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# Green Synthesis of Silver Nanoparticles Using *Mangifera Indica L.* (Musk) Peels Extract and Evaluation of Its Cytotoxic Activities

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**Abstract**

The aim of this study is to determine the bioactive compounds in *M. indica* peels extract which act as bioreducer agent with aqueous silver nitrate solution ($AgNO_3$) which act as precursor at different conditions. The phytoconstituents in the peels acted as reducing and stabilizing agent for Silver Nanoparticles (AgNPs) formation. UV-Vis spectroscopy, analysis were used to characterize the green synthesized AgNPs showed characteristic spectra at 430 nm. The size of the synthesized AgNPs is in the range from 1.5 to 9.5 nm, with an average size of 6.5 nm. SEM images showed that the green synthesized silver nanoparticles have a relatively spherical shape with a diameter of 38-62 nm. In addition, *M. indica* Peels extract and AgNPs showed efficient cytotoxic activities against (MCF-7) breast carcinoma cell line and (HeLa) cervical carcinoma cell line in comparison to Doxorubicin drug. The anticancer activity of AgNPs may be due to the number of phenolic compounds which was carried on the surface of silver nanoparticles. The results showed that synthesized AgNPs decreased % cell viability against MCF-7 and HeLa cell lines.

Keywords: *Mangifera indica* L. peels, HPLC analysis, phenolic compounds, green synthesis, silver Nanoparticles, cytotoxic activities

**Introduction:**

*M. indica* is a member of the Anacardiaceae family. It is one of the most important tropical fruits in the world. All aspects of the herb, including the bark, leaves, fruit peel and pulp, stems, and flowers, have medicinal properties and have historically been used to treat a variety of diseases and ailments [1]. Several studies have shown the pharmacological capacity of mango tree sections such as bark, leaves, fruit peel and pulp, stems, and flowers as anti-inflammatory, anticancer, antidiabetic, antioxidant, antibacterial, anthelmintic, antifungal, gastroprotective, hepatoprotective, anti-plasmodial, immunomodulatory, and anti-hyperlipemic [2]. Peels and kernels of vegetables and fruits are commonly thrown into the environment as waste products; however, these sections, like any other component of the plant, contain phytoconstituents that can be used therapeutically [3]. *M. indica* peels and seeds are the most important byproducts of mango production, accounting for 35percent and 60percent of total fruit weight, respectively [4]. Bio waste peels and seeds exhibit a wide range of promising biological activities, including antioxidant, antimicrobial, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, wound healing, and anti-ulcer, etc. [5]. As a result, it may be used for pharmacological and medicinal applications. Cancer is the 2nd major cause of death world - wide, behind only cardiovascular disease, which can be treated with chemotherapy, radiation, surgery, hormone treatment, and biological therapy [6,7]. Cancer diseases have grown in Egypt, with the incidence rate more than doubling in the last decade, especially hepatocellular carcinoma (HCC), due to a combination of biological (hepatitis B and C virus infection) and environmental factors (aflatoxin). Other factors such as tobacco smoking, pesticide exposure, and community based endemic infections such as schistosomiasis can also play a role in the etiology or development of the disease [8]. Herbal plants and plant-derived medicines have traditionally been used as a source of powerful anticancer agents in ancient societies around the world, and they are gaining popularity in modern society [9]. As a result, synthetic drugs are becoming less common due to their various side effects, high cost, and increased risk of recurrence [10].

Recently, the focus has shifted to nanotechnology. The advancement of nanoscale technologies has been widely recognized as a paradigm shift in cancer detection and treatment. Admittedly, the increased number of research attempts into the design and preparation of nanoparticles (NP) devices has resulted in the discovery of several

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substance forms with positive therapeutic and diagnostic effects in a single nano drug for the therapy of several types of cancer [11]. Physical, chemical, and biological methods can be used to build nanoparticles such as silver, gold, zinc, copper, palladium, and platinum; however, biological methods are the most preferred and recommended method because physical and chemical methods have several drawbacks [12]. The green method of using plant parts is the most convenient, fast, quick, cost efficient, and environmentally friendly. It has several benefits. The various plant parts contain several structurally distinct phytoconstituents such as phenols, flavonoids, saponins, glycosides, tannins, alkaloids, and anthocyanins, which serve as reducing and stabilizing agent for nano particle synthesis, thus eliminating the use of toxic solvents and chemicals [13]. The phytochemicals in the peels serve as reducing and capping agents and are responsible for the production of silver nanoparticles (AgNPs). There are various examples of AgNPs synthesis from plant peels, such as orange peels [14], dragon fruit peels [15], and banana peels [16]. Ag nanoparticles have a broad variety of uses as well as many pharmacological properties. Anticancer properties against MCF-7 cell lines, for example, as well as antimicrobial and antioxidant properties of various fruit peels waste (pomegranate, lime, banana, and apple (POBA)) [17], antibacterial function of dragon fruit peels against bacteria [18], antioxidant activity of Sambucus nigra L. products [19], antibacterial effects of phyllanthus emblica fruit [20], antioxidant, antibacterial, antidiabetic, and cytotoxicity potential of the outer peels extract of Ananas comosus [21], etc. They are also used in medical equipment (wound dressing, catheters, and bone cement), water purification, biosensors, bioimaging, cosmetics, nutritional processing, cell electrodes, integrated circuits, drinking water filters, and other applications [22,23]. *Mangifera indica* seed aqueous extract synthesized Ag NPs and AuNPs were reported [24,25].

However, the effectiveness of *M. indica* peels to synthesize Ag NPs is yet to be investigated, and this may be the first evidence of using methanolic extract of *M. indica* for AgNPs synthesis.

As a result, the primary objective of this study was to synthesize Ag NPs using methanolic extract of *M. indica* peels which is rich in phenolic compounds as a bioreducing agent to reduce Ag+ to Ag0, which were then analyzed and characterized using visual colour transition, pH analysis, Ultraviolet–Visible (UV–Vis) spectroscopy, Field emission scanning electron microscopy (FESEM), and Transmission electron microscopy (TEM). After characterization, the in vitro cytotoxic activities of the green synthesized Ag NPs were evaluated against (MCF-7) breast carcinoma cell line and (HELA) cervical carcinoma cell line then compared to the in vitro cytotoxic activities of pure methanolic extract of *M. indica* peels.

## Materials and Methods:

### Preparation of Peels Powders:

Fresh *M. indica* (Musk) peels (3Kg) were collected from a from Abu Hammad- Zagazig - Sharqia, Egypt in September 2019, with voucher number (No. 2012MT)., which were identified and authenticated by Dr. Alaaeldin Sayed Ewase, Ministry of Environment; Nature Conservation Sector, Biodiversity administration, Cairo, Egypt. The plant washed with distilled water and dried yield (250 gm). The powder was stored at 4ºC for further analysis.

### Extraction of *M. indica* (Musk) Peels:

Fresh dried *M. indica* peels (250 gm) defatted by using Soxhlet apparatus with (500 ml) of each of petroleum ether 60-80º, followed by *n*-hexane, finally by methanol. Yielded extracts were 5.78, 4.80, 3.75 gm, respectively. The dried plant crude extracts kept in refrigerator for further analysis.

### Chemicals Reagent:

All standards (ellagic acid, gallic acid, chlorogenic acid, mangiferin, naringenin, taxifolin, cinnamic acid, caffeic acid, syringic acid, pyrocatechol, protocatechuic acid, *p*-coumaric acid, vanillic acid, ferulic acid, methyl gallate, (+)-catechin, rutin and kaempferol) were used for identification and quantification were purchased from Sigma-Aldrich, the solvents used purchased from Merck (Germany).

### Chemical Studies

### 1. High Performance Liquid Chromatography

The HPLC apparatus used is Agilent 1260 series. The Kromasil $C_{18}$ column (4.6 mm x 250 mm 5 μm). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (100% A); 0–5 min (70% A); 5-8 min (60%A);8-12 min (30%A); 12-15 min (10%A) and 15-16 min (100% A).

HPLC analysis was performed in National Research Centre, (NRC), Giza, Egypt.

### 2- Biosynthesis of Silver Nanoparticles

Silver nanoparticles were created by combining silver nitrate ($AgNO_3$), distilled water, and a methanol extract of *M. indica* peels, by co-operation with the Nanotechnology Institute at Kufr El-Sheikh University in Egypt.

Silver nanoparticles were prepared from aqueous silver nitrate using a simple green route and *M. indica* peels extract as a reducing and capping agent [26]. The AgNPs were prepared from *M. indica* peels extract using the previously mentioned procedure with slight adjustments [27]. Diluted extract was prepared by adding 1 ml of concentrated methanolic extract drop by drop to 100ml of double distilled water. The silver nitrate (0.034g) was added to 200 ml of double distilled water to make a 1 mM silver nitrate solution. 200ml of 1mM $AgNO_3$ solution was put in 500 ml beaker, Then, 100ml of methanolic extract solution was dropped from burette and heated at 80ºC for 2 hours using a hot plate with a magnetic stirrer (1000 rpm).

As soon as, the methanolic extract solution was added, precipitate formed in the solution as dark brown color. Enough precipitate was found after the addition of 100ml of solution. The solution rested until reaching room temperature. It was separated using high-speed centrifugation at about 8000 rpm. The separated solid mass was washed several times with ethanolic alcohol to eliminate organic alcohol soluble impurities. Finally, the firm mass was dried in an oven set to 700 Celsius. The full drying of this solid mass produced a black-colored material (25mg), which was powdered in mortar and sampled for characterization.

### 3- Characterization of Silver Nanoparticles

### 3-1. pH analysis:

Silver nitrate aqueous solutions (1mM) and methanolic extract showed pH 5.91 pH was 6.98. respectively. The

changed pH of reaction mixtures was determined using digital pH meter (Eutech Cybersacn pH 300), during synthesis of silver nanoparticles.

**3.2. UV-Visible Spectra Spectrophotometer:**

The periodic scans of the optical absorbance between 300 and 700 nm with a double-beam UV-visible spectrophotometer (Carry 100 with tungsten halogen light sources) were performed to investigate the reduction of silver ions by methanolic extract. Which the biosynthesized Ag NPs solution was collected at room temperature, after various time intervals (15, 30, 45, 60 and 90 min.). UV-Visible Spectra was performed in National Research Center, (NRC), Giza, Egypt.

**3-3. Scanning Electron Microscopy (SEM):**

The shape, scale, and surface area of the AgNPs were investigated using SEM. The AgNPs solutions were ultrasonicated for 15 minutes at room temperature, and one drop of the sample was mounted on a glass slide. After drying, the glass slide was coated with gold and examined under a scanning electron microscope (Zeiss Evo-MA 10, Germany). Scanning Electron Microscopy (SEM)was performed in National Research Center, (NRC), Giza, Egypt.

**3-4. Transmission Electron Microscope (TEM):**

TEM analysis was done using Philips (technai 10). Thin films of sample were made on a carbon coated copper grid by simply dropping a very small amount of sample on the grid, excess solution was removed through blotting paper, and the film on the TEM grid allowed to dry in an incubator. In this technique, an electric beam is passed through an ultra-thin specimen, communicating with the specimen as it passes through. The presence of electrons passing through the specimen results in the formation of an image. An imaging system is used to magnify and focus the image. Transmission Electron Microscope (TEM)was performed in National Research Center, (NRC), Giza, Egypt.

**4-In *vitro* Assay for Cytotoxic Activity by (SRB assay):**

The human breast (MCF-7) and cervical (HeLa) carcinoma cell lines used in this study were obtained in the frozen state under liquid nitrogen (−180°C) from the American type of culture collection (ATCC, Minnesota, U.S.A.). The tumor cell lines were preserved by serial subculturing in the laboratory (National Cancer Institute, Cairo, Egypt). The cytotoxicity was assessed using the Sulphorhodamine-B (SRB) assay, as stated in [28]. SRB is a bright pink amin oxanthrene dye that comprises two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins in slightly acidic conditions, providing a sensitive index of cellular protein content. Briefly, at the initial concentration of 3x103 cell/well, cells were plated into 96-well microtiter plates in a 150 µl fresh medium and left to attach to the plates for 24 hrs. Different concentrations of 0, 5, 12.5, 25, 50 µg/ml of extracts were applied. After this, 3 wells were used for each extract concentration. For 48 hours, the plates were incubated. Cells were fixed with 50 µl of cold trichloroacetic acid,10% final concentration for 1 hr. at 40 ºC, then the plates were washed using distilled water (automatic washer Tecan, Germany) and stained at room temperature with 50 µl of 0.4 percent SRB dissolved in 1 percent acetic acid for 30 minutes. The plates were subsequently washed and air-dried with 1 percent acetic acid. finally, the dye was solubilized with 100 µl/well of 10M tris base (PH 10.5) and the optical density (O.D.) of each well was determined with an ELISA microplate reader spectrophotometrically at 570 nm (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted, and the mean values were determined for each extract concentration. The experiment was repeated 3 times. The results are expressed as the percentage of cell viability in comparison with the control cells (cells without extract). The cell viability of the control group without exposure to the extracts was defined as 100%.

**Results and Discussion**

**1-Investigation of Bioactive Compounds Using HPLC.**

The HPLC standards compounds are (ellagic acid, gallic acid, chlorogenic acid, caffeic acid, syringic acid, naringenin, taxifolin, cinnamic acid, pyrocatechol, *p*-coumaric acid, vanillin, ferulic acid, methyl gallate, (+)-catechin, rutin and kaempferol) used as references (Fig.1 and Table1).

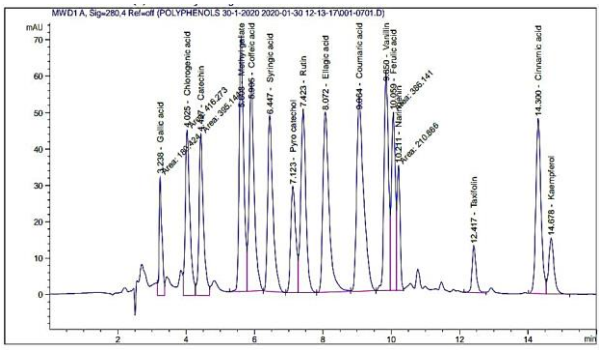


**Fig. (1): HPLC of Phenolics Standard**

**Table (1): HPLC of Phenolics Standard**

| Compounds | $R_t$ (min) | Area (%) | Conc. (µg/g) |
|---|---|---|---|
| Gallic acid | 3.238 | 180.42 | 16.8 |
| Chlorogenic acid | 4.025 | 416.27 | 28 |
| Catechin | 4.427 | 395.14 | 67.5 |
| Methyl gallate | 5.608 | 628.10 | 10.2 |
| Caffeic acid | 5.905 | 594.08 | 18 |
| Syringic acid | 6.447 | 514.34 | 17.2 |
| Pyro catechol | 7.123 | 294.70 | 29.2 |
| Rutin | 7.423 | 528.80 | 61 |
| Ellagic acid | 8.072 | 629.81 | 34.3 |
| Coumaric acid | 9.064 | 754.14 | 13.2 |
| Vanillin | 9.850 | 539.55 | 12.9 |
| Ferulic acid | 10.095 | 386.14 | 12.4 |
| Naringenin | 10.211 | 210.87 | 15 |
| Taxifolin | 12.417 | 106.21 | 13.2 |
| Cinnamic acid | 14.300 | 503.57 | 5.8 |
| Kaempferol | 14.678 | 164.35 | 12 |

**1.2. Investigation of Phenolics and Flavonoids Compounds of *M. indica* peels Using HPLC.**

The investigation of phenolic acids and flavonoids compounds of *M. indica* peels of (70%) methanolic extract data was recorded at (Fig. 2& Table 2) , which showed presence of (13) phenolic compounds compared to standard

samples using HPLC instrument where, the highest concentrations were, gallic acid (10416.39 µg/g), chlorogenic acid (2529.71µg/g), catechin (1378.18µg/g) , vanillin (357.99µg/g), naringenin (263.83µg/g) and taxifolin (185.23µg/g). while methyl gallate (70.91µg/g) pyrocatechol (55.29µg/g) and cinnamic acid (10.46µg/g) were the lowest concentrations, respectively.

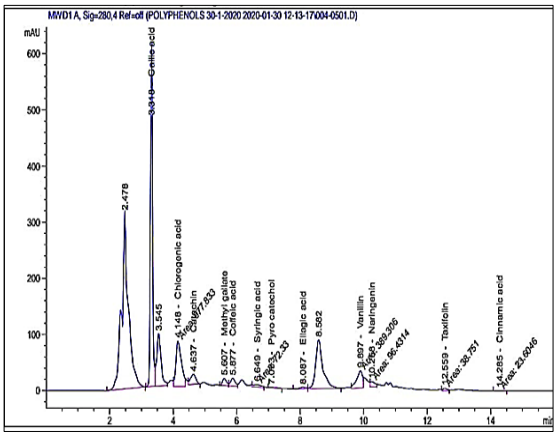


**Fig. (2): HPLC analysis of methanolic extract of *M.indica* L. peels**

**Table (2): Investigation of Bioactive Compounds of *M. indica* L. Peels Using HPLC.**

| Compounds | $R_t$ (min) | Area (%) | Conc. (µg/ml = µg / 26 mg) | Conc. (µg/g) |
|---|---|---|---|---|
| Gallic acid | 3.318 | 2908.54 | 270.83 | 10416.39 |
| Chlorogenic acid | 4.148 | 977.83 | 65.77 | 2529.71 |
| Catechin | 4.637 | 209.76 | 35.83 | 1378.18 |
| Methyl gallate | 5.607 | 113.53 | 1.84 | 70.91 |
| Caffeic acid | 5.877 | 137.02 | 4.15 | 159.68 |
| Syringic acid | 6.649 | 72.33 | 2.42 | 93.03 |
| Pyro catechol | 7.093 | 14.51 | 1.44 | 55.29 |
| Ellagic acid | 8.087 | 37.48 | 2.04 | 78.50 |
| Coumaric acid | 9.064 | 0.00 | 0.00 | 0.00 |
| Vanillin | 9.897 | 389.31 | 6.86 | 357.99 |
| Naringenin | 10.258 | 96.43 | 4.82 | 263.83 |
| Taxifolin | 12.559 | 38.75 | 0.27 | 185.23 |
| Cinnamic acid | 4.285 | 23.60 | 0.00 | 10.46 |

**2-Biosynthesis of AgNPs:**

The phytochemicals phenolics and flavonoids in the *M. indica* peels extract acted as the reducing agent and capping agents in the nanoparticles formation reported in this work. The observed colour change (light yellow to brown) when the extract was added to silver nitrate solution was an evidence of nanoparticles formation (Figs. 3). This can be attributed to the reduction of Ag + to $Ag^0$ excitation of surface Plasmon vibrations in the metal nanoparticles and changes in electronic energy level.

**3-Mechanism of Silver Nanoparticles Synthesis:**

The synthesis of AgNPs by methanolic extract of *M. indica* peels is due to the presence of large number of bioactive compounds like phenolic acid and flavonoids were investigated using HPLC, capable of donating electrons for the reduction of Ag+ ions to $Ag^0$. The carboxyl and hydroxyl groups form a protective coating on the surface of the Ag nanoparticles during the reaction. This shielding layer can induce steric hindrance around the particles, stabilizing them [29,30].

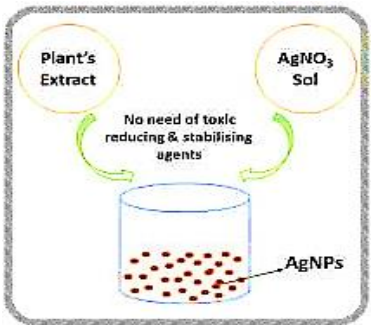


**Fig (3): Biosynthesis of Silver Nanoparticles**

A schematic diagram showing the silver ion reduction and stabilization to form a particle of nano size is shown in Fig. (4).

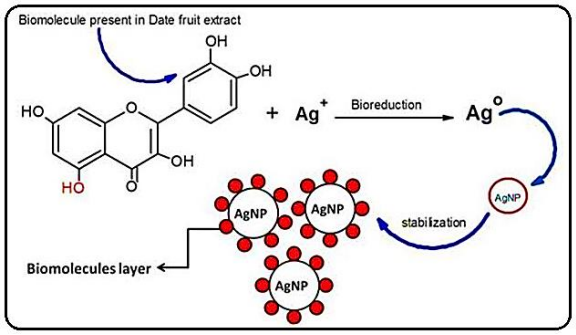


**Fig (4): Mechanism of Ag NPs Synthesis**

**4-Characterization of The Silver Nanoparticles**

**4.1. The Production of AgNPs:**

The first indication of the production of AgNPs is a change in the color of the reaction mixture [31]. Within a few minutes, the color changed from pale yellow to dark brown (Fig.5). These Change indicates the development of silver nanoparticles. With increasing time, the color intensity gradually increased (Table 3). A similar visual observation was made in Ag NPs synthesized from aqueous extract of *M. indica* peels [32].

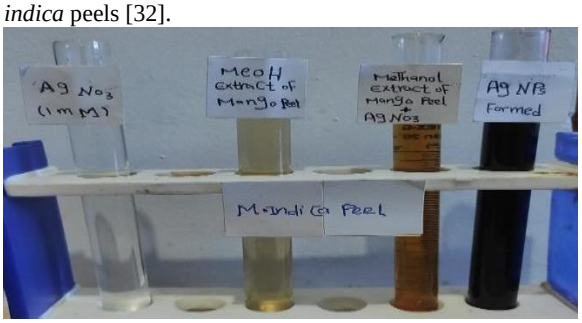


**Fig. (5): Change in Color of the reaction mixture during formation of Ag NPs.**

**Table (3): Change in Color of the Solution during Silver Nanoparticle Synthesis.**

| Sr. No | Solution | Color Change | | Color Intensity | Time |
|---|---|---|---|---|---|
| | | Before | After | | |
| 1 | Methanolic extract of *M. indica* peels | pale yellow | dark brown | +++ | few min. |
| 2 | 1mM $AgNO_3$ solution | Colorless | | | |

*Color intensity: (+) = Light color, (++) = Dark color, (+++) = Very dark color*

**4.2. Reduction of pH in Reaction Mixture:**

The pH of the reaction mixture decreased from 6.98 to 5.2 in the presence of methanolic extract of M. india L. peels, suggesting a reduction of 1 mM $AgNO_3$ during the formation of silver nanoparticles (Table 4). This result agreed with the involvement of Tecomella leaf extract reduced pH reduction during biogenic synthesis of silver nanoparticles [33]. When the original pH of the solution was increased, the color of the $AgNO_3$ solution changed from light yellow to dark brown. After 15 minutes of reactions, the color change will be an example of silver bio reduction induced by *M. indica* peels extract and the subsequent production of silver nanoparticles. This may be because the pH of the reaction solution influences the dissociated condition for capping functional groups in *M. indica* peels extract. The deprotonation of the capping functional groups is aided by the increased pH. Deprotonated functional groups can have a higher negative charge. As a result, the negatively charged groups bind to the silver nanoparticles and improve their stability due to the electrostatic repulsion [30].

**Table (4): Reduction of pH in reaction mixture During Formation of Silver Nanoparticles in presence of methanolic extract of *M. indica* peels**

| Sr. No. | Solution | (pH) Before reduction | (pH)After reduction |
|---|---|---|---|
| | 1mM $AgNO_3$ | 5.91 | No change |
| 1 | Methanolic extract of *M. indica* peels. | 6.52 | No change |
| 2 | Methanolic extract of *M. indica* peels +1 mM $AgNO_3$ | 6.98 | 5.2 |

**4.3. UV-Visible Spectrophotometer Analysis:**

UV–vis absorption spectroscopy is a main tool for studying the development of noble metal nanoparticles, which is based on surface plasmon resonance (SPR). The SPR dominates the optical absorption spectrum of metal nanoparticles, which varies with particle size, form, aggregation state, and dielectric medium [34]. The UV-Vis spectroscopy of the synthesized nanoparticles ranged from 412 to 430 nm. The methanolic extract of *M. indica* peels was able to synthesize silver nanoparticles due to the presence of suitable (SPR), the peaks near visible spectrum at 430 nm (Fig. 6).

It is understood that an absorption band appears at approximately 400–440 nm because of surface plasmon resonance in Ag nanoparticles [35,36]. In various time intervals, the peaks stand near 430 nm, showing the stability and uniformity of silver nanoparticle synthesis. The plasmonic peak of silver nanoparticles produced in the reaction mixture can be attributed to the absorbance peak near 430 nm. There is no change in peak position over time periods, but the difference in maximal absorbance with increasing time indicates that the rate of development of Ag nanoparticles is increasing. The absorption maximum of Ag NPs synthesized from *M. indica* seed aqueous extract was 450 [32]. In addition, Ag NPs synthesized from an aqueous extract of *M. indica* peels exhibited characteristic absorption bands at about 412-434 nm [30].

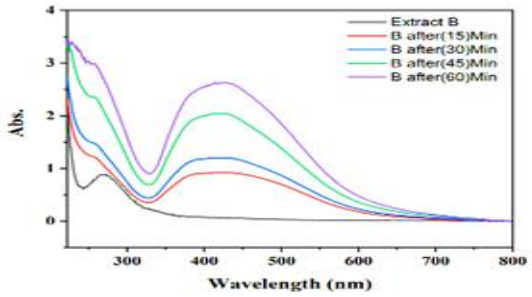


**Fig (6): UV-Visible Spectra of Silver Nanoparticles at 1mM Silver Nitrate in Different Time Course of Reaction with Methanolic Extract of *M. indica* peels.**

**4.4. Scanning Electron Microscopy (SEM):**

Images of high-density AgNPs synthesized by methanolic extract *M. indica* peel are seen in SEM analyses (Fig. 7). According to the SEM images, the green synthesized silver nanoparticles have a relatively spherical shape with a diameter of 38-62 nm. Silver nanoparticles were formed as a result of interactions between biomolecules capped with $Ag^0$ by hydrogen bonds.

Also, in the aggregated state, the nanoparticles were not in close contact, suggesting that the capping agent was stabilizing the nanoparticles.

Consequently, Spherical AgNPs were synthesized using an aqueous extract of Tanaetum vulgare fruit [37].

Furthermore, synthesized Ag NPs using aqueous extract of Piper nigrum (black pepper) were spherical [38].

**4.5. Transmission Electron Microscope (TEM):**

TEM is used to assess the morphology, shape, and size of nanoparticles. TEM images of synthesized Ag NPs at various magnifications are shown (Fig.8). The size of the synthesized AgNPs in the range from 1.5 to 9.5 nm, with an average size of 6.5 nm. The AgNPs were predominantly round shaped. This finding is similar to Sphere-shaped AgNPs with an average size of 7-27 nm obtained from an aqueous extract of *M. indica* peel [30].

Also, the size of the synthesized AgNPs was in the range 9–61nm with an average size of 26.85 nm, using *M. indica* seed aqueous extract which were spherical [32].

**5-Comparative Cytotoxic Activity:**

Cancer is a disease described by unregulated cellular proliferation and differentiation. Now, Cancer become a very common disease, with a high annual prevalence rate [39]. Cancer is treated by surgery, radiotherapy, or chemotherapeutic agents [40]. Chemotherapy has been used

to cure cancer for over five decades, either in conjunction with or parallel to radiotherapy or surgery [40]. Potential anticancer agents extracted from natural products are known to contain a variety of bioactive compounds, including roscovitine from red radish and flavopiridol from Amoore rohitukine, a tropical tree that has demonstrated considerable benefits in the treatment of cancer [41].

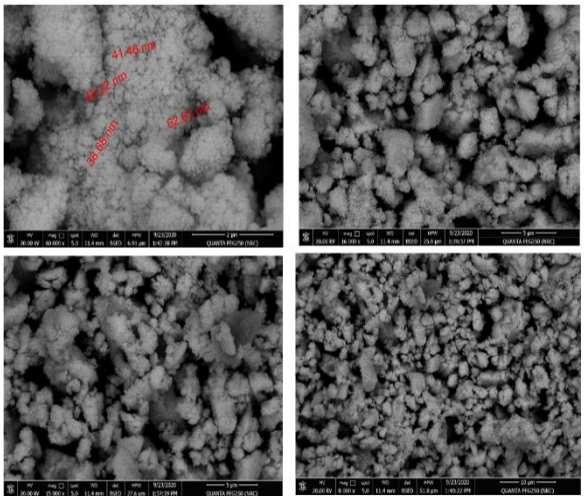

**Figure (7): SEM images of synthesized Ag NPs.**

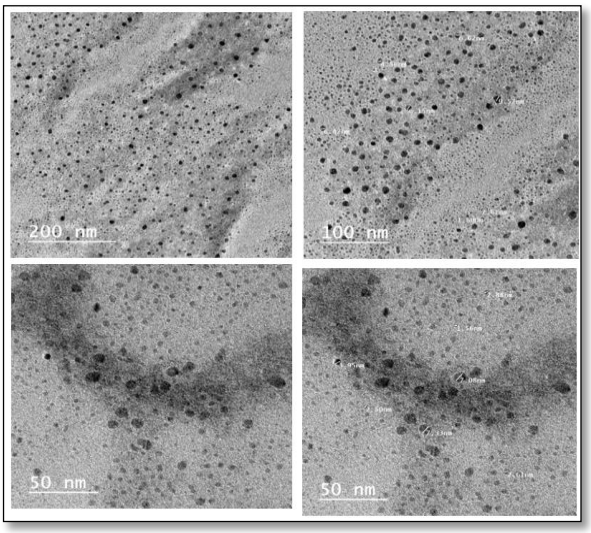


**Figure (8): TEM images of synthesized Ag NPs.**

The presence of phytoconstituents (tannins, alkaloids, phenolic and flavonoid compounds) in mango peel methanol extracts and the combination of these active substances with each other can increase the pharmacological and biological activity of the plant.

The cytotoxic effect of green synthesized AgNPs was evaluated by SRB assay against breast (MCF-7) and cervical (HeLa) carcinoma cell lines exposed to different concentrations (0, 5, 12.5, 25 and 50 µg /ml) of AgNPs (Fig.9,10). AgNPs showed dose dependent cytotoxicity effect on two cancer cell lines; both (MCF-7) and (HeLa) cell lines showed strong negative correlation between % cell viability and concentration of AgNPs; as concentration of AgNPs increased, the % cell viability decreased. Thus, one of the purposes of this research was comparative studies on the in vitro cytotoxic activities of pure methanolic extracts of *M. indica* peels and silver nano particles synthesized against breast (MCF-7) and cervical (HeLa) carcinoma cell lines.

### 5.1. Anti-tumor Activity of Methanolic Extract of *M. indica* peels and Silver Nano Particles Synthesized against (MCF-7) Carcinoma Cell Line:

Methanolic extract of *M. indica* peels showed best correlation with breast (MCF-7) cancer cell with IC50 39 µg/ml. At Concentration of 5 µg/ml, the methanolic extract of *M. indica* peels reduced the viability from 100 to 83.3% (16.7% death) and the dead cells produced by methanolic extract of *M. indica* peels reached 59.2% by 50 µg/ml while AgNPs correlation with breast (MCF-7) cancer cell with IC50 41.3 µg/ml. At concentration of 5 µg/ml, the silver nano particles reduced the viability from 100 to 87% (13% death) and the dead cells produced by AgNPs reached to 58% by 50 µg/ml (Fig. 9).

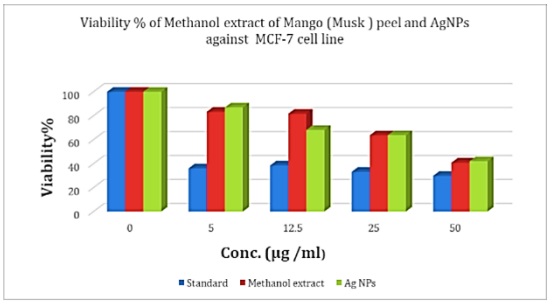


**Fig. (9): In *vitro* Cytotoxicity of Activity of Methanol Extract of *M. indica* peels and Ag NPs against (MCF-7) Carcinoma Cell Line.**

### 5.2. Anti-tumor Activity of Methanolic Extracts of *M. indica* peels and Silver Nano Particles Synthesized against (HELA) Carcinoma Cell Line:

Methanolic extract of *M. indica* peels showed best correlation with cervical (HeLa) cancer cell with $IC_{50}$ 24.5 µg/ml. At Concentration of 5 µg/ml, the methanolic extract of *M. indica* peels reduced the viability from 100 to 88% (12% death) and the dead cells produced by methanolic extract of *M. indica* peels reached to 53.5% by 50 µg/ml, while AgNPs correlation with cervical (HELA) cancer cell at concentration of 5 µg/ml, the silver nano particles reduced the viability from 100 to 99% (1% death) and the dead cells produced by AgNPs reached to 25.5% by 50 µg/ml (Fig.10).

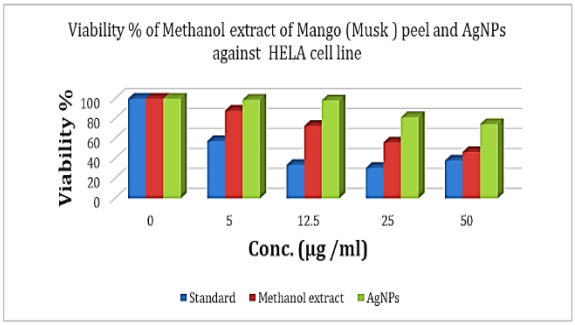


**Fig. (10): In vitro Cytotoxicity of Activity of Methanol Extract of *M. indica* peels and AgNPs against (HeLa) Carcinoma Cell Line.**

The increasing concentration, biologically AgNPs showed cytotoxicity towards the breast (MCF-7) cell line. The inhibitory concentration (IC50) of biosynthesized AgNPs against MCF-7 cells was created to be 86.7 µg ml−1 after 24 hours. When cancer cells were preserved with a high concentration of AgNPs (200 µg $ml^{-1}$), a high percentage of inhibition was detected [42]. Dose-dependent cytotoxicity was created in AgNPs-treated HeLa cells and raising the concentration of AgNPs resulted in grown cytotoxicity in HeLa cells. The IC50 value of biosynthesized AgNPs against HeLa cells is determined by 50% cell death at 300 µg/ml concentration [43].

Furthermore, these biologically synthesized silver nanoparticles have been shown to have an outstanding cytotoxic effect on HeLa cells [44]. As a result, AgNPs can participate in genes involved in cell cycle development, as well as cause DNA disruption and apoptosis in cancer cells [45]. Indeed, our findings show that AgNPs have a cytotoxic impact on cancer cell lines such as Breast (MCF-7) and Cervical (HeLa). On the other hand, the cytotoxic effect of AgNPs, is dependent on the dosage, concentration, time, and size of the AgNPs. The synthesized AgNPs showed decreased % cell viability against MCF-7 and HeLa cell lines but, the AgNPs show less cytotoxic effects than pure methanolic extract of *M. indica* peels. This is because phenolic compounds carried on the surface of silver nanoparticles have fewer anticancer activities than phenolic compounds in methanolic extract. The antitumor activity of *M. indica* peels extracts can be attributed to their high flavonoid and phenolic content. Flavonoids suppress cancer cell proliferation and tumor growth in animal models. The pattern of hydroxylation of the flavanone and flavone B ring, such as naringenin, taxifolin, and catechin, appears to have a critical impact on their activities, especially protein kinase inhibition and antiproliferation [46].

The powerful action of methanolic extract, which is related to its high constituents of isolated phenolic and flavonoid compounds with various hydroxyl groups in the flavonoid structure, in combination with a strongly conjugated -π- electron system, enables them to serve as free radical scavengers through hydrogen atom or electron donation activities. Furthermore, by cHeLating redox-active transition metal ions, they can prevent the formation of ROS (reactive oxygen species) such as hydroxyl radicals [47]. This has the potential to improve cancer detection by reducing phenomena like DNA oxidative damage.

## CONCLUSION

In the present study, in accordance with the saying best from waste, we have synthesized AgNPs from *M. indica* peels. The methanolic extract of *M. indica* peels rich with bioactive flavonoids and phenolic compounds which investigated using HPLC analysis. So, the bioactive compounds in the peel acted as reducing and stabilizing agent for the formation of AgNPs. The green synthesized AgNPs showed characteristic peak near 430 nm. In addition to AgNPs were predominantly round or spherical in shape with an average size of 6.5 nm.

It was demonstrated that the production of silver nanoparticles using green synthesis is an efficient, inexpensive and environmentally friendly than the classical chemistry. Therefore, synthesized AgNPs using methanolic extract of *M. indica* peels can be of both clinical and environmental applications. AgNPs using methanolic extract of *M. indica* peels was found to be active against the proliferation of human breast (MCF-7) and cervical (HeLa) cancer cell lines in concentration dependent manner.

This anticancer activity of AgNPs may be due to the number of phenolic compounds which was carried on the surface of silver nanoparticles.

The results showed that synthesized AgNPs decreased % cell viability against MCF-7 and HeLa cell lines but, AgNPs show less cytotoxic effects than pure methanolic extract of *M. indica* peels.

This is because phenolic compounds borne on the surface of silver nanoparticles have less effective on anticancer activity than phenolic compounds in methanolic extract.

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