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Research Article

Assessment of Antioxidant Activity and Anti-Cancer Efficiency of *Moringa oleifera* Leaves and its Biosynthesized Metal Nanoparticles

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Abstract

In recent years, with the advancement of nanotechnology, new methods have been invented to cure cancer. Moringa plant (*Moringa oleifera* leaves) is rich in phenolic and flavonoid compounds, which have anticancer and antioxidant properties. In this research, Chemical Composition, total polyphenols, total flavonoids and antioxidant activity % (DPPH) were determined, while, HPLC technique was used to fractionate phenolic and flavonoids components. The characteristics of metal nanoparticles of Zinc biosynthesized from aqueous extract of *M. oleifera* leaves were studied using Ultra Violet Visible Spectroscopy (U.V), Transmission Electron Microscopy (TEM), Scanning Electron Microscope (SEM), Energy dispersive x-ray analysis (EDX), Zeta size and zeta potential, the effect of dried aqueous extract and the synthesized of metal nanoparticles on normal cell (WI-38) and the growth and survival of cancer cell lines (MCF -7 and HepG-2) using different concentrations of (1, 10, 25, 50, 100, 250 and 500 µg/mL) were investigated.

Results: Results showed that the moisture content, crude protein, ether extract, crude fibers, ash and available carbohydrates of *M. Oleifera* leaves were 8.16, 28.40, 10.50, 7.50, 5.33 and 47.60% respectively. The total polyphenol contents of the aqueous extract and the ML/Zn-NPs were (86.44 and 84.05 mg GAE/g dry weight) respectively, and the total flavonoid content decreased from (20.04 to 16.55 mg QE/g dry weight) after nanoparticles formation, while the IC_{50} of DPPH increased marginally from 357 µg/ml (aqueous extract) to 366 µg/ml (ML/Zn-NPs). Chlorogenic acid was the predominant phenolic compound in both samples recording 568.33 ppm in (the aqueous extract) and increasing to 600.41 ppm in (ML/Zn-NPs). In the aqueous extract, daidzein was the predominant flavonoid (16.72 ppm), followed by quercetin (1.70 ppm) and rutin (0.40 ppm). After conjugation with Zn nanoparticles remarkable changes in the flavonoid profile were observed the concentration of daidzein decreased sharply to 6.44 ppm, while both rutin and quercetin increased to (3.05 and 2.34 ppm) respectively. Furthermore, the observed red-shift in the UV–Vis spectrum from (520 to 530 nm) indicates the appearance of surface plasmon resonance (SPR) as a result of the formation of Zn-NPs coated with organic phytochemicals. According to the results obtained from DLS, the sizes of zinc nanoparticles was 365.5 nm, while zeta potential of ML/Zn-NPs was -6.87 mV. Results of TEM reported that the particles size of MO/Zn-NPs ranged from approximately 45.21 to 86.98 nm. Meanwhile, the particles appeared predominantly spherical to near-spherical with an average diameter ranging between 0.041 and 0.070 µm. The EDX spectrum of ML/Zn-NPs revealed that the dominant peaks corresponding to oxygen (45.07 wt%), carbon (30.08 wt%) and zinc (14.44 wt%). Results showed dried aqueous extract and ML/Zn-NPs have equal effect on MCF7 at 500 µg/mL (98%). While (ML/Zn-NPs) had the highest inhibition effect against HepG2 tumor cell line at 500 µg/mL (96%). The IC_{50} values recorded for DAEML and ML/Zn-NPs being (161.20 and 88.54 µg/mL) on W1-38 were substantially higher than those observed in cancer cells, indicating reduced toxicity towards normal cells.

Conclusion: Obtained results indicated that, the zinc nanoparticles biosynthesized from aqueous extract of *Moringa oleifera* leaves had the highest anti-cancer effect on MCF -7 and HepG-2 cell lines in compared with those of the dried aqueous extract without any effect on normal cells.

Keywords: *Moringa oleifera* Leaves; Aqueous Extract; Metal Nanoparticles; U.V.; TEM; SEM; EDX; Zeta Size; Zeta Potential; WI-38; MCF -7 and HepG-2

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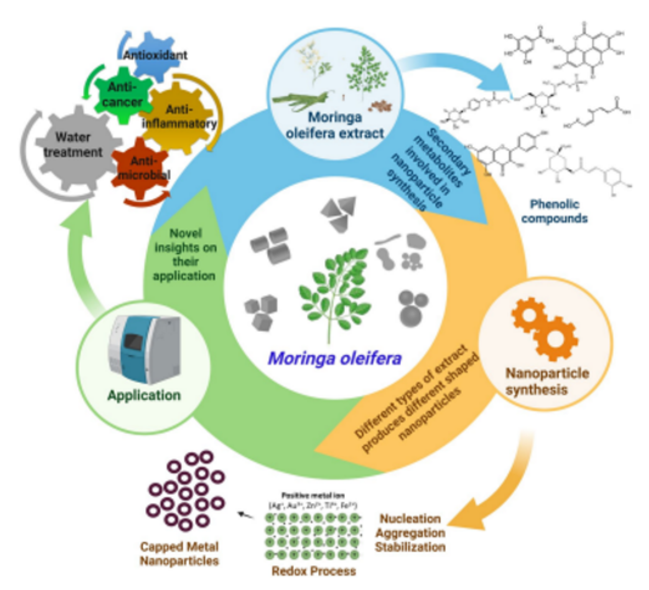


Figure a

Introduction

Moringa oleifera leaves are considered as a potential source of dynamic phyto-chemicals such as total phenolics, ascorbic acid oxidase, polyphenol oxidase, catalase, vitamins (A, C and E) and minerals. It was found that these phyto-chemicals exhibit antioxidant and scavenging activities. *M. Oleifera* leaves are wealthy in high protein content that is considered as perfect source of essential amino acids like methionine, cysteine, lysine and tryptophan. They exhibited their anti-cancer, anti-atherosclerotic, anti-microbial, antifungal, anti-diabetic, anti-rheumatoid arthritis, anti-depressant, anti-fertility and diuretic effects ... etc. [13].

One of the key therapeutic features of medicinal plants is their antioxidant capacity. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defence mechanisms, contributes to the development of many chronic diseases, including cardiovascular disorders, cancer, diabetes and neurodegenerative diseases. Antioxidants derived from plants, especially polyphenols and flavonoids, can scavenge free radicals, donate hydrogen atoms or electrons, and bind metal ions, reducing oxidative damage and supporting cellular health. As a result, the exploration of natural antioxidants from plant sources has received considerable scientific attention as per new trends [8].

Cancer is a fatal disease with a great mortality rate, which leads to a numerous of psychological and economic conflicts. Behaviors of the lifestyle which include smoking, physical inactivity and poor diet increase susceptibility of the body to cancer incidence especially in less economically developed countries. It is mainly happened as a result of one or more of the genetic alterations. Cancer not only occure as result of genetic change, but also due to hormonal modifications, cocarcinogen and tumor promoter effects [33].

Hepatocellular carcinoma and breast cancer are one of the most popular types of cancers. Depending on the sort of cancer and its progress, several techniques have been developed to cure these diseases including surgery, chemotherapy, hormonal, radiotherapy, stem cell transplantation and targeted medication delivery systems. Most of these available therapeutic options have several side effects. Cancer curing by mean of chemotherapy became limited because of the deleterious side effects and multi-curing resistance, so 74% of known anti-cancer agents and presently used today were derived from different plant species [25].

The studies focused lately on evaluation of *M. Oleifera* extract efficiency with respect to tumor-suppressive activity. Synthesis of metal nanoparticles attracted the attention because of their various characteristics, due to their small size (10 -100 nm) with their huge density and surface charge, have a high connect surface that supports binding to organic compounds such as DNA, RNA, antibodies, peptides and aptamers [37]. Gold, zinc, silver, iron, copper, palladium, platinum and nickel nanoparticles can be mentioned among the widely utilized metal nanoparticles that have been applied at the research level to cure cancer. There are many methods for the synthesis of different types of metal nanoparticles, which can be referred to chemical, physical and biological systems (synthesis by microorganisms and plants [26].

Most of the biologically active constituents absorbed slowly because of their high molecular weights which reduces their capability to cross through the cellular membrane, consequently decreases their efficiency and bioavailability. Therefore, the traditional medicinal plants have been incorporated with nanotechnology to settle the stability issues for increasing their bioavailability and lowering their toxicity. Also, integration of M-NPs into plant extracts enhanced the biological activities at lower concentrations as compared to plant extract only [31].

From this point of view, the study was designed to develop novel strategy for incorporating characterization of *Moringa oleifera* leaves particles, its antioxidant activity and Zn-NPs into *M. Oleifera* leaves extract to enhance its antioxidant efficiency. The cytotoxicity of the nano-extract against Hepatocellular carcinoma and breast cancer.

Materials and Methods

Materials

Moringa oleifera Leaves

Trees of *Moringa oleifera* were cultivated from April to August in Faculty of Agriculture farm, Mansoura University, then leaves were collected in July and August.

Chemicals and reagents

Zinc nitrate hexahydrate (Zn $(NO_3)_2$ $6H_2O$) was purchased from lab Egypt Supplies, Yassin Str., Al-Firdaws Towers Complex, Shubra El-Kheima Central, Egypt.

Media

RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

Cell line

Human cell lines including normal lung fibroblasts (WI-38), Hepatocellular carcinoma (HEPG-2) and Mammary gland Breast cancer (MCF-7) were obtained from American Type Culture Collection)ATCC(via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Methods

Preparation of Aqueous Extract of *Moringa oleifera* Leaves (AEML)

Moringa oleifera leaves were washed with deionized water to removed impurities, foreign materials and dust, then dried in the greenhouses (30-45°) for 3 days as reported by [32] in plant pathology farm, Faculty of Agriculture, Mansoura University, then dried leaves were grounded to fine powder. 30 g of dried *Moringa oleifera* leaves were immersed in 300 ml of boiled deionized water using magnetic stirrer (model Cat D-7813 staufen) for 1h45 at 50-52°C. The mixture was cooled until reached to room temperature and filtered with nylon mesh, then fill in falcon and centrifugation (model FC5706) at 4500 rpm for 12 min. The precipitate is removed and the filtered was stored at 4±1°C for further studies as mentioned by [30].

Preparation of metal nanoparticles (MO/Zn-NPs)

Metal of zinc nanoparticles were synthesized at Agricultural Chemistry Department, Faculty of Agriculture, Mansoura University, Egypt as citied by [30] with a slight modification. 50 ml of filtered *Moringa oleifera* Leaves extract was used to dissolve 0.3444 g of Zn $(NO_3)_2$ $6H_2O$ under magnetic stirrer for 18 hr. The zinc salts were completely dissolved in *Moringa oleifera* leaves extract without any additional heat processing. The mixture was covered with aluminum foil paper to avoid any photo-induced phenomenon and kept to room temperature. However, there was an obvious color change after 18 hr., but no precipitate suggesting that any formation of a zinc compound was in the shape of a suspension. Then stored at -18 °C further analysis carried out.

Characterization of Synthesized *Moringa oleifera* leaves zinc nanoparticles (ML/Zn-NPs)

UV – Vis spectrum analysis

The reduction of pure zinc ions of the resulting zinc nanoparticles were monitored using ATI Unicom UV–Vis Spectrophotometer vision software V 3.20 at different wavelengths ranging from 190 nm to 1100 nm at Spectral Analysis Unit, Department of Chemistry, Faculty of Science, Mansoura University, Egypt.

Zeta size of nano particles

Was estimated on a Zetasizer Nano-ZsgO at Electron Microscope Unit, Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

Zeta potential of nano particles

Zeta potential Analysis is a method applied by Malvern Instruments Ltd Zeta Potential Ver. 2.3 to estimate the surface charge of nanoparticles in suspensions without any acceleration at Electron Microscope Unit, Central Laboratory, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

Transmission electron microscope (TEM) measurements

The size, shape, surface, crystal structure and morphological data of zinc nanoparticles were characterized using transmission electron microscopy (TEM) (JEOL jam-2100) at Electron Microscope Unit, Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

Scanning electron microscope (SEM) measurements

Size, shape and morphology of the nanoparticles were determined using Scanning electron microscopy (SEM) (JEOL jsm 6510 lv) made in Japan at Electron Microscope Unit, Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

Energy dispersive X-Ray analysis (EDX)

The presence of element zinc and their concentration within the material by energy dispersive X-ray (EDX) spectrometry analysis with Oxford x-Max20 made in England, at Electron Microscope Unit, Central Laboratory, Faculty of Agriculture, Mansoura University, Egypt.

Determination of proximate chemical composition of *Moringa oleifera* leaves Powder (MLP)

Moisture content, crude protein, carbohydrates, crude fiber and ash of *Moringa oleifera* leaves powder were determined according to the methods described by [7].

Determination of total phenolic contents (TPC) of aqueous extract of *Moringa oleifera* leaves and ML/Zn-NPs

Total polyphenols were determined using Folin-Ciocalteu assay. Gallic acid was used to calculate the characteristic values as milligram gallic acid equivalent (GAE)/g of the dried plant, which carried out at Unit of Genetic Engineering and Biotechnology, Faculty of Science, Mansoura University, Egypt.

Determination of total flavonoids contents of aqueous extract of Moringa oleifera leaves and ML/Zn-NPs

Total flavonoids were estimated using aluminum chloride colorimetric assay at Unit of Genetic Engineering and Biotechnology, Faculty of Science, Mansoura University. The contents of flavonoids are articulated as milligram catechin equivalent per gram (mg QE)/g of the dry weight of the plant.

Identification and fractionation of phenolic compounds of aqueous extract of Moringa oleifera leaves and ML/Zn-NPs

Phenolic and flavonoid compounds were identified using high performance liquid chromatography (HPLC) Technique at the Institute of Food Science, Agricultural Research Center, Giza, Egypt.

Determination of radical scavenging (DPPH%) of aqueous extract of *Moringa oleifera* leaves and ML/Zn-NPs

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were carried out at Unit of Genetic Engineering and Biotechnology, Faculty of Science, Mansoura University.

Cytotoxicity and cell viability assay (MTT Assay)

In this study, the cytotoxic potentiality of the extract and their nanoparticles was done using the methods described by [15,34]. The dried aqueous extract of *Moringa oleifera* leaves (DAEML) and green-synthesized *Moringa oleifera*/Zn-nanoparticles (ML/Zn-NPs) were used to determine cytotoxic assessment on cell growth using MTT assay. Doxorubicin was employed as a standard anticancer drug for comparison. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C in a with 5% CO_2 incubator. The cells were seeded into 96-well plates at a density of 1.0×10^4 cells/ well and incubated for 48 h at 37°C under 5% CO_2 to facilitate the attachment of cell line to the plate wall**.** After incubation, the cell lines were treated with different concentration of the dried aqueous extract of *Moringa oleifera* leaves (DAEML) and their nanoparticles (ML/Zn-NPs) at levels of (1, 10, 25, 50, 100, 250, and 500 µg/mL) and incubated for 24 h. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 h. Subsequently, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the purple formazan formed. The absorbance was measured at 570 nm using a plate reader (EXL 800, USA). Cell viability was expressed as a percentage relative to untreated control cells according to the following equation:

$$\text{Cell Viability (\%)} = \left(\frac{\text{A570 treated}}{\text{A570 control}}\right) x\ 100$$

The biological evaluation was carried out at College of Pharmacy, Mansoura University, El-Mansoura, Egypt.

Statistical analysis

The obtained data were statistically analyzed as stated using the SPSS program (Statistical Package For Social Sciences program, version, 17 for windows). One-way analysis of variance (ANOVA) was used to test the differences between treatments**,** the least significant difference (L.S.D) and Duncan's methods was used to compare the differences between the means of treatment values [39].

Results and Discussion

Characterizations of nanoparticles

Ultra violet visible spectroscopy

UV-Vis spectroscopy is a simple, dynamic tool for monitoring nanoparticles (NPs) synthesis, which detecting their formation, growth and constancy by observing changes in their unique color (surface plasmon resonance - SPR). This analysis proves the success of the process of manufacturing nanoparticles (such as zinc or silver) and helps in characterize their size, shape and aggregation.

The obtained results showed that the UV–visible spectrum of the aqueous extract of *Moringa oleifera* leaves (AEML) exhibited two main absorption peaks at 255 nm (Abs = 0.824) and 520 nm (Abs = 1.176) as stated in Figure (1). The band observed at 255 nm is attributed to π→π* transitions of aromatic rings confirming the presence of phenolic and flavonoid compounds such as chlorogenic acid, gallic acid and quercetin… etc. These bioactive components are characterized by conjugated double bonds and aromatic systems responsible for strong UV absorption [4].

The second band at 520 nm corresponds to n→π* transitions of conjugated carbonyl and polyphenolic structures. This region reflects the presence of chromophoric groups with electron-donating ability indicating that the extract contains reducing and stabilizing agents essential for nanoparticle biosynthesis Figure (1). Hence, the UV–Vis spectrum of the aqueous extract confirms the abundance of phytochemicals with potential reducing power [4].

Results declared also, the UV–visible spectrum of the biosynthesized ML/Zn-NPs displayed two peaks at 255 nm (Abs = 0.823) and 530 nm (Abs = 1.424) as reported in Figure (1). The sharp and intense absorption around 530 nm represents the surface plasmon resonance (SPR) band of zinc nanoparticles confirming their successful formation. The red-shift of the visible peak from 520 nm in the pure extract to 530 nm in ML/Zn-NPs and the increase in absorbance intensity indicate a strong interaction between Zn^{2+} ions and the phytochemicals of *Moringa oleifera* leaves.

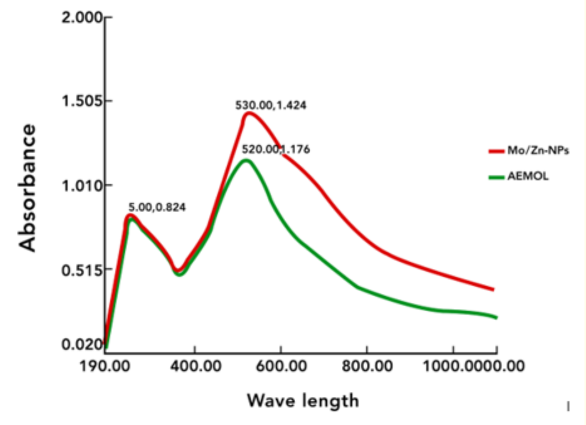


Figure 1: UV-Vis spectroscopic measurements of AEML and ML/Zn-NPs.

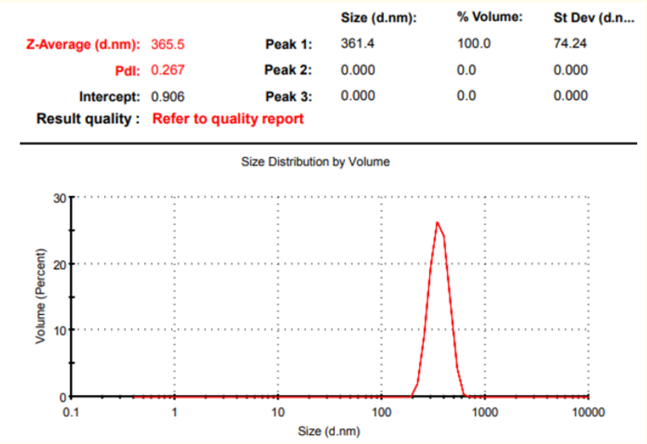
These biomolecules such as (phenolics, flavonoids, terpenoids and proteins) which act as natural reducing and capping agents. During the reaction zinc ions (Zn^{2+}) from zinc salts interact with hydroxyl (–OH) and carbonyl (C=O) functional groups present in these biomolecules. Phenolic hydroxyl groups donate electrons reducing Zn^{2+} to metallic Zn^0 nanoparticles. Simultaneously, other oxygenated groups in flavonoids and proteins form a protective layer around the nanoparticles preventing agglomeration and ensuring colloidal stability. The observed red-shift in the UV–Vis spectrum from (520 to 530 nm) supports this mechanism, indicating the appearance of surface plasmon resonance (SPR) due to the formation of Zn-NPs capped with organic phytochemicals.

This natural capping layer also enhances the stability and bioactivity of the nanoparticles making them suitable for biological and environmental applications. Accordingly, the high flavonoids and phenolics content in *Moringa oleifera* leaves extract revealed in the phytochemical analysis highly support the potential of *Moringa oleifera* leaves to bio-shorthand of zinc ions to zinc nanoparticles [4].

Zeta size valus of synthesized zinc nanoparticles (Zn-NPs)

The particle size distribution of the biosynthesized zinc nanoparticles (Zn-NPs) was analyzed using the Dynamic Light Scattering (DLS) technique (Malvern Zetasizer, version 7.01). The results revealed that the nanoparticles exhibited a Z-average diameter of 365.5 nm with a polydispersity index (PDI) of 0.267 indicating a narrow and homogeneous size distribution as showen in Figure (2). The PDI value below 0.3 suggests that the synthesized Zn-NPs are moderately monodisperse reflecting uniformity in particle size and stability of the colloidal suspension. A single prominent peak was observed at 361.4 nm confirming the presence of a single population of nanoparticles without significant aggregation or formation of larger clusters.

From data presented in Figure (2) the observed particle size range is consistent with previous reports on plant-mediated synthesis of Zn-NPs, where the involvement of *Moringa oleifera* leaf extract biomolecules such as polyphenols, flavonoids and proteins plays a crucial role as reducing and capping agents by preventing agglomeration through surface binding and charge repulsion mechanisms. Consequently, the obtained DLS data confirm the successful formation of stable, well-dispersed zinc nanoparticles within the nanometric scale.



			Size (d.nm):	% Volume:	St Dev (d.n...
Z-Average (d.nm):	365.5	Peak 1:	361.4	100.0	74.24
PdI:	0.267	Peak 2:	0.000	0.0	0.000
Intercept:	0.906	Peak 3:	0.000	0.0	0.000
Result quality :	Refer to quality report				

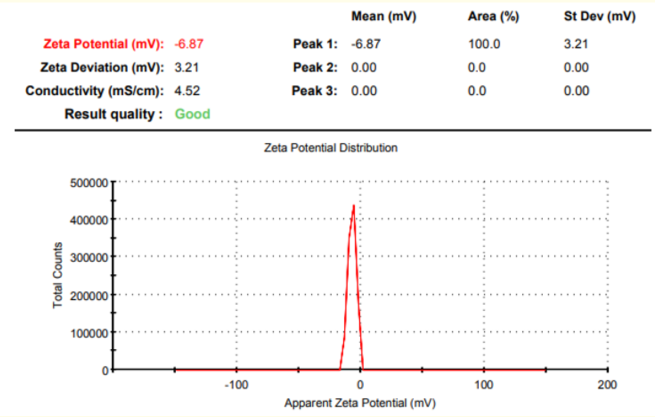
Figure 2: Zeta size distribution for Zn-NPs synthesized by *Moringa Oleifera* leaves extract.

This results is slightly higher than those reported by [22] who noticed that the average size of Zn-NPs which synthesized using *Cayratia pedata* leaf extract was 52.24 nm. The relatively larger particle size in the current study could be attributed to differences in synthesis parameters such as extract concentration, metal ion ratio, pH, temperature and reaction time, these variations in particle size among different studies are common in green synthesis methods due to the complex nature of plant metabolites involved in reduction and stabilization processes.

Zeta potential values of synthesized zinc nanoparticles (Zn-NPs)

Zeta potential is a main tool for understanding the condition of the nanoparticle surface and appreciating the long-term stability of the nanoparticle. Zeta potential technique was used to define the nanoparticles surface charge. Nanoparticles possess a surface charge that catches a thin layer of ions of opposite charge to the nanoparticle surface. Nanoparticles own double layer of ions moves as it divides throughout the solution, the electric potential at the boundary of the double coat is known as the zeta potential of the particles and its values that usually varied from +100 mV to -100 mV [29].

Figure (3), reported that the synthesized zinc nanoparticles using aqueous extract of *Moringa oleifera* leaves powder has zeta potential value of -6.87 mV. This negative surface charge indicates the presence of negatively charged functional groups such as hydroxyl and carboxyl groups, derived from phytochemicals in the plant extract, which are responsible for the stabilization of the nanoparticles.



			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-6.87	Peak 1:	-6.87	100.0	3.21
Zeta Deviation (mV):	3.21	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	4.52	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

Figure 3: Zeta potential distribution of the synthesized Zn-NPs using *Moringa Oleifera* leaves extract.

The nanoparticles with zeta potential values lower than +25 mV or greater than -25 mV usually have high levels of stability as declared by [20]. Extremely positive or negative zeta potential rates cause major repulsive forces, while repulsion through particles with equal electric charge block accumulation of the particles, thus guarantee easy dispersion. This results is agreement with [19,29] who reported that the zeta average size of Zn-NPs for ethanolic extract showed that the density and the aggregates of particles were more constant.

Transmission electron microscope (TEM) characterization for nanoparticles

The Zn^{++} ions with *Moringa oleifera* leaves extract were examined with TEM to confirm the presence of Zn-NPs. As shown in Figure (4), illustrates the morphology and size distribution of the synthesized ML/Zn-NPs at 100 nm, it could be observed that the particles size for *Moringa oleifera*/Zn-NPs ranged from approximately 45.21 to 86.98 nm, with an average diameter of about 55 nm, this is considered as Nano-particles size. The particles shape of *Moringa oleifera*/Zn-NPs have a spherical, smooth and granular shape.

Data showed also, the *Moringa oleifera*/Zn-NPs contains a larger number of small particles size, this is the reason that it gave higher effect on the different cancer cell lines. These resulted were in accordance with [29] who studied TEM technique for characterizing digital images of the prepared selenium and zinc nanoparticles of *Ginkgo biloba* dried leaves powder and *Melissa officinalis* dried leaves powder. They observed that the size of the zinc particles is ranged from (70.63 to 82.57 nm) and (72.30 to 86.46) nm for *Ginkgo biloba* dried leaves powder and *Melissa officinalis* dried leaves powder.

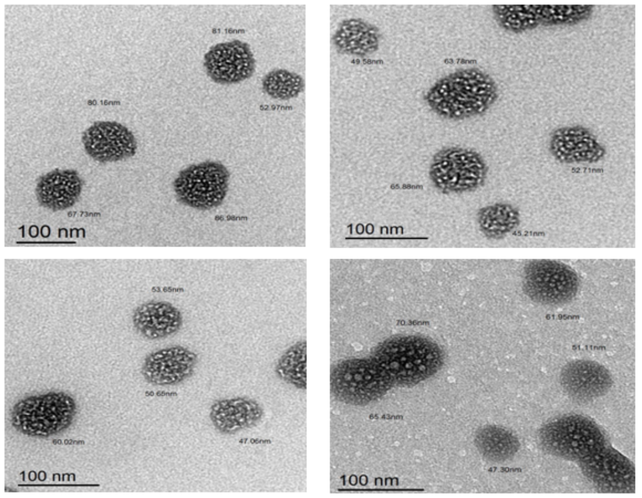


Figure 4: TEM micrographs characterization illustrating the size and morphology of Zn-NPs obtained with *Moringa Oleifera* leaves extract at 100 nm.

Scanning electron microscope (SEM) characterization for nanoparticles

The SEM analysis was performed to identify the uniformity and surface morphology of Zn-NPs. Figure (5), illustrate the SEM results of Zn nanoparticles obtained with *Moringa oleifera* leaves extract. Results revealed a highly dense distribution of uniformly shaped nanoparticles covering the entire surface. The particles appeared predominantly spherical to near-spherical with an average diameter ranging between 0.041 and 0.070 µm, as indicated by the measured points in the micrograph. The narrow size range reflects a relatively homogeneous nucleation process during synthesis and indicates the effective reducing and stabilizing actions of *Moringa oleifera* phytochemicals.

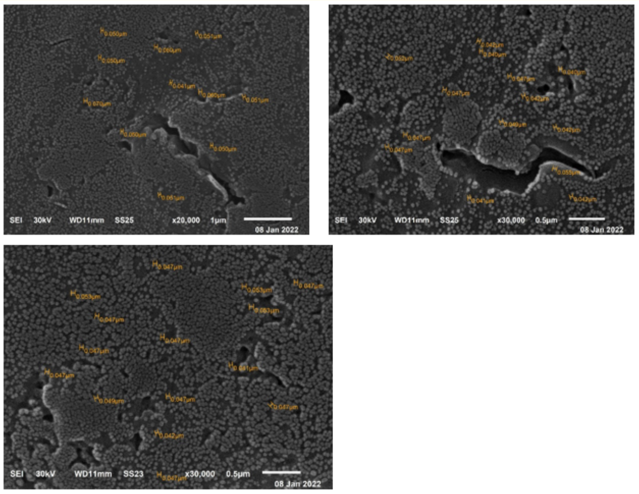


Figure 5: SEM micrograph of Zn-NPs nanoparticles from *Moringa Oleifera* leaves extract.

Data showed also, the uniform particles distribution and smooth surface morphology suggest the presence of an organic capping layer, consistent with biomolecules detected by EDX in similar studies. This organic coating plays a crucial role in preventing particle fusion and stabilizing the nanostructures. Our results is agreement with [14] who studied the SEM results of Zn nanoparticles extracted from *Moringa oleifera* leaves extract. They reported that the average size of nano zinc oxide was 0.064 µm.

Energy dispersive X-ray analysis (EDX)

Energy dispersive X-ray (EDX) spectrum shows the intensity of X-rays emitted by a material versus their energy, revealing its elemental composition by identifying characteristic energy peaks for each element, which are generated when an electron beam strikes a sample, causing throw of the nucleus electrons and fall off the upper shell electrons, releasing characteristic X-rays. This non-destructive method, often used with SEM/TEM, provides crucial chemical information, which the heights of the peaks indicates quantities and distribution of the elements [9].

The energy dispersive X-ray (EDX) spectrum of the nanoparticles synthesized using *Moringa oleifera* leaf extract revealed dominant peaks corresponding to oxygen (45.07 wt%), carbon (30.08 wt%) and zinc (14.44 wt%). The high carbon and oxygen signals are consistent with the presence of organic biomolecules from the *M. Oleifera* extract such as (polyphenols, proteins and flavonoids), which act as reducing, capping and stabilizing agents on the nanoparticle surface.

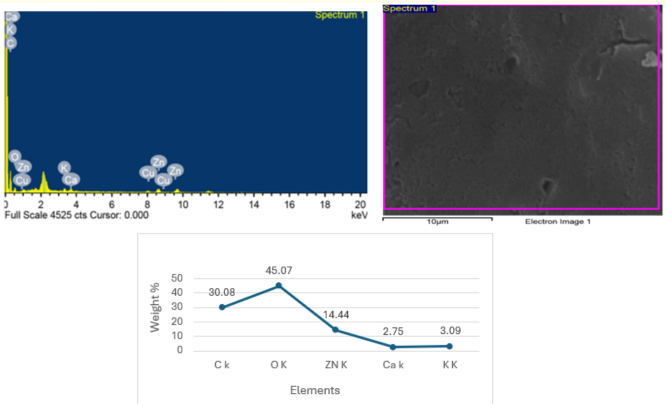


Figure 6: EDX spectrum of synthesized Zn-NPs nanoparticles.

These findings are in good agreement with the TEM and SEM data, providing a comprehensive understanding of the composition and morphology of the synthesized Zn nanoparticles. The distinct zinc signal confirms the successful incorporation and formation of zinc-based phases Zn as shown in Figure (6). Potassium and calcium are present at low levels, most likely representing residual ions originating from the plant extract or synthesis medium. These results support a phytochemical-mediated synthesis mechanism, in which plant metabolites reduce metal ions and subsequently stabilize the formed nanoparticles.

Proximate chemical composition of *Moringa oleifera* leaves powder (MLP)

Data in Table (1) revealed that the moisture, ash, crude protein, ether extract, crude fibers and available carbohydrates contents were identified in *Moringa oleifera* leaves powder. All results were calculated (on dry weight basis). Results in Table (1) showed that the content of *Moringa oleifera* leaves powder from moisture, crude protein, ether extract, crude fibers, ash and available carbohydrates were 8.16, 28.40, 10.50, 7.50, 5.33 and 47.60% (on dry weight basis) respectively. It could be noticed that *Moringa oleifera* leaves powder could be a good and economic source of crude protein, consistent with previous findings that identified Moringa as a high-protein functional food in low and middle income countries and countries suffering from malnutrition. These results was similar to those reported by [21] who reported that the crude protein of *Moringa oleifera* leaves powder was 28.11%, and higher than those mentioned by [36] who found that protein content of (MLP) was 22.7%.

Chemical composition of *Moringa oleifera* leaves powder (%)	Moisture	Crude Protein	Crude oil (ether extract)	Crude fibers	Ash	Carbohydrates
	8.16	28.40	10.50	7.50	5.33	47.60

Table 1: Proximate some chemical constitutes of *Moringa Oleifera* leaves powder (MLP) (on dry weight basis).

Also in the same Table (1), it could be noticed that the fibers content of (MLP) was 7.50%, this aids in improving digestive health and reducing the risk of chronic diseases. It helps with weight management, blood sugar control and lower the risk of certain cancers and heart diseases. Our findings were lower than those determined by [21,36] who revealed that the crude fibers of *Moringa oleifera* leaves powder were 20.5 and 19.61% respectively.

Results showed also the oil and ash content of *Moringa oleifera* leaves powder were 10.50 and 5.33% respectively, reflecting the presence of beneficial fatty acids that may contribute to the plant's biological activities and indicating a considerable amount of mineral elements in the leaves, these results was lower than those determined by [21,36] who reported that the ash content of (MLP) were 6.5 and 10.50% respectively.

As presented in the same Table (1), it could be observed that the carbohydrates content of *Moringa oleifera* leaves powder (MLP) was 47.60%, these higher carbohydrates content are crucial for providing the body with energy especially for the brain and muscles and they play a role in digestion and blood sugar regulation, these results was higher than those mentioned by [36] who noticed that the carbohydrates content of (MLP) was 41.2%.

So the variations in chemical constitutes would be refer to the differences in the phase of maturity of the plants as well as the soil fortified with several chemical fertilizers and geographical position of the plants as revealed by [21].

Total phenolic, total flavonoids and DPPH antioxidant activity of aqueous extract of *Moringa oleifera* leaves and ML/Zn-NPs

Phenolic compounds have redox properties for which it acts as antioxidants. The total phenolic content could be utilized as a basis for rapid examination of antioxidant activity due to their hydroxyl group which support the free radical scavenging ability. The antioxidant activity of flavonoids which contain flavanols, flavones and condensed tannins rely on the presence of free OH groups, mainly 3-OH. From data presented in Table (2), the biochemical characterization of both the aqueous extract of *M. Oleifera* leaves and the green-synthesized MO/Zn-NPs revealed noticeable variations in their total phenolic, flavonoid contents and antioxidant potential. The total polyphenol content of the aqueous extract (86.44 mg GAE/g dry weight) was slightly higher than the MO/Zn-NPs (84.05 mg GAE/g dry weight). These results was higher than those reported by [1,36] who observed that the total phenolic content of the AEML were (6.83 and 8.46 g/100g) respectively.

Results showed also in the same table, the total flavonoid content decreased from (20.04 to 16.55 mg QE/g dry weight) after nanoparticles formation. These results was higher than those determined by [1,36] who found that the total flavonoid content of *M. Oleifera* leaves extract were up to (2.12 and 18.05 g/100g).

Extracts	**Aqueous Extract of *Moringa oleifera* Leaves**	**MO/Zn-NPs**
Total polyphenols (TPP) (GAE mg/g dry weight)	86.44	84.05
Total flavonoids (TF) (QE mg/g dry weight)	20.04	16.55
Antioxidant activity DPPH (IC_{50} mg/ml)	0.357	0.366

Table 2: Total phenolic, total flavonoids and antioxidant activity of aqueous extract of *Moringa Oleifera* leaves and ML/Zn-NPs.

This minor reduction in both phenolic and flavonoid contents might be attributed to the participation of these bioactive molecules in the reduction and capping processes during the biosynthesis of zinc nanoparticles. Phenolic and flavonoid compounds are known to act as natural reducing and stabilizing agents, converting Zn^{2+} ions into Zn^0 and forming a protective organic layer around the nanoparticles. Consequently, part of these compounds becomes bound or transformed during nanoparticle formation, leading to the observed decrease in their measurable content.

Also in the same table, the IC_{50} of DPPH increased marginally from 0.357 mg/ml (aqueous extract) to 0.366 mg/ml (ML/Zn-NPs), reflecting a minor decrease in antioxidant activity. This trend aligns with prior studies on plant-mediated zinc nanoparticles, where active phenolics groups are partially consumed during nanoparticle formation, yet substantial antioxidant potential is retained [38]. Our obtained results was higher than those observed by [17] who decleared that the antioxidant activity of *M. Oleifera* leaves extract being 0.07582 mg/ml, but lower than those reported by [18] who mentioned that the IC_{50} of *M. Oleifera* leaves extract were up to 0.646.0 mg/ml.

Overall, the obtained results demonstrate that *M. Oleifera* leaf extract possesses a high level of phenolic and flavonoid compounds contributing to its antioxidant activity. The slight decline in these parameters after nanoparticle formation confirms the involvement of these biomolecules in promoting nanoparticle formation and preserving bioactive compounds, supporting the efficiency of *M. Oleifera* leaves as a potent bioreductant and capping agent in eco-friendly nanoparticle synthesis [16].

Fractionation and identification of phenolic compounds of aqueous extract of *Moringa oleifera* leaves and ML/Zn-NPs

HPLC analysis revealed qualitative and quantitative variations in the phenolic composition between the aqueous extract of *Moringa oleifera* leaves and the synthesized ML/Zn-NPs. Both samples contained eleven phenolic compounds with different concentrations as showen in Table (3). Chlorogenic acid was the predominant phenolic compound in both samples recording 568.33 ppm in the aqueous extract and increasing to 600.41 ppm in ML/Zn-NPs. This elevation may indicate that chlorogenic acid plays a major role in the reduction of Zn^{2+} ions and the stabilization of the synthesized nanoparticles due to its strong reducing and chelating abilities [11].

Also, in the aqueous extract ferulic acid and naringenin were the second most abundant phenolics which recorded (210.00 and 204.49 ppm) respectively, followed by pyrocatechol (106.12 ppm). These compounds decreased in the ML/Zn-NPs fraction (167.46, 175.12 and 64.52 ppm) respectively, suggesting their partial involvement in nanoparticle formation and capping processes.

Phenolic compounds	**Aqueous Extract of *Moringa Oleifera* Leaves (ppm)**	**ML/Zn-NPs (ppm)**
Gallic acid	22.04	24.80
Chlorogenic acid	568.33	600.41
Catechin	Nd	11.15
Methyl gallate	17.52	15.57
Coffeic acid	1.93	Nd
Syringic acid	3.10	4.73
Pyro catechol	106.12	64.52

Ellagic acid	95.00	149.26
Coumaric acid	0.90	7.45
Vanillin	Nd	Nd
Ferulic acid	210.00	167.46
Naringenin	204.49	175.12
Cinnamic acid	0.38	0.27
Hesperetin	Nd	Nd

Table 3: Fractionation and identification of phenolic compounds of aqueous extract of *Moringa oleifera* leaves and ML/Zn-NPs.

MO/Zn-NPs = ZnO/NPs synthesized by *Moringa oleifera* leaves extract; Nd: Not detectable.

On the other hand, gallic acid, catechin and coumaric acid were found in higher concentrations in the ML/Zn-NPs fraction than the leaves extract. This increase might be attributed to their strong affinity for Zn^{+2} ions or their stabilization during nanoparticle synthesis. Conversely, some minor phenolics such as caffeic acid and cinnamic acid either disappeared or were detected in trace amounts after nanoparticle formation possibly due to their oxidation or structural conversion during the biosynthesis reaction.

These results indicate that the phenolic compounds are actively involved in the bio-reduction and stabilization of zinc nanoparticles, where certain compounds are consumed during synthesis while others remain adsorbed on the nanoparticle surface enhancing their stability and bioactivity. Therefore, the enrichment of specific polyphenols in ML/Zn-NPs may enhance their antioxidant and potential therapeutic properties compared to the leaves extract.

These results differ from those reported in previous studies [2] stated that the fractions of the aqueous extract of *Moringa oleifera* Leaves contained catechin, epicatechin, trans-ferulic acid, rosmarinic acid, rutin hydrate and syringic acid with the concentrations of (207.21, 83.55, 62.42, 60.56, 11.29 and 3.44 mg/100g) respectively as the major polyphenols. Likewise, [28] who studied the fractions of aqueous extract of *Moringa oleifera* Leaves, which observed that the gallic acid as the most abundant phenolic compound followed by protocatechuic acid, quinic acid, p-coumaric acid, caffeic acid and naringenin being (1383, 447, 277, 117 and 64 μg/g) respectively.

In contrast, [24] reported that the aqueous extract of *Moringa oleifera* Leaves contained six polyphenolic compounds, including coumarin, ferulic acid, resorcinol and phenanthrene being (4.25, 4.65, 0.31 and 117.71 ppm) respectively. This variations between the present findings and previous reports could be attributed to differences in extraction solvents, plant origin, climatic conditions and analytical methodologies. Such factors strongly influence both the composition and concentration of phenolic constituents in plant-based extracts.

Flavonoid fractions of aqueous extract of Moringa oleifera Leaves and ML/Zn-NPs

High-performance liquid chromatography (HPLC) analysis of the aqueous extract of *Moringa oleifera* leaves and the synthesized ML/Zn-NPs revealed three major flavonoid compounds: daidzein, quercetin and rutin as presented in Table (4). In the aqueous extract, daidzein was the predominant flavonoid (16.72 ppm), followed by quercetin (1.70 ppm) and rutin (0.40 ppm). After conjugation with Zn nanoparticles remarkable changes in the flavonoid profile were observed the concentration of daidzein decreased sharply to 6.44 ppm, while both rutin and quercetin increased to (3.05 and 2.34 ppm) respectively.

Flavonoids	Aqueous Extract of *Moringa oleifera* Leaves (ppm)	ML/Zn-NPs (ppm)
Rutin	0.40	3.05
Daidzein	16.72	6.44
Querectin	1.70	2.34
Apigenin	Nd	Nd
Kaempferol	Nd	Nd

Table 4: Fractionation and identification of flavonoids compounds in the aqueous extract of *Moringa oleifera* leaves and ML/Zn-Nps.

Nd: Not detectable.

These variations may be attributed to the interaction between Zn^{+2} ions and the phenolic or hydroxyl groups of flavonoids leading to complex formation and partial oxidation or degradation during nanoparticle synthesis. Such interactions could also enhance the stabilization of certain flavonoids like quercetin and rutin, which act as reducing and capping agents during nanoparticle formation. The observed alteration in flavonoid composition after nanoparticle formation indicates that these bioactive compounds played a dual role as both reducing and stabilizing agents during the green synthesis of ML/Zn-NPs.

As showen in Table (4), the enrichment of quercetin and rutin in the nanostructure may enhance the antioxidant and antimicrobial activities of the nanoparticles, suggesting that the incorporation of flavonoids into the Zn-NPs matrix significantly contributes to their biological efficacy.

The obtained results differ from those reported by [2] who identified rutin and quercetin was the dominant compounds in *Moringa oleifera* extract, also [28] declared that the apigenin as the major flavonoid. While [24] claimed the *Moringa oleifera* Leaves extract contained 2 flavonoid compounds being quercetin and Kaempferol. These discrepancies may result from differences in geographical origin, climatic conditions, extraction solvents and analytical techniques used.

Inhibition of cancer cells using nanoparticles (in vitro)

The cytotoxicity of dried *Moringa oleifera* leaves extract (DMLE) and their nanoparticles with Zn metal was evaluated against (WI-38) normal cell line and (MCF-7 and HepG-2) tumor cell lines using different concentrations (1, 10, 25, 50, 100, 250 and 500 µg/mL).

Table (5), show the results of viability % and IC_{50} values of different tested samples against various cell lines compare with dox. Results showed that the dried *Moringa oleifera* leaves extract and *Moringa oleifera*/Zn nanoparticles (ML/Zn-NPs) have equal effect on MCF7 at 500 µg/mL (98%). While *Moringa oleifera*/Zn nanoparticles (ML/Zn-NPs) had the highest inhibition effect against HepG2 tumor cell line at 500 µg/mL (96%).

The tested samples have higher effects on MCF7 and HepG2 cell lines, in which dried *Moringa oleifera* leaves extract on HepG2 (500 µg/mL) exhibited high anticancer activity. DMLE and ML/Zn-NPs samples (50, 100 and 250 µg/mL) have good anticancer activity against MCF7 and HepG2 cell lines. Moreover, the other concentrations at (25, 10 and 1 µg/mL) have moderate effects to low inhibition percentage. From the obtained IC_{50} values, it was found that ML/Zn-NPs is the most effective sample against MCF7 and HepG2 cell lines which recorded (9.16 and 46.81) respectively, in addition to DMLE sample showed strong anti-tumor activity with (IC_{50}=17.30 and 67.96) respectively.

The enhanced anti-tumor activity of ML/Zn-NPs may be attributed to several factors including the nanoscale size, increased surface area and improved cellular internalization of zinc nanoparticles. Moreover, the synergistic interaction between Zn-NPs and *Moringa oleifera* phytochemicals such as flavonoids and phenolic compounds may contribute to increased intracellular reactive oxygen species (ROS) generation, mitochondrial dysfunction and induction of apoptosis in cancer cells.

Importantly, the cytotoxic effects of both DMLE and ML/Zn-NPs on the normal WI-38 cell line were considerably lower. The IC_{50} values recorded for DMLE and ML/Zn-NPs (161.20 and 88.54 µg/mL) were substantially higher than those observed in cancer cells, indicating reduced toxicity towards normal cells.

Sample	**Conc. (µg/ml)**	**Normal cell**	**Cancer cells Viability %**		**IC_{50}**		
		W138	**MCF7**	**HePG2**	**W138**	**MCF7**	**HePG2**
Dox	500	$0.70^{B} \pm 0.06$	$0.13^{A} \pm 0.03$	$0.27^{A} \pm 0.03$	7.62	3.90	4.98
	250	$2.30^{B} \pm 0.12$	$1.00^{B} \pm 0.15$	$1.23^{B} \pm 0.09$			
	100	$4.53^{C} \pm 0.20$	$2.37^{D} \pm 0.18$	$3.43^{C} \pm 0.12$			
	50	$7.86^{D} \pm 0.09$	$6.03^{F} \pm 0.19$	$7.50^{D} \pm 0.23$			
	25	$15.46^{E} \pm 0.33$	$14.20^{I} \pm 0.26$	$16.03^{F} \pm 0.15$			
	10	$46.43^{J} \pm 0.32$	$34.70^{M} \pm 0.26$	$40.53^{J} \pm 0.35$			
	1	$87.46^{O} \pm 0.27$	$75.13^{R} \pm 0.65$	$77.87^{N} \pm 0.58$			

AEML	500	24.63^{G} ± 0.59	1.70^{C} ± 0.06	11.37^{E} ± 0.32	161.2	17.30	67.96
	250	39.77^{H} ± 0.18	7.07^{G} ± 0.12	18.53^{G} ± 0.34			
	100	55.93^{K} ± 0.12	18.33^{J} ± 0.13	37.70^{I} ± 0.44			
	50	78.50^{M} ± 0.42	29.67^{L} ± 0.20	53.30^{L} ± 0.47			
	25	96.17^{P} ± 0.46	43.10^{O} ± 0.12	80.47^{O} ± 0.52			
	10	100.00^{Q} ± 0.0	61.73^{Q} ± 0.23	99.90^{Q} ± 0.10			
	1	100.00^{Q} ± 0.0	89.30^{T} ± 0.21	100.00^{Q} ± 0.00			
ML/zn-NPs	500	15.20^{E} ± 0.38	1.10^{CB} ± 0.12	3.53^{C} ± 0.09	88.54	9.16	46.81
	250	23.47^{F} ± 0.46	4.76^{E} ± 0.09	15.70^{F} ± 0.32			
	100	42.40^{I} ± 0.32	9.30^{H} ± 0.12	28.30^{H} ± 0.23			
	50	64.57^{L} ± 0.29	21.83^{K} ± 0.20	46.10^{K} ± 0.12			
	25	85.20^{N} ± 0.70	37.63^{N} ± 0.23	66.27^{M} ± 0.26			
	10	100.00^{Q} ± 0.0	52.97^{P} ± 0.15	91.67^{P} ± 0.18			
	1	100.00^{Q} ± 0.0	76.07^{S} ± 0.15	100.00^{Q} ± 0.00			

Table 5: Viability% and IC_{50} values of dried plant extract and their nanoparticles on different cell lines.

As shown in Figures (7-8), the strongest effect of the dried leaves extract on various tumor cell lines was observed at concentrations 500 µg/mL followed by 250 µg/mL. Other results stated that increase concentrations of DML extract led to increasing of the percentage inhibition on cancer cell growth. At a concentration of 300 mg/mL, dried MLE greatly stimulated apoptosis, suppressed tumor cell growth and reduced the level of internal reactive oxygen species (ROS) in human lung cancer cells. Furthermore, the DML extract showed greater cytotoxicity for tumor cells than normal cells as reported by [23]. [12] declared that the *Moringa oleifera* leaf extract prevented the growth of pancreatic cancer cells, the cells NF-κB signaling pathway. This impact was significant in all cells following display to ≥0.75 mg/ml of the extract and increases the efficiency of chemotherapy in human pancreatic cancer cells as mentioned by

Nanoparticles have received much attention recently due to their use in cancer therapy. Studies have confirmed that different metal oxide nanoparticles stimulated cytotoxicity in cancer cells, but not in normal cells. Among metal oxide NPs, ZnO-NPs possess powerful inhibitory effects against malignant cells because of their hidden toxicity, which they achieve through intracellular ROS generation and stimulating the apoptotic signaling pathway, making them a convenient choice for anticancer medications [5].

The obtained data are in agreement with those reported by [27] who observed that zinc oxide nanoparticles have a cytotoxic effect on cancer cells HT29 and PC3 at a concentration of 25 µg/

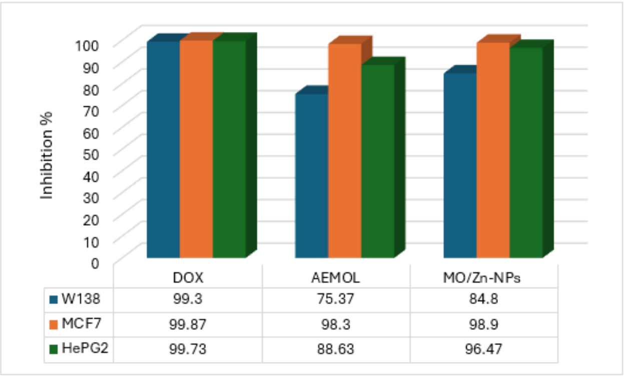


Figure 7: Inhibition % values at 500 µg/mL of dried plant extract and their nanomaterials against different cell lines.

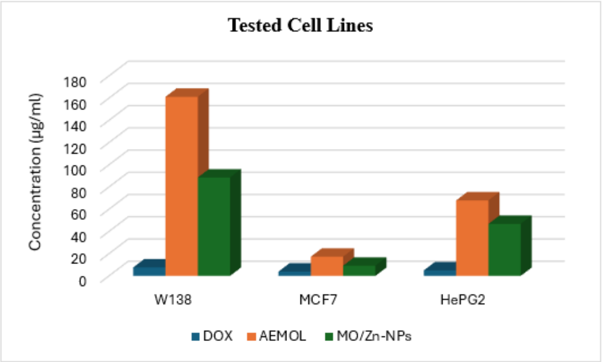


Figure 8: IC50 values at 500 µg/mL of dried plant extract and their nanomaterials against each of W138, MCF7 and HEPG2 cell lines.

mL which suppressed growth by stimulate ROS, weakening their mitochondrial function and decreasing their antioxidant capacity. Also, [10] mentioned that the ZnO-NPs are able to create significant cytotoxicity to human ovarian cancer cells through induction of intracellular (ROS) which they could directly impact a mechanical pathway of cells by apoptosis and autophagy. Especially, ZnO-NPs could associate with DNA and double-strand DNA breaks and eventually lead to cell damage. [6] stated that ZnO-NPs selectively encourage apoptosis in cancer cells (HepG2, BEAS-2B and A549) whereas posing no effect on normal cells, which is probably to be mediated by reactive oxygen species via p53 pathway, through which most of the anticancer medications stimulate apoptosis.

Other results showed that the metal oxide NPs with dried *Moringa oleifera* leaves extract has the strongest anti-tumor effect and increases the efficiency of drugs in cancer cells. The copper nanoparticles biosynthesized from the aqueous extract of *Moringa oleifera* leaves had the highest anticancer effect on MCF -7 and BT -549 cell lines [35]. [3] observed that silver of *M. Oleifera* leaves nano-extract improved the colon cancer which it inhibited the inflammatory reactions detected histopathologically in colon tissues and repaired the tissue to its normal configuration.

Conclusion

Eventually, it could be concluded that the green synthesis of nano-metals using plants were more safe and easy to prepare. The efficiency of aqueous extract of *Moringa oleifera* leaves in the rapid synthesis of nanoparticles (NPs) because of their bioactive components that have antioxidant activity played an significant role as anticancer agents and could be applied as a safe dynamic sources as a highly anti-carcinogenic effects on cancer cell lines.

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