferes with cellular homeostasis and becomes the initial stage in the activation of intrinsic apoptosis pathways. Excessive ROS accumulation can damage various important components of mitochondria, such as mitochondrial membranes, mitochondrial DNA (*mitochondrial DNA*/*mtDNA*), as well as various enzymes that play a role in energy metabolism. This damage causes a decrease in mitochondrial function and interferes with the cell's ability to maintain its physiological balance. If this condition persists, cellular damage will expand and trigger programmed cell death or apoptosis. In addition to playing a role in the mechanism of cell death, oxidative stress due to ROS accumulation is also known to contribute to the development of a variety of chronic diseases, including cancer, diabetes mellitus, and neurodegenerative diseases [49].

Excessive ROS accumulation can also disrupt the integrity of mitochondrial outer membranes through the induction of oxidative stress that triggers *mitochondrial outer membrane permeabilization* (MOMP) [50]. MOMP is an important stage in the intrinsic apoptosis pathway because it causes the release of various proapoptotic proteins, especially cytochrome-c, from the mitochondrial intermembrane space to the cytoplasm. Increased permeability of the outer membrane of mitochondria

is often considered a *point of no return* in the process of apoptosis because once proapoptotic proteins are released, the cell enters a series of irreversible death mechanisms [50]. Mitochondrial damage triggered by oxidative stress further activates the mitochondria-dependent intrinsic apoptosis pathway. These pathways are generally triggered by various internal factors, such as DNA damage, increased oxidative stress, and cellular metabolic disorders. Increased permeability of the outer membrane of mitochondria allows the release of cytochrome-c into the cytosol, which further binds to the adapter protein to form an apoptosome. The complex then activates the initiator caspase followed by the activation of the executor caspase as the main implementer of the apoptosis process. The sequence of processes suggests that mitochondria serve as the center of signal integration that determines cell survival or death [51]. The activation of the caspase pathway suggests that oxidative stress induced by AgNPs not only causes direct cellular damage, but also initiates a series of molecular signals that direct cells to apoptosis through an organized mechanism. This pathway has an important role in TNBC considering that an apoptosis-based approach has the potential to be an alternative strategy in inhibiting the proliferation of cancer cells that have limited therapeutic targets [46].

The activation of the intrinsic apoptosis pathway is also influenced by the regulation of Bcl-2 family proteins that play a role in controlling the permeability of mitochondrial membranes [52]. The balance between proapoptotic and antiapoptotic proteins has an important role in determining the fate of cells, i.e. maintaining physiological function or entering a programmed cell death pathway (Royhan et al., 2025). Bax protein is a member of the Bcl-2 family which is proapoptotic, while Bcl-2 acts as an antiapoptotic protein that maintains the integrity of the mitochondrial membrane and inhibits the release of proapoptotic proteins into the cytoplasm. Under physiological conditions, the expression of the two proteins is in a balanced state to maintain cellular homeostasis. However, oxidative stress conditions due to increased ROS can disrupt this balance, shifting cellular signals towards the process of apoptosis [53]. Increased ROS due to exposure to AgNPs is known to affect the regulation of Bcl-2 family proteins through increased expression of Bax proapoptotic protein and decreased expression of Bcl-2 antiapoptotic proteins. Such changes in the Bax/Bcl-2 ratio lead to increased permeability of the outer membrane of mitochondria which facilitates the release of cytochrome-c into the cytoplasm and strengthens the activation of intrinsic apoptosis pathways [52]. Increased Bax/Bcl-2 ratios are also often used as a molecular indicator to evaluate the effectiveness of anticancer agents. The increased Bax/Bcl-2 ratio shows the dominance of proapoptotic signals over antiapoptotic signals, thus increasing the tendency of cells to experience apoptosis rather than maintaining their survival. Several studies on AgNPs have shown that an increase in the Bax/Bcl-2 ratio correlates with decreased viability of cancer cells and increased apoptosis activity in TNBC cells [54]. The mechanism further strengthens the activation of intrinsic apoptosis pathways and contributes to the cytotoxic effects of AgNPs on TNBC cells [36].

In addition to affecting mitochondrial function, increased ROS production that exceeds the capacity of the cell's antioxidant system can also lead to damage to various cellular macromolecules, including DNA. DNA is one of the main targets of ROS because it is susceptible to structural changes due to oxidation reactions. Hydroxyl radicals (•OH) are known to have a very high level of reactivity and play an important role in oxidative DNA damage through direct interaction with DNA molecules [55] DNA damage due to ROS can be in the form of nitrogenous base oxidation, deoxyribose group damage, or disturbances in the structure of the DNA chain that produce various genetic lesions and disrupt genome stability [55]. The accumulation of DNA damage due to oxidative stress can cause *single-strand breaks* (SSBs), *double-strand breaks* (DSBs), and various other forms of DNA damage that can interfere with the normal functioning of cells. DNA damage that is not effectively repaired can result

in genome instability, disruption of the replication process, as well as changes in cellular function. Under these conditions, the cell will activate the *DNA damage response* (DDR) mechanism in an effort to maintain genome integrity [56]. The response to DNA damage involves the activation of sensor proteins, such as ATMs and ATRs, which act as key regulators in the DDR pathway [44]. Activation of the pathway can further stimulate the p53 protein known as the *guardian of the genome* because it plays a role in the regulation of DNA repair, cell cycle termination, senescense, and apoptosis. When the rate of DNA damage can no longer be repaired, p53 will direct the cell to a programmed cell death pathway through the mechanism of apoptosis [57].

Overall, apoptosis is a programmed cell death mechanism that plays an important role in maintaining tissue homeostasis and the balance of the number of cells in the body [71]. In cancer cells, including TNBC, disruption of apoptosis regulation is one of the main characteristics that allows cells to experience uncontrolled proliferation and resistance to anticancer therapies [58]. Exposure to green synthesized AgNPs is known to induce apoptosis in TNBC cells through an increase in ROS that triggers oxidative stress [59]. The accumulation of ROS causes disruption of mitochondrial function, changes in the Bax/Bcl-2 ratio, caspase activation, and DNA damage that ultimately leads to apoptosis [60 61]. Thus, *ROS-mediated apoptosis* is the main mechanism underlying the anticancer activity of green synthesized AgNPs against TNBC cells through various interrelated molecular targets.

6. Challenges, Future Perspectives, and Conclusion

Standardization of plant extracts is a major challenge in the development of plant-based AgNPs resulting *from green synthesis* as TNBC anticancer agents through *the ROS-mediated apoptosis mechanism*. Plant extracts contain bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, and terpenoids whose composition can vary depending on the type of plant, plant parts, extraction method, and environmental conditions [3]. The findings are reinforced by [13]. who explains that the type of natural bioreducer and the conditions of synthesis can affect the size, shape, stability, and biological activity of AgNPs. Therefore, the standardization of extracts through the determination of phytochemical profiles and characterization of functional groups is important so that the quality of the produced AgNPs is more consistent and *reproducible* [62].

In addition to the standardization of extracts, the *reproducibility aspect* is also an important challenge because the green synthesis process is greatly influenced by pH, temperature, incubation time, $AgNO_3$ concentration, and the ratio of extracts to metal precursors [62]. This statement is supported by [13] which confirms that changes in the physicochemical character of AgNPs can affect their biological activity. Therefore, characterizations such as UV-Vis, FTIR, XRD, SEM/TEM, DLS, and *zeta potential* need to be reported consistently in order for the relationship between nanoparticle characters and *ROS-mediated apoptosis* activity to be compared between studies.

In terms of mechanism, plant-based AgNPs have the potential to be cytotoxic agents because they are able to increase the excessive production of ROS in cancer cells [13]. Increased ROS can lead to oxidative stress, DNA damage, mitochondrial membrane disruption, as well as activation of apoptotic pathways. Similar results were also demonstrated by [54] who reported that *green-synthesized* AgNPs in MDA-MB-231 cells can enhance apoptosis through modulation of the Bax/p53/Bcl-2, PERK/CHOP, and HIF-1α pathways. Thus, green synthesis AgNPs not only act as silver nanoparticles, but also as a phytochemical-based nanomedicine system that has the potential to induce TNBC cell death through *ROS-mediated apoptosis*.

However, the toxicity aspect still needs to be considered. Green synthesis is indeed more environmentally friendly than conventional chemical methods, but AgNPs can still cause toxic effects on normal cells when used in high concentrations or long-term exposure [13]. In line with this, [62]

emphasizes that the main challenges in the development of AgNPs include *reproducibility*, scalability, and cytotoxicity. Therefore, selectivity tests on cancer cells and normal cells, hemocompatibility, biodistribution, pharmacokinetics, and *in vivo* tests are still needed before AgNPs can be directed as TNBC therapy candidates.

Opportunities for green nanotechnology-based cancer therapy are still very open, especially in the development of combination therapies for TNBC. Plant-based AgNPs can be developed as cytotoxic agents, drug delivery systems, and *radiosensitizers* to increase the effectiveness of therapies [54]. In this context, [54] reported that the combination of *green-synthesized* AgNPs with radiotherapy was able to increase cytotoxicity and apoptosis in MDA-MB-231 cells.

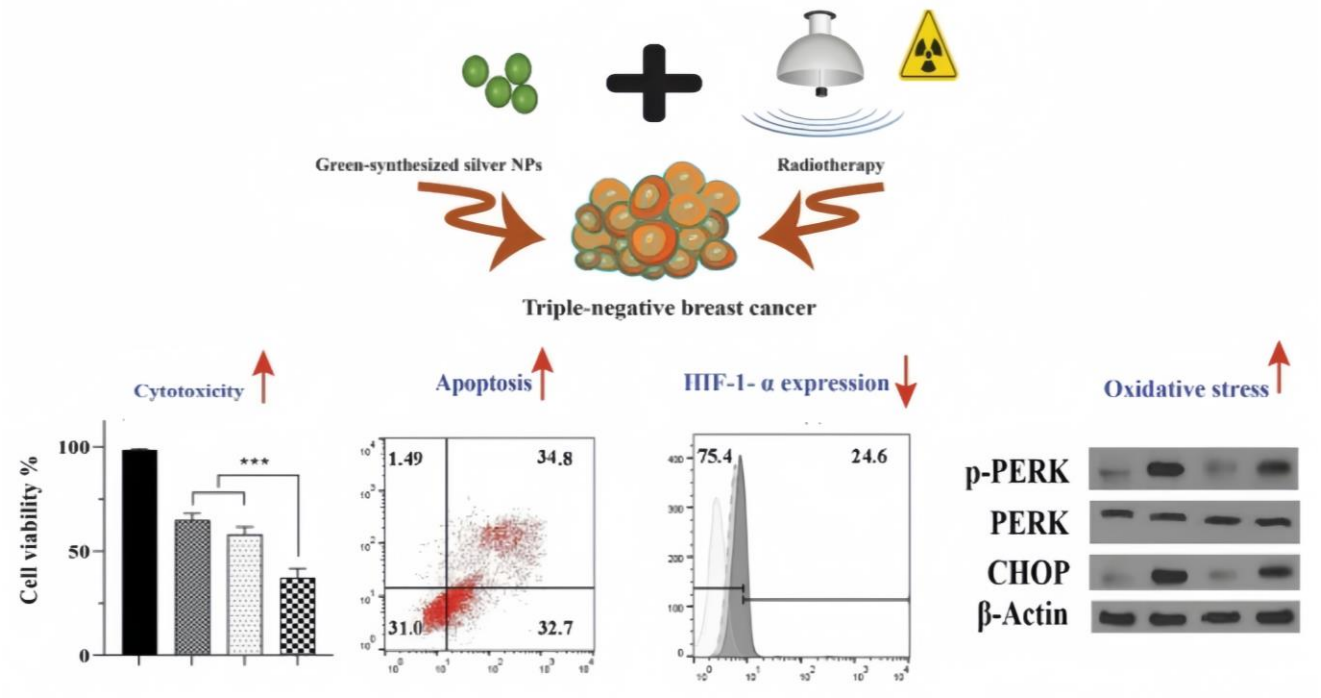


Figure 9. The potential *of green-synthesized* AgNPs as TNBC therapy through increased apoptosis and *oxidative stress* [54].

Thus, green synthesis plant-based AgNPs have a specific scientific identity as TNBC anticancer agents through *ROS-mediated apoptosis* mechanisms, but still require standardization, mechanism validation, and long-term safety evaluation in order to progress to clinical applications.

CONCLUSION

This literature review confirms that green-synthesized silver nanoparticles (AgNPs) derived from plant extracts possess significant cytotoxic potential against triple-negative breast cancer (TNBC) cells, primarily through reactive oxygen species (ROS)-mediated apoptosis. Plant secondary metabolites such as flavonoids, phenolics, alkaloids, and terpenoids function simultaneously as reducing and capping agents during synthesis, yielding AgNPs with sizes predominantly in the therapeutically relevant range of 10–30 nm, spherical morphology, and stable colloidal properties. These physicochemical characteristics directly influence the intracellular bioactivity of the nanoparticles. Upon internalization into TNBC cells through endocytosis, green-synthesized AgNPs induce a massive intracellular ROS surge that exceeds the cellular antioxidant capacity, triggering oxidative stress, mitochondrial membrane depolarization, mitochondrial outer membrane permeabilization, and release of cytochrome c. These events activate the intrinsic apoptotic pathway through upregulation of pro-apoptotic proteins Bax and p53, downregulation of anti-apoptotic Bcl-

2, and sequential activation of caspase cascades, ultimately resulting in programmed cell death. Furthermore, oxidative DNA damage mediated by hydroxyl radicals activates the DNA damage response and reinforces apoptotic signaling via p53. The phytochemical constituents of the capping layer contribute synergistically to these anticancer effects. Despite this considerable promise, challenges related to extract standardization, synthesis reproducibility, and long-term biosafety remain. Future research should focus on rigorous in vivo validation, pharmacokinetic profiling, and the development of targeted nanoformulations combining green-synthesized AgNPs with existing chemotherapeutic regimens to advance their translation toward clinical application in TNBC therapy.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Department of Natural Science, Faculty of Mathematics and Natural Science, Universitas Negeri Surabaya, for providing the academic environment and institutional support that facilitated the completion of this study. The authors also acknowledge all researchers and scholars whose published works formed the scientific basis of this literature review.

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