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EFFECT OF *MORINGA OLEIFERA* LEAF AND SEED EXTRACTS ON THE SYNTHESIS OF ZINC NANOPARTICLES AND THEIR ANTIBACTERIAL ABILITY AND CYTOTOXICITY

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Abstract

Moringa belongs to the *Moringaceae* family, composed of 13 species, 7 of which are tropical and 4 of which are subtropical. This plant is a veritable health fount of nutrients, including minerals, vitamins, and amino acids. It contains vitamin A as well as vitamin C. Polyphenols, calcium, iron, copper, zinc, magnesium, folic acid, and beta-carotene are present. *Moringa oleifera* is often considered in many medical applications. In traditional medicine, it has been used to cure various types of bacterial and fungal infections. Among the scurvy, cholesterol, tumors, cholera, asthma, spasmodic, and anxiety are treated by the same methods. Also, the seed is suitable for water filtration via normal coagulation in the water. Several characterization approaches were employed to analyze ZnO nanoparticles. The purpose of this study was to conduct anticancer activity and antibacterial investigations in *Moringa oleifera* plants in Saudi Arabia using zinc nanoparticle production. In this study, we have used the nutrient and Mueller Hinton agar as well as zinc chloride ($ZnCl_2$). The preparation of culture, bacteria, and extracts, as well the synthesis of zinc nanoparticles and anticancerous activities, were performed. The results of the current investigation support their potential to treat *Staphylococcus aureus, Bacillus subtilis*, and *Escherichia coli* with their antibacterial properties. The antibacterial activity of the plant extracts results showed the highest inhibition zone in *S. aureus* with *M. oleifera* seed extract in the amount of 27mm, and in *E. coli* with the extract of *M. oleifera* seeds, similar to the extract of *M. oleifera* leaves in *S. aureus* in the amount of 17.6 mm. Then *B. subtilis* with an inhibition zone in extract *M. oleifera* seeds was 10.8mm and followed by the same bacteria in the extract of *M. oleifera* leaves (9.3mm). The other results carried out with $ZnCl_2$NPs in the first experiment showed the highest inhibition in *B. subtilis* in the extract of *M. oleifera* leaves $ZnCl_2$NPs (27.93mm), followed by the extract of *M. oleifera* leaves $ZnCl_2$NPs in *E. coli* (27.6mm). Then, *S. aureus* in the extract *M. oleifera* leaves $ZnCl_2$NPs (26.53mm) and *B. subtilis* in the extract *M. oleifera* seeds $ZnCl_2$NPs (22.06mm). Our study concludes that *Moringa Oleifera* leaf extract can be utilized to make nanoparticles and can also be used for cancer treatment. The mineral salt can be used, and zinc nanoparticles are also acceptable. Antimicrobial and anticancer nanoparticles have been confirmed once they are used.

Key words: *Moringa oleifera;* Nanoparticles; $ZnCl_2$; Anticancerous activity.

Introduction

Nanoparticles (NPs) are tedious and have harmful solvents or reagents as a costly substratum with possible adverse effects, as well as complex instruments. They were synthesized with several physiochemical methods so far. Easy, low-cost, atmospheric synthesis, non-toxicity, and environmental compatibility of nanoparticles through eco-friendly, ecologic synthesis (Verma *et al.*, 2020). NP regulates atomic-level particles ranging from 1 to 100 nm in size because they differ significantly from mass materials in terms of size-related characteristics (Buzea *et al.*, 2007). Metallic nanoparticles (MNPs) such as silver and gold have previously been used in plant sciences for a wide range of applications, while zinc, calcium, and magnesium have also been used (Sanzari *et al.*, 2019). Zinc is largely discharged into the soil from minerals such as zinc oxides, sulphates, sulphides, carbonates, silicates, and phosphates, which are released from the parent rock. Further sources state that atmospheric processes, biotic activities, and, lastly, anthropogenic activity are all involved (Sturikova *et al.*, 2018).

The Moringaceae family (which includes the plant *Moringa oleifera*) is critical in many therapeutic fields and is found in tropical and subtropical places around the world. A variety of vital phenols, amino acids, proteins, vitamins, and β-carotene are all found in different sections of the organism (Anwar *et al.*, 2007; Irfan *et al.*, 2021). *Moringa oleifera* L., is endemic to Asia, Africa, the Caribbean Islands, and South America. Scientists have discovered that *moringa* has far more of a variety of vitamins, minerals, antioxidants, and biochemical compounds than any other plant species. In other words, it makes an excellent alternative to help cure certain diseases such as diabetes, cardiovascular disease, and even cancer (Zubair *et al.*, 2021). Vitamin- and amino acid-rich *Moringa oleifera* was said to be an excellent food source. The research suggested that moringa oleifera might improve immunity. Humans consume it, and the nutrients and carbohydrates come from the leaves and pods. The leaves and pods are also a high source of carotene and ascorbic acid (vitamin C), with a nice amino acid profile. The twigs are reported to be particularly appetizing to ruminants (Ogbe & Affiku, 2011). Limited studies were performed on the anticancer activity studies and the current study was constructed to carry out the anticancerous studies on *Moringa oleifera* plants in Saudi Arabia with the zinc synthesis of the nanoparticles.

Material and Method

Sample collection: Nutrient Agar (NA) and Zinc Chloride ($ZnCl_2$): Bacteria were isolated from biology labs in the science department at Princess Norah bint Abdirahman University. The bacteria used are *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*.

Mueller Hinton Agar (MHA) and Zinc Chloride ($ZnCl_2$): Bacteria were taken from a medical center in Riyadh and these bacteria are standard. The bacteria used are *Escherichia coli, Staphylococcus aureus, klebsiella pneumoniae, methicillin-resistant staphylococcus aureus, Enterococcus faecalis,* and *Pseudomonas aeruginosa.*

Plant collection in NA and MHA: Plants of *Moringa oleifera* were collected from Riyadh, Saudi Arabia. The leaves and seeds of *M. oleifera* were separated, washed with running water to remove dust, shaded dried, and powdered Plants of *Moringa oleifera* were bought from a local plant shop in Riyadh, Saudi Arabia.

Preparation of culture: NA-$ZnCl_2$: The cultures were purchased from the laboratory center.

MHA-$ZnCl_2$: suspend 79.8 grams of Mueller Hinton Agar in 2100 ml of distilled water. It was divided as follows: 11.4 g of Mueller Hinton Agar was added to each sex flask along with 300 ml of distilled water. After that, the sex flacks were pleased with the autoclave sterilization at 24 hours.

Preparation of bacteria: MHA-$ZnCl_2$: Part of the growth was taken to fill the knot with the inoculation loop of each microbial type and placed in a nutrient broth agar. Then it was placed in the incubator at 37°C for 12-24 hours.

Preparation of extract: 10 grams of leaf and seed powder from *M. oleifera* were weighed separately and placed in 100 ml of distilled water for each. After mixing, they were placed in a water bath at 70°C for 10 minutes. Then, it was filtered using filter paper (Whatman 185mm). After filtration, the extracts were stored at a temperature of 4°C until they were used.

Synthesis of $ZnCl_2$NPs: NA-$ZnCl_2$: 30 ml of leaves aqueous extract of *M. oleifera* was placed in a flask, then 70 ml of $ZnCl_2$. Also, the same method used in seed aqueous extract of *M. oleifera* with $Zncl_2$. After that, the two flasks put in a water bath shaker at 70°C for 24 hours to prevent Deposition of materials.

MHA-$ZnCl_2$: 10 ml of leaves aqueous extract of *M. oleifera* placed in flask, then added 90 ml of $ZnCl_2$. Also, the same method used in seed aqueous extract of *M. oleifera* with $ZnCl_2$. After, the two flasks put in a water bath shaker at 70°C for 24 hours to prevent the deposition of materials.

Antimicrobial assay: NA-$ZnCl_2$ "first experiment": The cultures of the three bacterial species were prepared for 24 hours in their specific cultures (nutrient agar) at 37°C. Part of the growth was taken to fill the knot with the inoculation loop of each microbial type and placed in a tube containing 9 ml distilled water, then it was shaken. The 9-ml water containing the bacteria was pulled using the sterile injection needle, and 3 drops (0.2 ml) of it were put into the specific cultures of bacteria. The bacteria were distributed equally throughout the environment. As a result, each bacterium has two cultures. Using the cork borer, each disk was punctured with three holes, and then 5 drops (0.35ml) of the extracts were placed in each hole. One Petri dish is for *M. oleifera* leaves $ZnCl_2$NPs extract and the other is for *M. oleifera* seeds $ZnCl_2$NPs extract for each type of bacteria. It was placed in the incubator at 37°C for 12-24 hours.

MHA-$ZnCl_2$ "second experiment": The prepared Mueller Hinton Agar culture was removed of the autoclave sterilization after 24 hours. There are 6 flasks and each determined for a specific type of bacteria. 1 ml of nutrient broth agar containing bacteria was taken and placed in the flask containing Mueller Hinton agar after confirming the appropriate temperature for the bacteria, then it was shaken. The Mueller Hinton agar with bacteria culture was poured into the Petri dishes; each type of bacteria has two Petri dishes for $ZnCl_2$NPs. After the hardening of the culture, it was punctured with four holes using a cork borer. 5 drops (0.35 ml) of the extracts were placed in three holes. One Petri dish is for *M. oleifera* leaves $ZnCl_2$NPs extract, and the other is for *M. oleifera* seeds $ZnCl_2$NPs extract, for each type of bacteria) . In the fourth hole, 5 drops (0.35 ml) of sterilized distilled water were placed. Thus, there were 12 Petri dishes for all 6 types of bacteria. Every bacterium has 2 petri dishes, each containing a specific type of nano-extract. The first one contains an extract of *M. oleifera* leaves $ZnCl_2$NPs, and the second petri dish contains an extract of *M. oleifera* seeds $ZnCl_2$NPs. It was placed in the incubator at 37°C for 12-24 hours.

Transmission electron microscopy: In this study, we investigated the transmission electron microscopy (TEM) approach in *M. Oleifera* employing a technique similar to that used in earlier investigations (Albrahim *et al*., 2021).

MTT cytotoxicity assay: The MTT assay was used to assess the cytotoxicity of ZnNPs against MDA and MCF-7 cell lines grown in 96-well plates. Different doses of ZnNPs were applied to cell lines and examined after 72 hours of incubation at 37°C in 5% CO_2.

Results

The current study results confirm their ability with their anti-bacterial effect against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. Study their ability to have an antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis*, and Escherichia coli in the first experiment. In the second experiment, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *methicillin-resistant Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*.

To achieve the first goal in the first experiment, an amount of 30 ml of each plant extract showed the ability to alter the color reaction mixture to different colors. With 30 ml of the leaf aqueous extract of M. oleifera, the color

changed from brown to a range of brown. With 30 ml of a seed aqueous extract of M. oleifera, the color does not change (Fig. 1). This color alternative is points man for the conversion of zinc chloride ions to zinc chloride nanoparticles. The color alternative is gained due to the excitation of surface plasmon resonance in the synthesized NPs. To achieve the first goal in the second experiment, an amount of 10 ml of each plant extract showed the ability to alter the color reaction mixture to different colors. With 10 ml of the aqueous leaf extract of *M. oleifera*, the color changed from brown to orange brown. With 10 ml of the aqueous seed extract of *M. oleifera* the color does not change (Fig. 2). These color alternatives are points for the conversion of zinc chloride ions to zinc chloride nanoparticles. The color alternative is obtained due to the excitation of surface plasmon resonance in the synthesized NPs.

Current results showed that the aqueous extract of plants had antibacterial activity in the first experiment (Table 1). The highest inhibition zone in *Staphylococcus aureus* with the extract of *M. oleifera* seeds was 27 mm. Then there is a similarity in the inhibition zone between the extract of *M. oleifera* leaves in *Staphylococcus aureus* and the extract of *M. oleifera* seeds in *Escherichia coli* in the amount of 17.6 mm. Next in *Bacillus subtilis* with inhibition zone in extract *M. oleifera* seeds by the amount of 10.8 mm. Then, in the same Bactria, the inhibition zone in the extract of *M. oleifera* leaves is 9.3 mm. In *Escherichia coli*, the inhibition zone in the extract of *M. oleifera* leaves is 7.6 mm (Fig. 3).

Current results showed that zinc chloride nanoparticles synthesized by plant extracts have antibacterial activity in the first experiment, "NA-$ZnCl_2$" (Table 2). The extract of plants synthesized $ZnCl_2$NPs had the highest effect on the bacteria *Bacillus subtilis*, where there is a higher inhibition zone in the extract of *M. oleifera* leaves $ZnCl_2$NPs by the amount of 27.93 mm. Also, in the same bacteria, the inhibition zone in extract *M. oleifera* seeds $ZnCl_2$NPs is 22.06 mm; in *Escherichia coli*, the inhibition zone in extract *M. oleifera* leaves $ZnCl_2$NPs is near *Bacillus subtilis*, and the amount of the inhibition zone is 27.6 mm. After that, in *Staphylococcus aureus*, the inhibition zone in an extract of *M. oleifera* leaf $ZnCl_2$NPs is 26.53 mm.

In the second experiment, "MHA-$ZnCl_2$", results showed that zinc chloride nanoparticles synthesized by plant extracts have antibacterial activity. The heist in *Staphylococcus aureus* had an inhibition zone in the extract of *M. oleifera* leaves $ZnCl_2$NPs of 24.2mm, and in the same bacteria, the inhibition zone in the extract of *M. oleifera* seeds $ZnCl_2$NPs is 23.2mm. The following bacteria are *Escherichia coli*, with the inhibition zone in the extract of *M. oleifera* leaves $ZnCl_2$NPs being 21.6mm and the inhibition zone in the extract of *M. oleifera* seeds $ZnCl_2$NPs being 19.8mm. The following bacteria are *klebsiella pneumoniae*, which is equal to *Escherichia coli* in the inhibition zone of extract *M. oleifera* seeds $ZnCl_2$NPs is 19.8 mm, and the inhibition zone in extract *M. oleifera* leaves $ZnCl_2$NPs is 15.9mm. The last *Enterococcus faecalis* inhibition zone in the extract of *M. oleifera* seeds $ZnCl_2$NPs is 15.4mm and the inhibition zone in the extract of *M. oleifera* leaves $ZnCl_2$NPs is 14.9mm. There are two resistant bacteria: *methicillin-resistant Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table 3).

Table 1. Inhibition zone diameter (mm) aqueous extracts of plants.

Bacteria	1	2
Staphylococcus aureus	17.6 mm	27 mm
Bacillus subtilis	9.3 mm	10.8 mm
Escherichia coli	7.6 mm	17.6 mm

*(1) Aqueous extract of *M. oleifera* leaves (2) aqueous extract *M. oleifera* seeds

Table 2. Inhibition zone diameter (mm) of extract $ZnCl_2$NPs "first experiment".

Bacteria	1	2
Staphylococcus aureus	26.53 mm	19.3 mm
Bacillus subtilis	27.93 mm	22.06 mm
Escherichia coli	27.6 mm	17.26 mm

* (1) Extract of *M. oleifera* leaves $ZnCl_2$NPs (2) extract *M. oleifera* seeds $ZnCl_2$NPs

Table 3. Inhibition zone diameter (mm) of extract $ZnCl_2$NPs "Second experiment".

Bacteria	1	2
Staphylococcus aureus	24.2 mm	23.2 mm
klebsiella pneumoniae	15.9 mm	19.8 mm
Escherichia coli	21.6 mm	19.8 mm
methicillin resistant staphylococcus aureus	no inhibition	no inhibition
Enterococcus faecalis	14.9 mm	15.4 mm
Pseudomonas aeruginosa	no inhibition	no inhibition

* (1) Extract of *M. oleifera* leaves $ZnCl_2$NPs (2) extract *M. oleifera* seeds $ZnCl_2$NPs.

For the production of zinc nanoparticles, approximately 90 ml of zinc chloride ($ZnCl_2$) (mmol/ml) was added to 10 ml of the aqueous extracts (from Example 1), causing the color to shift from white to milky white. The color shift of the liquid demonstrated the reduction of zinc ions to zinc nanoparticles. Fig. 4 depicts the findings of this investigation.

TEM was used to investigate the shape, surface structure, and size of zinc nanoparticles. Fig. 5 shows the phytochemical screening and TEM pictures that were created for the current investigation of the plant *M. oleifera*.

MTT was used to assess the cytotoxicity of ZnNPs against MDA and MCF-7 cell lines. The IC50 values were determined for various ZnNPs. Fig. 6 describes the effect of cancer cells treated with Zn nanoparticles.

Before			After	
Zinc chloride composite $ZnCl_2$	leaves aqueous extract of *M. oleifera*	seeds aqueous extract of *M. oleifera*	extract of *M. oleifera* leaves $ZnCl_2$NPs	extract *M. oleifera* seeds $ZnCl_2$NPs

Fig. 1. Synthesis of NPs in first experiment.

Before			After	
Zinc chloride composite $ZnCl_2$	leaves aqueous extract of *M. oleifera*	seeds aqueous extract of *M. oleifera*	extract of *M. oleifera* leaves $ZnCl_2$NPs	extract *M. oleifera* seeds $ZnCl_2$NPs

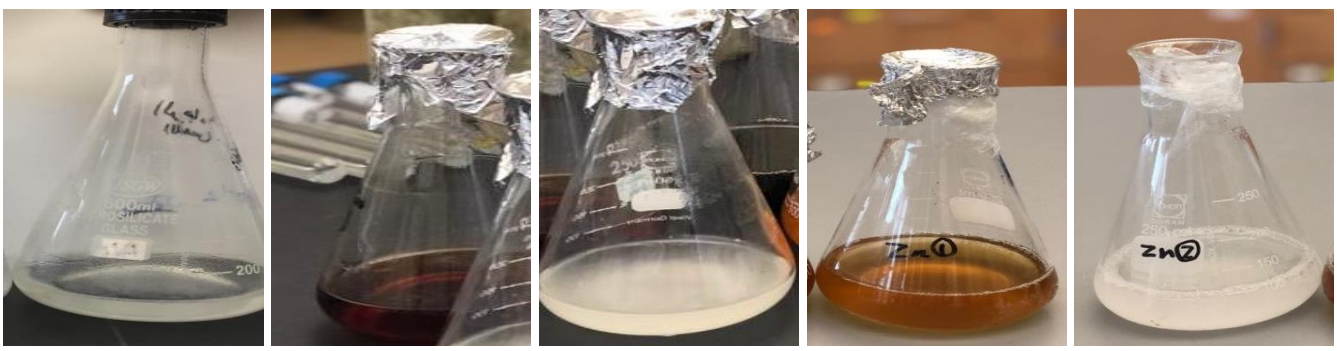

Fig. 2. Synthesis of NPs in Second experiment.

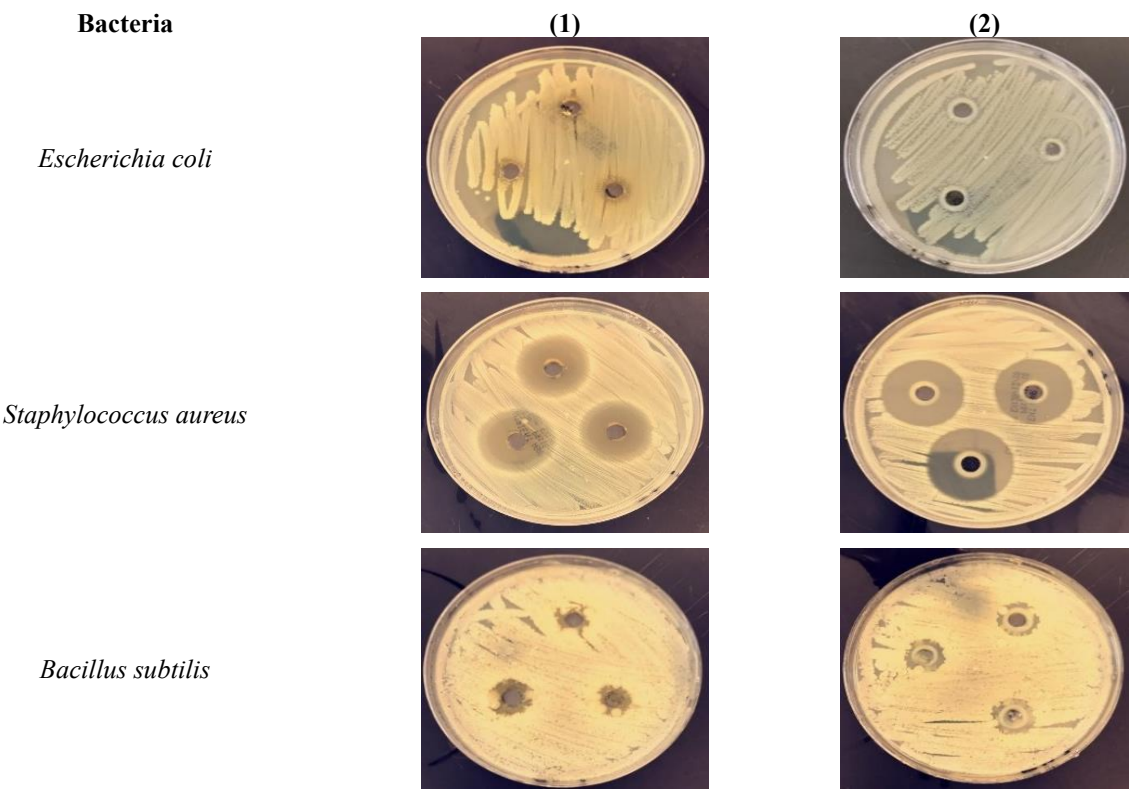

Fig. 3. Inhibition zone of aqueous extracts of plants.

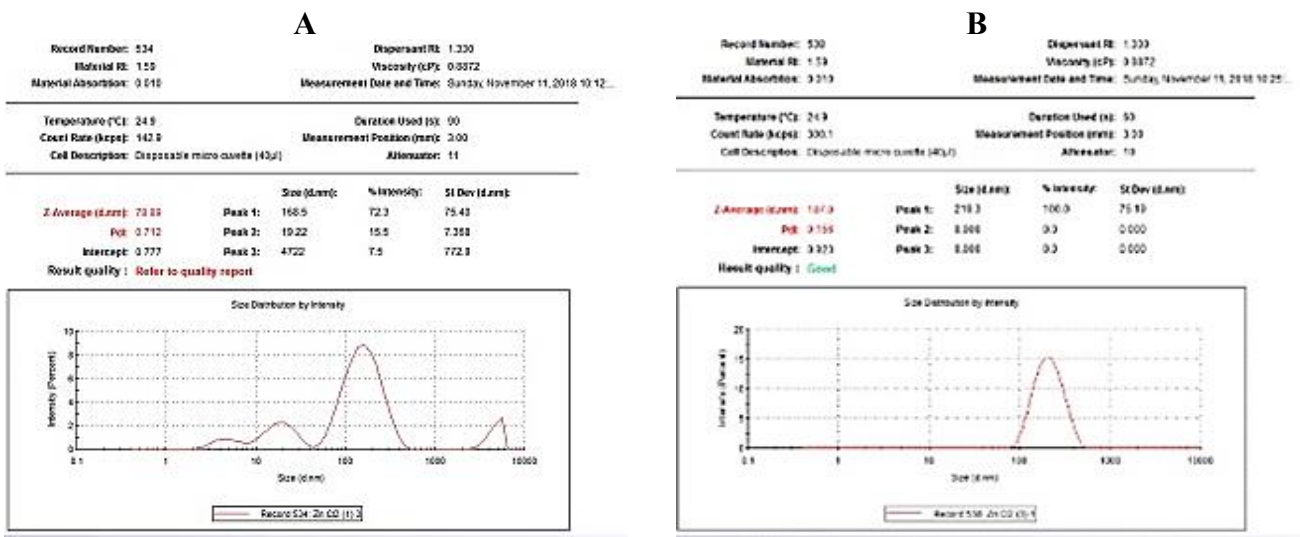

Fig. 4. *Moriga oleifera* leaf extracts A and B were used to obtain size distribution and zinc nanoparticles.

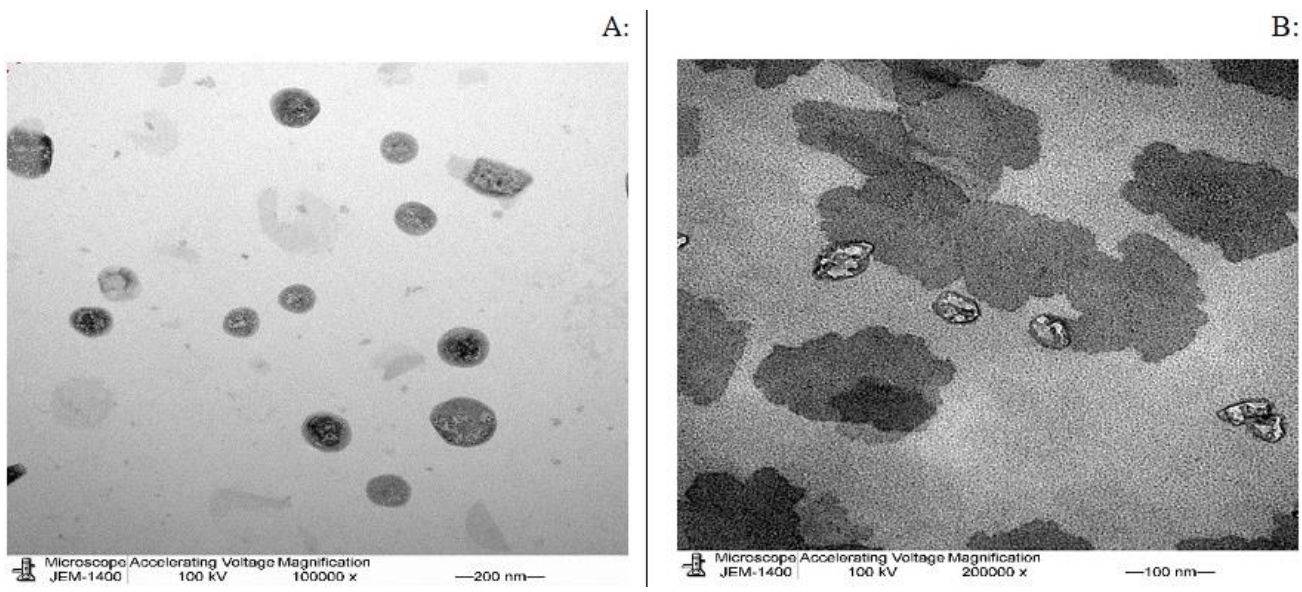

Fig. 5. TEM micrograph of zinc nanoparticles prepared from *Moriga oleifera* leaf extracts A and B. The magnifications are 100,000 (A) and 200000 (B), respectively, and the scale bars represent 200 nm (A) and 100 nm (B).

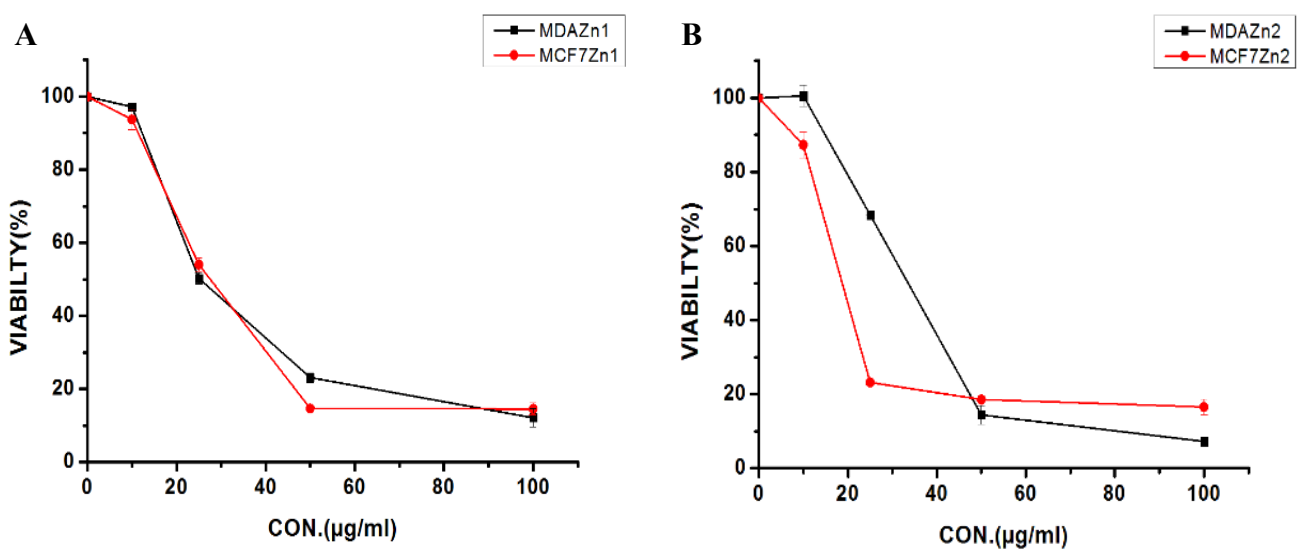

Fig. 6. The IC50 of the cancer cell treated with zinc nanoparticles obtained using *Moriga oleifera* leaf extracts A and seed extract B.

Discussion

Multiple studies have demonstrated the effectiveness of plant use in microbial inhibition and, thus, have become widely spread in medical fields. The current study aims to evaluate the zinc chloride nanoparticles extracted from the leaves and seeds of *M. oleifera* as a result of the reduction and stability of the biological factors that form the nanoparticles of zinc chloride. Another aim of the study is to confirm and characterize the bio-Zinc chloride nanoparticles.

One of the primary benefits of room-temperature NP synthesis and the utilization of plant extracts is that they partially fulfill the green synthesis requirement (Jones *et al.*, 2011). *Moringa oleifera*, better known as the drumstick tree or horseradish tree, is both nutritious and therapeutic. This plant contains several minerals, vitamins, and amino acids, among other beneficial elements. An Indian who was born in the northwestern part of India known as the Himalayas *Moringa oleifera* is found in many African, Asian, and South American countries, as well as in other regions like the Caribbean and Pacific Islands. *Moringa oleifera* is the most well-known species in the genus *Moringaceae* (Sreelatha & Padma, 2009). This plant is utilized as a nutritious supplement with numerous pharmacological qualities, including significant antioxidant effects. In addition, the healing properties of moringa have been recognized in traditional Indian and Persian medicine for several illnesses, such as stomach ulcers, skin problems, hay fever, tiredness, and bronchitis. One of the studies have found that the leaf extracts of *M. oleifera* has antioxidant properties in vitro and in vivo due to the presence of many phenolic acids and flavonoids. It was said that there were numerous components present in *moringa* leaves, including chlorogenic acid, gallic acid, kaempferol, and quercetin glycosides (Vongsak *et al.*, 2013).

As a result of Matinise *et al* finding's, they determined that *Moringa oleifera* leaf extract can generate zinc oxide nanoparticles with various sizes ranging from 12.27 to 30.51 nm, and to do so, they used several methods. The XRD and EDX tests revealed that the pure wurtzite ZnO phase is synthesized at a temperature of about 500°C in air after being annealed. The XRD studies revealed that the nanoparticles are polycrystalline (Matinise *et al.*, 2017). Ezhilarasi studies confirmed that a simple and environment-friendly technique was used to produce NiO nanoparticles by employing *Moringa oleifera* plant extract as the fuel. XRD and FTIR both confirmed the development of the NiO phase. UV and visible light stimulate the development of hydrogen peroxide, which kills bacteria, thus forming electron-hole pairs.

The synthesized nanoparticle showed good antibacterial properties, and it was found to be effective against Gram-positive bacterial strains (which include bacteria that have the gram-positive designation, such as some varieties of streptococci) rather than Gram-negative bacterial strains (which include bacteria that have the gram-negative designation, such as *Escherichia coli*). *Moringa oleifera* plant extract-prepared NiO nanoparticles exhibited cytotoxic action on HT-29 cells (Ezhilarasi *et al.*, 2016).

For the first time, scientists at McMaster University demonstrated that green synthesis of zinc oxide nanoparticles was possible by using a mixture of Zn (NO_3) $26H_2O$ as a precursor and *Moringa oleifera* flowers, seeds, and leaf extract to chelate or oxidize using a chelating reduction/oxidizing agent. This discovery was supported by different characterization techniques, including XRD, ATR-FTIR, and UV-VIS. Three different areas of *Moringa oleifera* give rise to similar but slightly different optical and structural ZnO-based nanoparticles with distinct optical properties. The different bioactive components contained in this tree could be used to synthesize ZnO-NPs and other multifunctional nanoscale oxides, including the roots and peels (Ngom *et al.*, 2021).

Zinc, a vital plant nutrient, can be carcinogenic in excessive doses (Moyo *et al.*, 2011). For sperm cells to grow properly, zinc must be ingested in adequate amounts in the diet. Zinc content in the leaves of *M. oleifera* ranges from 25.5–31.03 mg/kg, which is the daily dietary zinc requirement (Barminas *et al*., 1998). The role of $ZnCl_2$ in *M. oleifera* has been reported, as acid activation had a more practical effect on changing the structure of the precursor material. H_3PO_4 was shown to be more successful than zinc chloride in modifying the structure of lignocellulosic materials impregnated with $ZnCl_2$ at temperatures up to 500^{o}C, whereas phosphoric acid was found to be more effective than $ZnCl_2$ at modifying the structure of agro-industrial waste. Researchers also discovered acid activation was better at creating mesoporous adsorbents and larger surface areas than either $ZnCl_2$ or KOH (dos Santos Bispo *et al.*, 2021). ZnNPs' cytotoxic effect on MDA and MCF-7 cell lines was evaluated using MTT. Which allowed the study's findings on cancer cells to be validated. IC50 values were found for a variety of ZnNPs.

Conclusions

Based on this study, we confirmed and concluded that *Moringa oleifera* leaf extract and seed extract can be utilized to make nanoparticles of $ZnCl_2$, and they can also be used for cancer treatment. The mineral salt can be used, and zinc nanoparticles are also acceptable. Antimicrobial and anticancer nanoparticles have been confirmed once they are used.

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