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# Green synthesis of nanohydroxyapatite through *Elaeagnus angustifolia* L. extract and evaluating its anti-tumor properties in MCF7 breast cancer cell line

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## Abstract

**Background** One of the most common types of cancer in women is breast cancer. There are numerous natural plant-based products, which exert anti-tumoral effects including *Elaeagnus Angustifolia* (EA). It modulates cell-cycle process, heat-shock proteins expression, anti-proliferative properties, apoptosis induction, blocking of angiogenesis, and cell invasion inhibition. The current study aimed to synthesize and evaluate the anticancer effects of hydroalcoholic EA extract (HEAE), Nanohydroxyapatite (nHAp) and nHAp synthesized through EA (nHA-EA) in MCF-7 breast cancer cell line.

**Methods** In the present study, HEAE preparation and green synthesis of nHA-EA was done and phase composition, functional groups, and crystallin phase of nHA-EA and nHAp were determined using Fourier-transform infrared (FTIR) and X-ray diffraction (XRD). The characteristics of synthesized nanoparticles including structural and morphological parameters were investigated using scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) techniques. Then, by using MTT-assay (Dimethylthiazoldiphenyltetrazolium), the in vitro cytotoxic and half maximal inhibitory concentration (IC<sub>50</sub>) of EA extract, nHAp, and nHA-EA in the MCF-7 breast cancer cell line was evaluated. Next, we assessed the expression of apoptosis-related genes Bax, Bcl2 and p53 using quantitative reverse-transcriptase polymerase-chain-reaction (qRT-PCR) and migration of MCF-7 cells by scratch assay.

**Results** The FTIR results demonstrated formation of nHAp and its interaction with HEAE during synthesis process. The XRD results of the synthesized nanoparticles showed similar XRD pattern of nHA-EA and nHAp and purity of synthesized nanomaterials. The average IC<sub>50</sub> of HEAE, nHAp, and nHA-EA extract after treatment of cancer cells for 24 h was 400 µg/mL, 200 µg/mL, and 100 µg/mL, respectively. Our results revealed that nHA-EA significantly reduced the migration and invasion of the MCF-7 cells, in comparison to the nHAp and EA extract. Moreover, level of Bax/Bcl2 and p53 was significantly higher in the nHA-EA extract group in comparison to the EA extract and nHAp group.

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**Conclusion** Taken together, our results demonstrated that bioactive constituents of EA medicinal plant in form of nHA-EA particles, can effectively exerts potential anticancer and chemo preventive effect against breast cancer growth and can be proposed as a promising beneficial candidate for BC therapy. However, further investigations are required to discover what bioactive compounds are responsible for the chemo preventive effect of this extract.

**Keywords** MCF-7 breast cancer, *Elaeagnus Angustifolia*, Hydroxyapatite nanoparticle, Apoptosis

## Introduction

One of the most common types of cancer in women is breast cancer (BC) [1], and it is considered a leading cause of death in patients with cancer all around the world [2]. Despite the recent advances in BC diagnostic and therapeutic methods, due to the high rate of mortality and chemoresistance, BC cancer patients' treatment is still a matter of debate [3].

There are numerous natural plant-based products and extracts, which exert anti-tumoral effects and could be useful for the design and development of new anti-cancer drugs [4]. *Elaeagnus Angustifolia* (EA) (also called oleaster, Russian olive, wild olive, silver berry), is a tree, that belongs to the Elaeagnacea (Araliaceae) family, and its fruits are characterized by small size and reddish-brown color. Different type of EA is commonly growing in Asia, Europe, and some regions of North America [5, 6]. The fruit of EA has been utilized as a medicinal plant, which acts as a potential therapeutic agent in numerous disorders through modulation of the immune system and oxidative stress balance [5]. It also exerts anti-cancer, antibacterial, and antifungal effects and has gastro- and hepatoprotective efficacy [7–10]. It has been shown that EA fruit is a rich source of different vitamins, carbohydrates, proteins, and minerals [6, 11, 12]. Moreover, EA fruits are a good source of beneficial compounds such as coumarins, tannins, phenolic acids, and flavonoids [5, 13, 14]. Different studies are showing that EA exerts anticancer effects via modulating the cell-cycle process, heat-shock proteins expression, anti-proliferative properties, apoptosis induction, blocking of angiogenesis, and cell invasion inhibition [15–17].

Nanomedicine has drawn a lot of interest because of its various and effective utilization in medicine, particularly in drug delivery [18]. Due to the high dispersion stability of nanoparticles (NPs), they have attracted considerable research interest in biomedical applications and drug delivery systems [19, 20]. The biosynthesis of NPs, using natural sources such as plants, without the utilization of any hazardous substances, reduces potential health and environmental threats [21, 22]. Moreover, plant extract allows the preparation of NPs with controlled and defined shape and size [23].

Hydroxyapatite (HAp,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), has various biomedical applications in many areas of medicine, due to its high bioactivity and biocompatibility. According to the method applied for HAp preparation, various mechanical properties bioactivity level, and dissolution behavior in the biological environment is expected [24]. It has been shown that HAp exerts anti-cancer effects via increasing drug release and consequently greater growth inhibition properties. Taken together, HAp enhances the chemotherapeutic efficacy of various agents including Cisplatin, Methotrexate, and Adriamycin. This could be because HAp mediates drug penetration into the tumor and improves drug delivery [25] improvement. It has been shown that the green synthesis of NPs has opened up new possibilities in material development, and there is research conducted on the green synthesis of HAp [26].

A different source of flavonoids including, catechin, epicatechin, gallic acid, gallo catechin, epigallocatechin, kaempferol, quercetin, luteolin, isorhamnetin, and isorhamnetin-3- $\beta$ -D-galactopyranoside have been isolated from EA [27]. Furthermore, various phenolic components such as 4-hydroxybenzoic acid and caffeic acid have also been found in EA [28]. It has been shown that flavonoids contain phenolic hydroxyl groups, they may play a role in metals chelating efficiency, lowering lipid peroxidation process, and increasing antioxidant and free radical scavenging capacity [29, 30]. According to these findings, in the current study, nano hydroxyapatite (nHAp) was synthesized with the EA extract, which shows reducing/stabilizing effects, as well as capping properties. This synthesis method is inexpensive and simple, has long-lasting stability, and is appropriate for macro-scale procedures [7, 31–33].

Therefore, the current study aimed to synthesize and evaluate the anticancer effects of hydroalcoholic EA extract (HEAE), nHAp, and nHAp synthesized through EA (nHA-EA) in MCF-7 breast cancer cell line. The synthesized nanoparticles were characterized, and their structural, morphological, and optical properties were determined via different analytical tools, including x-ray diffraction (XRD), fourier-transform infrared (FT-IR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

**Materials & methods**

In the present study, all chemicals and reagents used to synthesize nHAp and nHA-EA were of analytical grade. Calcium nitrate tetrahydrate [Ca (NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O], diammonium hydrogen phosphate [(NH<sub>4</sub>)<sub>2</sub> HPO<sub>4</sub>], sodium hydroxide (NaOH), and were purchased from Sigma Aldrich. All chemicals solution was prepared using deionized water.

**Plant material and extract preparation**

The Russian Olive was obtained from South Khorasan, Birjand, Iran. It was identified as *Elaeagnus Angustifolia* L. by Dr. F. Askari (Assistant Professor of Traditional Pharmacy, School of Pharmacy). The voucher specimen was deposited in the Herbarium Center of the School of Pharmacy, Birjand University of Medical Sciences (221).

**Hydroalcoholic EA extract preparation & green synthesis of nHA-EA**

We have discussed synthesis of nHAp and nHA-EA in the previous studies [29, 34]. Briefly, EA pulps were obtained from fresh fruits and HEAE was prepared using the maceration procedure as below. 40 gr of dried EA pulp powder with 320 mL methanol and 80 ml distilled water were mixed. Finally, the extracts were filtered and concentrated using a rotating vacuum and HEAE was stored at 4 °C.

nHA-EAs were synthesized using the sol-gel technique, using calcium nitrate tetrahydrate and diammonium hydrogen phosphate, (molar ratio: 1.67). V/V HEAE (10 ml, 10%), diammonium hydrogen phosphate (5 ml, 0.3 M), and calcium nitrate tetrahydrate (15 ml, 0.3 M) were dissolved in ionized water. The Calcium nitrate tetrahydrate solution was introduced to the HEAE solution, then stirred upon slow warming to 50 °C, for 0.5 h. Then, diammonium hydrogen phosphate solution was added to the above-mixed solution at the flow rate

of 1 ml/min. NaOH solution was used for pH adjustment (pH=11). At 50 °C for 90 min, the suspension was agitated (Fig. 1). The resulting solution was subjected to the centrifuge and then gently rinsed with deionized water and ethanol (nHA-EA). Finally, nHAp solution without HEAE was synthesized under the same situations described for comparison.

**Characterization**

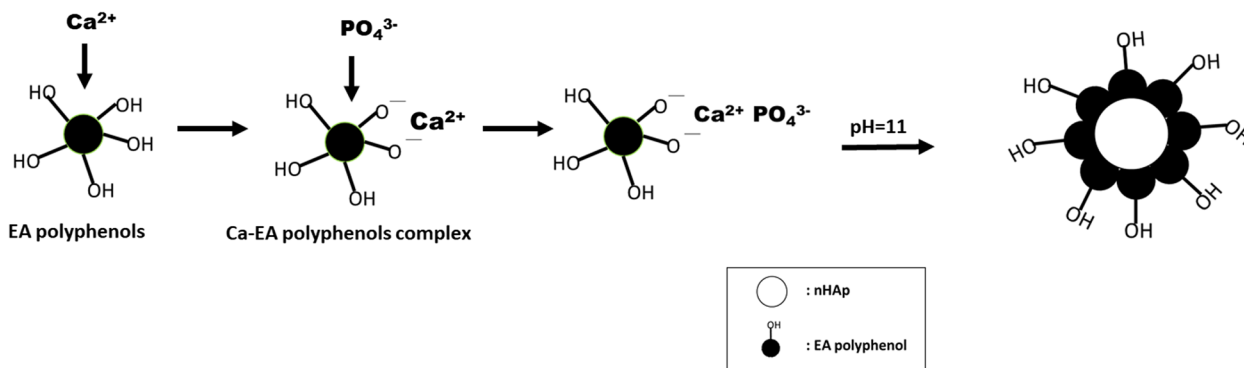
The phase composition, functional groups, and crystallin phase of nHA-EA and nHAp were determined using FTIR and XRD. The characteristics of synthesized nanoparticles including structural and morphological parameters were investigated using SEM and TEM techniques.

**Cell culture**

The human MCF-7 cells were purchased from the Pasteur institute (Tehran, Iran). The cells were cultured in dulbecco’s modified eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% streptomycin/ penicillin and maintained at 37 °C in a 5% CO<sub>2</sub> atmosphere.

**MTT-assay**

The MTT-assay (Dimethylthiazoldiphenyltetrazolium) was used to assess the in vitro cytotoxic and half maximal inhibitory concentration (IC<sub>50</sub>) of EA extract, nHAp, and nHA-EA in the MCF-7 breast cancer cell line. Cells were treated with different concentrations of EA extract, nHAp, and nHA-EA for 24 h. Then, the medium was removed from the wells and MTT substrate (Sigma, Germany) was added to each well, and the plate was incubated for 4 h. Then, the resulting crystals were dissolved in dimethylsulfoxide (DMSO) and incubated for 1 h. Finally, the absorbance was measured at 540 nm using a microplate reader. The experiments were performed in triplicate.



**Fig. 1** A schematic representation of the nHA-EA preparation process

**Scratch assay**

Briefly, MCF-7 cells were seeded in a 12-well plate at a concentration of  $4 \times 10^5$  cells /well. Then, a narrow scratch was created with a 100  $\mu$ l sterile pipette tip through a monolayer of adherent cells growing on the bottom of a cell culture plate, 24 h after cell seeding. Then, cell debris was removed. Plates were treated and after incubation at different time points were imaged and analyzed by using Digimizer 5.4.9 software.

**Quantitative reverse-transcriptase polymerase-chain-reaction (qRT-PCR)**

In this study, a quantitative RT-PCR assay was used to measure transcript levels of different genes. First, total RNA was extracted from the cells using the Pars Tous kit, (Tehran, Iran) according to the manufacturer’s instructions. Then, RNAs were converted to complementary DNA (cDNA) using the commercial cDNA synthesis kit (Parstous kit). Quantitative RT-PCR was performed with specific forward and reverse primers for target genes (Table 1). The cDNA amplification was performed by using the StepOne instrument (Applied Biosystems, Foster City, CA). The gene expression levels were normalized to a housekeeping control gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**Statistical analysis**

All values are expressed as mean  $\pm$  standard error of the mean. GraphPad Prism (version 9) was used to conduct the analyses. Statistical comparisons were determined using student’s t-test or one-way analysis of variance (ANOVA) followed by tukey’s multiple comparison test. The differences were considered to be statistically significant at  $P < 0.05$ .

**Results**

**FTIR**

The FTIR technique in the middle infrared spectral ( $400\text{--}4000\text{ cm}^{-1}$ ) wavelength was used to evaluate the functional groups of HEAE, nHAp and nHA-EA (Fig. 2). In the HEAE spectra, the continued peak at  $3367\text{ cm}^{-1}$  is defined to O–H vibration and peaks at  $1060\text{--}1283\text{ cm}^{-1}$  are the C–O–C characteristic peaks. In the nHAp, the

intense peak around at  $564\text{--}601$  and  $1030\text{ cm}^{-1}$  indicated the  $\text{PO}_4^{3-}$  bands. Moreover, the O–H vibrations on the water molecule were found around  $3000$  to  $3600\text{ cm}^{-1}$ . In the nHA-EA spectra, the extended peak around at  $3443$  shows water absorption in the synthesized nanoparticles. nHA-EA spectra analysis showed distinct bands of  $\text{PO}_4^{3-}$  at  $564$ ,  $604$ , and,  $1029\text{ cm}^{-1}$ . The extra peak at  $870$  showed the presence of C=O stretching of carboxylic acid, and clearly suggests the interaction between carboxyl group and nHAp. These FTIR functional groups findings clearly demonstrates formation of nHAp and its interaction with HEAE during synthesis process.

**XRD**

The X-ray diffraction patterns results of the synthesized nanoparticles are presented at Fig. 3, nHA-EA and nHAp showed similar XRD pattern. There was no peak related to the calcium hydroxide and calcium phosphates, which indicates synthesized nanomaterials purity. The seven peaks observed at  $26^\circ$ ,  $32^\circ$ ,  $39^\circ$ ,  $46^\circ$ ,  $49^\circ$ ,  $53^\circ$  and  $64^\circ$ , were corresponded to the plane (002), (112), (310), (222), (123), (004), and (233), respectively, and related to the hydroxyapatite, which find to be matched with ICDD: 01–084-1998.

**SEM and TEM**

Data obtained from SEM and TEM images revealed nanorod particles (Figs. 4 and 5). Notably, nHAPs have  $17\text{--}29\text{ nm}$  width and  $62\text{--}89\text{ nm}$  length, whereas nHA-EA nanorods have  $17\text{--}23\text{ nm}$  width and  $93\text{--}146\text{ nm}$  length.

**Cell proliferation**

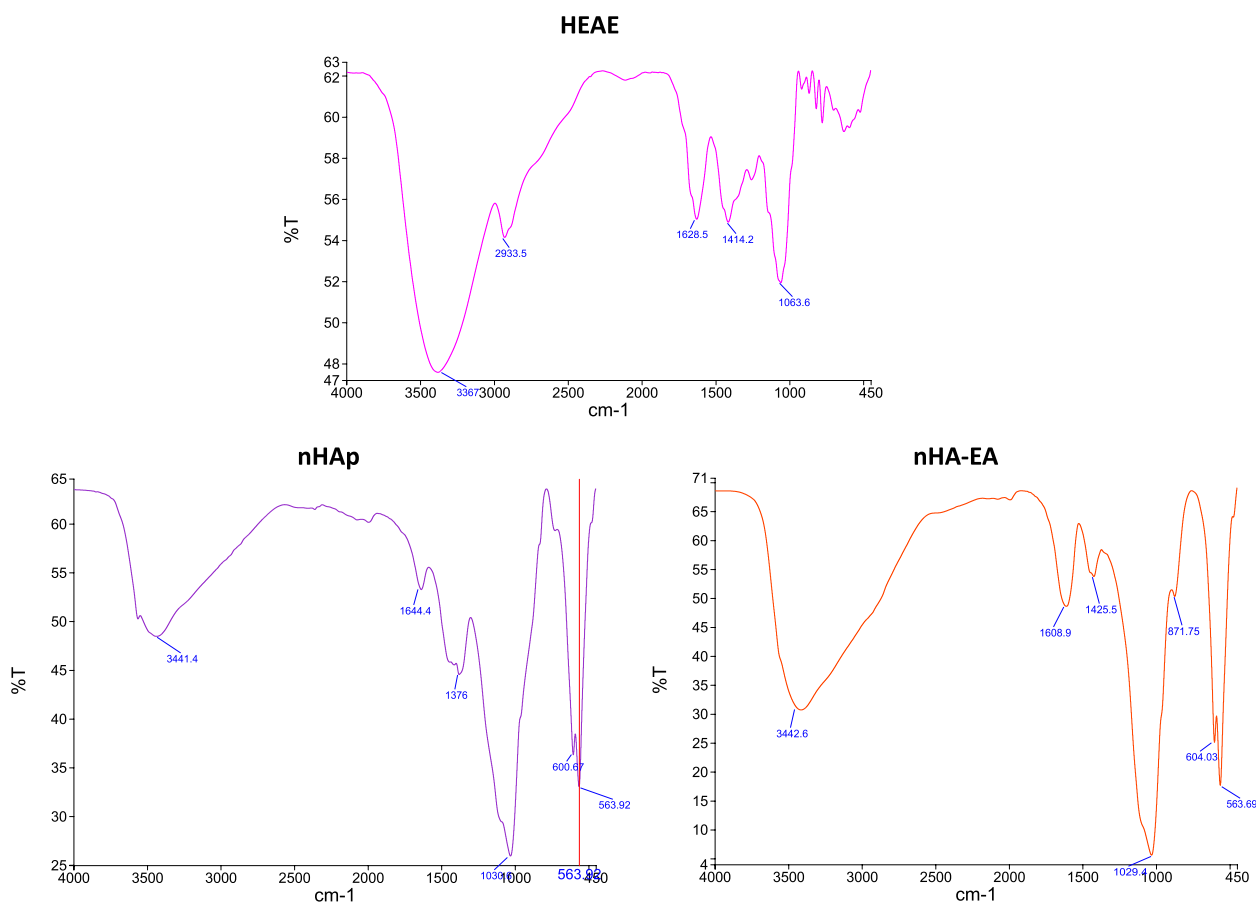
In the current study, an MTT assay was performed to evaluate the in vitro cytotoxic and  $\text{IC}_{50}$  of EA, nHAp, and nHAEA extract in the MCF-7 breast cancer cell line. Our results indicated that the average  $\text{IC}_{50}$  of EA, nHAp, and nHAEA extract after treatment of cancer cells for 24 h was  $400\text{ }\mu\text{g/mL}$ ,  $200\text{ }\mu\text{g/mL}$ , and  $100\text{ }\mu\text{g/mL}$ , respectively (Fig. 6).

**Scratch assay**

Regarding the potential effects of EA nanoparticles on breast cancer cell migration, in the present study, we

**Table 1** Primers used for real-time polymerase chain reaction

Gene Symbol	Gene Name	Forward Primer	Reverse Primer
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase	TCAAGATCATCAGCAATGCCTCC	GCCATCACGCCACAGTTTC
Bax	Bcl-2-associated X protein	TGACGGCAACTTCAACTGGG	CTTCAGTGACTCGGCCAGGG
Bcl2	B-cell lymphoma 2	GTCATGTGTGTGGAGAGCGTC	CCGTACAGTTCCACAAAGGCATC
p53	Tumor suppressor protein	ACACGCTTCCCTGGATTGG	CTAGGATCTGACTGCGGCTC



**Fig. 2** FTIR spectra of HEAE, nHAp and nHA-EA

examined the breast cancer cells invasiveness inhibitory effects of nHA-EA using the scratch assay. Our results revealed that nHA-EA significantly reduced the migration and invasion of the MCF-7 cells, in comparison to the nHAp and EA extract (Fig. 7).

#### qRT-PCR

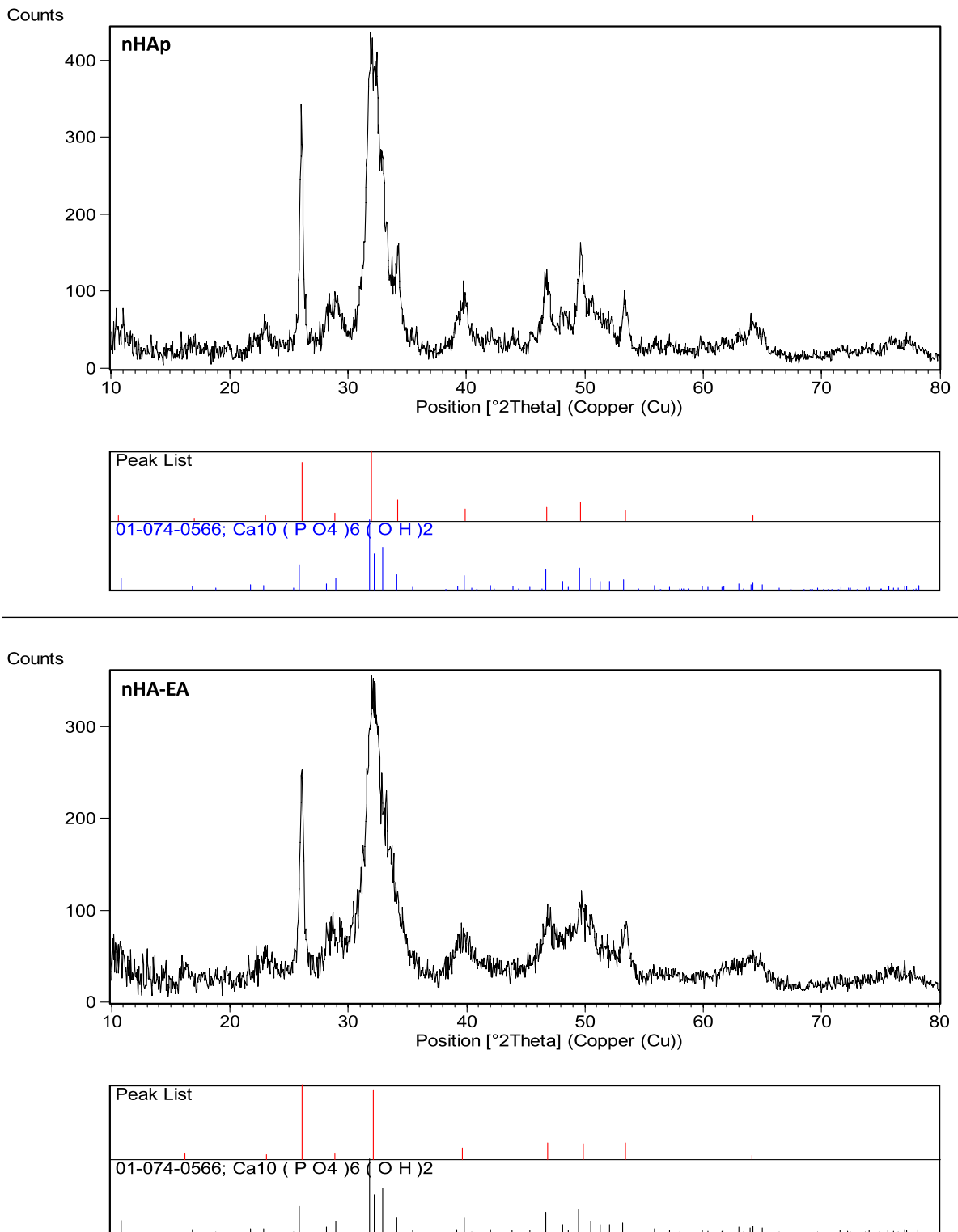
To determine the anti-cancer effects of EA extract, nHAp, and a combination of EA extract and nHAp in the MCF-7 cells, we evaluated the expression level of Bax/Bcl2 and p53 as key markers in the carcinogenesis process. Our results revealed that the level of Bax/Bcl2 and p53 are significantly higher in the nHA-EA extract group in comparison to the EA extract and nHAp alone (Fig. 8).

#### Discussion

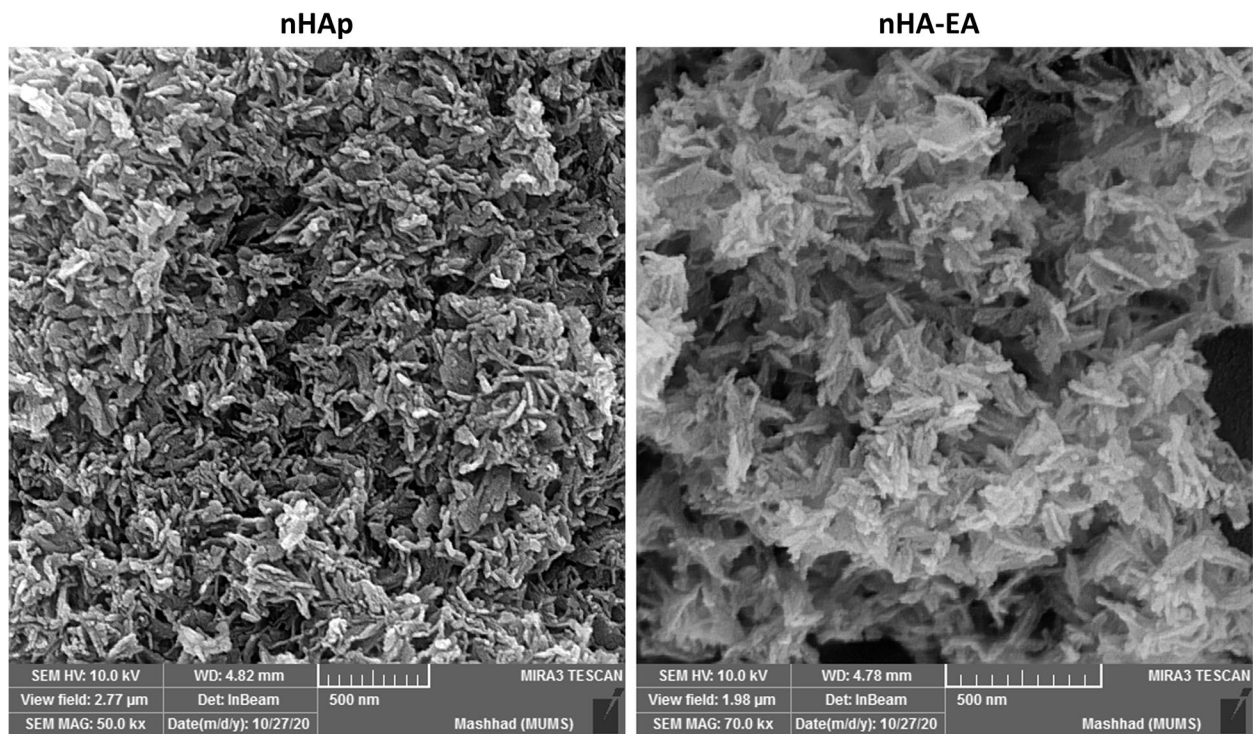
In the past few years, there have been reports of nanoscale HAp that exhibit superior biocompatibility compared to regular HAp. Furthermore, these nanoscale HAp particles have shown anti-cancer properties and play a crucial role in controlling the behavior of breast

cancer cells [35]. There are multiple methods for synthesizing nHAp, such as co-precipitation, hydrothermal, sol-gel, and others. Additionally, the utilization of metal nanoparticles extracted from plants is considered environmentally friendly. As a result, the use of natural materials in the production of nHAp has captured the interest of numerous researchers, contributing to the development of a significant field of study within the realm of nanotechnology science [29].

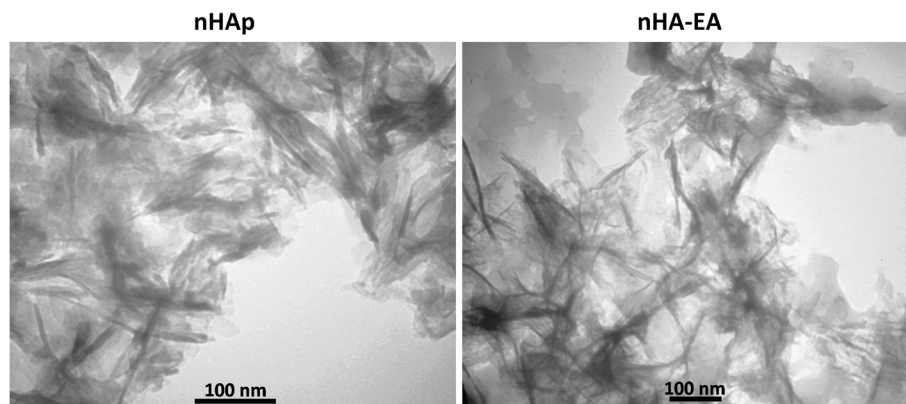
A wide range of natural plant-derived products and extracts possess anti-tumoral properties, presenting potential value in the creation and advancement of novel anti-cancer nanoparticles. There are growing body of evidences showing that natural products have an important role in treatment of wide variety of human disease, including types of cancer [30, 36]. EA, as an herbal medicine plant with different properties, have been used extensively for a long time to treat different disorders [13, 33]. There are various bioactive constituent in EA, including phenolic acids and flavonoids, which exerts a critical role in cancer development and progression



**Fig. 3** XRD patterns of nHA and nHAEA



**Fig. 4** SEM images of nHAp and nHA-EA

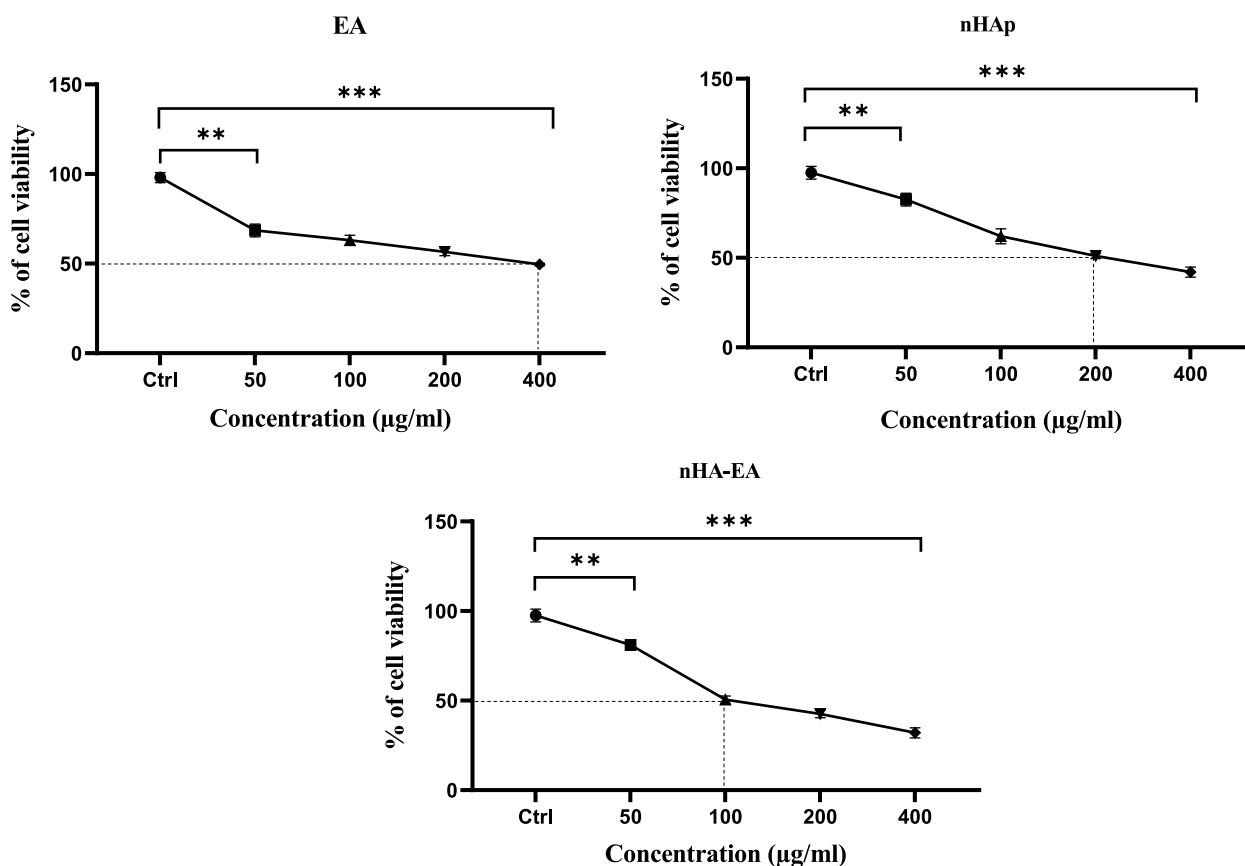


**Fig. 5** TEM images of nHAp and nHA-EA

inhibition [13, 27]. It has been shown that bioactive compounds of EA could modulate different biological processes of cells including cell cycle progression, apoptosis and DNA repair [28, 37]. In recent years, EA has received considerable attention for cancer therapy because of its promising effectiveness and potential therapeutic effects as chemo preventive and antitumor natural product. It has been shown that flavonoids with different signaling pathways which are correlated with carcinogenesis, including cellular proliferation, apoptosis, angiogenesis,

and metastasis. Moreover, apigenin, as a phytoestrogen aglycone has shown to suppress apoptosis, cell cycle and invasion in malignant cells, alone or in combination with other chemotherapeutic agents [38].

In this study, nHAp was synthesized with EA extract, which could act as a reducing, stabilizing, and capping agent. This synthesis is easy, cheap, durable, and suitable for largescale processing. To determine the anticancer effects of nHA-EA in MCF-7 cells were treated with nHA-EA.



**Fig. 6** MTT assay images show cytotoxic effects and IC<sub>50</sub> values of EA, nHAp and nHA-EA

According to result of this study, nHA-EA had more growth inhibitory properties on MCF-7 breast cancer cell line than other groups. Our results revealed that, nHA-EA decreased the viability and proliferation of MCF-7 cells and significantly increased the expression level of p53 and Bax/Bcl2 genes.

Flavonoids have been demonstrated to increase the expression of p53 and induce cell cycle arrest specifically in the G2/M phase in cancer cells. Additionally, they are recognized for their ability to inhibit the expression of Ras proteins and modulate heat-shock proteins in different types of cancers, particularly in leukemia and colorectal cancer.

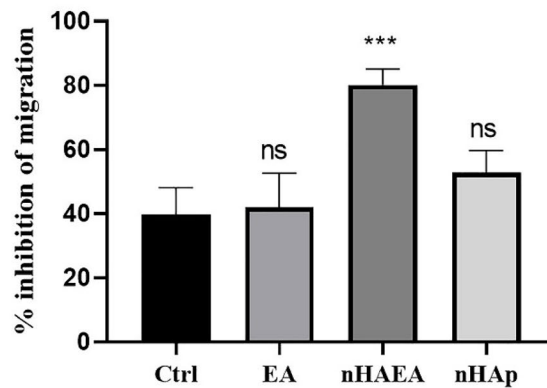
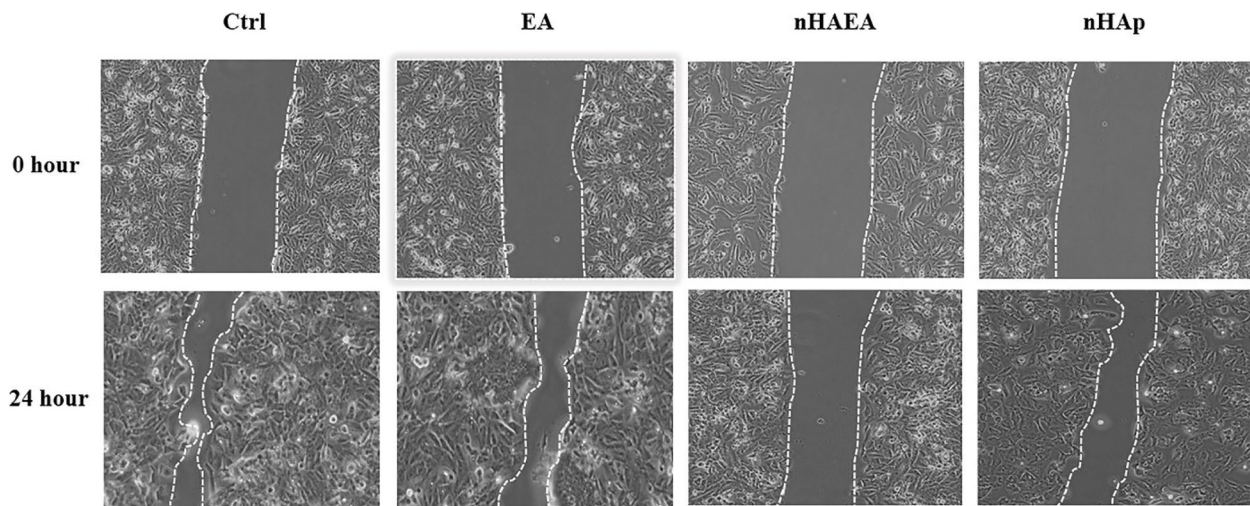
The flavonoids in the EA extract surround the nHAp like a cap. As a result, they may increase the anti-cancerous properties of nHAp. Quercetin, a prominent flavonoid found in EA, is a significant anti-proliferative compound. In addition, it plays a role in enhancing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by increasing the expression of Bax and suppressing the activity of Bcl2 protein [39].

These results are in accordance with previous studies showing the suppressive effects of EA on different type of cancer including glioblastoma and breast cancer [38, 40]. Although, the results of the current study are not consistent with other investigation, showing that EA extract did not exerted any significant antiproliferative activity against cancerous cells, which may be due to the different concentration of extract and its characteristics [41].

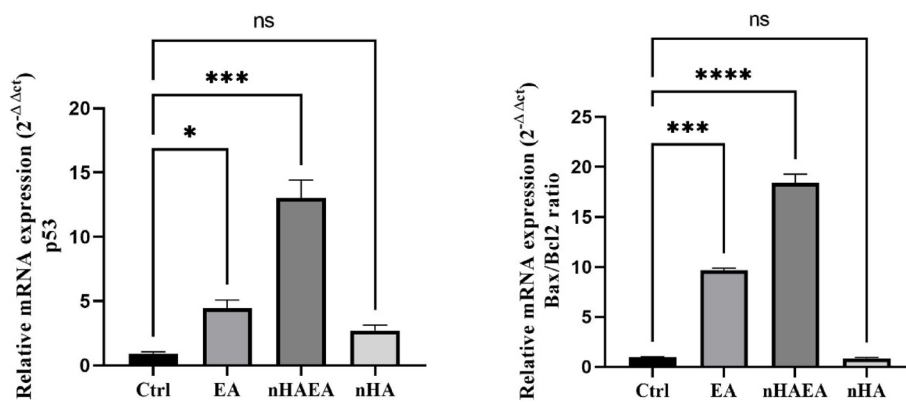
Uncontrolled cell division and growth and escape from apoptosis are well-known hallmarks of malignant cells and important mechanism involved in cancer treatment resistance; therefore, targeting specific cancer deregulated pathways could be an important way for providing novel therapeutics strategies [42, 43].

Moreover, we revealed that the ethanolic extract of EA could decrease migration of MCF-7 cells and induces apoptosis in this cell line. Our results are in agreement with other studies that confirmed EA exerts its anti-tumor impacts via upregulation of p53 and Bax/Bcl2.





**Fig. 7** The effects of EA, nHAp and nHA-EA on the migration of the MCF-7 cells after 24 h



**Fig. 8** EA, nHAp and nHA-EA triggered MCF-7 cells apoptosis genes as showed by qRT-PCR

The observed anti-proliferative effects of EA extract on breast cancer cells in our study could mediate via increasing the expression of p53 as a tumor suppressor gene. It has been shown that bioactive constituents

of in EA, including flavonoids, triterpenoid, lignanoid, benzenoid, pro-anthocyanosides, polysaccharides and phenolic acids, plays an important role in preventing cancer development and progression. Investigations

revealed that these constituents could regulate various biological pathways including apoptosis, DNA repair, inflammatory processes and cell cycle progression [13, 33, 44]. p53 regulates the expression of Bcl2, a proapoptotic factor, in which triggers apoptosis process [45]. Moreover, there are studies showing that EA extract induces apoptosis via different mechanisms including, human epidermal growth factor receptor 2 gene (HER2) and Jun N-terminal kinases (JNK) inhibition [39]. Thus, these results support the role for EA extract in the Bcl2-associated intrinsic pathway of apoptosis via p53 upregulation.

It is well known that invasion and migration, which are key underlying mechanisms of metastasis plays an important role in breast cancer disease. Metastasis is a process in which malignant cells leaving primary tissue, disseminate, circulate and induces secondary tumors in distant sites [46].

In the present study, analysis of scratch assay revealed that, nHA-EA significantly inhibited migration and invasion in MCF-7 cell line. The anti-migratory and anti-metastatic potential of EA herbal plant has been confirmed in a study conducted by Jabeen et.al. showing that, EA extract inhibited the invasiveness of HER2-positive breast cancer cell lines. They showed that, this effect is associated with JNK signaling suppression and mesenchymal-epithelial transition (MET) inhibition. They also reported that suppression of JNK signaling pathway is associated with induction of apoptosis process [39]. In another study conducted by Saleh et.al, it has been found that EA extract suppresses the invasiveness and migration of oral carcinoma cell lines. They reported that this extract inhibits cell invasion via increasing the amplification of E-cadherin, an important modulator of MET process. The also indicated that this reduction in migratory properties of oral carcinoma cell lines is mediated via inhibition of extracellular-signal-regulated kinases 1 and 2 (ERK1/ERK2) pathway [47].

## Conclusion

Taken together, our results demonstrated that bioactive constituents of EA medicinal plant in form of nHA-EA particles, can effectively exerts potential anticancer and chemo preventive effect against breast cancer growth and can be proposed as a promising beneficial candidate for BC therapy. Moreover, we showed that nHA-EA triggers cell apoptosis in MCF-7 cells via increasing proapoptotic gene. Additionally, our findings indicate the role of nHA-EA as a natural product in targeting p53 as a key marker in BC treatment. However, further investigations are required to discover what bioactive compounds are responsible for the chemo preventive effect of this extract.

## Abbreviations

EA	Elaeagnus Angustifolia
HEAE	Hydroalcoholic EA extract
nHAp	Nano hydroxyapatite
nHA-EA	nHAp synthesized trough EA
FTIR	Fourier-transform infrared
XRD	X-ray diffraction
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
BC	Breast cancer
NPs	Nanoparticles
Ca (NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	Calcium nitrate tetrahydrate
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	Diammonium hydrogen phosphate
MTT	Dimethylthiazoldiphenyltetrazolium
cDNA	Converted to complementary DNA
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
ANOVA	One-way analysis of variance
DMEM	Dulbecco's Modified Eagle's Medium
qRT-PCR	quantitative reverse-transcriptase polymerase-chain-reaction
DMSO	Dimethylsulfoxide
FBS	Fetal bovine serum
MET	Mesenchymal-epithelial transition
HER2	Human epidermal growth factor receptor 2 gene
JNK	Jun N-terminal kinases
ERK1/ERK2	Extracellular-signal-regulated kinases 1 and 2
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
IC50	Half maximal inhibitory concentration

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## Authors' contributions

SK, EA contributed to research planning, case examination, and editing the manuscript. MMB contributed to examination and writing. AZ and MN contributed to statistical analysis, research planning, collect and enter data.

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## Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Experimental research and field studies on plants including the collection of plant material are complying with relevant guidelines and regulation for plant ethics.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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