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Anti-Cancer Activity of a Mixture of Ag Nanoparticles and Clove Extract Synthesized by DC Sputtering and Cold Plasma Techniques Versus Breast Cancer Cells

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Abstract

Cancer treatment represents a major challenge in the medical field due to its complexity and the diverse nature of the disease. Conventional treatments, such as chemotherapy, have numerous side effects that negatively impact patients. Plasmonic nanomaterials and herbal plant extracts have recently been exploited as a new future therapeutic approach to combat cancer with minimal side effects. Silver nanoparticles (AgNPs) have unique chemical and physical properties, such as biocompatibility and plasmonic behavior, which can be used as effective anticancer agents. Clove extract (*Syzygium aromaticum*) is a medicinal plant with many therapeutic properties, such as antibacterial, antifungal, antiviral, and antiseptic properties. In this study, the plasma phenomenon was adopted through DC sputtering and cold atmospheric plasma jetting (CAPJ) technique as a simple, inexpensive, and environmentally friendly physical technique for the preparation of cold nanoparticles (AgNPs) and extraction of clove emulsion. The anticancer effect of silver nanoparticles, clove emulsion, and their mixture was studied against MDA-MB-231 breast cancer cells and normal REF cells in vitro. AgNPs were synthesized at different preparation currents (6, 8, and 10 mA), and some of their physical properties were analyzed using UV-vis spectroscopy, XRD, and SEM. The localized surface plasmon resonance (SPR) peak is visible, indicating plasmon behavior. The crystalline structure and spherical shape of the nanoparticles were observed from XRD and SEM images, respectively. FTIR spectroscopy of the emulsified clove extract was performed to analyze its chemical compounds. Eugenol compounds were observed in the infrared spectrum of the emulsion extract of cloves that had undergone the filtration and drying process. The anticancer activity was investigated by performing a cytotoxicity test (MTT test) against the breast cancer cell line MDA-MB-231. The maximum growth inhibition value of MDA cancer cells was obtained by adding a mixture of cold colloidal nanoparticles with clove emulsion extract at a mixing ratio of 100:100 and an incubation period of 48 hours. Our results confirm the potential of AgNPs and clove emulsion mixture as a promising anticancer therapy.

Keyword: AgNP • clove emulsion extract • breast cancer • plasma preparation techniques.

Introduction

Nanotechnology is a revolutionary science that has been adopted to develop various nanoparticles and nanostructures for many industrial (Technology) and medical (Healthcare) applications [1-3]. Nanoparticles of various materials and shapes have been used for medical purposes in diagnosis and therapy[4]. Silver nanoparticles (Ag NPs) are one of the most explored nanomaterials in the medical sciences, especially in cancer diagnosis and treatment[5]. Ag NPs have gained significant interest due to their unique properties, which include a high surface area-to-volume ratio, biomedical compatibility thanks to their low toxicity[6], and the ability to interact with tissues and cells in a precise manner[7, 8].

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These particles can be modified to suit a wide range of medical applications such as drug delivery, medical imaging, and thermotherapy, making them a powerful tool in the fight against cancer[9].

Furthermore, Silver nanoparticles have many advantages due to their unique chemical and physical properties, such as highly tunable, stable optical properties, and easily variable surface chemistry[10]. These properties make it a good candidate for wide-ranging medical applications such as drug delivery, diagnosis, and treatment of various diseases. The unique physicochemical properties of silver nanoparticles have allowed them to be used in various health therapeutic functions such as anticancer, antimicrobial properties, antioxidant activity, and biomolecules[2]. In addition to the ability to modify and encapsulate, the surface of silver nanoparticles can be modified in various ways, including encapsulating them with chemical compounds or anticancer drugs. These particles can act as effective drug carriers, delivering drugs to cancer cells specifically and precisely, enhancing the effectiveness of treatment and reducing side effects[11]. Silver nanoparticles can be coated with anticancer drug compounds or other materials capable of directly targeting cancer cells. These particles are precisely directed to cancerous sites in the body, where the drug is released locally. This ensures that the therapeutic dose is concentrated

only in cancer cells, reducing the side effects of treatment on healthy cells[12]. In some research, silver nanoparticles are being used as a vehicle to deliver therapeutic genes to cancer cells[13]. This can involve introducing genes that repair damaged cells or disabling genes responsible for tumor growth[14]. This is done by interacting with the DNA inside cancer cells using these particles[15]. Biochemical stimulation, silver nanoparticles are also used to interact with enzymes or other biological materials to stimulate biochemical reactions that enhance the immune system's response or directly destroy cancer cells[16].

Herbal medicine has been recognized for centuries and in ancient civilizations as an effective medicine for many diseases. Cloves (*Syzygium aromaticum*) are a popular herbal medicine that can be used to reduce pain and fight inflammation, thanks to the chemical composition of clove oil, which contains eugenol[17]. Several studies have found that cloves have many medicinal properties, such as antibacterial, antifungal, antiviral, and antiseptic properties[18, 19]. Recently, many studies have been conducted to explore the amazing properties of cloves as an antioxidant, antimicrobial, and antiviral[20-22], as well as its anticancer activity[23, 24]. Moreover, cloves have been exploited in green synthesis technology of metal nanoparticles, and their properties have been explored in industrial and pharmaceutical applications. This technology has enabled the fabrication of synthetic (environmentally friendly) nanoparticles with less toxicity, allowing their safe integration into biomedical applications. The biologically active secondary metabolites of these extracts helped improve the biological activity of the prepared nanoparticles[25].

Cold plasma technology has many advantages, such as enhancing the biological activity of clove extract in medical treatments. This technology improves the properties of clove extract by improving the extraction process and increasing the concentration of active chemical compounds such as eugenol and other plant compounds responsible for biological activity[26]. The interaction of cold plasma with plant materials can modify the chemical structure of plant compounds, improving their effectiveness in fighting cancer or supporting immune system function[27]. Cold plasma generates different active bodies as reactive oxygen species (ROS), such as free radicals, which are effective in interacting with plant compounds and enhancing their therapeutic effects. Active oxygen can help improve the antibacterial and antifungal effects, which is an important factor in the treatment of many diseases[28]. Reducing toxic effects and improving safety, cold plasma may help reduce the toxicity of plant extracts, as it promotes the breakdown of potentially toxic compounds or converts them into less toxic forms[29]. This could contribute to improving safety when used in medical treatments. Reducing toxic effects and improving safety is one of the benefits of the cold plasma technique that may help reduce the toxicity of plant extracts, as it promotes the decomposition of potentially toxic compounds or converts them into less toxic forms[30]. Several nanomaterials and nanostructures prepared by cold plasma technology have also shown significant improvement in their antibacterial and biosensor properties[31-33].

The present aim of the study is to explore the anti-breast cancer activity in vitro of both silver nanoparticles prepared using the DC sputtering technique, clove emulsion extract through the cold plasma technique, and their mixture. **Figure 1** shows the stages of the process in this research.

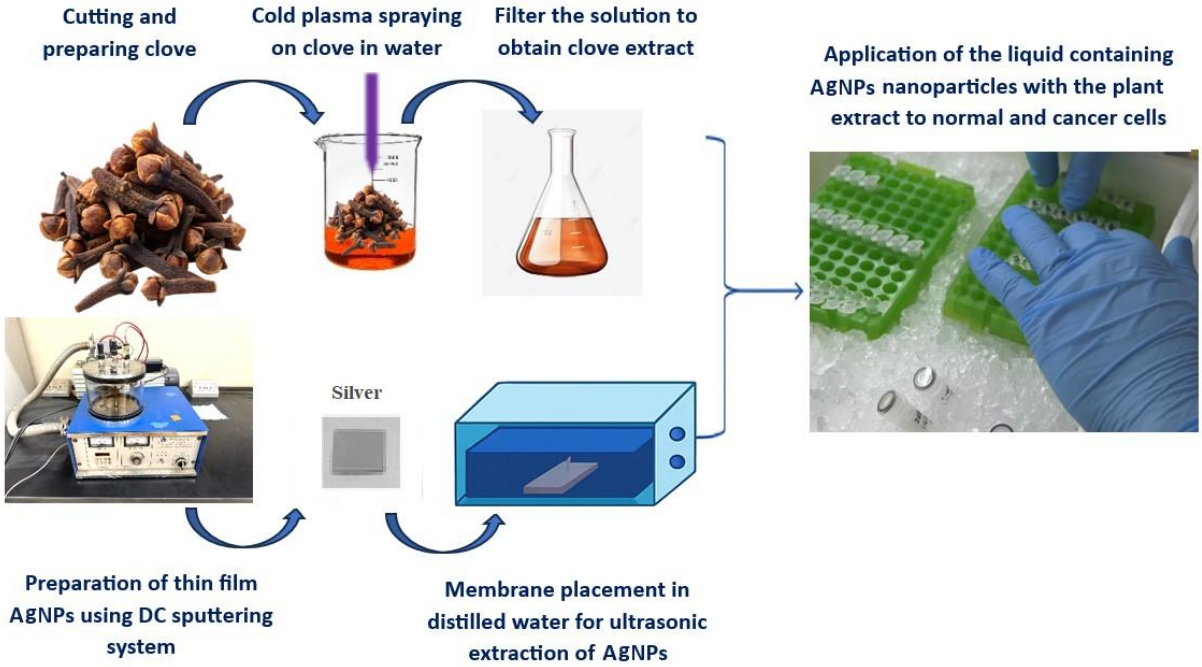

Figure 1. The schematic diagram shows the complete workflow for preparing silver nanoparticles via a DC spray system and clove extract via a cold plasma (plasma jet) system, and preparing a mixture for testing its anticancer activity.

Material and Methods

Plasma Diagnostics

In this study, optical emission spectroscopy (OES) was adopted as a method for analyzing the spectrum produced by silver nanoparticles. This spectrum is formed due to argon gas atoms colliding with the target of silver and being deposited on the slide in the DC sputtering system. High-purity silver and gold (99.995%) were used for deposition. These targets were obtained from Kurt J. Lesker Company, USA. A spectrometer (200-1025 nm) was used to characterize the silver spectrometry (Flame-S-XR1 model) with an optical accuracy of 1.69 nm. It is manufactured by Ocean Optics Spectrometer (USA). Reference data from NIST [34]. Atomic Spectra has been adopted as a database to compare it with the silver tops that appeared during work.

In addition, Optical Emission Spectroscopy (OES) revealed various spectral lines indicating repeated and rapid ionization events of the same argon atom. Strong atomic emission lines were observed at wavelengths of 284.4 nm, 328.17 nm, and 665.08 nm, corresponding to the following electronic transitions:

Ar II ($3s^2 3p^4(^1S)4s$) → ($3s^2 3p^4(^3P)6p$)

Ar II ($3s^2 3p^4(^3P)4p$) → ($3s^2 3p^4(^3P)4d$)

Ar II ($3s^2 3p^4(^1S)3d$) → ($3s^2 3p^4(^3P_2)4f$)

Similarly, prominent emission peaks for silver were identified at 328.1 nm, 338.55 nm, 486.27 nm, and 656.02 nm, which are attributed to the following transitions:

338.55 nm: Ag I ($4d^9 5s(^3D)5p$) → $4d^9 5s(^3D)5d$

486.27 nm: Ag I ($4d^{10}5p$) → ($4d^{10}5d$)

656.02 nm: Ag II ($4d^9(^2D_{5/2})6s$) → ($4d^8(^3F)5s5p(^3P°)$)

These emission lines reflect both neutral silver atoms and singly ionized silver ions (Ag I and Ag II), providing a comprehensive picture of the plasma–material interaction. The presence of both Ag I and Ag II species indicates active sputtering and ionization under the applied conditions.

The emission spectrum was recorded in the range of 200–900 nm using a high-resolution spectrometer, with argon serving as the carrier and sputtering gas. The data confirmed the excitation and ionization states of silver and argon within the plasma. These processes can be generally described by the ionization reaction:

$$Ag \rightarrow Ag^{++} + e^{-2} \quad \ldots (1)$$

The spectral peaks observed in **Figure 2** validate the generation of highly energetic species and confirm the successful excitation of silver atoms during deposition. OES thus served as a vital tool in monitoring plasma quality, species generation, and energetic transitions, which are essential for optimizing nanoparticle synthesis in sputtering systems[35].

The DC sputtering system was used in this research to study the important characteristics of the emission spectra. The emission spectrum of the plasma mixture (Ar) for the Ag foil was analyzed by OES (Ocean Flame); the emission spectra were obtained in the wavelength range of 200-900 nm. All the slides were exposed to plasma for a fixed period of 30 sec, with a gas ratio and a constant voltage of 350 V and different currents (6, 8, and 10 mA). A flow meter was used to regulate the gas flow rate for the argon.

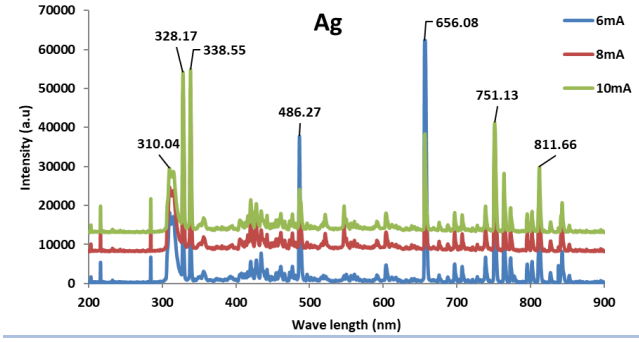

Figure 2. Optical emission spectra of (DC) sputtering plasma for Ar on Ag slide with different currents (6, 8, and 10 mA).

Ag NPs Prepared via DC Sputtering Technique

Figure 3 illustrates the Ag NPs preparation steps using the DC sputtering system available in technique in Advanced Nano Lab, Department of Physics, Mustansiriyah University. A DC sputtering system containing a target (Ag) was used to prepare a thin silver layer on a glass substrate with pure and uniform metallic Ag nanoparticles. Three Ag membranes were deposited with DC sputtering under a pressure of 1.5×10^{-1} mbar, and then argon gas was passed to the system under a voltage of (1kV) and a flow gas (1.5) L/min. Argon gas ionization was used to generate plasma, which was used to extract particles from the target Ag and deposited on a glass substrate with dimensions (1×1 cm^2) previously cleaned by ultrasound. Three silver films were prepared using three currents (6, 8, 10 mA) with a deposition time of 30 s in order to optimize the thickness and density of nanoparticles. After deposition, the slide was placed in a beaker containing 20 ml of distilled water. The beaker was then placed in a water bath at 80 °C to ensure nanoparticle extraction from the slide surface. The thin film changed into a scraped solution in distilled water, resulting in a colloidal solution containing the nanoparticles. The colloidal solution of silver nanoparticles was ready for investigation of its anticancer activity, alone and after mixing with clove emulsion extract.

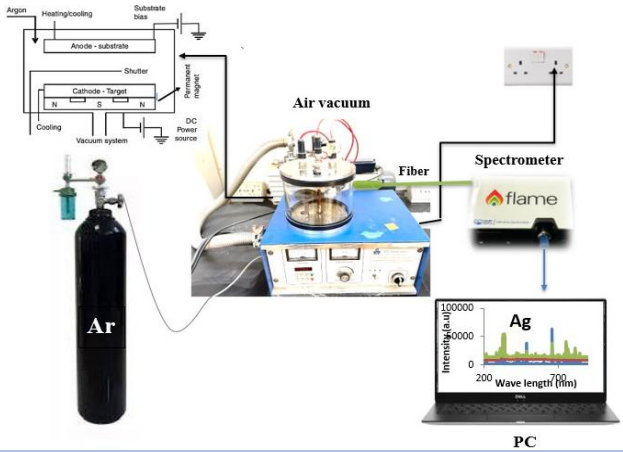

Figure 3. Schematic diagram of the silver nanoparticle preparation process using a DC sputtering system.

Several concentrations of colloidal silver nanoparticles were prepared by dilution to 25%, 50%, and 100%, as described in refs[36, 37].

Clove Oil Extract via Cold Plasma Technique

A plasma jet in a cold atmosphere was used to extract nanoemulsion from clove essential oil through exposing it to a cold atmosphere plasma jet. This non-thermal plasma technique provides several advantages in the extraction of nanoemulsions for different herbal medicines, such as preserving the therapeutic efficacy of the herbal medicines. Thus, the destruction of its chemical and biological composition is prevented, allowing for enhanced bioavailability and therapeutic effects, and its anticancer activity can be improved. The nanoemulsion was extracted from pre-cleaned cloves cut into small pieces weighing 50 g and kept in a beaker containing 30 ml of distilled water and exposed to cold plasma flow for 30 min. The cold plasma system is set to optimal conditions and operated at room temperature. The concentrated extract of clove nanoemulsion was obtained by a filtration process. **Figure 4** illustrates the cold plasma system used to extract clove nanoemulsion.

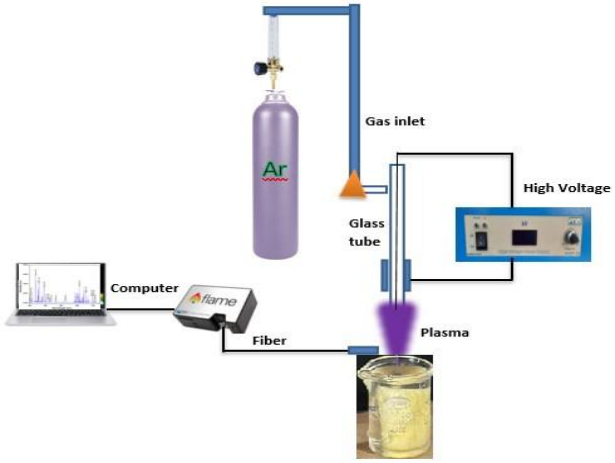

Figure 4. Schematic diagram of the cold plasma system used to prepare the clove nanoparticles.

In Vitro cytotoxicity tests

The anticancer activity was evaluated by performing cytotoxic effect assay on both normal mouse embryonic fibroblast (REF) cells and MDA-MB-231 breast cancer cells by adding different proportions of colloidal silver nanoparticle solution, clove extract emulsion, and their mixture. For this purpose, MTT Assay was performed to identify the cell viability (i.e., Inhibiting cell growth or inducing cell death) on both normal and cancer cells. Samples of cell lines have been prepared at the Biotechnology Center of Al-Nahrain University. Initially, trypsin-EDTA was applied to improve trypsin activity by weakening intercellular and cell-surface adhesion and to prepare single-cell suspensions that aid in counting, flow cytometry, and further cell culture. The cells were then seeded at a density of 10,000 cells per well in a 96-well microplate after adding RPMI medium supplemented with 10% fetal bovine serum (FBS). The cell inhibition efficiency was verified by adding several serial dilutions of colloidal silver nanoparticle concentrations (100%, 50%, and 25%). Incubation was applied at 37°C with two time periods of 24 and 48 hours. Cell viability was examined using spectrophotometry by staining the cells with 50 µg of crystal violet dye to improve visualization. The wavelength of 570 nm is adopted in the spectrophotometer, which corresponds to the maximum absorption of crystal violet dye[38].

Results and Discussions

Several physical characterizations were performed to examine the optical, structural, and morphological properties of AgNPs, in addition to using Fourier transform infrared (FTIR) to analyze the chemical composition of the clove emulsion extract. Statistical analysis of the anticancer activity of the prepared samples was performed using GraphPad Prism version 8.0 software to estimate the behavior and effect of the proposed CAP system on MDA-MB-231 breast cancer cells under in vitro conditions. The "ComboSyn" version 0.1 was also used to analyze data on active ingredient concentrations, calculate synergistic or inhibitory coefficients, and determine whether the effects resulted from a cumulative or synergistic interaction between the components used. The experimental data from the GraphPad Prism v8.0, where *P < 0.5, **P < 0.1, ***P < 0.01, and ****P < 0.001 should be examined. Notably, the stars' symbols state the degree of significance: * (P≤0.05), ** (P≤0.01), *** (P≤0.001), and **** (P≤0.0001).

Characterization of AgNPs

UV-vis Absorption

Ultraviolet-visible spectroscopy was performed to determine the optical properties of silver nanoparticles (AgNPs) prepared by the DC sputtering technique at different currents (6, 8, and 10 mA). This analysis is based on the absorption of light by AgNPs due to the Surface Plasmon Resonance (SPR) phenomenon, which is characteristic of metallic nanomaterials. From the absorption spectrum, a major absorption peak for silver is observed at a wavelength of about 400-450 nm. This indicates the red shift of the plasmon resonance peak of silver nanoparticles with increasing nanoparticle size, which is attributed to the manufacturing conditions[39].

The existence of this peak is attributed to the phenomenon of surface plasmon resonance (SPR), in which free electrons on the surface of nanoparticles interact with electromagnetic waves of light. This is clear evidence of the existence of AgNPs because they are considered important particles in the phenomenon of plasmon resonance. The lack of peak transformation is clear evidence of the stability and non-agglomeration of nanoparticles. Furthermore, the observed absorption profile supports the successful synthesis of AgNPs. The absorption range at which nanoparticles (AgNPs) appear is suitable in multiple fields, such as medicine, where their properties can be exploited in medical imaging and treatment. They can also be used as catalysts in chemical reactions, opening the way for the development of new and effective technologies such as cancer diagnosis and

treatment. **Figure 5** shows the visible spectrum of silver nanoparticles[35].

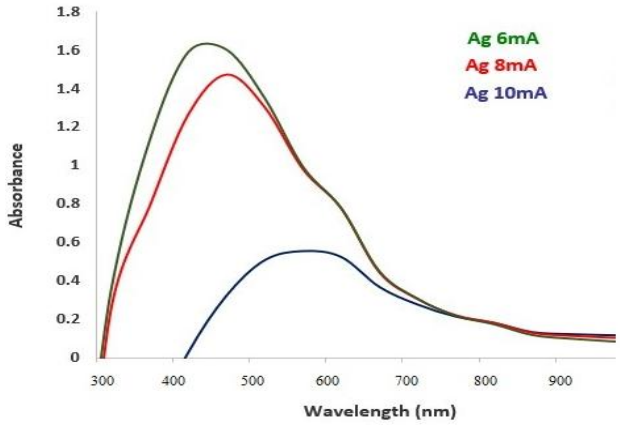

Figure 5. UV-vis absorption spectra of AgNPs prepared by the DC sputtering technique at different currents (6, 8, and 10 mA) with a constant voltage of 350 V[35].

Raman Spectroscopy Analysis

Raman spectroscopy is a useful analytical tool for examining molecular vibrations and structural characteristics of nanomaterials. In contrast to infrared spectroscopy, Raman analysis exhibits significantly reduced sensitivity to water interference, rendering it particularly suitable for aqueous nanoparticle formations.

Figure 6 presents the Raman spectra of silver nanoparticles synthesized using DC sputtering at three distinct discharge currents (6, 8, and 10 mA) over 30 seconds each. The spectra exhibit distinct peaks at 1114.23 cm^{-1}, 1308.74 cm^{-1}, 1384.04 cm^{-1}, and 1543.19 cm^{-1}, which may relate to vibrational modes linked to surface-bound molecules or residual functional groups from stabilizing agents.

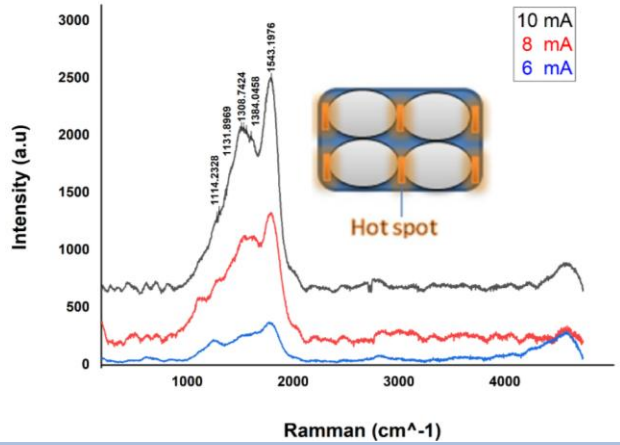

Figure 6. Raman spectrum of AgNPs for currents 6, 8, and 10 mA[35].

The augmentation observed in the Raman signal intensity is ascribed to the effects of Surface-Enhanced Raman Scattering (SERS), which intensifies with increasing nanoparticle size and surface roughness. The spherical form of AgNPs facilitates robust electromagnetic field localization in "hot spots" between closely spaced particles, hence enhancing the Raman signal. This amplification mechanism is essential for various biomedical applications, including biosensing and cancer diagnostics, while its therapeutic role (e.g., in cancer cell ablation) may necessitate more validation.

X-ray Diffraction

XRD analysis is considered a fingerprint of materials, as each material has its a unique fingerprint or pattern. Comparing the XRD pattern of a material with a database of known patterns can identify the material and determine its characteristics. XRD analysis was used to determine the structure and crystal size of the silver nanoparticles.

Figure 7 shows the AgNPs XRD patterns were prepared using a DC sputtering plasma system at (6, 8, and 10 mA) for durations of 30 sec. The results showed a diffraction pattern that proved the sample was crystalline. They also observed crystalline peaks that looked like silver nanoparticles (AgNPs) with a face-centred cubic (FCC) crystal structure. There are three obvious peaks at 2θ: 38.1° and 44.3°, in the XRD pattern. The (111) and (200), crystallographic planes of silver AgNPs (JCPDS No.04 - 0783) are what make these peaks. The results also suggest that the sample is quite pure, which means it might be employed in medicine and technology, such as cancer diagnosis and treatment.

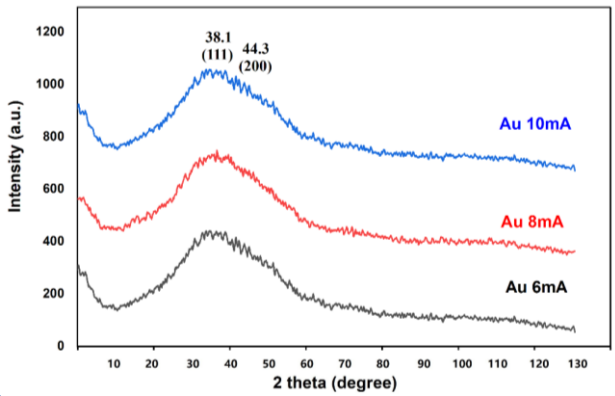

Figure 7. X-ray patterns of silver nanoparticles prepared by the DC Sputtering technique[35].

Field Emission Scanning Electron Microscope (FE-SEM) of AgNPs

Figure 8 depicts FE-SEM images of the specimens after preparation by DC sputtering plasma at (6, 8, and 10 mA) for a duration of 30 sec. FE-SEM analysis was performed to study the morphological properties of silver nanoparticles deposited on the glass slide. Figure 8c displays FE-SEM images of the AgNPs prepared at 6 mA for 30 sec. This image displays the AgNPs, which have an average diameter of 36.5 nm according to ImageJ software. Figure 8b, the uniform nanoparticles structure with an average diameter of roughly 27.55 nm was synthesized by increasing the current to 8 mA for 30 sec. The dark areas observed between the structures of the nanoparticles can be attributed to the presence of black spots, which in turn confirms the presence of electromagnetic attraction that is a catalyst in the process of killing cancer cells. In Figure 8a, it is seen that some of the nanoparticle constructions agglomerate when the current is increased to 10 mA, and the average diameter decreases to 18.13 nm.

These particles are considered ideal for various applications in medical fields such as cancer diagnosis and treatment. The

uniform size distribution of the nanoparticles indicates the success of the DC sputtering preparation technique in producing homogeneous nanoparticles.

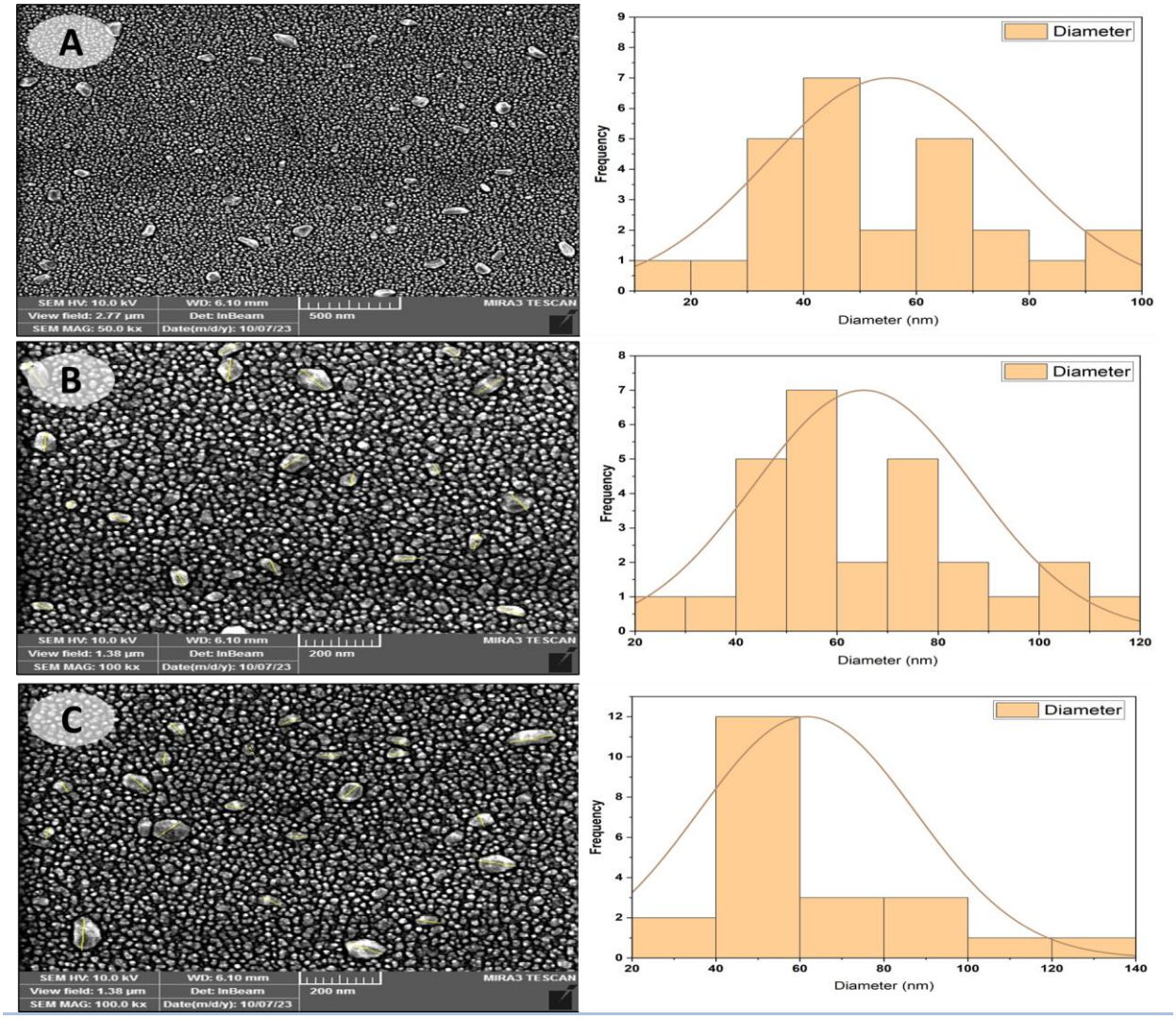

Figure 8. SEM images of silver nanoparticles AgNPs prepared with different current values. A. (10 mA), B. (8 mA) and C. (6 mA).

FTIR Characterization of Clove Emulsion Extract

Fourier transform infrared (FTIR) spectroscopy was used to determine the molecular structures of clove emulsion extract. **Figure 9** shows the FTIR spectra of the clove extract, revealing molecular vibrations similar to various biologically active components. Several distinct peaks can be observed in the FTIR spectrum, which are related to a major component of cloves, eugenol. These peaks are: a peak at 3403 cm-1, which is related to O-H stretching, indicating hydroxyl groups (likely from eugenol)[40]; a peak at 1380 cm-1, which is related to O-H stretching, indicating hydroxyl groups (likely from eugenol)[41]; a peak at 845 cm-1, which is related to C-H bending, indicating aromatic structures[42]; and finally, a peak at 757 cm-1, which is related to the absorption band associated with the out-of-plane bending of aromatic C-H bonds, which is found in compounds such as eugenol, a major component of cloves[41]. Eugenol has been shown to have numerous pharmacological properties, such as antibacterial, anticancer, antidiabetic, antioxidant, hypolipidemic, anti-inflammatory, and neuroprotective effects[17].

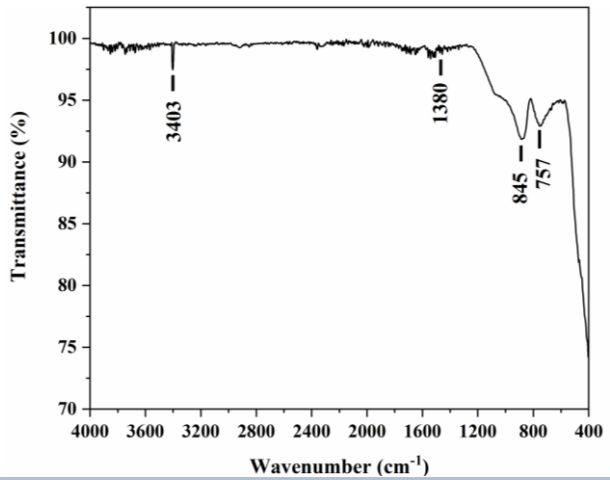

Figure 9. FTIR spectrum of clove oil emulsion extract to identify functional groups.

Growth Inhibition of Normal Cell Line (REF) by Silver Nanoparticles (AgNPs), Clove Emulsion Extract, and Their Mixture

In vitro cytotoxicity evaluation was performed by adding silver nanoparticles (AgNPs) to the REF cell line to confirm their effect on treating normal cells. A well-known equation[43] has been adopted to calculate cell viability and growth inhibition (GI):

$$(\mathrm{GI}) = \frac{control - treated}{control} \times 100\% \quad \ldots (2)$$

The growth inhibition rate of the REF cell line was evaluated at incubation times of 24 and 48 h, which indicates the cytotoxic effect of silver nanoparticles, clove emulsion extract, and their mixture, as shown in **Table 1**.

The growth inhibition rate of REF cells under the influence of silver nanoparticles was calculated, and its value increased with increasing concentration and incubation time. The cytotoxic effect of clove emulsion extract at the mentioned concentrations (25%, 50%, and 100%) mg/ml on REF cells was observed, which also increased with increasing concentrations. In general, the growth inhibition percentage (GI%) values obtained from the clove emulsion extract were lower compared to those obtained from the silver nanoparticles. The cytotoxic effect of the mixture of silver nanoparticles and clove emulsion extract added at equal concentration (25:25%, 50:50%, and 100:100%) on REF cells was observed, which also increased with increasing concentrations. A significant decrease in the growth inhibition rate was observed as a result of the combination of silver nanoparticles and clove emulsion compared to each of them alone, as shown in **Figure 10**.

Table 1. Impact of AgNPs, clove emulsion extract, and their mixture on growth inhibition of REF cell line after incubation for 24 and 48 hours.

REF cell line				
Concentration of AgNPs (µl)	**Incubation time (hours)**			
	24 h		**48**	
	GI%	**± SD**	**GI%**	**± SD**
25%	23.26	0.46	24.5	2.5
50%	25.2	0.46	26.6	1.5
100%	28.2	0.5	28.9	1.2
REF cell line				
Concentration of Clove mg/ml	**Incubation time (hours)**			
	24		**48**	
	GI%	**± SD**	**GI%**	**± SD**
25%	16.4	1.96	17.23	2.67
50%	18.6	3.1	20.31	5
100%	22.5	2.1	25.84	4.1
REF cell line				
Concentration of AgNPs + Clove (µl: mg/ml)	**Incubation time (hours)**			
	24		**48**	
	GI%	**± SD**	**GI%**	**± SD**
25%	15.3	1.86	13.6	1.4
50%	18	2.45	15.46	2
100%	18.9	2.1	17.58	1.95

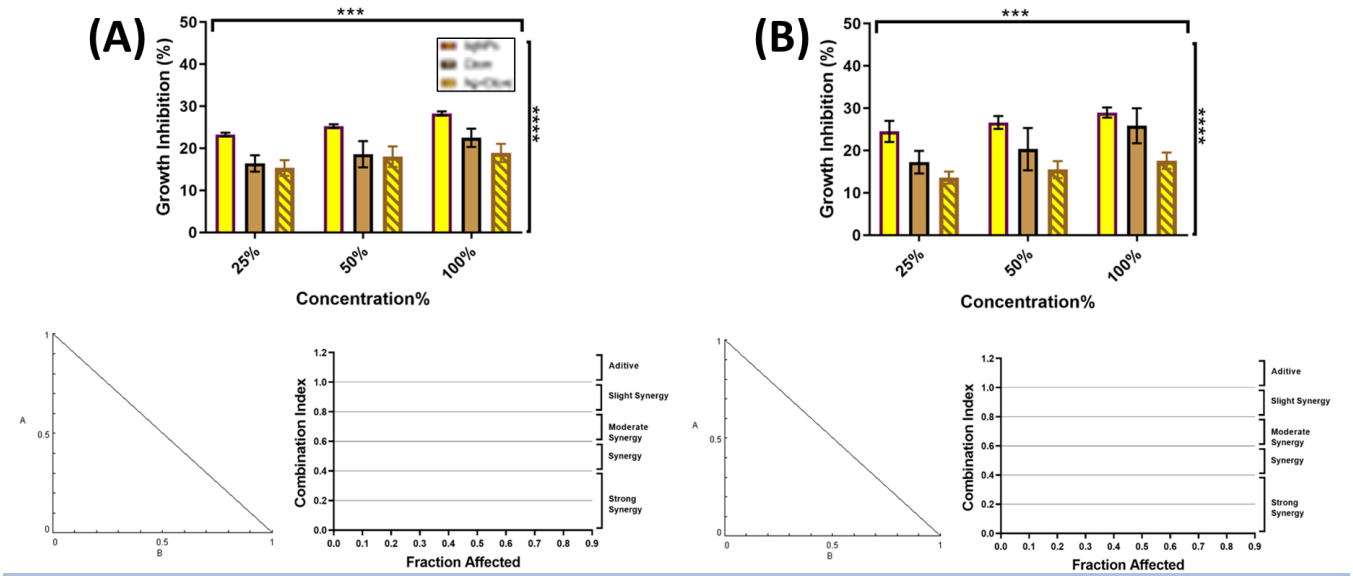

Figure 10. A. The effect of AgNPs and clove extract on the normal cell line REF after 24 hours of exposure, and B. The effect of AgNPs and clove extract on the normal cell line REF after 48 hours of exposure.

Growth Inhibition of Breast Cancer Cell Line (MDA) by Silver Nanoparticles (AgNPs), Clove Emulsion Extract, and Their Mixture

The growth inhibition rate of the MDA cell line was evaluated at incubation times of 24 and 48 h, which indicates the cytotoxic effect of silver nanoparticles, clove emulsion extract, and their mixture, as shown in **Table 2**. The cytotoxic effect of silver nanoparticles (AgNPs) on the MDA cell line was increased by higher concentrations of AgNPs. The growth inhibition rate of MDA cells under the influence of silver nanoparticles was calculated, and its value increased with increasing concentration and incubation time. The cytotoxic effect of clove emulsion extract at the mentioned concentrations (25%, 50%, and 100%) mg/ml on REF cells was observed, which also increased with increasing concentrations. In general, the growth inhibition percentage (GI%) values obtained from the clove emulsion extract were lower compared to those obtained from the silver nanoparticles. The cytotoxic effect of the mixture of silver

nanoparticles and clove emulsion extract added at equal concentration (25:25%, 50:50%, and 100:100%) on MDA cells was observed, which also increased with increasing concentrations. A significant decrease in the growth inhibition rate was observed as a result of the combination of silver nanoparticles and clove emulsion compared to each of them alone.

Various mechanisms contribute to the inhibition of MDA cancer cell growth, which include DNA damage, disruption of mitochondrial function, and oxidative stress[36, 44]. Clove emulsion extract also has a significant effect in inhibiting the growth of several cancer cell lines, which is attributed to multiple mechanisms, including "cell cycle arrest, apoptosis, and a potential effect on angiogenesis (the formation of new blood vessels that tumors need to grow) and metastasis"[45, 46]. The combination of silver nanoparticles and clove emulsion extract will certainly improve the inhibition of MDA cancer cell growth because it will act through the combination of their two mechanisms. As shown in **Figure 11**.

Table 2. Impact of AgNPs, clove emulsion extract, and their mixture on the growth inhibition of MDA cell line after incubation for 24 and 48 hours.

MDA cell line				
Concentration of AgNPs (µl)	**Incubation time (hours)**			
	24		**48**	
	19.01	2.38	22.66	1.6
25%	31.38	3.56	34.83	2.17
50%	40.21	2.7	45.93	2.4
100%	19.01	2.38	22.66	1.6
MDA cell line				
Concentration of Clove mg/ml	**Incubation time (hours)**			
	24		**24**	
	GI%	**± SD**	**GI%**	**± SD**
25%	14.53	2.05	15.86	1.18
50%	20.16	2.15	21.83	1.45
100%	25.4	1.9	27.1	3
MDA cell line				
Concentration of AgNPs + Clove (µl: mg/ml)	**Incubation time (hours)**			
	24		**24**	
	GI%	**± SD**	**GI%**	**± SD**
25%	52.56	3.29	61.87	1.39
50%	66.43	3.35	71.81	1.73
100%	78.23	4.53	80.93	5.04

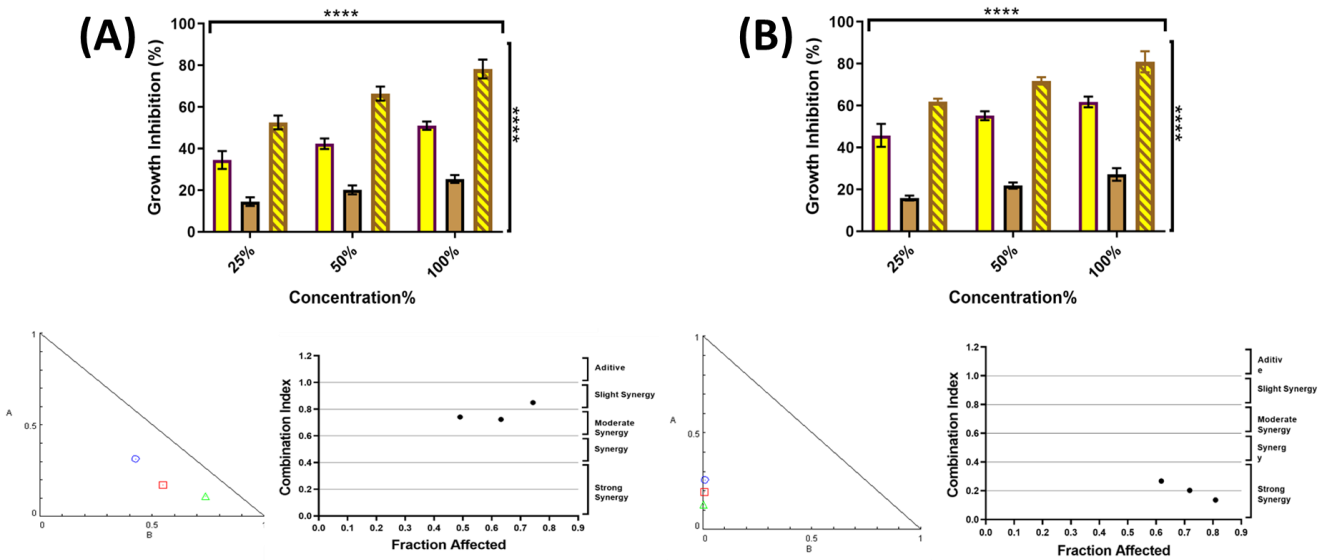

Figure 11. A. The effect of AgNPs and clove extract on cancer cell line MDA after 24 hours of exposure. and B. The effect of AgNPs and clove extract on cancer cell line MDA after 48 hours of exposure.

Conclusion

In this research, the anticancer activity of silver nanoparticles, clove emulsion extract, and their mixture on the MDA-MB-231 breast cancer cell line was investigated by performing a cytotoxicity test (MTT test). The plasma phenomenon has been adopted in two noncomplicated and inexpensive preparation techniques: DC sputtering and cold plasma to synthesize silver nanoparticles and clove emulsion extract, respectively. This study highlights the potential of environmentally friendly preparation methods to improve the physicochemical properties of synthesized plasmonic nanomaterials and medicinal herbal plant extracts for their exploitation in biomedical applications. The results of the cytotoxicity test of the prepared samples were encouraging in killing cancer cells, and their toxicity to living cells was moderate. This research presents a new approach combining nanomaterials and herbal plants for cancer treatment that could be adopted as an alternative to conventional radiotherapy, offering fewer side effects.

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Declaration of Competing Interests

The authors declare that they have no conflicts of interest.

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