Preparation and identification of nanomaterial from myrtle and its use in treating lung cancer

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Abstract

Myrtle leaves are considered one of the most promising industrial food wastes as dietary fibers in food products. They also contain aromatic oils with health benefits that are used to treat many diseases that affect the human body.

1. In this study, a new nanomaterial was prepared and characterized by the sol-gel method from inexpensive and environmentally friendly primary plant sources (Leaves of Myrtus) with zinc oxide, Diagnosis of the nanomaterial prepared by the sol-gel method using several techniques, namely FTIR, EDX, XRD, and XRF ,Using the nanomaterial prepared by the sol-gel method in treating lung cancer, as the results showed that it has a high killing rate ranging from 69-82%, respectively, of cancer cells after 48 hours, while low concentrations (50) micrograms/ml or less have a percentage Toxic effect less than 50%.

Introduction:

1. Myrtus:

The scientific name is (Myrtaceae) or (Myrtus communis) and it is a family of woody flowering plants that includes about 5,500 species divided into 144 genera, 17 tribes, and 2,500 species found mainly in the Neotropics **(1)**, Myrtus is the only genus found in Europe and North Africa. And Asia, especially in the Mediterranean region in southern Europe to the far west and the Sahara Mountains and as far east as West Asia **(2)**.

One of the most frequently mentioned species of myrtle in traditional books is Myrtus, also referred to as the Mediterranean shrub M. ommunis, also known as the common myrtle and is native to the Mediterranean region. The length of the plant is 2.4-3 meters, and the branches form a full head that is densely covered with leaves and small fruits. It has small green leaves. The length of its evergreen leaves ranges from 2 to 5 cm. It has a bitter taste. The flowers of the myrtle plant are star-shaped, white or pink, and very fragrant **(3)**. See Figure No. (1) etc. Some studies have shown that myrtle oil has a significant inhibition on the growth of cancer cells in the laboratory, at a concentration of 200 micrograms/ml, where the inhibition was 67% for prostate cancer cells and 95.2% for breast cancer cells **(4)**.

Figure No. (1) Myrtle plant

2. Malignant neoplasms (cancer):

Malignant tumors remain a disease that threatens human life and health throughout the world. It is also one of the leading causes of human death **(5-6)**. Due to the 2019 novel coronavirus (COVID-19) pandemic, there has been a negative impact on the diagnosis and treatment of malignant tumors **(7,8)**. The number of diagnosed cancer cases has reportedly decreased significantly worldwide, with a significant increase in diagnosed cases expected in the near future **(9)**. There is therefore an urgent need to identify highly potential biomarkers to improve early cancer diagnosis and enhance survival outcomes

3. Lung Cancer:

Lung cancer or bronchial cancer in humans has been considered the primary cause of death from cancer for both sexes in the last ten years in all parts of the world, where more than 228 thousand cases of lung cancer have been recorded. In 2019 in Iraq, lung cancer ranked second after breast cancer. It is among the top ten most common cancerous diseases in Iraqi society, with more than 2,000 cases recorded in the past few years **(10)**.

Most lung cancers begin in the membrane lining the bronchial tubes. They often form next to the lungs and sometimes in the cells of the glands located under the bronchial membrane. Lung cancer is considered life-threatening because it often spreads by metastasis. But when cancer spreads to another part of the body, such as breast cancer or bowel cancer to the lung: Cancer that extends to the lung is designated "as a secondary cancer, so the patient must verify whether the cancer began in the lung or has spread to it, because determining the treatment steps depends on the place where the infection began" **(11).**

4. Classification of lung cancer:

Lung cancer has been broadly classified histologically into two main types. The first type is non-small cell lung cancer (NSCLC), which represents 85-95% of lung cancer types. The second type is small cell lung cancer (SCLC), which represents 10-15% of all other types of lung cancers. It is very necessary to distinguish between these two types because each type is treated differently (12). See Figure No. (2)

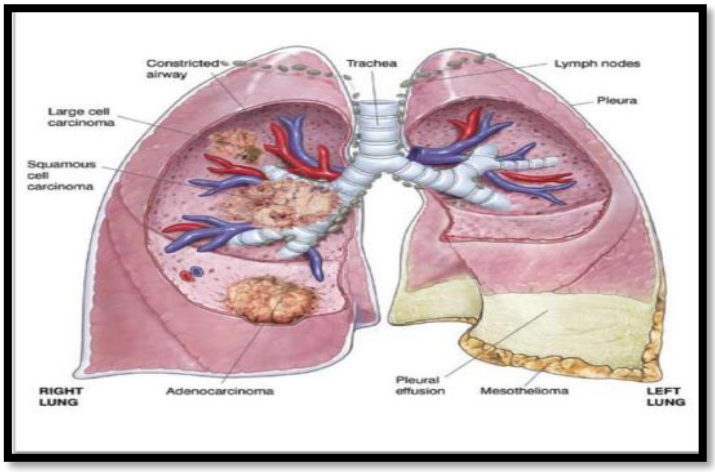

Figure No. (2) shows the locations of lung cancer

5. Treatment of cancerous tumors:

For more than 2000 years, cancer has been known and ancient civilizations have mentioned it, but with certain symptoms and manifestations, and it later became clear that it is cancer **(13)**. From that time until now, researchers and scientists have still rushed to find suitable ways to treat cancer, but despite this extensive research and studies, only There is not yet a completely curative treatment for this malignant disease, there are currently some common treatments that are used to treat tumors. These treatments have contributed to prolonging the patient's life without a completely curing treatment. On the other hand, studies are still ongoing to find treatments with low toxicity and effectiveness. High **(14)**, and among the treatments currently used to treat malignant tumors are:

- Surgical treatment: It is used in this type of tumor treatment in the case of removing solid tumors if the tumor (cancer) has not spread to other parts of the body through metastasis. Although the tumor is removed by surgical intervention, it may return. It appears after several years due to the survival of one or more cancer cells, which can cause the tumor to reappear again **(15)**.
- Radiation therapy: This type of treatment is used for tumors that have not spread to the rest of the body. This treatment leads to the elimination of cancer cells, which have a high ability to divide, by influencing their genetic material. Radiotherapy has disadvantages, as it cannot distinguish between a cancer cell and a normal cell, it kills both types **(16)**.
- Chemotherapy: This type of treatment is distinguished from radiation and surgical therapy in its ability to reach all cells of the body, but it is similar to radiation therapy in terms of its effect on normal cells, as chemotherapy affects normal cells by generating mutations in the genetic material of the cell, which leads to the emergence of other types of cancer **(17)**.
- Immunotherapy: Strengthening the work of the immune system and directing it against cancerous cells is the most important goal of immunotherapy. This is done by stimulating the immune response against cancer cell antigens. This is due to the failure of the host's immune system to function because it cannot control the proliferation of cancer cells. Therefore, Strengthening the immune system is one of the effective ways to treat cancer **(18)**.
- Biological gene therapy: This treatment aims to use viral genetic therapy by using the virus to

eliminate cancer cells in a specialized manner **(19)**.

- Treatment using medicinal plants and nanotechnology: Since traditional treatments for cancerous tumors are not completely curative treatments for the disease, regardless of genetic and immunological treatments, the use of medicinal plants in treating tumors is one of the treatments with a promising role that is still under research, so the world's attention has shifted. Today we have discovered new therapeutic mechanisms that have an effective ability to treat malignant tumors, as well as reduce the rate of side effects and toxic effects of these treatments **(20).**

Materials and methods of working:

Table (1)shows the chemicals used

Origin	The originating company	Molecular weight	Molecular formula	Chemical materials	NO
Iraq	Lab, Samarra pharmaceutical	18	H_2O	Deionized water	.1
Germany	Capricorn	-	-	Trypsin/EDTA	.2
USA	Santacruz Biotechnology	78.13	$SO(CH_3)_2$	DMSO	.3
Germany	Capricorn	-	-	RPMI 1640	.4
USA	Bio-World	414.32	C 18 H 16 BrN 5 S	MTT stain	.5
Germany	Capricorn	-	-	Fetal bovine serum	.6
Germany	Sigma	81.4	Zno	Zinc oxide	.7

1. Preparation of nanomaterial from myrtle:

The dried myrtle leaves were washed to clean them of dust and impurities, then dried in an oven at a temperature of 50 degrees Celsius for 24 hours, and then ground using an electric grinder.

Using the modified sol-gel method **(21)**, by adding 4 grams of the herbal material (myrtle) to the zinc oxide solution (5 grams of zinc oxide in 200 ml of ion-free water) the mixture was stirred on a magnetic stirrer for 10 hours. The mixture was placed in the shaking incubator for four hours at a temperature of 40 degrees Celsius and a speed of 100 revolutions per minute. It was observed that the solution changed from green to pale green or yellow, and then the sediment was separated by a centrifuge at a speed of 3000 revolutions/minute for 20 minutes. Then the precipitate was washed several times with deionized water, then the precipitate was dried in an electric oven at a temperature of 60 degrees Celsius, and then the precipitate was ground after drying using a ceramic mortar to obtain a fine powder that would be used later.

Several measurements were used to examine the prepared nanomaterial:

1- X-ray diffraction measurement (XRD).

2- FT-IR spectrum measurement

3- XRF fluorescence analysis

4- X-ray scattering measurement (EDX).

2. Preparation of solutions, culture media, and dyes:

The solvents used in the preparation of the nanoscale

- Distilled water free of ions

- DMSO (dimethyl sulfoxide)

3. Stock Solution for Tissue culture

Solutions were prepared according to the method **(22)** for tissue culture. These solutions and media were mixed under sterile conditions Antibiotics

* Streptomycin Solution The solution was prepared by dissolving 1 gm of streptomycin in 5 ml of distilled water, then 0.125 µl was taken from it and added to 250 ml of culture medium and stored at -20°C.

* Ampicillin Solution

The solution was prepared by dissolving 1 g of ampicillin in 5 ml of distilled water, then 0.25 microlitre was taken from it and added to 250 ml of culture medium and stored at a temperature of (-20) Celsius, and it is preferable to use it immediately.

* Nystatin Solution

0.062 microliter of nystatin solution was taken and added to 250 ml of the medium after filtering the medium. The solution is stored at a temperature of (-20) Celsius, and it is preferable to use it immediately.

* Phosphate Buffer Saline (PBS)

It consists of the following ingredients dissolved in 1000 ml distilled water:

0.2 gm	KCl
8 gm.	**NaCl**
0.92	**Na_2HPO_4**
0.2	**KH_2PO_4**

Sterilize the solution in an autoclave at 121°C for 15 minutes, then store it at 4°C until use.

4. Trypsin – EDTA

The solution was prepared by dissolving the following ingredients in 250 ml of distilled water **(23)**:

2.53 gm	Trypsin- EDTA powder
0.55 gm	Bicarbonate sodium
0.25 ml	Ampicillin solution
0.125 ml	Streptomycin solution
0.062 ml	Nystatin

The solution was placed in a magnetic stirrer for half an hour at room temperature, and antibiotics were added to it, then sodium bicarbonate to adjust the pH to 7.0. The solution was then sterilized by passing it through a membrane filter (0.22 microns). After filtration, nystatin was added to it. Store the solution in sterile, tightly closed bottles at a temperature of 4°C until use.

4. Used serums

* Bovine fetal serum

Bovine calf serum was obtained from the Iraqi Center for Cancer Research and Medical Genetics (Capricorn, Germany). The serum was sterilized using a filter with pores of a diameter of (0.22 µm) and under sterile conditions. 10% of the sterile bovine calf serum was mixed with the culture medium RPMI -1640 Sterilized, in sterile conditions, and stored in sterile glass containers until used.

5. RPMI-1640 Rose Well Park Memorial Institute

This medium for preparing half a liter consists of the following ingredients:

8.2 gm	RPMI-1640 powder with hepes with L-glutamin
.1 gm1	Sodium bicarbonate
0.5 ml	Ampicillin solution
0.25 ml	Streptomycin solution
0.124 ml	Nystatin
50 ml	Fetal Calf Serum

The volume was increased to 500 ml using distilled water, then the solution was sterilized by passing it through membrane filters (0.22 microns) before adding nystatin, then incubated at 37°C for 72 hours to ensure it was free of contaminants.

6. Methyl Thiazolyl Tetrazolium (MTT) dye:

The solution was prepared according to method **(**24-25**)** by dissolving 0.005 g of dye powder in 1 ml of warm buffer solution (PBS) in a beaker. The beaker was placed on a vibrating magnetic plate, and then the dye was filtered by passing it through a membrane filter (Syringe filter type 0.2 µm) to remove the blue crystals that formed from it. The dye was prepared and stored in sterile conditions and in a dark place to avoid oxidation in the light. The dye is prepared simultaneously for better results.

7. Lung cancer cell line A1549 (LCLA1549):

The lung cancer cell line is a cancer cell line from the alveolar basal epithelial cells of the human lung. It was first developed in 1972 and taken from the lung tissue of a 58-year-old Caucasian man with lung cancer. 549A cells are used as models for studying lung cancer and developing treatments against it.

Perpetuating and preserving normal and cancerous cell lines ex vivo.

Measuring the cytotoxic effects of five concentrations of the nanomaterial prepared from the myrtle herb on a lung cancer cell line.

8. Preparation of concentrations of nanomaterials prepared from myrtle:

The concentrations of the nanomaterial prepared from myrtle **(**26**)** were:

By dissolving 0.1 g of crude extract powder in 1 ml of serum-free medium (SFM) prepared as previously mentioned and sterilized using a filter with holes with a diameter of 0.22 micrometers. The original stock was prepared from it and sterilized using a filter. Five concentrations of each extract were prepared using RPMI - 1640 free media, which are (6.25, 12.5, 25, 50, 100) micrograms/ml under sterile conditions. All prepared concentrations were used immediately after completing the preparation process and when preparing the concentrations used for cytotoxic assay tests.

9. Maintenance of Cell Lines:

The cell lines **(**27**)** were maintained by observing these cells, and when they formed a complete monolayer, they were subcultured. This was done by removing the old growth medium and then adding 3 milliliters of trypsin-EDTA solution in an amount of (2-3) milliliters for (3- 5) Minutes later, then moving the bottle gently so as not to affect the vitality of the cells and incubating it in an incubator at a temperature of 37°C for a few minutes to break up

the adherent cells and also loosen their adhesion to the wall of the bottle to obtain single cells, provided that their exposure to the solution does not continue for more than 10 minutes (depending on the nature of the line) **(28)**. Cellular. Approximately 20 ml of fresh growth medium (RPMI-1640) was added to the bottle containing the disassembled cells to neutralize the effect of the trypsine-EDTA solution. The bottle was stirred well and then the contents of the bottle were evenly distributed with another new bottle so that the level of the culture medium with the cells was equal between the two bottles after Complete information was written on it about the type of cells, the new passage number, and the date of the secondary culture procedure. The bottles were monitored daily to ensure that they were free of any contamination and that the cells were in good condition **(29)**, by examining them with an inverted-phase microscope, and when a layer formed Integrated monocultures of cells make the vials ready for use. This process is called the subculturing process.

Normal and cancerous cell lines differ in the time they take to grow into a full cell. The normal cell line REF takes three days, while the lung cancer cell line (LCLA1549) takes one day. A change in the color of the culture medium indicates a change in its pH and requires changing it, i.e. pouring it and adding a new culture medium to maintain cell growth.

10. MTT-based cytotoxic assay

• The cell suspension was prepared by treating the layer of cells grown in a 50 cm3 tissue culture bottle with a trypsin-ferric solution, then adding 20 ml of culture medium containing 10% serum. The cell suspension was mixed well, and 0.2 ml was transferred after each good mixing into the holes of a flat-bottomed tissue culture calibration dish using a micropipette **(30)**.

• The dish was left in the incubator at a temperature of 37 °C for a period ranging between (12-18) hours until the cells adhered to the hole, after which the old culture medium in the holes was disposed of and 0.2 ml of the previously prepared concentrations were added to each of the extracts, four replicates for each concentration. Four replicates were also made for Control, and the plates were incubated at a temperature of 37°C.

• After the specified exposure time for incubation had passed, the dish was removed from the incubator, the culture medium was removed, then the cells were washed with the phosphate buffer solution prepared in paragraph [10], then 0.1 ml of MTT stain was added to each hole and left for 3 hours, then the contents were discarded. After the end of the time in the incubator, the cells attached to the bottom of the hole are dyed yellow, and then we add 0.1 ml of DMSO solution. The results are read using the ELISA Microplate Reader at a wavelength of 492 nm.

• The growth inhibition percentage for each concentration of plant extracts was calculated according to what was stated in **(31)** through the following equation:

Inhibition rate % = (control reading - treatment reading for each concentration) / control reading x 100%

The growth stimulation rate was also calculated according to **(32)** as follows:

Proliferation Rate (PR)% = (B/A) x 100

11. Statistical analysis:

The data obtained were statically analyzed using an unpaired t-test with GraphPad Prism 6 Values are presented as mean ± SD of triplicate measurements **(33)**.

Results and discussion:

The nanomaterial prepared from myrtle and zinc oxide was characterized using the following techniques:

1. **X-ray diffraction measurement (XRD) of the prepared nanomaterial**

The nanomaterial prepared from myrtle and zinc oxide was diagnosed using an **(Debye Scherer equation) (34).**

Figure (3) shows the X-ray diffraction (XRD) spectrum of the prepared nanomaterial.

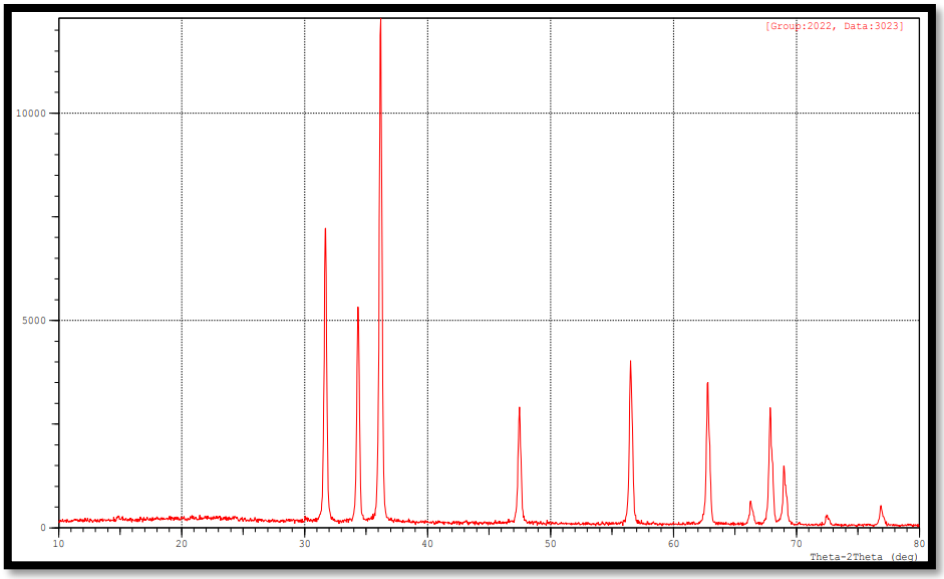

Figure (3) shows the X-ray diffraction (XRD) spectrum of the prepared nanomaterial.

Table (2) gives the information obtained from the X-ray diffraction spectrum of the three most intense beams, which was used to calculate the average crystalline size of the prepared nanomaterial sample, as it was found to be equal to 37 nanometres, which is less than 100 nanometres. This means that the prepared nanomaterial is within Nano scale space.

Table (2): shows data for the three strongest bands in the X-ray diffraction spectrum.

Number of peaks	2Theta (degree)	I/I1	FWHM	Nanoparticles(nm)
1	**36.1969**	**100**	**0.2255**	**37.47**
2	**31.7161**	**57**	**0.2218**	**35.86**
3	**34.3681**	**43**	**0.2203**	**37.88**

According to the data in the table above, the particle size of the prepared nanomaterial in the first package is 37.47 nanometers, in the second package it is 35.86 nanometers, and in the third package it is 37.88 nanometers.

2. Fourier transformed infra-red:

The infrared spectrum (FT-IR) of the model: (A) myrtle before adding zinc oxide, shown in Figure (4), was compared with model (B) myrtle after adding zinc oxide, shown in Figure (5), where a spectrum measurement showed Infrared radiation (FT-IR) before adding zinc oxide, many bands, as shown in Table (3), which shows the frequency of each band and the type of bond before adding, and model (B) of myrtle after adding zinc oxide shows the connection of the oxide group with the existing active groups. In myrtle herb, as shown in Table (4).

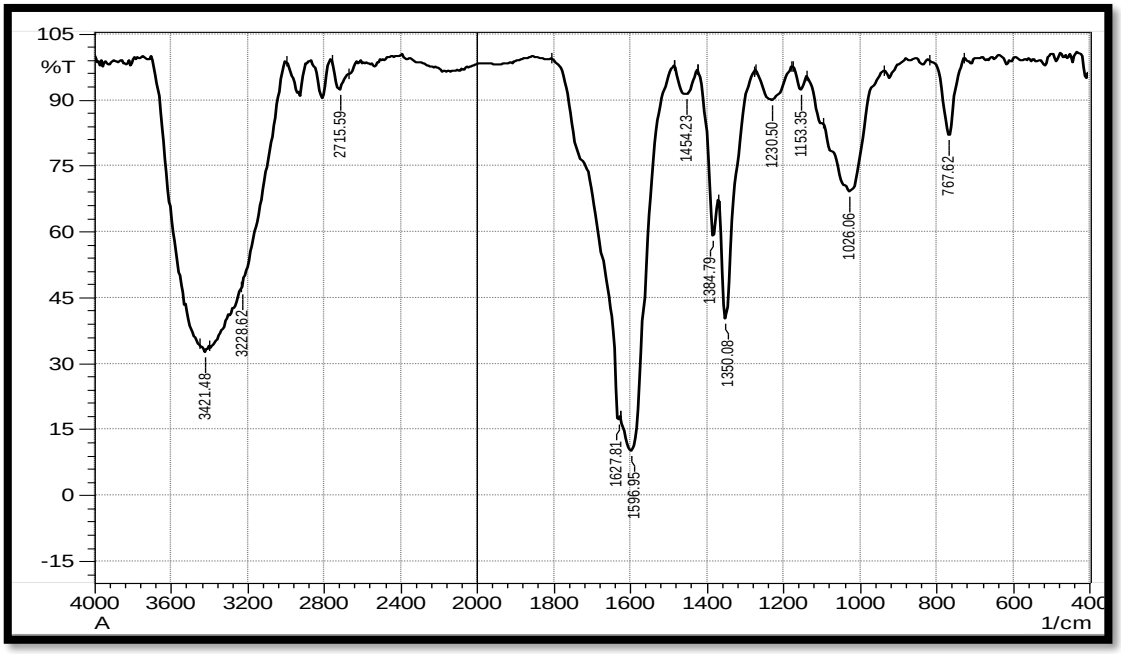

Figure No. (4) Infrared spectrum of myrtle before adding zinc oxide

Table (3): Infrared spectrum (FT-IR) bands of myrtle before addition

Frequency(cm^{-1})	Bond type
3421	Stretch(O-H)
2715	Stretch (C-H) aliphatic
1627	(C=N) Azomethine
1596	(C=C) Alcaine
1454	Bending(CH_2)
1384	Bending(CH_3)
1153	Bending(C-OC)
1026	Bending(C-OH)
767	Bending(C-H)

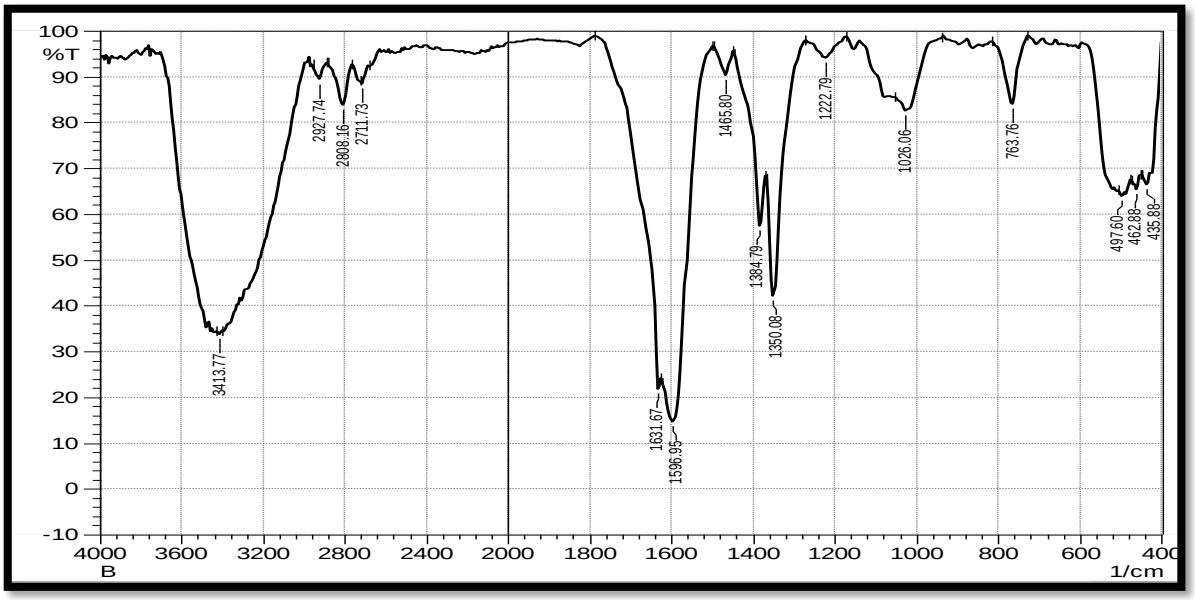

Figure No. (5) Infrared spectrum of myrtle herb after adding zinc oxide

Table (4): Infrared spectrum (FT-IR) bands of myrtle after adding zinc oxide

Frequency(cm^{-1})	Bond type
3413	Stretch(O-H)
2808	Stretch(C-H) aliphatic
1631	(C=N) Azomethine
1596	(C=C) Alcaine
1465	Bending(CH_2)
1384	Bending(CH_3)
1222	Bending(C-OC)
1026	Bending(C-OH)
763	Bending(C-H)
497	Stretch(Zn-O)

Through Figures (4) and (5) and the values shown in Tables (3) and (4), we notice that all the values for the two samples are identical except for the bond stretch (Zn-O) in the second sample, which confirms the loading of zinc oxide on the herb powder by the sol-gel process. To prepare the nanomaterial.

3. X-ray fluorescence spectroscopy analysis:

The aim of using this technique is to determine the elemental composition of the nanomaterial prepared from myrtle and zinc oxide. According to the results, the most abundant elements in the prepared sample were Zn, Si, and Ca, with average values of 78.59%, 2.38%, and 1.40%, respectively, as shown in Table (5), which shows the percentage of elements contained in the prepared nanomaterial, as well as Figure No. (6) which shows the X-ray fluorescence spectroscopy analysis.

Table (5): Percentage of elements contained in the prepared nanomaterial

Element	Content	Detection limit	Error
Si (%)	2.38	0.00	0.16
Al (%)	0.59	0.00	0.04
Mg (%)	0.00	0.00	0.00
P (%)	0.03	0.00	0.00
S (%)	0.00	0.00	0.00
K (%)	0.18	0.00	0.00
Ca (%)	1.40	0.00	0.01
Ti (%)	0.12	0.00	0.00
V (%)	0.03	0.00	0.00
Cr (%)	0.18	0.00	0.00
Mn (%)	0.00	0.00	0.00
Fe (%)	0.42	0.00	0.00
Co (%)	0.29	0.00	0.00
Ni (%)	0.97	0.00	0.07
Cu (%)	0.10	0.00	0.01
Zn (%)	78.59	0.00	9.67
As (%)	0.00	0.00	0.00
Se (%)	0.00	0.00	0.00
Sn (%)	0.00	0.00	0.00
Sb (%)	0.00	0.00	0.00
Ag (%)	0.01	0.00	0.00
Mo (%)	1.17	0.00	0.00
Zr (%)	0.02	0.00	0.00
Rb (%)	0.01	0.00	0.00
Sr (%)	0.01	0.00	0.00
Ba (%)	0.10	0.00	0.00
W (%)	1.22	0.00	0.17
Ta (%)	0.72	0.00	0.09
Au (PPM)	0.00	0.00	0.04
Hg (PPM)	0.00	0.00	0.00
Pb (%)	0.00	0.00	0.00
Cd (%)	0.00	0.00	0.00

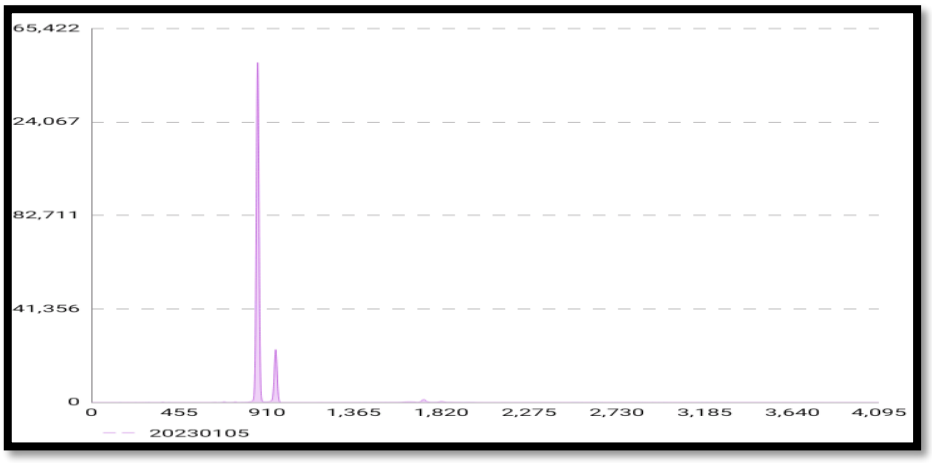

Figure No. (6) which shows the analysis of X-ray fluorescence spectroscopy.

From the above, it became clear that the nano sample prepared from the myrtle herb loaded with zinc oxide contains multiple oxides such as zinc oxide ZnO, silicon oxide SiO, and calcium oxide CaO, in addition to other oxides present in very small percentages. In addition to the fact that the prepared sample is nano, these oxides play a role. It is important in the treatment of multiple cancer diseases and its role is clear in influencing the cellular structure of cancer cells **(35)**. In this study, the toxic effect of this prepared substance on lung cancer cells will be clarified.

4. X-ray dispersion measurement (EDX):

This technique is used for element analysis to determine the chemical composition of samples and also studies the interaction between the source of excitation of the atomic structure of elements to be uniquely identified from each other **(36)**. This technique was used to determine the components of the prepared nanomaterial, and it was shown from Figure (7), Figure (8), and Table (6) that the highest component percentage of the prepared sample was zinc (76.6%), followed by oxygen (13.7%). %), followed by carbon (9.7%). This result confirms the connection of the myrtle herb, whose elements are mostly oxygen and carbon, with zinc oxide.

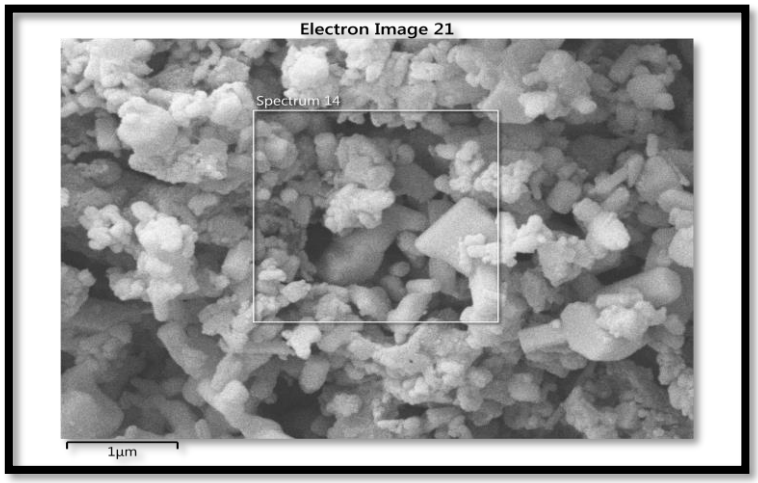

Figure No. (7) Surface of the prepared nanomaterial by X-ray scattering spectroscopy EDX

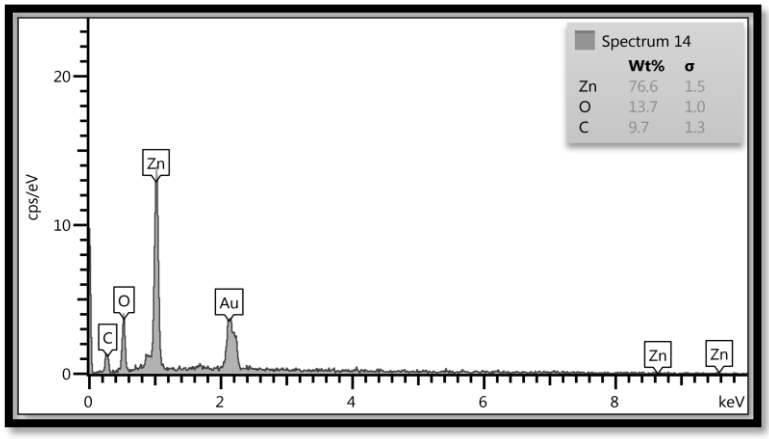

Figure No. (8) X-ray scattering spectrum EDX of the prepared nanomaterial

Table (6): Percentages of the elements that make up the sample of the material prepared from myrtle and zinc oxide

Element	Weight%
Zn	76.6
O	13.7
C	9.7

5. Toxic effect of the prepared nanomaterial on the growth of cells of the lung cancer cell line A1549:

The ability of the nanomaterial prepared from myrtle and zinc oxide to inhibit the growth of cancer cells outside the body of the organism was tested. These cells were treated with different concentrations of the prepared nanomaterial, starting from the lowest concentration (6.25) micrograms/ml and ending with the highest concentration (200) micrograms/ml. The results showed that high concentrations (100-200) micrograms/ml have a high killing rate ranging from 69-82% respectively for cancer cells after 48 hours, while low concentrations (50) micrograms/ml or less have a lower toxic effect rate. From 50%, and as shown in Figure (9), the toxic effect of the prepared nanomaterial on the lung cancer cell line A1549.

It is clear from the previous figure that the (X) axis represents the concentrations of the nanomaterial prepared from acetate and zinc oxide, and the (Y) axis represents the toxicity to cancer cells (lung cancer A1549), and we notice that as the concentration increases, the percentage of toxicity to cancer cells increases, meaning their spread decreases. At the concentration (6.25 micrograms/ml, the cytotoxicity increased with a rate and standard error of N=3 0.8353± 4.733, and at the concentration (12.5) micrograms/ml the cytotoxicity increased with a rate and standard error of N=3 1.559± 16.93, and at the concentration (25) micrograms/ml Cytotoxicity increased at a rate and standard error of N=3 2.494± 28.47, and at a concentration of (50) micrograms/ml the cytotoxicity rate increased at a rate and standard error of N=3 2.315± 49.17, and at a concentration of (100) micrograms/ml the cytotoxicity rate increased at a rate Standard error N=3 2.083± 69.27, and the concentration (200) micrograms/ml, the rate of cytotoxicity increased by a large percentage, standard error N=3 2.300± 82.93.0. The results showed consistency with previous studies **(37-38)**, as the concentration of the nanomaterial increases, the efficiency and inhibition of cancer cells increases **(39)**.

Figure No. (10) shows a group of cancer cells before they were exposed to the prepared nanomaterial, and Figure No. (11) shows a group of cancer cells after they were exposed to the prepared nanomaterial.

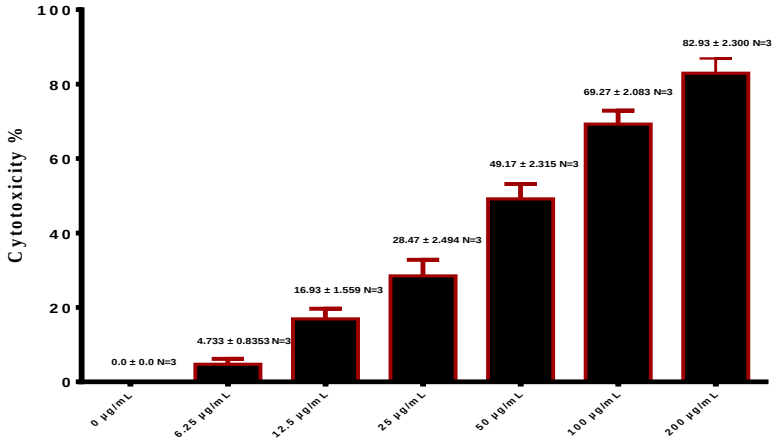

Figure No. (9) Toxic effect of the prepared nanomaterial on the A1549 lung cancer cell line.

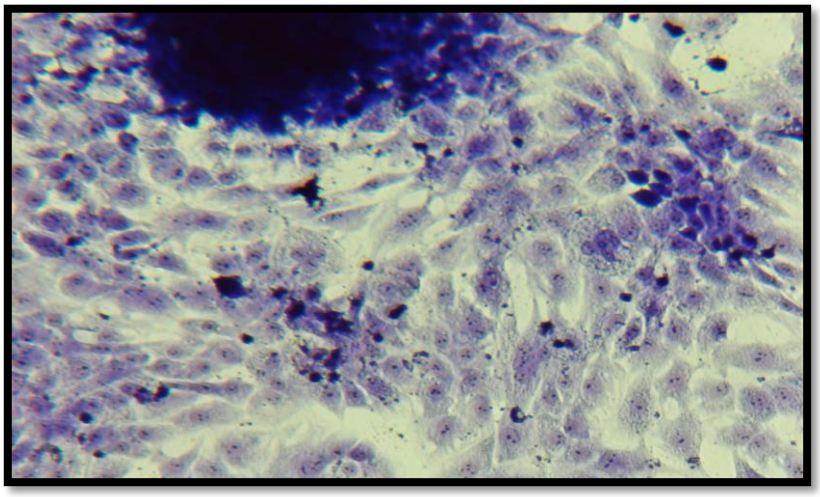

Figure No. (10) A group of cancer cells before being exposed to the prepared nanomaterial

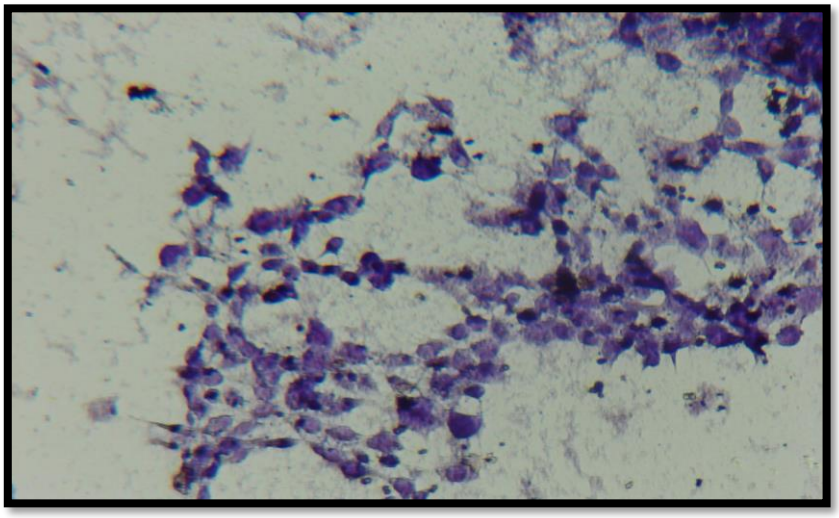

Figure No. (11) A group of cancer cells after being exposed to the prepared nanomaterial

Figures (10 and 11) show the effect of the prepared nanomaterial on the A154 lung cancer cell group, where the cancer cells were regular before adding the prepared nanomaterial to them. After adding the prepared nanomaterial, the cancer cells showed a clear change in their shape as they began to swell and there were also Black dots indicating that the cancer cells swelled to a larger size than their normal size and began to explode. Figure (11) is very consistent with previous studies **(40)** which showed that the shape of the cancer cells after exposure to Nano therapy is small in number and irregular in shape, in addition to the presence of swollen cells, as well as the presence of black areas that indicate the remains of swollen cells that exploded after exposure to the nanomaterial or Nano therapy.

Conclusions:

1. The study proved that the material prepared by the sol-gel method is a nanomaterial, and the size of the nanoparticles was calculated by X-ray diffraction (XRD) using the Debye-Spark equation.

2. The material prepared by the sol-gel method was characterized by FT-IR, where the infrared spectrum showed the active groups of the myrtle herb before and after the addition of zinc oxide, as it showed the zinc oxide group after the addition.

3. The diagnosis by Oxides plays an important role in the treatment of multiple cancer diseases, and their

role is evident in influencing the cellular structure of cancer cells.

4. EDX measurement of X-ray scattering showed that the highest component of the prepared sample was zinc (76.6%), followed by oxygen (13.7%), followed by carbon (9.7%). This result confirms the connection of myrtle, which contains most of its elements. Oxygen and carbon with zinc oxide.

5. The toxic effect of the prepared nanomaterial on the growth of cells of the lung cancer cell line A1549 proved that high concentrations (100-200) micrograms/ml of the prepared nanomaterial have a high killing rate ranging from 69-82%, respectively, of cancer cells after 48 hours. Low concentrations (50) micrograms/ml or less have a toxic effect of less than 50%.

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Competing interests:

The authors declare that they have no competing interests.

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