Novel Alhagi maurorum leaves mediated synthesis of titanium nanoparticles for human breast carcinoma applications: a preclinical trial

Type
Research paper

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In this study, titanium nanoparticles (TiNPs) were synthesized in an aqueous medium using Alhagi maurorum extract as stabilizing and reducing agents.

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Ultraviolet–visible spectrophotometry (UV-Vis), fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and Energy Dispersive X-ray Spectrometry (EDS) were the techniques to characterize the biosynthetic of TiNPs. According to the XRD analysis, TiNPs were synthesized using A. maurorum extract. To survey the anti-human breast cancer effects of TiNPs, MTT assay was used on the common breast cancer cell lines i.e., breast cancer (Breast adenocarcinoma (MCF7), infiltrating ductal cell carcinoma (Hs 319.T), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453) cell lines.

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16.08 nm was measured for TiNPs crystal size. SEM images exhibited a uniform spherical morphology in range size of 12.16 to 43.46 nm for the biosynthesized nanoparticles respectively. The cell viability of breast carcinoma cells decreased dose-dependently in the titanium nanoparticles presence. The IC50 of A. maurorum and titanium particles on MCF7 cell line were 680 and 359 µg/mL, on Hs 319.T cell line were 507 and 191 µg/mL, on UACC-732 cell line were 477 and 217 µg/mL, and on MDA-MB-453cell line were 507 and 191 µg/mL, respectively. TiNPs had high anti-breast cancer activities dose-dependently against MCF7, Hs 319.T, UACC-732, and MDA-MB-453 cell lines.

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Novel *Alhagi maurorum* leaves mediated synthesis of titanium nanoparticles for human breast carcinoma applications: a preclinical trial study

**Abstract**

In this study, titanium nanoparticles (TiNPs) were synthesized in an aqueous medium using *Alhagi maurorum* extract as stabilizing and reducing agents. Ultraviolet–visible spectrophotometry (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and Energy Dispersive X-ray Spectrometry (EDS) were the techniques to characterize the biosynthetic of TiNPs. According to the XRD analysis. To survey the anti-human breast cancer effects of TiNPs, MTT assay was used on the common breast cancer cell lines i.e., breast cancer (Breast adenocarcinoma (MCF7), infiltrating ductal cell carcinoma (Hs 319.T), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453)) cell lines. 16.08 nm was measured for TiNPs crystal size. SEM images exhibited a uniform spherical morphology in range size of 12.16 to 43.46 nm for the biosynthesized nanoparticles respectively. The cell viability of breast carcinoma cells decreased dose-dependently in the titanium nanoparticles presence. The IC50 of *A. maurorum* and titanium particles on MCF7 cell line were 680 and 359 µg/mL, on Hs 319.T cell line were 507 and 191 µg/mL, on UACC-732 cell line were 477 and 217 µg/mL, and on MDA-MB-453 cell line were 507 and 191 µg/mL, respectively. TiNPs had high anti-breast cancer activities dose-dependently against MCF7, Hs 319.T, UACC-732, and MDA-MB-453 cell lines. The best result of anti-breast cancer effects was seen in the case of the Hs 319.T cell line.

**Keywords:** Titanium nanoparticles; *Alhagi maurorum* leaf; Green synthesis; Chemical characterization; Human breast cancer.
Introduction

Breast carcinoma is a cancer sort that commences in the breast tissue. Being a woman is the most important risk factor for breast cancer. Although men also get this cancer, women are more than 100 times more likely to get it. Other breast cancer symptoms can include breast deformity, dimples, discharge from the nipple, or scaling of part of the skin. In severe conditions of the disease, these symptoms can include jaundice, breath shortness, swollen lymph nodes, and bone pain. Risk factors include the first menstruation at an early age, hormone replacement therapy during menopause, lack of physical exercise, ionizing radiation, drinking alcohol, and obesity. Breast cancer treatments are targeted therapy, chemotherapy, radiation therapy, and surgery. In people whose cancer has distributed to other body parts, cures are mainly done to improve the quality of life and comfort of the person. Raloxifene or Tamoxifen can be administrated to inhibit human breast cancer in people that are more likely to get it. Chemotherapeutic drugs have severe side effects for the patients, so finding new formulations with low side effects are the priority of the Food and Drug Administration. The previous reports have revealed the metallic nanocomposites have unique anticancer efficacies with low side effects.

Nowadays, nanotechnology has been developed in several ways due to its wide range of applications. The applications in medical diagnosis, food, medicine, biotechnology, environment, energy, chemistry, physics, etc. have been considered, which introduces this technology as an interdisciplinary and cross-sectorial field. One of the fields of nanotechnology is the production of nanoparticles to prevent and treat various diseases. The tendency to produce nanometer-sized materials and to use them is increasing day by day due to the interesting industrial properties of these materials, but nanoparticles from the chemical procedures used today, because of the hazardous and toxic chemicals use and environmental damage caused by them, they have caused a great deal of concern. The production of nanoparticles using the principles of green chemistry has found a special place in research and for this purpose, various types of biological systems are used; microorganisms, diatoms, and optical eukaryotes are among these systems, but these systems are less used due to their high cost of preparation and maintenance.
Plants and agricultural products have been given special attention as cheap and renewable sources for the preparation of biological nanomaterials.\textsuperscript{6-10} The surface area to volume ratio of nanoparticles makes them potent agents in biological aspects.\textsuperscript{7} Metallic nanoparticles with various properties and a wide range of activity have been well known.\textsuperscript{8} TiO\textsubscript{2} nanoparticles are one of the new metallic nano-compounds with a wide variety of applications in different fields of science. TiNPs have exhibited optical, dielectric, photo-catalytic properties. TiNPs also show biological activity such as anticancer, antibacterial, and antifungal activity.\textsuperscript{9-13} These properties depend on different factors such as surface area, morphology, crystallinity, and crystal phase effect on the operational performance of TiNPs.\textsuperscript{14}

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. Traditional medicine has a unique role in producing herbal therapeutic supplements and drugs.\textsuperscript{15-17} \textit{Alhagi maurorum} is an herb with many pharmaceutical properties. It belongs to the \textit{Plantae} kingdom, \textit{Fabales} order, \textit{Fabaceae} family, and \textit{Alhagi} genus. The several parts of \textit{Alhagi maurorum} and its products have been used in different world regions for the treatment of diseases such as several cancers, dropsy, rheumatism, asthma, bronchitis, loss of appetite, amenorrhoea, dysmenorrhoea, diarrhea, indigestion, skin eruptions, and worms.\textsuperscript{18-26} Since ancient times people have used \textit{Alhagi maurorum} to cure many diseases related to the respiratory, liver function, cardiovascular, gastrointestinal, immune, and urinary, and genital systems.\textsuperscript{18-20, 22} The chemical antioxidant compounds isolated from \textit{Alhagi maurorum} are as fallow: 2-nonadecanone, Nonacosane, actinidiolide, 6,10,14-trimethyl-2-pentadecanone, \textit{trans-β}-ionone, pentacosane, furanacetic acid, 9-octylheptadecane, drimenol, squalene, tetracosane, docosane, eicosane, octadecane, and drimenol.\textsuperscript{19-24} The therapeutic features of this plant due to its chemical antioxidant composition were mentioned above.
According to the above explanations, we green-synthesized the titanium nanoparticles using *Alhagi maurorum* in first, then we measured its anti-breast adenocarcinoma efficacies in vitro condition.

**Experimental**

**Material**

Bovine serum, antimycotic antibiotic solution, 2,2-diphenyl-1- pirkilhydrazil (DPPH), dimethyl sulfoxide (DMSO), decamplmaneh fetal, 4- (Dimethylamino) benzaldehyde, hydrolyzate, Ehrlich solution, and borax-sulfuric acid mixture, DMED, all were afforded from the US Sigma-Aldrich company.

**Extraction of *A. maurorum* leaf aqueous extract**

To obtain the aqueous extract of the plant, 250 gr of the dried branches of the *A. maurorum* leaves were poured into a container containing 2000 mL boiled water, and the container lid was tightly closed for 4 h. Then, the container’s content was filtered, and the remaining liquid was placed on a bain-marie to evaporate. Finally, a tar-like material was obtained, which was powdered by a freeze dryer.

**Preparation and synthesis of TiONPs**

A 25 mL of *A. maurorum* extract (10g/L) was added to 100 ml of 0.1M aqueous solution of TiCl₄at 120°C temperature and stirred for 120 min in the dark. The solution color turned to light brown that indicates the formation of TiNPs. The obtained mixture was incubated for 12h at 40 °C. Thereafter the mixture was centrifuged at 10,000 rpm for 20 min and the
upper phase was removed. The participate was washed with deionized water three times and once with ethanol.

**Chemical Characterization techniques**

TiNPs were characterized by some techniques (UV-Vis., FT-IR, XRD, SEM, and EDS). THERMO UV-vis spectrophotometer (200-800 nm), Shimadzu FT-IR 8400 (in the range of 400–4000 cm⁻¹, KBr disc), GNR EXPLORER instrument (voltage of 40 kV, a current of 30 mA, and Cu-Kα radiation 1.5406 Å), and MIRA3TESCAN-XMU, to report the XRD, FE-SEM Images and EDS result.

**Evaluation of anti-human breast cancer properties of titanium nanoparticles**

The breast cancer (Breast adenocarcinoma (MCF7), infiltrating ductal cell carcinoma (Hs 319.T), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453)) cell lines and the normal cell line (HUVEC) in the MTT assay were used as follows. They were in RPMI1640 liquid culture medium containing 10% inactivated bovine fetal serum (FBS), 2 mM glutamine, solution of penicillin (100 units per ml) and streptomycin (100 μg / ml) at 37 °C and 5% CO₂ and 95% moisture were cultured and passaged so that the cells reached the desired number in terms of number and morphology (after 3-4 passages). After separating the cells from the flask surface, trypsin-EDTA (Gibco BRL, Scotland) counted and evaluated cell viability, and 3,000 cells were cultured in 96-well wells with or without TiCl₄, A. maurorum leaf aqueous extract, and titanium nanoparticles. Morphological changes and general characteristics of the cell were assessed 24 hours using an inverted microscope (Motic, AE31 model, China).
The effect of cytotoxicity of the TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles was evaluated by MTT Methy Thiazol Tetrazolium colorimetric test. MTT (Sigma-Aldrich, USA) is a water-soluble tetrazolium salt based on the activity of living cell mitochondrial dehydrogenase succinate enzyme, which converts the yellow solution of MTT to insoluble purple crystals of formazan (Because the dehydrogenase content of cells of one type is relatively constant, the amount of formazan produced is proportional to the number of cells) Which is soluble in dimethyl sulfoxide²⁸.

The cells began to grow in 75 cm² square T-flasks in 15 ml of medium with an initial number of 1-2 10 10⁶ cells. After three days and covering the bottom of the flask with the cell, the cell layer adhering to the bottom of the flask was separated enzymatically using trypsin-Versen and transferred to a sterile test tube was centrifuged at 1200 rpm for 10 minutes (Germany). Sigma, 3-30k model,). The cells were then resuspended in fresh culture medium with a pasteurizer pipette and 100 μl of cell suspension with or without TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles were added to each well of the 96-well plate so that there were 3,000 cells in each well. The plates were incubated for 24 hours in an incubator (Germany, Memmert) to return the cells to normal from the stress of trypsinization. Then, suitable dilutions of the desired TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles were prepared and 100 μl of each dilution was added columnar to the plate wells and the cells were incubated for 37 hours at 37 ° C and 5% CO₂. After 72 hours of adding the TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles to the cells, 20 μl of 5 mg/ml MTT solution was added to each well. The plates were incubated for 4 hours after the required time, the culture medium containing MTT was carefully removed and 100 μl of DMSO was added to each well to
dissolve the resulting formazan. After 10 minutes and shaking the plates using the plate shaker, the light absorption at 570 nm was read by ELISA Reader (Awareness Technology Inc, Stat Fax 2100, USA). Cells containing no TiCl4, A. maurorum leaf aqueous extract, and titanium nanoparticles were considered as controlled light density and wells without cells and only RPMI1640 medium with bovine fetal serum were considered as blank 28.

\[
\text{Cell viability (\%)} = \frac{\text{Sample A.}}{\text{Control A.}} \times 100
\]

Finally, linear regression was performed to obtain the IC50 level, which represents the concentration of the extract, which causes 50% inhibition of cancer cell growth[25]:

Qualitative Measurement

The obtained findings were analyzed by SPSS (version 20) software using one-way ANOVA, followed by Duncan post-hoc test (\(P \leq 0.01\)).

Results and Discussion

Characterization of TiNPs

**UV–visible spectroscopy analysis**

Fig.1 presents the UV-Vis. spectrum of biosynthesized TiNPs using A. maurorum extract. The result of UV-Vis. spectroscopy confirms the formation of TiNPs. The peak at 220 nm belongs to the biosynthetic TiNPs. This observation is in good agreement with the previous report of UV-Vis. for a biosynthesized TiNPs 27.
**FT-IR analysis**

Fig.2 exhibits the spectra of TiNPs. The peaks at 503 and 582 cm$^{-1}$ belong to the bending vibration of Ti-O. These peaks for TiO$_2$ nanoparticles have been reported previously with a small difference in the wavenumber $^{29}$. FT-IR technique is an acceptable method to evaluate the secondary plant metabolites as the capping and reducing agents of titanium chloride precursor to TiNPs. FT-IR spectrum of TiNPs shows the presence of different-IR bands that correlate to the presence of various functional groups in *A. maurorum* extract that covers the TiNPs. A broad peak around 3400 relates to O-H, peak at 2941 cm$^{-1}$ shows an aliphatic C-H stretching band. These peaks can be considered for some phytochemical compounds found in *A. maurorum* extract such as phenolic, flavonoid, triterpenes $^{21,30,31}$. 

![Figure 1.UV–Vis. spectra of biosynthesized TiNPs.](image)
Figure 2. FT-IR Spectrum of biosynthetic TiNPs.

**XRD analysis**

Fig. 3 shows the XRD pattern of TiNPs. The result, same to FT-IR spectra, approves the synthesis of zinc oxide. The peaks at 25.37, 38.59, 48.10, 53.97, 55.10, 62.75, and 75.17 corresponding to (101), (112), (200), (105), (211), (204), and (215) planes. These peaks are matched as well as to those of TiO2 nanoparticles reported previously. The crystallinity of the TiNPs has been accepted using the XRD diffractogram. The average crystal size of TiNPs was calculated using X-ray diffraction according to the Scherrer equation (eq.1). The TiO2 nanoparticles had an average crystal size of 16.08 nm.

\[
D = \frac{k\lambda}{\beta\cos\theta} \quad \text{eq.1}
\]
**SEM analysis**

Fig 4 shows the FE-SEM images of TiNPs. The images exhibited a spherical morphology for the biosynthetic of TiNPs. This morphology for biosynthesized TiNPs has been reported previously \(^{14}\). The particle size of TiNPs varied from 12.16 to 43.46 nm. The figure also confirms the uniformity, well dispersed, and homogeneous of the TiNPs. The biosynthetic metallic nanoparticles such as TiNPs, ZnNPs, MnNPs, SnNPs, and AgNPs usually show a tendency to aggregate \(^{27, 32-35}\). This property also is observed for the biosynthetic of TiNPs in this research. Various particle sizes, from 10 to 100 nm, have been reported for TiNPs that were synthesized using green chemistry procedures \(^{11, 13, 14, 27}\).
Figure 4. SEM images of TiNPs.

**EDS analysis**

The EDS analysis is shown in Fig. 5. EDS is a qualitative method to screen the elemental composition profile of compounds. It is based on the interaction of an X-ray beam with the sample to recognize the atomic structure of the sample. The spectrum shows the presence of titanium, oxygen, and carbon in the synthetic nanoparticles. The signal before 1Kev (for TiLα), after 4.5 Kev (TiKα), and below 5 Kev (for TiKβ). These signals are as well as match those of biosynthesized TiNPs that were reported previously 29. The presence of oxygen is approved by the signal around 0.5 Kev. This signal is related to the oxygen of TiO₂ and the phytochemical of *Maurorum* extract. The presence of carbon on the surface of TiNPsis was also confirmed with the signal before 0.3 Kev.
Anti-human breast carcinoma effects of the titanium nanoparticles

Despite many advances in disease control and treatment, cancer remains one of the global challenges to human health. The most common treatment for cancer is chemotherapy. An important point in the treatment of cancer with chemotherapy is the acquired resistance of the tumor to drugs, and this has created major problems for the treatment of cancer. Because the rate of mutation and genetic instability in cancer cells is very high and genetic changes occur rapidly in them, these cells become resistant to drugs. Therefore, further research on discovering new treatment strategies to overcome the drug resistance of cancers seems necessary. Meanwhile, nanotechnology has created a promising field in the field of cancer treatment. In recent years, the anti-cancer and anti-angiogenic effects of metallic nanoparticles have been considered and the results have shown that metallic nanoparticles can be considered as a potential anti-cancer agent. Reddy et al. investigated the antioxidant, antibacterial and anticancer effects of metallic nanoparticles produced using Piper longum extract.
Many parameters such as surface functions' nature and texture and size are important in the anticancer effects of metallic nanoparticles, of course, the efficacy of the size is the main. Many reports have been indicated whatever the size of the nanoparticles is low, their ability to poring and destroying the cancer cells is more. In detail, it has been reported the nanoparticles with a size lower than 50 nm have the best condition for anticancer effects. As shown in the FE-SEM figure, the size of nanoparticles is less than 40 nm.

In our research, the treated cell lines with TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles were tested by a well-known cytotoxicity test, i.e. MTT test for 72 h regarding the cytotoxicity activities on normal (HUVEC) and breast cancer (Breast adenocarcinoma (MCF7), infiltrating ductal cell carcinoma (Hs 319.T), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453) cell lines (Table 1 and 2). TiCl₄, *A. maurorum* leaf aqueous extract, and titanium particles didn’t show any toxicity on HUVEC cells in the MTT assay.

The cell viability of breast carcinoma cells decreased dose-dependently in the TiCl₄, *A. maurorum* leaf aqueous extract, and titanium nanoparticles presence. The IC50 of *A. maurorum* and titanium particles on MCF7 cell line were 680 and 359 µg/mL, on Hs 319.T cell line were 507 and 191 µg/mL, on UACC-732 cell line were 477 and 217 µg/mL, and on MDA-MB-453 cell line were 507 and 191 µg/mL, respectively. The best findings were reported about the Hs 319.T cell line.

Likely significant anti-cancer activities of *A. maurorum* leaf aqueous extract and titanium nanoparticles are related to their antioxidant properties. Similar reports have shown the antioxidant molecules such as metallic nanocomposites reduce the tumor volume by destroying free radicals. In detail, the free radicals high presence in the normal cells cause several mutations in their RNA and DNA and increase the growth and proliferation of cancerous cells. The free radicals in skin, throat, ovarian, testicular, bladder, colon, small intestine, gastrointestinal stromal, stomach, breast, lung, vaginal, prostate, pancreatic, liver, gallbladder, hypopharyngeal, fallopian tube, thyroid, esophageal, parathyroid, bile duct, and rectal cancers reveal the notable place of these radicals in causing tumorigenesis and angiogenesis.
TABLE 1 The anti-breast carcinoma properties of TiCl₄, A. maurorum leaf aqueous extract, and TiNPs against human breast carcinoma cell line.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>HUVEC</th>
<th>MDA-MB-453</th>
<th>MCF7</th>
<th>UACC-732</th>
<th>Hs 319.T</th>
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<tr>
<td>TiCl₄ (0)</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
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<tr>
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<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
</tr>
<tr>
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<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
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<tr>
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<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
</tr>
<tr>
<td>TiCl₄ (7)</td>
<td>99.6±0.89ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
<td>100±0ᵃ</td>
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</tr>
<tr>
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<td>99.4±0.89ᵃ</td>
<td>98.2±1.3ᵃ</td>
<td>99.4±0.54ᵃ</td>
<td>98.1±2.2ᵃ</td>
</tr>
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<td>TiCl₄ (31)</td>
<td>94.2±0.44ᵃ</td>
<td>97.2±0.83ᵃ</td>
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<td>97.6±0.89ᵃ</td>
<td>96.4±0.89ᵃ</td>
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<td>94±0.7ᵃ</td>
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<td>91.2±0.44ᵃ</td>
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<td>76.6±1.14ᵇ</td>
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<td>72±0.7ᵇ</td>
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<td>34±0.7ᵇ</td>
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<td>29.6±1.14ᵇ</td>
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<td>96±0.7ᵃ</td>
<td>92.6±1.14ᵃ</td>
<td>92.2±1.3ᵃ</td>
</tr>
<tr>
<td>TiNPs (15)</td>
<td>100±0ᵃ</td>
<td>92.4±0.89ᵃ</td>
<td>91.2±0.83ᵃ</td>
<td>86.6±0.89ᵃ</td>
<td>87.4±0.89ᵃ</td>
</tr>
<tr>
<td>TiNPs (31)</td>
<td>99.2±0.83ᵃ</td>
<td>85±1ᵃ</td>
<td>85.4±0.89ᵃ</td>
<td>79.2±0.44ᵇ</td>
<td>78.4±0.54ᵇ</td>
</tr>
<tr>
<td>TiNPs (62)</td>
<td>97.4±0.89ᵃ</td>
<td>76.6±1.14ᵇ</td>
<td>76.4±0.89ᵇ</td>
<td>70.6±1.14ᵇ</td>
<td>67.4±0.89ᵇ</td>
</tr>
<tr>
<td>TiNPs (125)</td>
<td>94.4±0.54ᵃ</td>
<td>65.2±0.44ᵇ</td>
<td>66.1±2.2ᵇ</td>
<td>59.4±0.54ᵇ</td>
<td>56.2±1.3ᵇ</td>
</tr>
<tr>
<td>TiNPs (250)</td>
<td>90.2±1.3ᵃ</td>
<td>53.2±0.83ᵇ</td>
<td>57.6±0.89ᵇ</td>
<td>48±0.7ᵇ</td>
<td>44±1ᵇ</td>
</tr>
<tr>
<td>TiNPs (500)</td>
<td>85±1.22ᵃ</td>
<td>38±0.7ᵇ</td>
<td>42±0.7ᵇ</td>
<td>35.6±1.14ᵇ</td>
<td>29.2±0.83ᵇ</td>
</tr>
<tr>
<td>TiNPs (1000)</td>
<td>78.6±0.89ᵇ</td>
<td>16.2±0.44ᶜ</td>
<td>24.6±1.14ᵇ</td>
<td>12.2±0.44ᶜ</td>
<td>10.6±0.89ᶜ</td>
</tr>
</tbody>
</table>

The various words report significant differences between examined groups (P≤0.01).
### TABLE 2

The IC50 of TiCl4, *A. maurorum* leaf aqueous extract, and TiNPs in cytotoxicity and anti-breast carcinoma tests.

<table>
<thead>
<tr>
<th></th>
<th>TiCl4 (µg/mL)</th>
<th><em>A. maurorum</em> (µg/mL)</th>
<th>TiNPs (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 against HUVEC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IC50 against MDA-MB-453</td>
<td>-</td>
<td>564±0b</td>
<td>297±0d</td>
</tr>
<tr>
<td>IC50 against MCF7</td>
<td>-</td>
<td>680±0a</td>
<td>359±0d</td>
</tr>
<tr>
<td>IC50 against UACC-732</td>
<td>-</td>
<td>477±0c</td>
<td>217±0g</td>
</tr>
<tr>
<td>IC50 against Hs 319.T</td>
<td>-</td>
<td>507±0bc</td>
<td>191±0g</td>
</tr>
</tbody>
</table>

The various words report significant differences between examined groups (*P*≤0.01).

### 4 CONCLUSION

In this research, the titanium nanoparticles were attained from the reaction between 25 milliliters of the extract and 100 ml of 0.1M aqueous solution of TiCl4. FE-SEM, UV–Vis, EDS, XRD, and FT-IR methods were utilized to evaluate nanoparticles characteristics. The results of these techniques revealed that titanium nanoparticles had been synthesized in the best way. Based on the FT-IR spectrum the presence of a great number of antioxidant compounds produced appropriate conditions for the reduction of titanium. In the FE-SEM technique, the range size of titanium nanoparticles was assessed to be 12.16 to 43.46 nm, which is favorable. The titanium nanoparticles had appropriate anti-human breast carcinoma activities dose-dependently against breast cancer (Breast adenocarcinoma (MCF7), infiltrating ductal cell carcinoma (Hs 319.T), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453)) cell lines without any cytotoxicity on the normal cell line (HUVEC). After clinical study titanium nanoparticles containing *A. maurorum* leaf aqueous extract can be utilized as an efficient drug/supplement in treating breast cancer and diseases in humans.
Ethics approval: No humans or animals are included in this study.

Consent for publication: All the authors consented to the publication of this article.

Availability of data and materials: The data used to support the findings of this study are included in the article. Additional data or information can be requested by contacting the corresponding author.

Competing interests: No potential competing interests relevant to this study were reported.

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