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APPLICATION OF PLANT-BASED METAL NANOMEDICINE IN TREATMENT OF VARIOUS TYPES OF CANCER: A REVIEW

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ABSTRACT

Background: Plant-based nanomedicine is a rapidly growing field of research that utilizes plant-derived nanoparticles for the treatment of cancer. Nanoparticles from plant extracts have a number of advantages over synthetic nanoparticles, including biocompatibility, low toxicity, and ease of synthesis **Objective:** This article comprehensively explores the synthesis, characterization, and anticancer activities of silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO-NPs), and gold nanoparticles (AuNPs) synthesized using various plant extracts. **Methods:** The research method used in this article is Systematic Literature Review (SLR). The research method used in this article is the Systematic Literature Review (SLR). The data are collected using Harzing's Publish or Perish. The included literature sources discuss the synthesis and characterization of plant-based nanoparticles as anticancer, are published within the last 5 years, and are indexed in Google Scholar. **Results:** Various synthesis methods influence the properties and cytotoxic effects of silver, zinc oxide, and gold nanoparticles derived from plant extracts. Characterization techniques provide insights into their synthesis, morphology, crystalline structure, surface chemistry, and stability. **Conclusion:** The results of this study reveal that silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO-NPs), and gold nanoparticles (AuNPs) synthesized from plants have great potential in the treatment of various types of cancer.

Keywords: *cancer therapy, gold nanoparticles, nanoparticle characterization, plant-based nanomedicine, silver nanoparticles, zinc oxide nanoparticles.*

INTRODUCTION

According to WHO (2022) cancer is the leading cause of death worldwide, with an estimated 10 million deaths in 2020.[1] The most common causes of cancer deaths in 2020 were lung (1.80 million deaths), colon and rectum (916,000 deaths), liver (830,000 deaths), stomach (769,000 deaths); and breast (685,000 deaths). Traditional cancer treatments, such as surgery, chemotherapy, and radiotherapy, can be effective, but often cause significant side effects. Nanomedicine, or the application of nanotechnology to the medical field, has the potential to revolutionize cancer treatment by providing targeted drug delivery to tumor cells, reducing systemic side effects, and increasing the effectiveness of existing therapies.

Plant-based nanomedicine is a rapidly growing field of research that utilizes plant-derived nanoparticles for the treatment of cancer. Nanoparticles from plant extracts have a number of advantages over synthetic nanoparticles, including biocompatibility, low toxicity, and ease of synthesis[2]. Plant-based nanoparticles can also be loaded with various anticancer drugs, phytochemicals, and other therapeutic agents, making them a versatile platform for cancer therapy.

One of the main advantages of plant-based nanomedicine is its ability to specifically target tumor cells.[2] Plant nanoparticles can be engineered to recognize and bind to specific receptors on tumor cells.[3] This allows targeted delivery of therapeutic agents to the tumor site, minimizing exposure of healthy tissue and reducing systemic side effects. In addition to targeted delivery, plant-based nanomedicine can also increase the effectiveness of existing cancer therapies.[4] For example, plant nanoparticles can be used to increase the solubility of water-soluble drugs, increase drug uptake by cells, and protect drugs from degradation.[5,6]

In this article, various methods of synthesis and characterization of plant-based nanomedicines, especially metal nanoparticles derived from silver (AgNPs), zinc oxide (ZnO-NPs), and gold (AuNPs), are evaluated for potential applications in cancer treatment.

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METHODS

The research method used is the Systematic Literature Review (SLR), which involves studying and analyzing various methods of synthesis and characterization of plant-based nanomedicines, especially metal nanoparticles derived from silver (AgNPs), zinc oxide (ZnO-NPs), and gold (AuNPs), to evaluate the potential applications in cancer treatment. The data for this study is sourced from previously conducted research rather than collected directly. The diagram below visually represents the different stages of the research method used in this systematic literature review. Each box and arrow in the diagram on Figure 1. depicts the flow from identifying records through database searching to the final inclusion of studies.

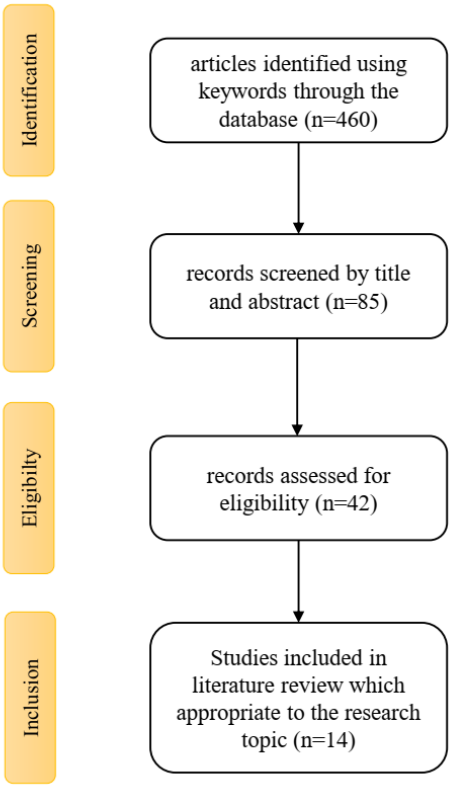

Figure 1. PRISMA flow diagram

The articles are collected using Harzing's Publish or Perish, this a stage where the necessary data for research is gathered for subsequent analysis. The keywords were entered to collect "Synthesis and characterization of plant-based silver nanoparticles as anticancer", "Synthesis and characterization of plant-based zinc oxide nanoparticles as anticancer", and "Synthesis and characterization of plant-based gold nanoparticles as anticancer".

The literature that has been obtained will be evaluated based on the following questions:

1. Does the literature discuss synthesis and characterization of plant-based nanoparticles as anticancer
2. Does the literature provide and discuss data relevant to the topic under consideration?
3. Is the literature indexed in google scholar?
4. Was the literature published within the last 5 years?

Each literature source will be assessed based on the above questions.

1. Yes: for journal papers that align with the questions in the quality assessment.
2. No: for journal papers that do not align with the questions in the quality assessment.

RESULTS

Recent research has delved into the synthesis of nanoparticles across various plant sources, focusing particularly on silver, zinc oxide, and gold nanoparticles. Several studies have been carried out to synthesize silver nanoparticles. Silver nanoparticles (AgNPs) have been synthesized using *Artocarpus integer*, *Cynara scolymus* (Artichoke), *Teucrium polium*, *Zanthoxylum rhetsa*, *Ginkgo biloba*.[7-11] The synthesis, characterization and anticancer activity of silver nanoparticles have been summarized in **Table 1.**

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Table 1. Synthesis, characterization and anticancer activity of silver nanoparticles

Types of Cancer	Plant	Characteri-zation	Form	Size (nm)	Action Mode	Cell Model	Reference
Breast Cancer	*Artocarpus interger*	UV-Vis, FTIR, TEM, TGA	Spherical	5.76-19	Interfere with DNA, damage cells, cytotoxicity	MCF-7, MG-63	[7]
Breast Cancer	*Cynara scolymus*	FTIR, SEM, XRD, UV-Vis	Spherical	98.47±2.04	Mitochondrial apoptosis	MCF7	[8]
Prostate Cancer	*Salvia miltiorrhiza*	UV-Vis, FTIR, XRD, SEM, EDX	Spherical, oval, hexagonal trigular	100	Apoptosis and cytotoxicity by bax, pathway intrinsic Bcl2	LNCAP cell line	[9]
Stomach Cancer	*Teucrium polyum*	UV-Vis, FTIR, XRD, SEM	Spherical	70-100	Induces apoptosis in cells cancer and cytotoxicity	MNK45 cell line	[10]
Lungs Cancer	*Zanthoxylum rhetsa*	UV-Vis, AFM, TEM, and SEM	Spherical	10 to 68	Cytotoxicity	A549 cell line	[11]
Cervix Cancer	*Ginkgo Biloba*	UV-Vis, TEM, DLS, zeta potential	Spherical or oval	20-90	Cytotoxicity, mitochondrial apoptosis	HeLa and SiHa cells	[12]

Multiple research projects have been conducted to create zinc oxide nanoparticles through synthesis methods. Zinc oxide nanoparticles have been synthesized using *Hertia intermedia*, *Hypericum triquetrifolium*, *Solanum nigrum* *Cissus quadrangularis*.[13-16] The synthesis, characterization, and anticancer activity of silver nanoparticles have been summarized in **Table 2.**

Table 2. Synthesis, characterization and anticancer activity of zinc oxide nanoparticles

Types of Cancer	Plant	Characteri-zation	Form	Size (nm)	Action Mode	Cell Model	Reference
Colon, neuroblastoma, breast, kidney embryonic cancer	*Hertia intermedia*	FESEM, UV-Vis, FTIR,	Spherical	20–80	Cytotoxicity, pro-apoptotic Bax, anti-apoptotic Bcl-2	Caco-2, SH-SY5Y, MDA-MB-231, and HEK-293	[13]
Lungs cancer	*Hypericum triquetrifolium*	UV-Vis, FTIR, XRD, DLS, SEM, EDX, TEM	Fusiform nanoflowers	length = 312.28 ± 78.93 and width = 48.69 ± 9.71.	Cytotoxicity	Cell A549	[14]
Cervix cancer	*Solanum nigrum*	UV-Vis, FTIR, XRD, DLS, SEM, EDX, TEM, zeta potential	Rectangular	30.8 - 86.8	Cytotoxicity through induction of apoptosis	HeLa cells	[15]
Pancreas Cancer	*Cissus quadrangularis*	UV, FTIR, XRD, TEM, SAED, and EDAX	Spherical	75 to 90	Cytotoxicity, apoptosis	MIA PaCa-2 cells	[16]

Several ways of synthesizing gold nanoparticles (Au NPs) have been studied. Gold nanoparticles have been synthesized from the *Rabdosia rubescens*, *Scutellaria barbata*, *Mentha Longifolia*, and *Albizia lebbeck*.[17-20] The synthesis, characterization, and anticancer activity of gold nanoparticles have been summarized in **Table 3.**

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Table 3. Synthesis, characterization and anticancer activity of gold nanoparticles

Types of Cancer	Plant	Characterization	Form	Size	Action Mode	Cell Model	Reference
Cancer lungs	*Rabdosia rubescens*	UV-Vis, DLS, TEM, EDX, FTIR, SAED, and AFM	nanosphere polydespers	130 nm	Apoptosis	Cell A546	[17]
Cancer pancreas	*Scutellaria barbata*	UV-Vis, TEM, SAED, AFM, and FTIR	Spherical	154 nm	Cytotoxicity	PANC-1 cells	[18]
Cancer breast	*Mentha Longifolia*	UV-visible and FT-IR, XRD, SEM, EDX, and TEM analysis .	Spherical	36.4 nm.	Cytotoxicity	MCF7, Hs 578Bst, Hs 319.T, and UACC-3133 cells	[19]
Bowel cancer	*Albizia Lebbeck*	UV-vis, XRD, SAED, FTIR, HR-TEM	Spherical	20-30 nm	Cytotoxicity, induces apoptosis	HCT-116	[20]

DISCUSSION

Synthesis and Characterization

1. Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) made with plant extracts are promising in nanotechnology because they're eco-friendly and have many uses in medicine. Studies have looked at different plants like *Artocarpus integer, Cynara scolymus, Salvia miltiorrhiza, Teucrium polium, Zanthoxylum rhetsa*, and *Ginkgo biloba.* These studies show how well plants can turn silver ions into nanoparticles.

The use of different plant extracts not only affects the synthesis yield, but also influences nanoparticle size and stability. The choice of extraction method and reaction conditions significantly impacts the final nanoparticle characteristics, such as size distribution and surface chemistry.

Different plants contain varying types and concentrations of bioactive compounds such as polyphenols, flavonoids, and proteins. These compounds act as reducing agents and stabilizers during the nanoparticle synthesis process. The specific composition of these compounds in each plant extract can influence how efficiently silver ions are reduced to nanoparticles and how effectively the nanoparticles are stabilized against aggregation.[21]

As for the characterization, from all the studies, UV-Vis spectrophotometry consistently detected absorption peaks within the range of 425-445 nm, which served as a reliable indicator of the formation of silver nanoparticles. [22]

Beside UV-Vis, FTIR (Fourier-transform infrared) spectroscopy is a powerful technique used to identify the functional groups present on the surface of silver nanoparticles (AgNPs) and to understand the role of biomolecules in their synthesis and stabilization. When plant extracts are used in the synthesis of AgNPs, FTIR spectra can provide insight into the interactions between the silver ions and the bioactive compounds in the extracts.

All the studies consistently identify characteristic FTIR bands that confirm the presence of silver nanoparticles. Common functional groups identified in silver nanoparticles using FTIR spectroscopy include hydroxyl groups (-OH), which show broad absorption bands around 3200-3600 cm^{-1}, indicating the presence of alcohols, phenols, or water. Carbonyl groups (C=O) appear as sharp peaks around 1650-1750 cm^{-1}, suggesting aldehydes, ketones, carboxylic acids, or esters. C-O stretching peaks around 1000-1300 cm^{-1} indicate ethers, esters, and alcohols. N-H groups are found around 1500-1600 cm^{-1}, denoting primary and secondary amines. C-H stretching peaks around 2850-2960 cm^{-1} are associated with alkanes.[23] These functional groups provide vital information about the surface chemistry and interactions of silver nanoparticles.

TEM and SEM provided in-depth insights into the size, shapes, and distribution properties of the silver nanoparticles. These techniques allowed researchers to observe and analyze the precise physical characteristics such as particle size variation, spherical or irregular shapes, and how uniformly the nanoparticles were dispersed in the samples. This detailed analysis aids in understanding how these

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nanoparticles interact at a microscopic level, which is crucial for assessing their potential applications in biomedicine.

2. Zinc Oxide Nanoparticles (ZnO-NPs)

The synthesis of zinc oxide nanoparticles (ZnO-NPs) using various plant extracts also represents a burgeoning field in nanotechnology, leveraging natural sources to create eco-friendly and potentially bioactive nanoparticles. Studies by Soltanian et al. (2021), Al Sharie et al. (2020), Thomas et al. (2022), and Sathappan et al. (2021) demonstrate diverse methodologies and outcomes in terms of nanoparticle size, morphology, and application potential.

Each study employs distinct plant extracts and synthesis conditions. Soltanian et al. (2021) utilized *Hertia intermedia* extract under mild conditions (pH 8, 60 °C), yielding spherical nanoparticles (20-80 nm). Al Sharie et al. (2020) used *Hypericum triquetrifolium* extract under alkaline conditions (pH 12), producing flower-like nanostructures (275.46 nm). Thomas et al. (2022) adopted *Solanum nigrum* extract, emphasizing green synthesis (60 °C, pH adjusted with NaOH) and multi-characterization techniques. Sathappan et al. (2021) utilized *Cissus quadrangularis* stem extract under similar alkaline conditions (pH 12), resulting in spherical nanoparticles (75-90 nm).

The variations in pH and extraction solvents among different methods for synthesizing zinc oxide nanoparticles (ZnO-NPs) using plant extracts directly impact critical aspects of nanoparticle formation and characteristics. pH can influence the nucleation and growth stages, affecting the final size and morphology of the nanoparticles. Alkaline conditions often result in larger and more complex nanostructures compared to neutral or acidic conditions.[24] Optimizing the solvents used in the green synthesis of nanoparticles is also crucial for effectively extracting bioactive compounds that are essential for nanoparticle nucleation and production.[25]

The characterization of ZnO nanoparticles (ZnO-NPs) typically involves examining their optical properties. UV-vis spectroscopy measures the absorbance of light by ZnO-NPs across a spectrum of wavelengths, which are often recorded in the wavelength range of 250–800 nm.[26] Each study reviewed demonstrates the utility of UV-vis spectroscopy in assessing the quality and consistency of ZnO-NPs, highlighting the effectiveness of different plant extracts in synthesizing nanoparticles with desirable properties.

FTIR is also a powerful tool for characterizing the surface chemistry of zinc oxide nanoparticles (ZnO-NPs). The FTIR spectra of ZnO nanoparticles typically display several key absorption bands corresponding to different functional groups and chemical bonds. A broad absorption band around 3200-3600 cm^{-1} is often observed, indicating the presence of hydroxyl groups (-OH), which can be due to adsorbed water or surface hydroxyl groups on the ZnO nanoparticles.[27] The characteristic absorption band of Zn-O stretching vibrations is usually found in the range of 400-600 cm^{-1}, confirming the formation of ZnO nanoparticles.[28] Peaks around 1000-1300 cm^{-1} can suggest the presence of ethers, esters, or alcohols, indicating the functionalization or stabilization of ZnO-NPs by organic compounds. [29] These functional groups are crucial for understanding the surface chemistry and interaction of ZnO nanoparticles with their environment, which can influence their properties and applications in biomedicine.

Other than UV-Vis and FTIR, X-ray diffraction (XRD) is also a widely used technique to characterize the crystalline structure of zinc oxide nanoparticles (ZnO-NPs). XRD analysis provides crucial information on the phase purity, crystallite size, and lattice parameters of the synthesized nanoparticles. The XRD patterns of ZnO nanoparticles typically exhibit several distinct diffraction peaks corresponding to the hexagonal wurtzite structure of ZnO. The most prominent peaks are usually observed at $2\theta^{o}$ values around 31.62, 34.33, 36.12, 47.33, 56.31, 62.64, 66.03, 67.64, and 68.73, which correspond to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystallographic planes, respectively. These peaks are consistent with the standard diffraction data for ZnO (JCPDS Card No. 36-1451).[30]

The broadening of these diffraction peaks can be used to estimate the average crystallite size of the nanoparticles using the Scherrer equation. The equation relates the full width at half maximum (FWHM) of the peaks to the crystallite size, indicating that narrower peaks correspond to larger crystallites, while broader peaks suggest smaller crystallites. XRD analysis can also reveal information about the strain and defects within the ZnO nanoparticles. Any shift in the peak positions or changes in peak intensity can

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indicate the presence of lattice distortions, impurities, or other structural anomalies. [31]

All the studies utilizing X-ray diffraction (XRD) for the characterization of zinc oxide nanoparticles (ZnO-NPs) confirm the presence and quality of ZnO by providing key insights into their crystalline structure. Overall, XRD is an essential technique for confirming the crystalline nature of ZnO nanoparticles, determining their phase purity, and estimating their crystallite size, all of which are critical parameters for their application in biomedicine.

In the studies on zinc oxide nanoparticles (ZnO-NPs) synthesis, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are also essential techniques for the detailed characterization of zinc oxide nanoparticles (ZnO-NPs). TEM achieves high-resolution images, allowing for the precise determination of nanoparticle size and morphology. Studies consistently report ZnO nanoparticles in the nanoscale range. TEM images provide direct measurements of particle size and distribution, revealing shapes such as spherical, rod-like, or hexagonal forms, which significantly influence optical and electronic properties. High-resolution TEM (HR-TEM) further confirms the crystalline nature and specific crystal planes of ZnO, which is crucial for understanding structural properties and behavior in various applications. TEM also shows the degree of nanoparticle agglomeration, important for applications requiring high surface area and reactivity. [32]

SEM, on the other hand, provides detailed surface morphology and topographical information. SEM images reveal surface texture and structure, critical for catalysis and sensor technologies. They show particle distribution and uniformity on substrates, essential for consistent performance in coatings and composites. SEM helps identify the aggregation state of ZnO nanoparticles; while some aggregation is common, excessive aggregation can reduce effective surface area and alter properties. SEM also reveals complex nanostructures like nanoflowers, nanorods, or nanospheres, which can benefit applications requiring high surface area. Together, TEM and SEM offer a comprehensive characterization of ZnO nanoparticles, ensuring they meet desired specifications for applications in biomedicine field. [33]

3. Gold Nanoparticles (AuNPs)

The synthesis of gold nanoparticles (Au-NPs) using various plant extracts is a burgeoning field within green nanotechnology, promising eco-friendly and biocompatible alternatives to traditional chemical methods. Different studies highlight the synthesis and characterization of Au-NPs using extracts from various plants such as *Rabdosia rubescens*, *Scutellaria barbata*, *Mentha Longifolia*, and *Albizia lebbeck.* These plant-mediated approaches not only offer sustainable synthesis but also incorporate bioactive compounds from the plants, which may enhance the nanoparticles' biomedical applications.

Each study employs distinct methods and plant extracts, leading to variations in nanoparticle characteristics. The main advantages of these green synthesis methods are their environmental friendliness, simplicity, and incorporation of bioactive compounds that may enhance the nanoparticles' functionality. Additionally, these methods typically operate under mild conditions, reducing the need for harsh chemicals or high-energy inputs. However, limitations include potential variability in nanoparticle size and shape due to differences in plant extract composition, and the need for thorough purification to remove organic impurities.

For charactirization, UV-Vis spectroscopy is a common technique employed to confirm the synthesis of gold nanoparticles (AuNPs) and study their optical properties. When AuNPs are formed, they exhibit a phenomenon known as surface plasmon resonance (SPR). This SPR results in a characteristic absorption peak in the UV-Vis spectrum, typically in the range of 500-600 nm, depending on the size, shape, and surface chemistry of the nanoparticles. [34]

In the studies mentioned earlier, UV-Vis spectroscopy was used to confirm the formation of AuNPs by observing the appearance of this characteristic absorption peak. Each study reported absorption peaks within the typical range for AuNPs, generally between 500 to 600 nm. Zhang et al. (2019) observed a characteristic peak around 520 nm for AuNPs derived from *Rabdosia rubescens*. Wang et al. (2019) noted absorption peaks in the reddish-yellow range, indicating the presence of AuNPs synthesized from *Scutellaria barbata*. Li et al. (2021) reported a surface plasmon resonance peak at 512 nm for AuNPs produced from *Mentha Longifolia* leaf extract.

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Similarly, Malaikolundhan et al. (2020) observed absorption peaks at 535 nm for AuNPs synthesized from *Albizia lebbeck*. These peaks are indicative of the collective oscillation of conduction band electrons in the gold nanoparticles, known as surface plasmon resonance, confirming their successful synthesis.

Aside from XRD, SAED (Selected Area Electron Diffraction) analysis of gold nanoparticles (AuNPs) also provides valuable insights into their crystal structure and orientation. This technique involves directing an electron beam onto a specific area of the sample, which results in diffraction patterns that correspond to the atomic arrangement within the nanoparticles. From SAED patterns, researchers can determine the crystallinity, lattice spacing, and phase purity of AuNPs. This characterization is essential for confirming the crystalline nature of synthesized nanoparticles and understanding how different synthesis parameters influence their structural properties, which are critical for various applications in nanotechnology and materials science. Zhang X et al, Wang et al, and Malaikolundhan H et al utilized SAED primarily to confirm the crystalline structure of their synthesized gold nanoparticles, complementing other characterization techniques such as TEM, FTIR, and UV-vis spectroscopy to provide a comprehensive understanding of the nanoparticles' properties.

Anticancer Activity

1. Silver Nanoparticles (AgNPs)

Majeed, S. et al. (2019) evaluated the anticancer activity on MCF-7 cells, MG-63, and the cytotoxic effect on normal fibroblast cells 3T3. The cells were obtained from the National Center for Cell Science (NCCS) Pune, India, and cultured in media containing fetal bovine serum (10%), streptomycin (100 U/ml), glutamine (1%), and penicillin (100 U/ml) in a carbon dioxide (CO_2) environment. The toxicity of nanoparticles was evaluated through the MTT test with various concentrations (10, 30, 50, 70, 90, 120 µg/ml) in cell culture media. Each well was incubated with the desired concentration of nanoparticles for 24 h at 37 °C. After incubation, the wells were washed with phosphate buffer and MTT tetrazolium solution was added to each well, then incubated for 2-3 hours. Next, the crystals formed were dissolved by adding DMSO and their optical density was measured at 570 nm. The anticancer activity of the nanoparticles was evaluated by measuring the ability to inhibit the growth of MCF-7 and MG-63 cancer cells, with IC50 values of 90 µg/ml and 70 µg/ml, respectively. The results showed that silver nanoparticles had good anticancer properties against cancer cells, but their toxicity against normal 3T3 fibroblast cells was lower.

Erdogan, O. et al (2019) tested the cytotoxic effect of AgNPs and/or PDT with the MTT test and migration with the scratch test. The apoptosis-inducing ability of AgNPs and/or PDT was investigated by analysis of intracellular ROS, antioxidant enzyme levels (SOD, CAT, GPx and GSH), Hoechst staining and Bax/Bcl-2 analysis using western blotting. The average AgNP particle size produced was 98.47 ± 2.04 nm with low polydispersity (0.301 ± 0.033). The zeta potential value of AgNPs shows -32.3 ± 0.8 mV. These results clearly demonstrate the successful formation of AgNPs for cellular uptake. Mitochondrial damage and intracellular ROS production observed upon treatment with AgNPs (10µg/mL) and PDT (0.5 mJ/cm^2) showed a significant decrease in cell migration, Bax expression, and Bcl-2 suppression. Significantly, the biosynthesized AgNPs showed broad-spectrum anticancer activity with PDT therapy and therefore represented an increase in ROS generation by modulating the induction of mitochondrial apoptosis in MCF7 breast cancer cells.

Zhang, K. et al (2019) tested the anticancer potential of AgNPs on prostate adenocarcinoma (LNCaP) cell lines. The anticancer potential of silver nanoparticles (AgNPs) was investigated through MTT-based cytotoxicity assay. The test results showed that increasing the concentration of AgNPs significantly inhibited the growth of LNcap cells, with a concentration of 50 mg/ml showing significant cell death. These AgNPs can also result in the production of reactive oxygen species (ROS), which induce oxidative stress and apoptosis. In addition, AgNPs also induce damage to cell organelles, such as mitochondrial depolarization, and result in fragmentation of the cell nucleus which is a typical sign of apoptosis. In this study, AgNPs also affected the expression of proteins involved in the regulation of apoptosis, namely reducing the expression of antiapoptotic proteins (Bcl-2, Bcl-xl) and increasing the expression of apoptotic proteins (Bax). In addition, caspase-3 expression was also significantly increased in LNcap cancer cells treated with AgNPs. These results indicate that AgNPs synthesized from *S.*

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miltiorrhiza plant extract effectively induce apoptosis via the intrinsic apoptotic pathway in prostate cancer cells.

Hashemi, S. F. et al (2020) tested the cytotoxic effects of various concentrations of *T. polium*-AgNPs evaluated on human gastric cancer cell culture MNK45 using the MTT assay. The MTT test results showed a dose-dependent decrease in the proliferation of MNK45 gastric cancer cells with a significant decrease at a concentration of 130 µg/ml, where the viability of these gastric cancer cells was reduced to 26.1%. The IC50 value of T. polium-AgNPs against MNK45 cancer cells was seen at a concentration of 68.2 µg/ml after exposure for 48 hours. This demonstrated a dose-dependent decrease in MNK45 gastric cancer cell proliferation. These results indicated that AgNPs synthesized from T. polium aqueous extract triggered cell death in human cancer cells MNK45. AgNPs have been investigated as a tool for the development of new cancer therapies, due to their unique properties that can improve therapeutic efficacy and safety. In vitro studies show that AgNPs can enter cancer cells, damage mitochondria, and produce reactive oxygen species (ROS) that trigger cancer cell death. In this study, AgNPs from *T. polium* extract also showed strong cytotoxic activity against human gastric cancer cells MNK45, which could be caused by various bioactive compounds in T. polium extract interacting with the surface of AgNPs.

Nayaka, S. et al (2020) assessed anticancer activity on A549 cells using the MTT salt method, which is based on the reduction of MTT salt by a mitochondrial enzyme called lactate dehydrogenase. The analysis results show that AgNPs have a cytotoxic effect that increases with increasing concentration. A concentration of 200 µg/ml AgNPs was most effective in destroying A549 cancer cells, with an IC50 value of around 65.17 µg/ml. The mechanism of action of AgNPs on cancer cells involves interactions with cell components, production of reactive oxygen species (ROS), and disruption of cell membrane integrity. ROS can also attack DNA and interfere with DNA damage repair, ultimately resulting in cancer cell death.

Xu, Z. et al. (2020) observed the impact of AgNPs on cell growth and apoptosis analyzed through MTT, MTS, and colony formation assays; Hoechst stain 33258; as well as flow cytometry. Intracellular ROS levels and oxidative stress were evaluated using appropriate commercial kits. Levels of apoptosis-related proteins were determined via western blotting.

Xu, Z. et al. made a series of GB-AgNPs which have different particle sizes (Ag nanoparticles synthesized from ginkgo extract), and the smallest particle size was 40.2 ± 1.2 nm with a low degree of dispersion (0.091 ± 0.011), and a zeta potential value of -34.56mV. Compared with the control group, GB-AgNP treatment inhibited cell growth and induced apoptosis in HeLa and SiHa cells. GB-AgNP treatment also caused a significant increase in intracellular ROS levels, release of cytochrome c (Cyt C) from mitochondria to the cytosol, and cleavage of caspase-9 and -3 in both types of CCa cells. Moreover, NAC, which is a ROS scavenger, abolished the effect of GB-AgNPs on HeLa and SiHa cells. This study revealed that GB-AgNPs inhibited cancer cell growth and induced apoptosis by increasing intracellular reactive oxygen species (ROS) production and activating the caspase-dependent mitochondrial apoptosis pathway in CCa cells. Therefore, GB-AgNPs may be a potential alternative drug for CCa therapy.

2. Zinc Oxide Nanoparticles (ZnO-NPs)

Soltanian, S. et al. (2021) showed that ZnO-NPs had a cytotoxic effect against Caco-2 (IC50 177 µg/mL), SH-SY5Y (IC50 184 µg/mL), MDA-MB-231 (IC50 168 µg/mL), and HEK cell lines. -293 (IC50 240 µg/mL) through MTT assay. Through the DCFH-DA assay, significant production of reactive oxygen species (ROS) was measured after 24 h of treatment with 200 µg/mL ZnO-NPs, indicating the presence of oxidative stress caused by ZnO-NPs. Induction of apoptosis/necrosis in cells treated with ZnO-NPs was determined using annexin V-PE/7-AAD staining. In addition, analysis of the expression of the pro-apoptotic gene Bax and the anti-apoptotic gene Bcl-2 by real-time PCR showed a 10-fold increase in Bax expression and a 16-fold decrease in Bcl-2 expression after cells were exposed to ZnO-NPs. All these results confirm that the ZnO-NPs synthesized in this study are potential candidates for inducing ROS and oxidative stress that impact cytotoxicity in cell lines.

Al Sharie, et al., (2020) revealed that HT-ZnO nanoflowers caused a dose-dependent decrease in the viability of A549 cells, human alveolar basal epithelial adenocarcinoma cells, with an IC50 value of approximately 20.45 µg/mL. The effects of HT-

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ZnO nanoflowers were also evaluated on the migration and colony formation abilities of A549 cells, confirming their cytotoxic activity. Thus, the results of this study indicate that zinc oxide nanoflowers were successfully synthesized using methanol extract from H. triquetrifolium and have potential as agents that can trigger reactive oxygen (ROS) production and oxidative stress leading to cytotoxicity in various cell lines.

Thomas, S., et al, (2022) revealed ZnO nanoparticles derived from Solanum nigrum exhibited notable anti-cancer activity against HeLa cell lines through the apoptotic pathway. To assess the cytotoxicity of these nanoparticles, they conducted the MTT test, wound healing test, DAPI staining, and double staining with acridine orange and ethidium bromide. Additionally, they analyzed the expression patterns of β-catenin, p53, caspase-3, and caspase-9 using reverse transcriptase-PCR. The study's findings revealed that the cytotoxic effect of ZnO nanoparticles from Solanum nigrum on HeLa cell lines was dose-dependent, achieved by inhibiting β-catenin and elevating the levels of p53, caspase-3, and caspase-9.

Sathappan, S. et al., (2021) revealed cytotoxicity of Cq-ZnO nanoparticles (NPs) was tested on MIA PaCa-2 cells at concentrations of 25, 50, and 100 µg/ml. After 24 hours, Hoescht 33342 staining revealed significant morphological changes, including cell clustering and membrane disruption at 100 µg/ml. NPs, with their high surface area-to-volume ratio, exhibit enhanced physical and chemical activity compared to bulk materials. Due to their small size and large specific surface area, ZnO NPs increased intracellular Zn^{2+} levels, leading to excessive generation of reactive oxygen species (ROS), plasma membrane leakage, mitochondrial dysfunction, and cell death.

3. Gold Nanoparticles

Zhang, X. et al. (2019) used MTT assay to determine the IC50 dose of RR-AuNPs, and its apoptotic effect was evaluated through the detection of caspase activation and ROS generation. The anticancer effect of RR-AuNPs was confirmed by DAPI staining, TUNEL assay, and protein expression analysis of apoptosis signal molecules. The results showed that RR-AuNP showed a strong absorption peak at a wavelength of 550 nm, and RRAuNP was in the form of polydesperous nanospheres with a size of 130 nm. The IC50 dose of RR-AuNP against A549 lung carcinoma cells was detected at 25 mg/ml. DAPI staining analysis, TUNEL assay, and immunoblotting confirmed that RR-AuNPs at doses of 25 mg/ml and 50 mg/ml had strong anticancer and apoptotic effects. This suggests that RR-AuNPs may be an effective molecule in the treatment of lung cancer.

Wang, L. et al (2019) synthesized Gold nanoparticles from *Scutellaria barbata*, it is demonstrated significant cytotoxic activity against human pancreatic cancer cell lines (PANC-1) in a dose-dependent manner, as assessed by the MTT cytotoxicity assay. The nanoparticles induced apoptosis in cancer cells, as evidenced by increased expression of apoptotic-related proteins, as analyzed through quantitative real-time PCR. Moreover, the synthesized gold nanoparticles increased the production of intracellular reactive oxygen species (ROS) in pancreatic cancer cell lines, potentially contributing to their anticancer activity. These findings highlight the potential of *Scutellaria barbata*-derived gold nanoparticles as effective agents against pancreatic cancer, suggesting a promising avenue for further research and drug development.

Li, S., et al (2021) test the anti-breast cancer effect on human breast cancer cells, we used the MTT assay on several types of common breast cancer cells, namely breast adenocarcinoma (MCF7), breast carcinoma (Hs578Bst), breast ductal infiltrating cell carcinoma (Hs319.T), and lobular infiltrating cell carcinoma of the breast (UACC-3133). Conversion is performed in short reaction times with good to excellent results along with excellent change frequency (TOF). Moreover, this nanocomposite catalyst is easily recovered and can be reused up to 12 times without a significant decrease in its catalytic activity. Gold nanoparticles had high anti-breast cancer activity in a dose-dependent manner against MCF7, Hs 578Bst, Hs 319.T, and UACC-3133 cells. The best results of anti-breast cancer effect were seen in the case of UACC-3133 cells. It is shown that gold nanoparticles can be used for the treatment of several types of breast cancer in humans.

Malaikolundhan, H. et al. (2020) explained L-AuNPs caused cytotoxicity at an IC50 concentration of 48 mg/ml and also induced apoptosis with increased ROS production, decreased mitochondrial

membrane potential, changes in apoptotic morphology by AO/EtBr, as well as changes in the expression of pro-apoptotic and anti-apoptotic proteins analyzed in HCT-116 colon cancer cells. The findings of this study prove that AL-AuNPs have potential anticancer activity against colon cancer cells (HCT-116).

ETHICAL APPROVAL

There is no ethical approval.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, investigation, resources, writing—original draft preparation, review, and editing Jihan Nurafifah Hernawan; writing—review and editing, Ratnaningsih Eko Sardjono.

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