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Investigating the Role of Acacia Nilotica Nanoparticles on Promoting Apoptosis in Human Breast Cancer Cell Line (MDA-MB-231)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

MDA-MB-231 is a model of a human breast cancer cell line. It represents a suitable cell line for breast cancer research worldwide, including anti-cancer studies. Natural products are rich in phytochemicals that have anti-cancer, antioxidant and anti-inflammatory effects. The aim of this study was to characterize the Acacia nilotica nanoparticles (AN-NPs) from the extract of Acacia nilotica (AN) using transmission electron microscopy (TEM), zeta sizer, X-ray diffraction (XRD) and Fourier transform infrared (FT-IR). Cytotoxic activity was assessed using the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The morphological changes of the cells were examined using an inverted microscope. The results showed that at serial concentrations (5, 10, 20, 50 and 70 µg/ml) of AN extract and AN-NPs, a cytotoxic effect and

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morphological degeneration and damage of the cells were observed. The effect varied depending on the exposure time and AN extract and/or AN-NP concentration on MDA-MB-231. The results showed cytotoxic effects, morphological degeneration, damage and more efficacy against breast cancer cells. We can conclude that AN extract and AN-NP are an effective choice for the development of pharmacological treatments against cancer.

Keywords: Acacia nilotica; breast cancer; inflammation; MDA-MB-231; nanoparticles.

HIGHLIGHTS

- MDA-MB-231 cells line is highly invasive and metastatic model in vitro breast cancer.
- AN-NPs stimulate apoptosis in human breast cancer cell line MDA-MB-231.
- Flavonoid and phenolic glycosides may be potential therapeutic target to MDA-MB-231.

1. INTRODUCTION

The second major cause of death is cancer. Cells that can no longer eliminate themselves proliferate uncontrolled causing cancer. When a gene that regulates growth is altered in a cell, cancer develops [1]. Chemotherapy is one type of cancer-treating that acts to suppress or slow the growth of cancer cells which grow and divide rapidly. But the healthy cells, which have rapidly divided, such as the mouth line, intestines, and hair growth cells, can be harmed by this way of therapy. Furthermore, this way may cause side effects on healthy cells. After the chemotherapy is over, most of the side effects disappear [2].

MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line [3].

Numerous medicinal plants act as anticancer herbs in various organs' experimental and/or clinical cancers/ tumors. Some of those cancers are leukemia, sarcoma, lymphoma, and carcinoma [4]. Acacia nilotica, also known as the gum Arabic tree, belongs to the Fabaceae family. It is popular in Africa and Asia and the tree has roots, pods, stems, and leaves. The extract has many phytochemical components as flavonoids, phenols, and tannins. An inspiring range of medicinal uses with potential antioxidant activity is found in Acacia nilotica (AN). AN act as an anti-cancer, antimutagenic, spasmogenic, vasoconstrictor, antipyretic, anti-asthmatic, cytotoxic, anti-diabetic, antiplatelet aggregator, and anti-plasmodial. Additionally has inhibitory activity against Hepatitis C virus (HCV) and human immunodeficiency virus (HIV)-I and antioxidant activities, in addition to being employed in the treatment of various ailments in the indigenous medicine system [5].

Cancer diagnosis and treatment pathways have been altered by nanotechnology. In cancer treatment, the favored size of nanoparticles (NPs) is (1–100 nm). Using NPs as a drug delivery system has unique characteristics particular to tumors and tumor environments. They can facilitate permeability, reduce toxicity, and target tumors more precisely [6].

A nanoparticle drug delivery system is used to reduce the lack of selectivity of anticancer drugs. Most cancer chemotherapeutics are administered either orally or intravenously to realize systemic distribution for effective treatment. Though, as a reason for the lack of selectivity, these drugs cause significant damage to rapidly proliferating normal cells [7]. Therefore, in the present study, we aimed to characterize the Acacia nilotica Nanoparticles (AN-NPs) from the Acacia nilotica (AN) extract using Transmission Electron Microscopy (TEM), Zeta sizer, X-ray Diffraction (XRD), and Fourier Transform Infrared (FT-IR) techniques. In addition to investigating the effect of AN-NPs and AN extract on the proliferation and promoting apoptosis of breast cancer cells using the MDA-MB-231 cell line.

2. MATERIALS AND METHODS

2.1 Plant Material

Acacia nilotica pods (fruit) purchased from local markets in Khartoum city, Sudan. Aerial parts of AN were washed with distilled water and let for 48hr to entirely dry. Aerial parts (Pods) of AN were ground into soft powder, to make them suitable for preparing the nanoparticles.

2.2 Synthesis of Nanoparticles using the Peel of Acacia nilotica

100 mg of AN powder was added to 20 ml of absolute Ethanol and poured gradually on 500 ml of boiling distilled water within ultrasonication for 1hr without heat. Then, the flask was transferred to the magnetic stirrer for 1hr to ensure NPs separated. The solution was freeze-dried and collected the NPs powder. The formed AN-NPs have been analyzed by Zeta sizer (ZEN 3600, Malvern, UK) and characterized using transmission electron microscopy (TEM) (JEM-1011, JEOL, Akishima, Japan). Furthermore, the AN- NPs synthesis was confirmed by UV-V as a spectrophotometer in the range of 200-1000 nm wavelength. The absorption spectra were recorded with Perkin-Elemer Lambda 40 B double-beam spectrophotometer using 1 cm matched quartz cells [8].

2.3 Cell Culture Techniques

All chemical products were obtained from Gibco company. Breast cancer cell line MDA-MB-231 was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were prepared to begin the experiment by thawing the cryopreserved MDA-MB-231 cell line under aseptic conditions. Inside the safety cabinet, the contents were transferred into a 15 ml centrifuge tube, and 5 ml pre-warmed media was added to avoid cell shock and mixed gently. The cells were centrifuged at 1600 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended by 3 ml of media, then transfer into a T25 flask. The cells were checked under an inverted microscope and incubated at 37°C in a 5% CO^2 incubator for 24hr. Cells were passaged upon reaching >70% confluence (approximately two to three times per week).

2.4 Automated Cell Counting

Under an inverted microscope, the cell morphology and confluency were checked. The media of the T25 flask was discarded and washed the cell's surface with 1 ml PBS. 1 ml of trypsin-EDTA was added and incubated at 37°C in a 5% CO2 incubator for 3 to 5 min to ensure the cells have dispatched. 2ml of DMEM was added to the flask, pipetting up and down to distribute the content. The content was transferred to a centrifuge tube for 5min and at 1500 r.p.m. The supernatant was discarded, and the pellet was resuspended with 10% DMEM. Place 10 µl of the cells in an Eppendorf tube, then add to10µl of trypan blue and, mix well. On a hemocytometer, the cells were loaded and then counted.

2.5 Experiment Design

This experiment used AN as crude and NPs on MDA-MB-231 as therapeutic agents. The MDA-MB-231 cells were cultured in 96-well plates at a density of 5x104 cells/ml for each well with 10% DMEM, and incubated at 37°C in a 5% CO2 incubator for 24 hr. On the day of treatment, the confluences of cells were checked (70%-80%). Then cells were washed with 200µl PBS. Different concentrations of AN extract and AN-NPs were added to the cells in a 96-well plate as the following concentrations (0, 5,10, 20, 50, 70) which were prepared from stock concentration. Then one plate was incubated for 24 hr and the other one was incubated for 48 hr.The stock was prepared by dissolving 10mg of AN or AN-NPs into 700µl DMSO with 1300 µl of DMEM to compose plant final concentration 5mg/ml and both (AN and NPs) distributed into the wells as follows:

Plate 1 (24hrs dose):

Group 1- First triple wells seeded with MDA-MB-231 cells without treatment dose (control).
Group 2 - Second triple wells (5 µg/ml conc.)
Group 3 - Third triple wells (10 µg/ml conc.)
Group 4 - Fourth triple wells (20 µg/ml conc.)
Group 5 - Fifth triple wells (50 µg/ml conc.)
Group 6 - Sixth triple wells (70 µg/ml conc.)

Plate 2 (48hrs dose):

Group 1- First triple wells seeded with MDA-MB-231 cells without treatment dose (control).
Group 2 - Second triple wells (5 µg/ml conc.)
Group 3 - Third triple wells (10 µg/ml conc.)
Group 4 - Fourth triple wells (20 µg/ml conc.)
Group 5 - Fifth triple wells (50 µg/ml conc.)
Group 6 - Sixth triple wells (70 µg/ml conc.)

2.6 MTT Assay

MTT assay is a colorimetric assay that relies on the reduction of a yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to purple formazan crystals by metabolically active cells. It is used to measure cell viability, proliferation, and cytotoxicity by measuring cellular metabolic activity [9]. Following culture and treatment as outlined above, 20µl/ml of MTT solution was distributed to each well in the two plates, this step was applied in the dark area. Then incubated for 2 to 4hr, the plate content was discarded and, 100µl of

Isopropanol-HCL for each well. The plate was then placed on the shaker for 10min. Finally, the absorbance was measured at 560 nm by a microplate reader (Biotek, ELX 800, USA).

2.7 Statistical Analysis

Statistical analyses were performed using GraphPad Prism, Analysis of variance (ANOVA) with Dunnett's tests was used to analyze statistically significant differences. The significance level was defined as ($p < 0.05$) for all tests.

3. RESULTS

The biosynthesis of NPs using microorganisms, or plant extracts has emerged as an alternative approach. There are several reasons for the interest in biosynthetic methods for NPs. They are simple, inexpensive in comparison to their effectiveness, safe, and environmentally friendly.

The study revealed the determined average particle size of nanoparticles by dynamic light scattering ZetaSizer (Malvern instrument). It is a technique used for determining the size and distribution profile of Acacia Nilotica. As shown in (Fig. 1), the NPs size is 151.5 d. nm, this result approves with homogenous distribution and variety size with no agglomerate of resulting nanoparticles, which was clearly observed from the appearance of one peak.

Furthermore, AN-NPs were imaged using TEM-JEM 1400, As shown in (Fig.2) the TEM image revealed that the majority of AN-NPs were clearly spherical in morphological shape, with sizes ranging from 63.7 to 33.9 nm.

FT-IR spectra of various AN extracts and AN-NPs were obtained between 4000 and 400 cm−1 with a resolution of 3753.36 cm−1. A scan was recorded using a PerkinElmer FT-IR spectrophotometer. According to the FTIR result shown in Fig. 3, the extract exhibited absorption bands, that can be used to define the composition of the functional group in the NPs generated from it (cm^{-1}). The O-H stretching vibration was attributed to peaks at 3753.36, 3753.93 cm^{-1}. Furthermore, the amine group (NH) stretching was recognized to peaks at 3400.94 and 3372.14 cm^{-1}. The stretching vibration of methylene group (CH2) was seen at 2926.98 and 2926.86 cm^{-1}. C=O stretching was identifiable by the prominent peak at 1704.84–1706.87 cm^{-1}. The C=C stretching was recognized to peaks between 1380.01 and 1636.02 cm^{-1}. The C=C stretching peak at 1345.95 and 1324.43 cm^{-1}. The C-F group was identified as the source of the peak at 1036.51– 161 1031.66cm^{-1}. C-Br was recognized at 759.88–585.05 and 757.90-618.8 cm^{-1}.

X-ray diffraction was utilized to characterize the crystalline structure of the dry powder of natural nanoparticles and crude AN sample. From 0° to 100°, the diffraction intensities were observed at two angles, at 2h of 24°, and 38°, which assigned for the Bragg reflections of (1400) and (800) (shown in Fig.4A). Figure 4B shows that there were two diffraction peaks, at 2h of 15, and 31, which were assigned for the Bragg reflections of (1000) and (950). Thus, AN and AN-NP powdered exhibit sharp peaks with X-ray diffraction, which indicates that AN and AN-NP are crystalline in nature.

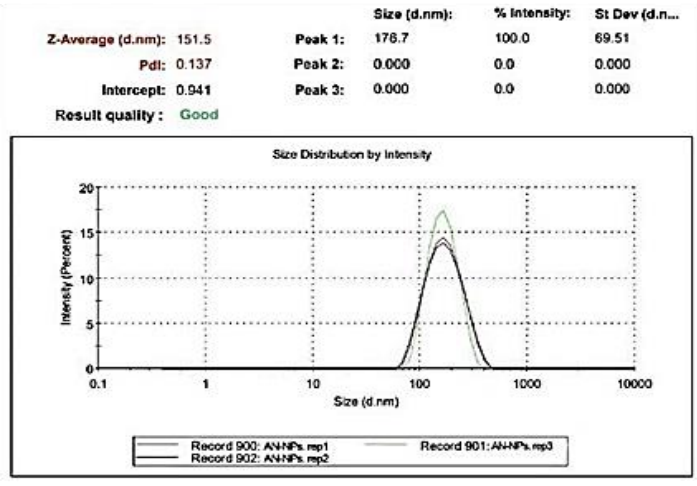
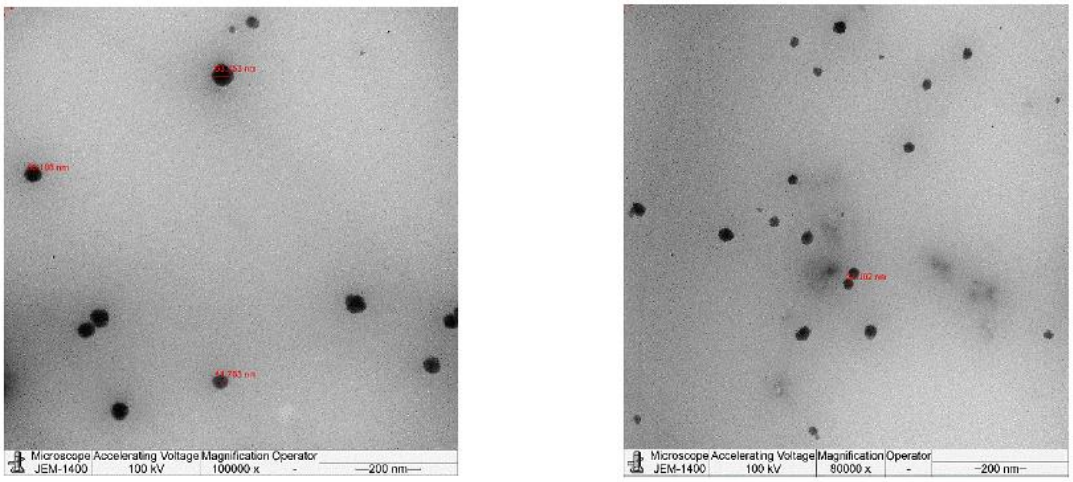


Fig. 1. Presents a Zeta sizer graph which was measure the average size of AN-NPs



(a) **(b)**

Fig. 2. TEM image of AN-NPs (scale bar:200nm)

3.1 Determination of Morphological Changes in Cells

The cell morphological alterations were observed with an EVOS fl Fluorescence Microscope from AMG. The AN extract showed antiproliferative activity in breast cancer cells. Therefore, it was investigated the ability of cytotoxic effects of AN-NPs on inducing apoptosis in MDA-MB-231 cells. As shown in (Fig.5A- 5B), the untreated, MDA-MB-231 cells exhibited normal morphological features with epithelial-like morphology that is very similar to mammary epithelial morphology with a normal rate of proliferation.

In contrast, exposure to AN or AN-NPs produced typical apoptotic features in a dose-dependent way. The morphological alterations varied dependent on the concentration and time of the treatment. As shown in (Fig. 5C-5D), the MDA-MB-231 cell that was treated with 5µg/ml for 24hrs presented slight morphological changes that begin to occur compared to the control group with healthy cells, after 48hr, as shown in (Fig. 7E- 7F) cells, begin to clump together with greater effect in AN-NPs.

As shown in (Fig. 6C-6D) presents MDA-MB-231cells after 24hr exposure of 10 µg/ml, a change in cell volume and shape compared to a control group with healthy cells, as well as after 48hr dead cells started to spread.

Table 1. Shows the functional groups of AN extract using PerkinElmer FT-IR spectrophotometer

S.NO	Peak Values cm^{-1}	Possible Functional groups	Possible compounds	Intensity
1	586.57	C-I	Alkyl	Strong
2	618.81	C-I	Alkyl	Strong
3	757.90	C-Cl	Alkyl	Strong
4	822.55	C-Cl	Alkyl	Strong
5	870.86	C-Cl	Alkyl	Strong
6	1031.66	C-Cl	Alkyl	Strong
7	1080.30	C-F	Ester	Strong
8	1201.38	C-C	Alkyl	Strong
9	1324.43	C-C	Alkyl	Strong
10	1448.20	C-C	Cyclopentadienyls	medium
11	1525.20	CH2	Amid II	Strong
12	1615.47	CH2	Vinyl Ketone	Strong
13	1706.87	C=O	Alkynes	medium
14	2926.86	C-H	Ketones	Weak
15	3372.14	OH	Alcohol	Weak
16	3753.93	OH	Alcohol	Weak

Table 2. Shows the functional groups of AN-NPs using PerkinElmer FT-IR spectrophotometer

S.NO	Peak Values cm^{-1}	Possible Functional groups	Possible compounds	Intensity
1	585.05	C-I	Alkyl	Strong
2	759.88	C-Cl	Alkyl	Strong
3	874.90	C-Cl	Alkyl	Strong
4	1036.51	C-Cl	Alkyl	Strong
5	1204.72	C-C	Alkyl	Strong
6	1345.95	C-C	Alkyl	Strong
7	1447.27	C-C	Alkyl	medium
8	1524.21	CH2	Amid II	Strong
9	1617.08	CH2	Aryl (**phenyl)**	medium
10	1704.84	C=O	B-diketones	Strong
11	1873.73	C-O	Alkynes	Strong
12	2126.57	C-O	Alkynes	Weak
13	2342.62	C≡O	Acetals	Weak
14	2926.98	C-H	Ketones	Weak
15	3400.94	OH	Alcohol	Weak
16	3753.36	OH	Alcohol	Weak

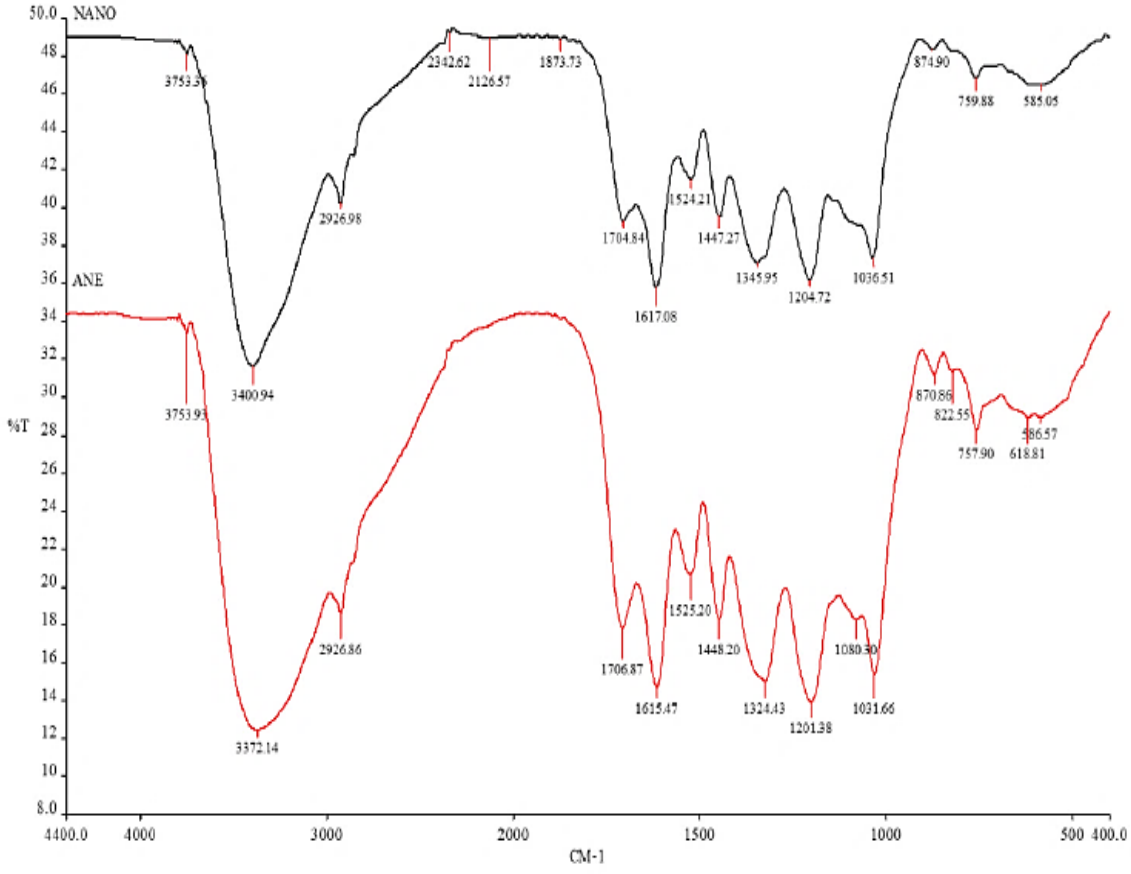
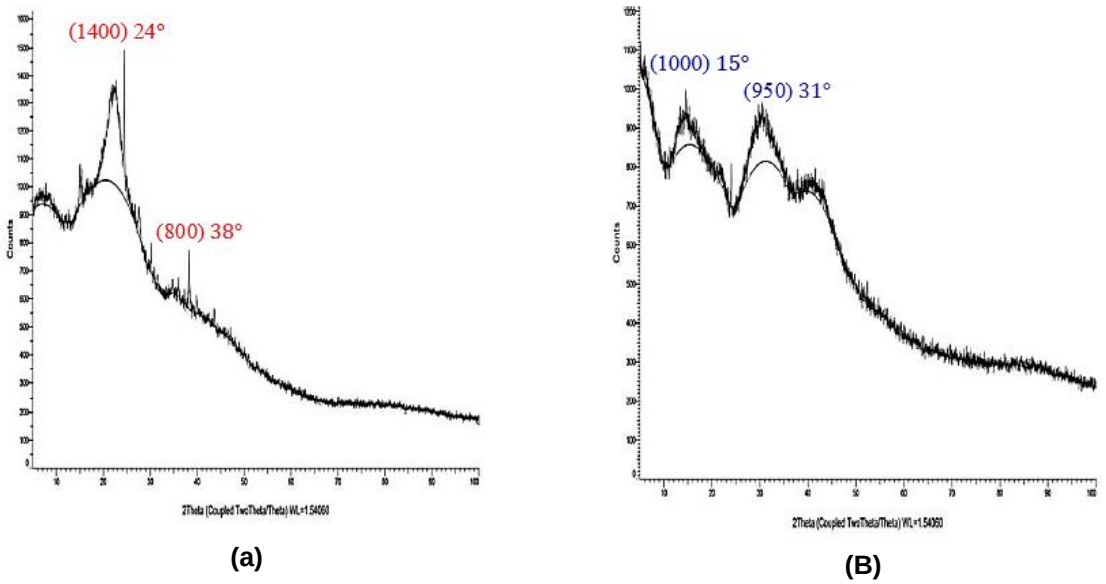


Fig. 3. FTIR spectrum analysis of phytochemicals in the plant extracts which shows a trace of AN-NPs and AN extract



(a) (B)

Fig. 4. XRD spectra analysis of (A) Acacia Nilotica extract and (B) Acacia Nilotica nanoparticles

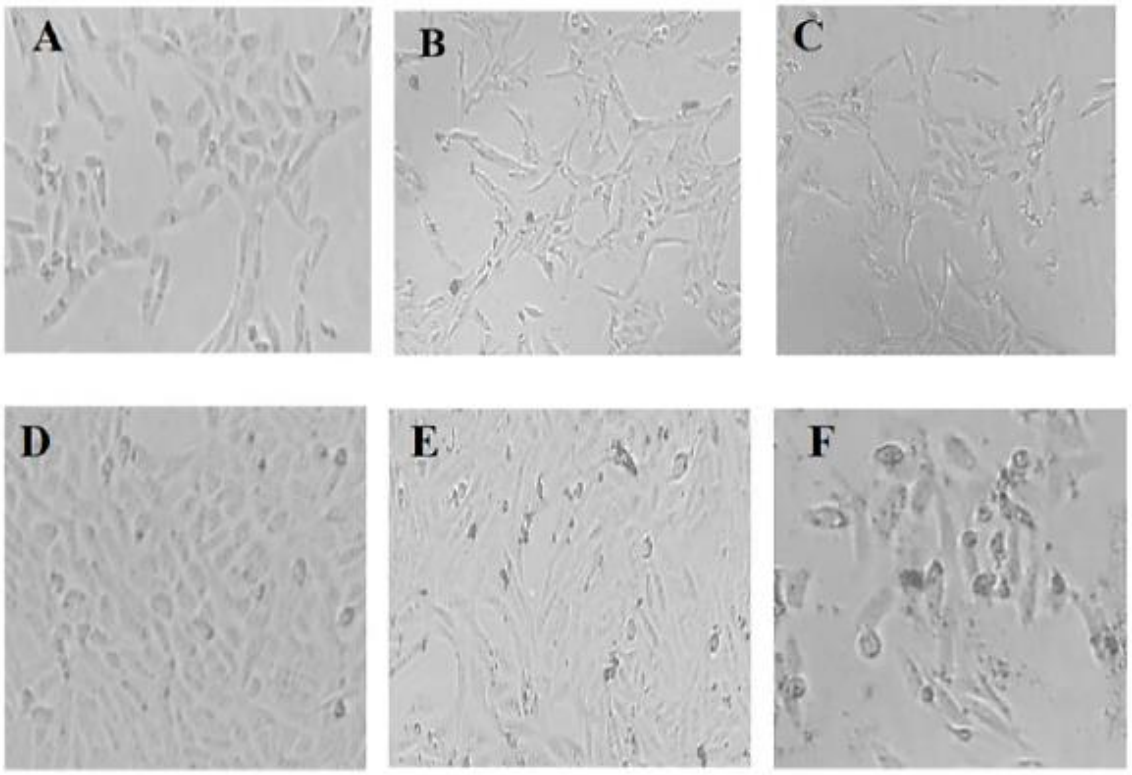


Fig. 5. Shows the morphological changes of MDA-MB-231 cells treated with 5µg/ml of AN crude and AN- NPs after 24 and 48h of treatment. (A) 24hr control group. (B) Cells were treated with 5µg/ml of AN crude s for 24hr. (C) Cells were treated with 5µg/ml of AN-NPs for 24hr. (D) 48hr control group. (E) Cells were treated with 5µg/ml of AN crude for 48hr. (F) Cells were treated with 5µg/ml of AN-NPs for 48hr

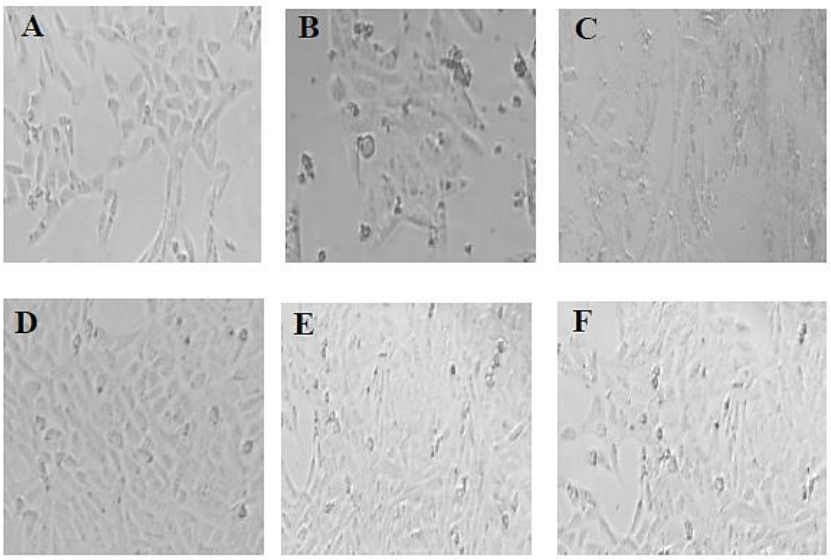


Fig. 6. Shows the morphological changes of MDA-MB-231 cells treated with 10µg/ml of AN crude and AN- NPs after 24 and 48h of treatment. (A) 24hr control group. (B) Cells were treated with 10µg/ml of AN crude s for 24hr. (C) Cells were treated with 10µg/ml of AN-NPs for 24hr. (D) 48hr control group. (E) Cells were treated with 10µg/ml of AN crude for 48hr. (F) Cells were treated with 10µg/ml of AN-NPs for 48hr

The (Fig.7C-7D) shows MDA-MB-231 cells after 24hrs exposure of 20 µg/ml, small colonies formed and gaps between cells compared to a control group with healthy cells, however after 48hr a clear reduction of cell volume and cell shrinkage were observed. When treated MDA-MB-231 with 50 and 70 µg/mL of the AN and AN-NPs, most of the cells died, shrinkage, lost their typical morphology, and appeared smaller and rounded. However, after 48hr exposure, the apoptotic fragments were massively spread.

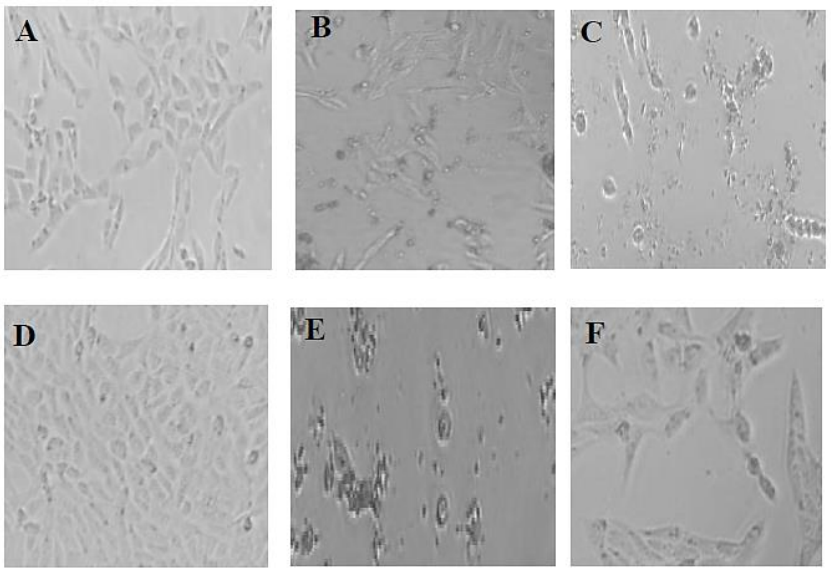


Fig. 7. Shows the morphological changes of MDA-MB-231 cells treated with 20µg/ml of AN crude and AN- NPs after 24 and 48h of treatment. (A) 24hr control group. (B) Cells were treated with 20µg/ml of AN crude s for 24hr. (C) Cells were treated with 20µg/ml of AN-NPs for 24hr. (D) 48hr control group. (E) Cells were treated with 20µg/ml of AN crude for 48hr. (F) Cells were treated with 20µg/ml of AN-NPs for 48hr

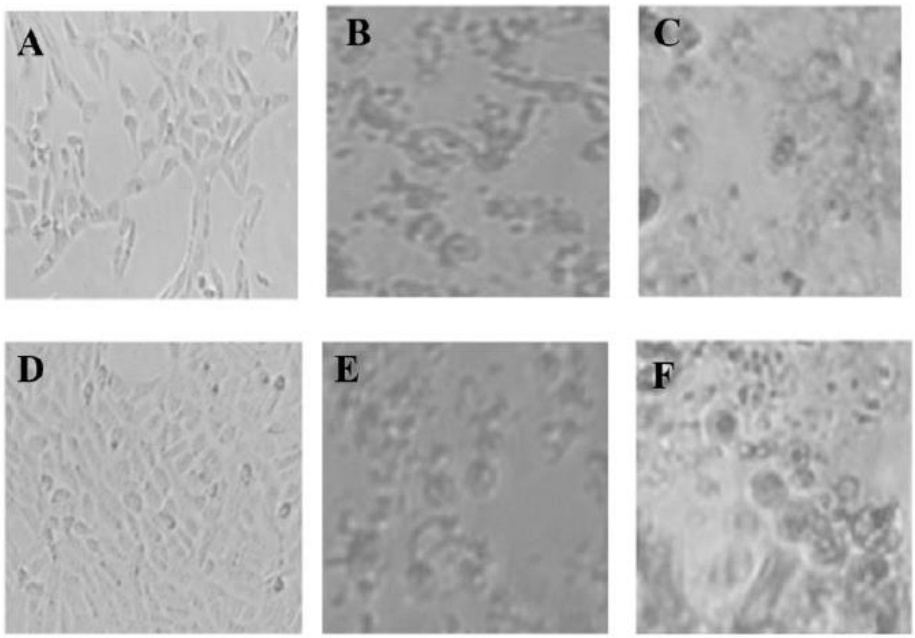


Fig. 8. Shows the morphological changes of MDA-MB-231 cells treated with 50µg/ml of AN crude and AN- NPs after 24 and 48h of treatment. (A) 24hr control group. (B) Cells were treated with 50µg/ml of AN crude s for 24hr. (C) Cells were treated with 50µg/ml of AN-NPs for 24hr. (D) 48hr control group. (E) Cells were treated with 50µg/ml of AN crude for 48hr. (F) Cells were treated with 50µg/ml of AN-NPs for 48hr

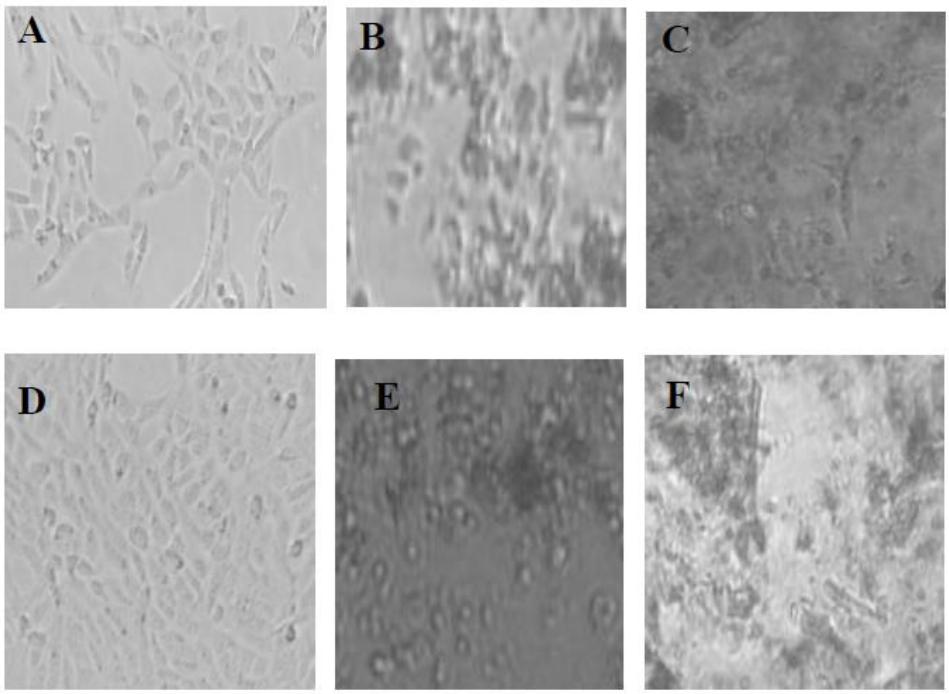


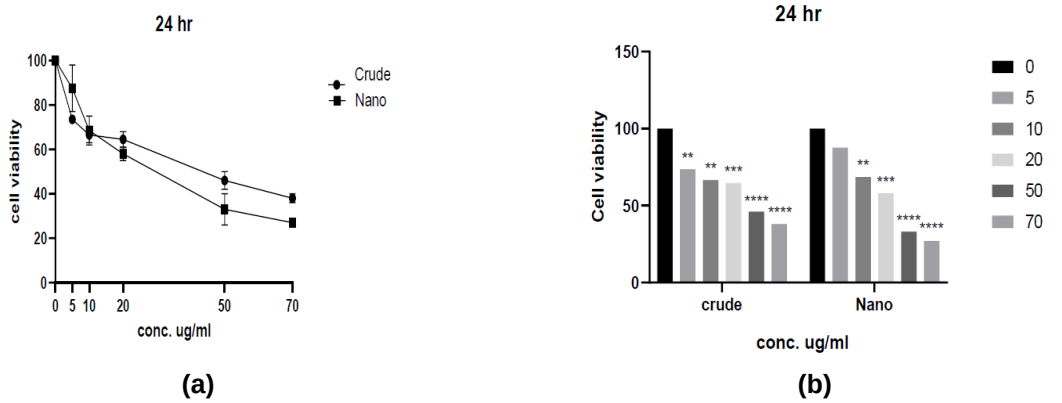
Fig. 9. Shows the morphological changes of MDA-MB-231 cells treated with 70µg/ml of AN crude and AN- NPs after 24 and 48h of treatment. (A) 24hr control group. (B) Cells were treated with 70µg/ml of AN crude s for 24hr. (C) Cells were treated with 70µg/ml of AN-NPs for 24hr. (D) 48hr control group. (E) Cells were treated with 70µg/ml of AN crude for 48hr. (F) Cells were treated with 70µg/ml of AN-NPs for 48hr

3.2 Cytotoxicity Assessment of AN and AN-NPs on MDA-MB-231 Cell Line

The cytotoxic effects of the AN and AN-NPs on the viability of the MDA-MB-231 cells are presented as percent cell viability in (Fig.10-11). MDA-MB-231 cells were treated with different concentrations of AN extract and AN-NPs at 0, 5, 10, 20, 50, and 70 µg/mL for 24h and 48h. The cytotoxicity was determined using the MTT reduction assay. All values were presented as the mean of three independent experiments.

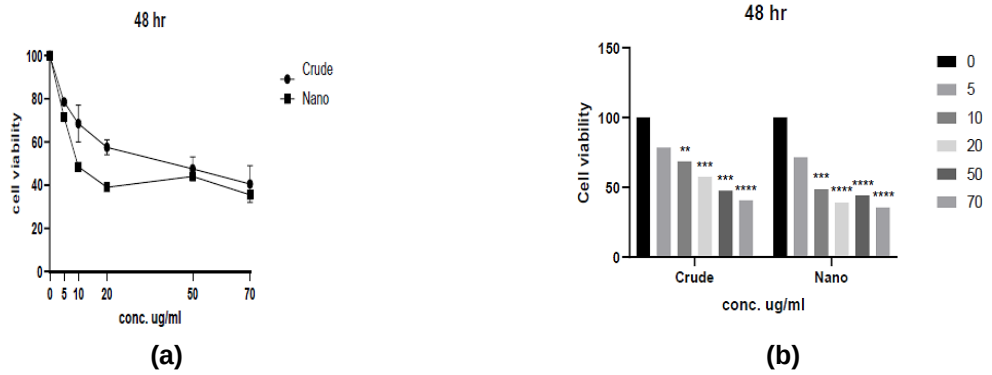
For the 24hr incubation period, the MTT results have shown that as the concentration of AN or AN- NPs increased, the cytotoxicity was observed in dose-dependent way. Two-way ANOVAs of the data in (Table.3) revealed that AN or AN-NPs concentration is significant source of variation (P<0.05). Dunnett's tests comparing responses of cells with AN or AN-NPs to control measures revealed statistically significant responses (P<0.05) with all examined concentrations except 5 µg/ml AN-NPs. However, the effect on suppressing the proliferation of the MDA-MB-231 cells was highly significant on cells treated with 50 and 70 µg/ml. Furthermore, the cell viability was more suppressed by AN-NPs (25% to 38% reduction) which means higher efficiency of AN-NPs to suppress the MDA-MB-231 cells than AN crude.

As for the data in (Table.4) 48hr incubation period, Two-way ANOVAs revealed that both AN or AN- NPs concentration and time are significant sources of variation (P<0.05). Dunnett's tests comparing responses of cells with AN or AN-NPs to control measures revealed statistically significant responses (P<0.05) with all examined concentrations except 5 µg/ml in both treatment ways. Nonetheless, the effect on suppressing the proliferation of the MDA-MB-231 cells was highly significant on cells treated with 10, 20, 50 and 70 µg/ml. Besides, the suppression effect was statically higher by AN-NPs compared with AN crude (18% to 46% reduction) which means higher efficiency of AN-NPs to suppress the MDA-MB-231 cells.



(a) (b)

Fig. 10. Presents the comparison of cell viability among different concentrations of AN crude and AN-NPs in MDA-MB-231 cells after treatment for 24 hours



(a) (b)

Fig. 11. Presents the comparison of cell viability among different concentrations of AN crude and AN-NPs in MDA-MB-231 cells after treated for 48 hours

Table 3. Shows the statistical response of MDA cells to a different concentration of AN crude and AN-NPs for 24hr using Dunnett's multiple comparison test.

Dunnett's multiple comparisons tests	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
crude					
0 vs. 5	26.5	7.576 to 45.42	Yes	**	0.0064
0 vs. 10	33.5	14.58 to 52.42	Yes	**	0.0011
0 vs. 20	35.5	16.58 to 54.42	Yes	***	0.0007
0 vs. 50	54	35.08 to 72.92	Yes	****	<0.0001
0 vs. 70	62	43.08 to 80.92	Yes	****	<0.0001
Nano					
0 vs. 5	12.5	-6.424 to 31.42	No	ns	0.2564
0 vs. 10	31.5	12.58 to 50.42	Yes	**	0.0017
0 vs. 20	42	23.08 to 60.92	Yes	***	0.0002
0 vs. 50	67	48.08 to 85.92	Yes	****	<0.0001
0 vs. 70	73	54.08 to 91.92	Yes	****	<0.0001

Table 4. Shows the statistical response of MDA cells to a different concentration of AN crude and AN-NPs for 48hr using Dunnett's multiple comparison test

Dunnett's multiple comparisons tests	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
Crude					
0 vs. 5	21.5	-1.406 to 44.41	No	ns	0.0682
0 vs. 10	31.5	8.594 to 54.41	Yes	**	0.0073
0 vs. 20	42.5	19.59 to 65.41	Yes	***	0.0007
0 vs. 50	52.5	29.59 to 75.41	Yes	***	0.0001
0 vs. 70	59.5	36.59 to 82.41	Yes	****	<0.0001
Nano					
0 vs. 5	28.5	5.594 to 51.41	Yes	*	0.0143
0 vs. 10	51.5	28.59 to 74.41	Yes	***	0.0001
0 vs. 20	61	38.09 to 83.91	Yes	****	<0.0001
0 vs. 50	56	33.09 to 78.91	Yes	****	<0.0001
0 vs. 70	64.5	41.59 to 87.41	Yes	****	<0.0001

4. DISCUSSION

Breast cancer was recorded as the most widespread cancer worldwide. Cancer occurs by the imbalance of cell proliferation and death [10]. Therefore, it is required to suppress and regulate this disruption through treatment. The plant-based drug which is rich in phytochemical compounds has been used as an anti-cancer treatment due to its safety, low toxicity, and availability [11]. NPs have recently been considered potential medication carriers. Drugs' pharmacokinetic characteristics are altered using nanocarriers to increase their efficacy and reduce their negative effects. Additionally, the capacity for deep tissue penetration contributes to an increase in the treatment's improved permeability [12]. The ability to deliver anticancer agents to targeted tumors, the ability to image tumors, the capacity to store thousands of drug molecules, and the ability to overcome solubility, stability, and resistibility issues are the key benefits of using NPs as anticancer agent carriers [14].

This study, aimed to evaluate the efficiency of NPs using *A. Nilotica* extracts by determining the antiproliferative activity against the human breast cancer cell line MDA 123. Our results confirmed a probable relevance between antioxidant activity and significant cancer cell inhibition with the use of plants in the form of nanoparticles. Various studies on cancer cells (e.g., MCF-7, Hep-2, and Hela cell lines) using plants have shown that the significant potential role of them in cancer [13].

AN-NPs have massive anti-cancer features and characterize by being natural products making them safe and, important in the field of cancer treatment. As shown in TEM results (Fig.2), AN-NPs range from 63.7 to 33.9 nm which is in favored size 1–100 [14]. As reported by Zhao et

al. [11], ex vivo optical imaging studies in mice showed that spherical nanoparticles spend less time in the gastrointestinal tract than rod-shaped nanoparticles. According to the results of Eleazu et al. [15], flavonoid has antioxidant effects on humans, contributing to protection against carcinogenic diseases. According to our FT-IR result (Fig. 3), flavonoid is one of the phytochemicals contained in the plant. This agrees with our result of the functional group test (FT-IR) in Fig.3. Based on the dielectric results of Karlsson et al. [12], antioxidants had little effect on the oxidation of polyethylene in its crystalline region but suppressed oxidation in its amorphous region. However, our study showed that AN-NPs are crystalline (Fig. 6B), demonstrating the ability of crystalline nanoparticles to utilise the antioxidant activity of AN extract to effectively suppress the breast cancer cell line MDA-MB-231.

Morphological changes of the cells are important to determine apoptosis [16]. The cell death induced by AN extract and AN-NPs was investigated on the basis of morphological changes. MDA-MB- 231 cells treated with AN extracts and AN-NPs showed different characteristics of apoptosis, including cell shrinkage, cell rounding and cytoplasmic condensation, as demonstrated by EVOS fl fluorescence microscope. Serial doses of AN extract and AN-NPs (5, 10, 20, 50, 70 g/ml) caused distinct changes in the cells. Starting with a modest change in the shape of the cells at a dose of 5 g/ml, small colonies and gaps at concentrations of 10 and 20 g/ml [17]. At a concentration of 50 g/ml, early phases of apoptosis set in very clearly and there was a clear morphological change. At the highest concentration, 70 g/ml, apoptotic fragmentation was observed. In this study, the MTT assay was used to determine the cytotoxic activity of AN nanoparticles against the MDA-MB-231 cells. The viability of the cells decreases in a dose-dependent manner when the treatment dose is increased. The reduced cell viability is statistically significant.

As shown in (Fig. 10), administration of 70 µg/ml for 24 hours reduced the cell viability of breast cancer cells to about 73%, which is the highest efficiency in suppressing the MDA cell line compared to other concentrations [16]. Sundarraj et al [17] found that the ethanolic extract of Acacia nilotica significantly increased the apoptosis percentage to 46.68% in MCF-7 cells compared to control cells. These results indicate that the extract of Acacia nilotica in the form of nanoparticles has a significant effect in promoting apoptosis in human breast cancer cells (MDA-MB-231).

5. CONCLUSION

In conclusion, it could be stated that the extract of Acacia nilotica as nanoparticles, has a significant role in promoting apoptosis in human breast cancer cells (MDA-MB-231). Therefore, AN extract and AN-NPs can be regarded as effective choices for creating pharmacological treatments against cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition, all protocols, and cell culture handling at the Department of Zoology, Faculty of Science, Helwan University was approved by the Committee on Research Ethics for Laboratory Animal Care (approval 108number: HU/Z/010-19).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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