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RESEARCH ARTICLE

Isolation and Purification of Phytosterols from *Euphorbia Milii* Plant Cultivated in Iraq by PHPLC and Evaluation of Anticancer Activity

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ABSTRACT

Combined, plant sterols and stanols, substances structurally similar to cholesterol, are referred to as phytosterols. In plants, phytosterols serve the same purposes as cholesterol does in animals and people. This study's primary goal was to compare two different cell line types to the extracted steroid and nano-steroid. PHPLC was used to isolate the novel steroid molecule from the *Euphorbia milii* plant in its pure form, and FT.IR was used to confirm its identity. The chemical had a retention time of 13.87. By using the chitosan-maleic complex process, nano-steroids were created. To evaluate produced nanoparticles, the Philips PW1730 XRD instrument was utilized. The morphological characteristics are ascertained using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Using the MTT assay, the anti-cancer activity of steroids and nano-steroids against prostate cancer cells (PC3), breast cancer cells (MCF-7), and healthy cell lines was investigated. Apoptotic indicators were examined using AO/EtBr double labeling and DNA fragmentation. Using flow cytometry, the dispersion of the cell cycle was ascertained. Recent research has confirmed that the cytotoxic effect on cell lines of prostate and breast cancer was significantly improved by the amounts of steroids and nano-steroids. These results suggest that steroids and nano-steroids may have a dose-dependent effect in stopping the proliferation of breast and prostate cancer cells where it induces apoptosis and halts development in the G0/G1 phase of the cell cycle. Based on the current data, treating cancer cells of different types could potentially benefit from the use of the steroid and nano-steroid combination as a therapeutic method.

Keywords: Apoptosis, MTT assay, Nano-steroid and the steroid, Phytosteols, PHPLC

Introduction

The world is constantly looking for new pharmaceuticals and medications. Worldwide, efforts are always on to find new drugs, adjustments, and improvements with the goal of curing various diseases, particularly cancer. Both traditional medicine and new medication research in the modern era have historically benefited greatly from medicinal plants. [1,2]

Euphorbia milii is one of several common plants that have been demonstrated to have medicinal properties a frilly and medical herbal species universally recognized as the "crown of thorns" in the medicinal genus *Euphorbia*. [3] In popular medicine, it is broadly used in countries like: China, Nepal, Brazil, and other equatorial districts as a treatment for cancer, warts, hepatitis, and eyesores. [4] Modern research states numerous pharmacological properties of *E. milii* such as analgesic, anti-inflammatory, antimicrobial, anti-tumor as well as many others. [5]

Given their well-known health benefits, a wide range of bioactive compounds originating from plants have been taken by people more and more. These substances, which are often referred to as phytochemicals, consist of sterols, organic acids, carotenoids, phenolic compounds, and alkaloids. In general, plant

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sterols are useful substances that can only be found in plants.[6] Plant sterols, or phytosterols, are structural elements of the cell membrane that help control the membrane's permeability and fluidity. They are byproducts of the intricate mechanism via which squalene is used in isoprenoid production. Phytosterols are a group of around 200 distinct chemicals. The three most prevalent are stigmasterol, campesterol, and b-sitosterol. They have proven to offer protection against a range of chronic illnesses, including diabetes, liver, heart disease, and certain cancers. Studies on the prevention of cancer indicate that a diet high in phytosterols can lower the chance of developing certain cancers.[7]

As a disease, cancer is described as a condition where some bodily cells grow uncontrollably and spread to the body's other organs. The trillions of cells that make up the human body are home to cancer, which can spread to any location.[8] Without the person's knowledge, many malignancies such as breast, lung, stomach, and esophagus exist in all sections of the body. These types of cancer may mainly disappear owing to the immune response of the body.[9] If the body doesn't resist cancer cells, the tumor progresses and leads to harm the body, which in turn, causes cancer. Several symptoms can be noticed during cancer infection, like weight inconsistency like unexpected weight loss or weight increase; different colors of the skin (yellowing, darkening, or redness), uncured wounds, changes to present moles represent marks of skin variations. A persistent cough and respiratory issues are some potential symptoms.[10]

The aim of this study was to isolate phytosterols from *E. milii,*by Preparative High Pressure Liquid Chromatography(PHPLC) and Identification,characterization of the isolated phytosterols by FT-IR then loaded them on chitosan nanoparticles to develop their anticancer activity against breast and prostate cancer cell lines. The results proved that the loaded nanoparticles have cytotoxicity influence against cancer cell line higher than the phytosterols alone.

Materials and methods

Extraction of Phytosterols

The Extraction of Phytosterols was conducted using established procedures.[11]

Isolation & purification of phytosterols by preparative HPLC

A 0.5 gram sample of phytosterol was introduced into the Preparative High-Performance Liquid Chromatography (PHPLC) after being melted in a minimum amount of chloroform using: Acetonitrile: absolute methanol (70:30) in an experimental mobile phase

- Column: mediterranea C18, 5 μm 15×2.12 cm.
- Flow rate: 5 ml / min.
- Injection volume: 1 ml.
- Detection: UV. Detector at λ 280 nm.

Identification of Phytosterols by Fourier transform infrared spectroscopy (FTIR)

By matching the chemical vibrations and bands in the spectrum to the distinctive functional group of the chemical class and structure, the functional groups of the isolated substance were recognized using FTIR. The removed chemicals were inspected at the BPC analytical laboratory in Iraq using a Shimadzu 1800. Every measurement was done at 27 ˚C.

Chitosan-Maleic-steroid Synthesis

Step 1: 400 mg/ml of maleic anhydride in THF was added after 600 mg/ml of nano-chitosan in THF was agitated at room temperature. The combination Chitosan-Maleic was produced by refluxing this mixture for two hours at 70 ˚C.

Step 2: Using an ultrasonic probe (150 W) for 5 minutes, 600 mg/ml of steroid or maleic was distributed in THF. This mixture was added to the chitosan-maleic complex made in step 1 and refluxed for three hours at 50 ˚C. To remove THF, the resulting product was centrifuged for 10 minutes at 10,000 rpm. The same process was used to separate the nanocomposite chitosan-Maleic-steroid after it had been dissolved in 300 ml of deionized water. This substance was dissolved in water for five minutes with an ultrasonic probe before being filtered through a μm filter.[12]

Characterization of chitosan- steroid nanocompound

The X-ray diffraction technique was used to analyze the crystalline structure of materials At the University of Tehran, Iran. An XRD instrument type Philips PW1730 was used to examine formed nanoparticle X-ray diffraction. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to determine the morphological properties.

Maintenance of cell line cultures

The effects of steroids and nano-steroids were examined on MCF-7 and PC3 cancer cell lines as well as MCF-10 normal cells used.[13]

Anticancer activity of compounds

The cytotoxic activity was conducted using established procedures,[14] for steroids and nano-steroids on MCF-7, PC3 and MCF-10.

Acridine Orange-Ethidium Bromide staining

AO/PI was used to test the steroids and nano-steroids capacity to cause apoptosis. Briefly stated, cells were exposed to the IC50 preparations of the steroids, Nano steroids, for 24 hours after being seeded on 12-well plates for 24 hours. After two PBS washes, two fluorescent dyes were put into each well. A fluorescent microscope was utilized to view the cells.[15]

Steroids, Nano steroids and their impact on DNA of cell lines

In order to study the cell cycle in cancer cell lines that had been exposed to steroids and nano-steroids, flow cytometry was used. Briefly stated, cells (5104 cells mL^{-1}) were displayed for 24 hours to the steroids and nano-steroids' IC50 concentration. Following cell fixation, extra ethanol was flushed away with PBS washing. The exposed cells were stained with propidium iodide (PI; 10 mg mL^{-1}) for 1 hour at 37 °C. To prevent DNA molecules from interacting with the PI stain, RNase A (10 mg mL^{-1}) was used. Using flow cytometry, the DNA content of the treated cells was examined.[16]

Analytical statistics

The resulting data were statistically processed using an unpaired t-test (GraphPad Prism 6), and they were presented as the mean and standard error of the three replicates per experiment.[17]

Results and discussion

Isolation & purification of Phytosterol from fraction C by Preparative High Pressure Liquid Chromatography

Preparative HPLC is typically related to high flow rates and big columns. Whereas the goal of a preparative HPLC run is to isolate and purify a useful product, the goal of an analytical HPLC run is to determine a component both quantitatively and qualitatively. The field of application for preparative HPLC has expanded due to the growing need in the chemical and pharmaceutical industries for the production of highly pure valuable compounds in varied amounts.[18] For this reason, preparative HPLC was utilized in this study to isolate a single, extremely pure sterol compound from a plant.

Since the crude extract is a very complex mixture, the old task of natural product chemistry is to isolate the energetic compounds from active crude natural product extracts. This is typically done through a series of successive purification steps until the active compound is obtainable in pure form for structure illumination. Preparative HPLC[19] was primarily used to meet the requirements for the isolation and purification system of a large number and good quantity of natural chemicals as shown in Figs. 1 to 3.

Retention time 13.43 matches with a Retention time of beta-sitosterol standard as shown below:

Retention time 13.18 matches with a Retention time of the stigmasterol standard as shown below:

So the novel compound with a Retention time of 13.87 was isolated in pure form by PHPLC & identified by FTIR.

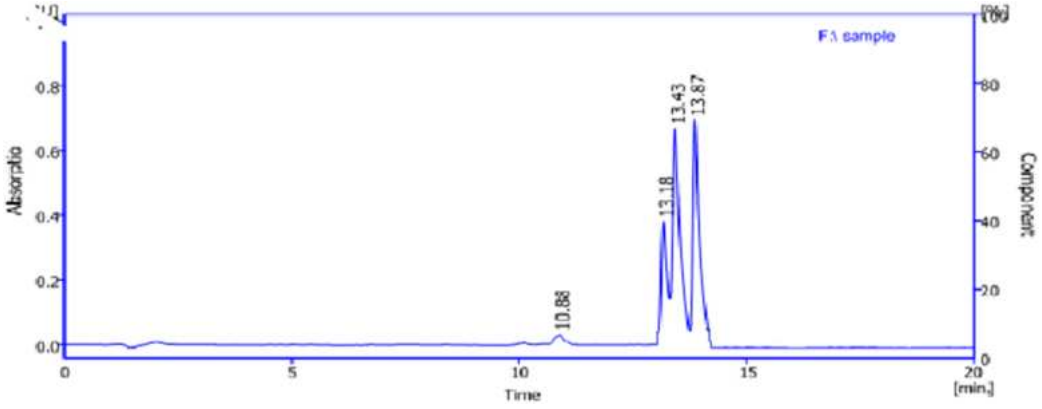

Fig. 1. HPLC of fraction C.

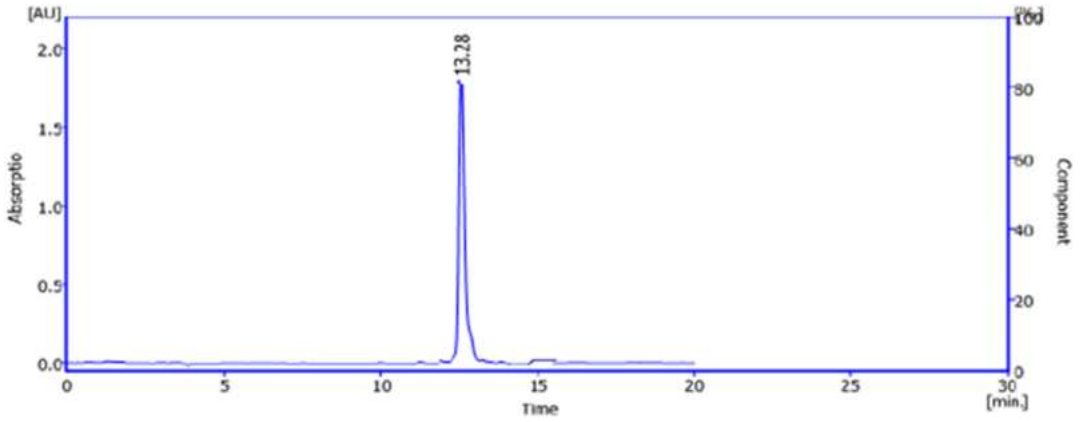

Fig. 2. HPLC for beta-sitosterol standard.

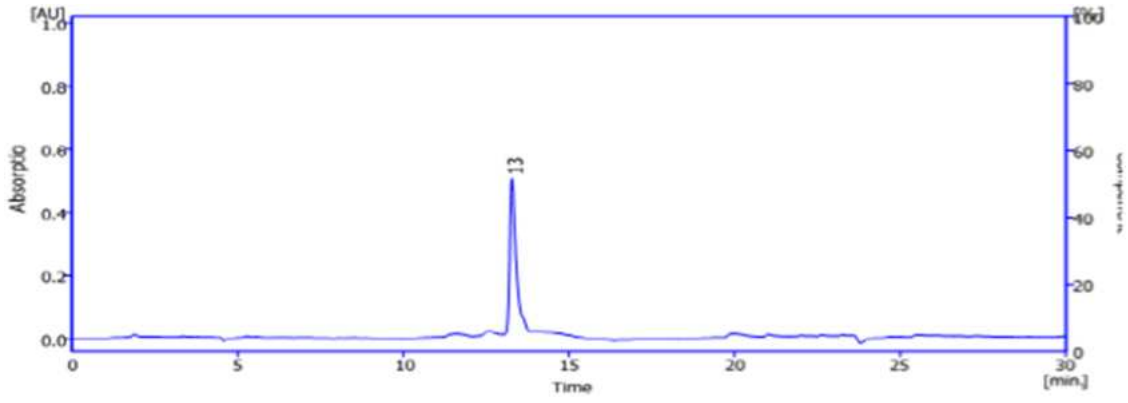

Fig. 3. HPLC for stigmasterol standard.

Identification of isolated Phytosterol compounds by Fourier transforms infrared spectra (FTIR)

A band at 3425 cm^{-1} in the FTIR spectrum displayed in Fig. 4 and Table 1 is attributable to the characteristic of the hydroxyl groups OH band stretch, whereas the bands at 2935 cm^{-1} relate to the aliphatic C-H groups of CH_2 stretching vibration. The existence of the aromatic ring is responsible for the band at 1639 cm^{-1}, which is linked to the C=C groups stretching vibration. Furthermore, bands at 1462 cm^{-1} show that the C-H vibration is present. The band is vibrating at 1377 cm^{-1}. O-C. In addition, the O-H in-plane bending vibration of the OH group is responsible for the band at 1168 cm^{-1}.[20]

Characterization of Chitosan-Flavonoid nano-compound

X-ray diffraction analysis (XRD)

The XRD measurement shows six main peaks at: 27.5109, 31.9159, 45.5955, 56.5884, 66.3349 and 75.3053°. However, the presence of peak at 27.3108° can be considered a solid evidence of the success of the reaction as it coincides with the peaks of the nano-chitosan[21] and the shifting of this peak from

Table 1. FTIR vibrations of isolated steroids with the assignment to their related functional group.

Functional group	Frequency wave NO. for isolated compound	Assignment
O-H	3425	Typical of the OH band stretch of hydroxyl group
C-H	2935	Aliphatic C-H stretch
C=C	1639	Aromatic stretch
C-H	1462	C-H vibration
C-O	1377	C-O vibration
O-H	1168	O-H in plane bending vibration of OH group
C-H	1060	C-H vibration

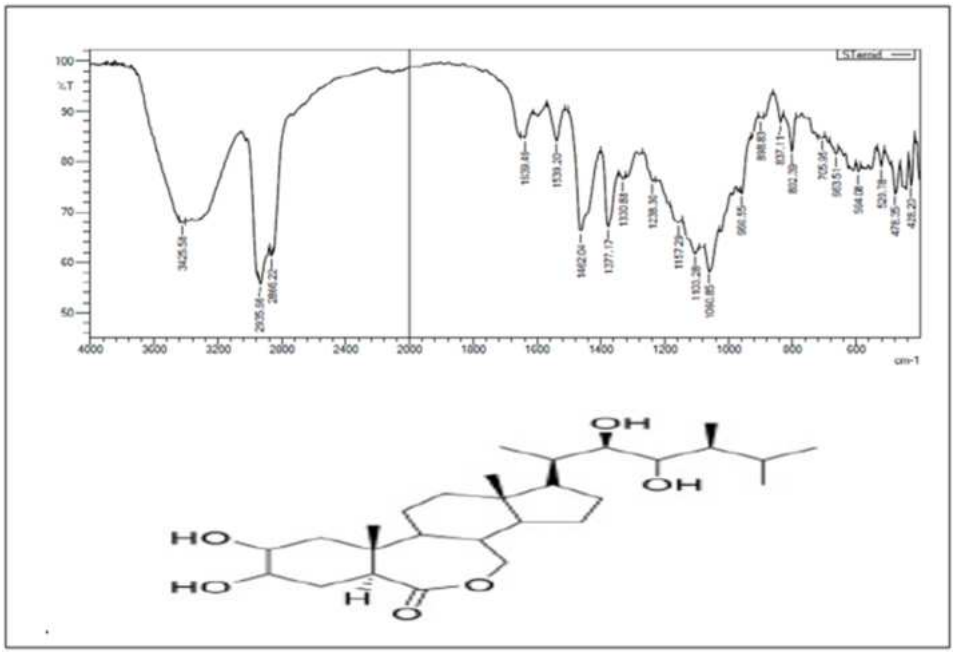

Fig. 4. FTIR spectrum and structure of isolated phytosterol.

their respective positions in the nano-chitosan is evidence of the formation of the natural organic nano compound. Moreover, the presence of the other aforementioned peaks is evidence of the presence of steroid within the material as shown in Fig. 5.

The average particle size of this material was calculated using the Scherrer equation for each peak, where it was found that its value is 37.5 nm, indicating the formation of the required natural organic compound with nano-sized sizes as shown in Table 2.

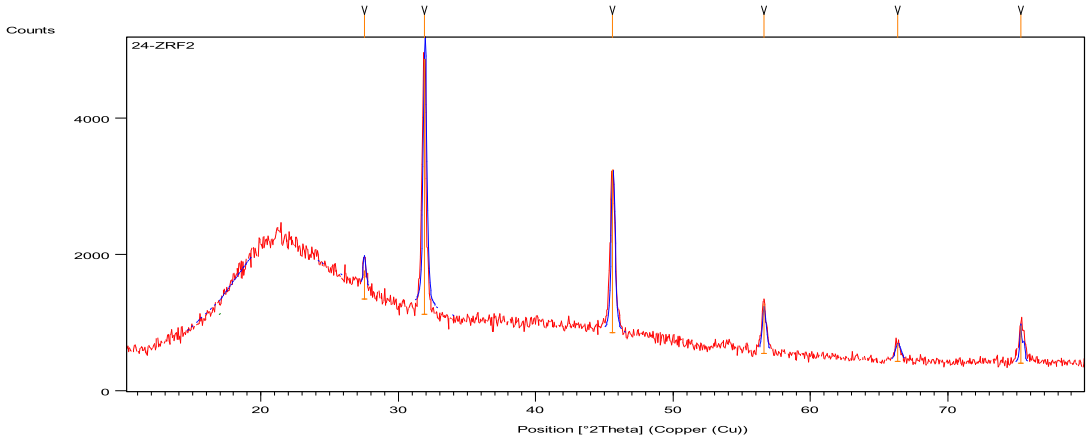

Fig. 5. XRD of Chitosan-steroid nano-compound.

Table 2. XRD data and particle size of Chitosan-steroid nano-compound.

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Particle size (nm)	Average particle size (nm)
27.5109	421.25	0.1968	3.24225	43.44	
31.9159	3749.89	0.2952	2.80410	29.26	
45.5955	2387.73	0.2952	1.98962	30.51	37.50
56.5884	728.52	0.2460	1.62645	38.34	
66.3349	262.26	0.3936	1.40916	25.20	
75.3053	558.78	0.1800	1.26098	58.27	

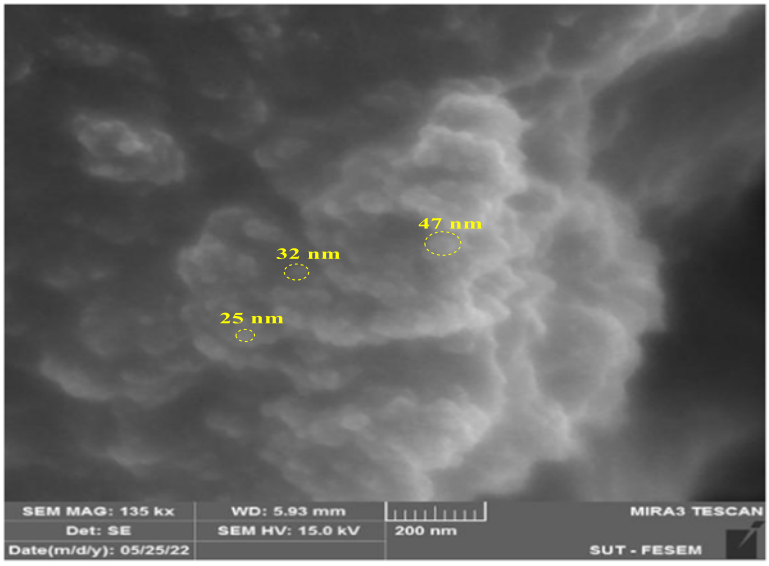

Fig. 6. SEM of chitosan-steroid nano-compound.

Scanning electron microscope analysis (SEM)

Agglomerated irregular sphere-like particles were detected by the SEM measurement. Furthermore, as can be shown in Fig. 6, the measurement confirmed that the generated chitosan-steroid nanoparticle's size was within the nanoscale range, with the as-prepared particles measuring between 25 and 47 nm. It was detected that the particle size estimated using Scherer's equation (37.50 nm) and the average particle size ascertained by SEM are extremely similar.

Transmission electron microscope analysis (TEM)

The characteristics of the steroids-coated chitosan nanoparticles (NPs) were examined using TEM. According to the results displayed in Fig. 7-A,B, chitosan nanoparticles (NPs) have an average size of roughly 25–65 nm and are spherical or semispherical in shape. Contrarily, steroids have a different appearance. As a result, the combination of Chitosan NPs and steroids results in coated, spherical nanoclusters. These findings validate findings from,[22] as seen in Fig. 7-C.

Anticancer activity of compounds

This test determined the examined cell lines' viability in response to steroid and nanosteroid treatment. When MTT enters metabolically active cells, it converts into insoluble purple MTT-formazan crystals, which are then dissolved in a solvent and assessed spectrophotometrically.[23] In contrast to MCF10, the steroids and nano steroids in this study showed more action against MCF-7 and PC3. When cells were treated with steroids and nano steroids, as shown in Figs. 8 to 10 and Tables 3 to 5, there was dose-dependent cellular toxicity. However, at 3.1 μg/mL concentrations, our results showed that the cytotoxic (Viability %) of the generated steroids and nano steroids was unaffected. The amounts of synthetic and nano steroids caused a considerable increase in cytotoxicity. Additionally, this experiment confirmed

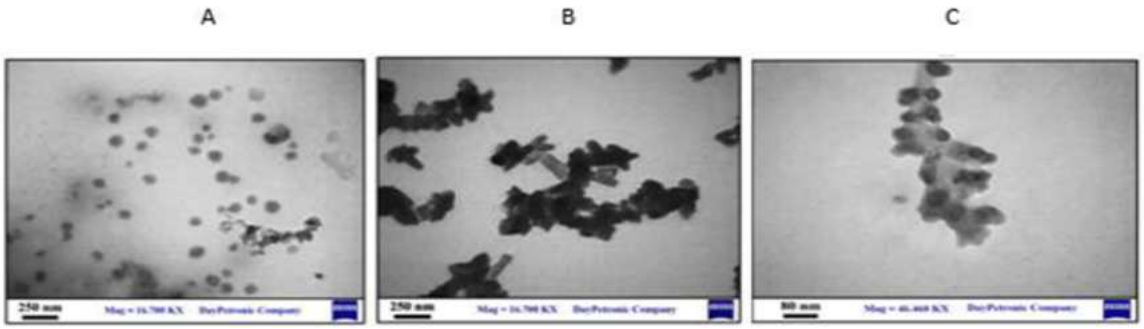

Fig. 7. TEM images of A, Chitosan nanoparticles. B, Steroid., C, steroid coated Chitosan NPs.

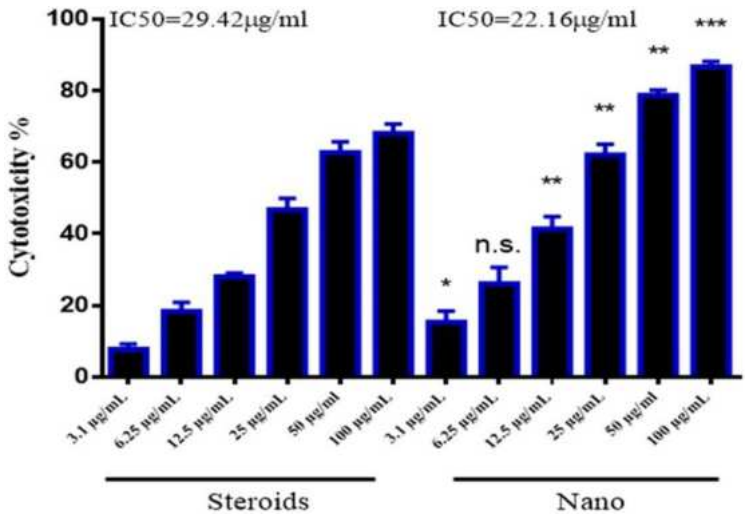

Fig. 8. Anticancer activity of Steroids and nanoSteroids against MCF-7 cells.

Table 3. Anticancer activity of steroids. and nanosteroids. against MCF-7 cells.

	Cell line		
Concentration μg/ml	MCF7/steroid	MCF7/nanosteroid	LSD value
3.1	7.66 ± 0.88 F b	15.33 ± 1.76 F a	5.47 **
6.25	18.33 ± 1.45 E b	26.00 ± 2.64 E a	4.66 **
12.5	28.00 ± 0.57 D b	41.33 ± 2.02 D a	5.93 **
25	46.66 ± 1.85 C b	62.00 ± 1.73 C a	6.11 **
50	62.66 ± 1.76 B b	78.67 ± 0.88 B a	5.04 **
100	68.00 ± 1.52 A b	86.67 ± 0.88 A a	5.38 **
LSD value	4.277 **	5.451 **	—

Means with different big letters in the same column and small letters in the same row are significantly different. ** ($P \leq 0.01$).

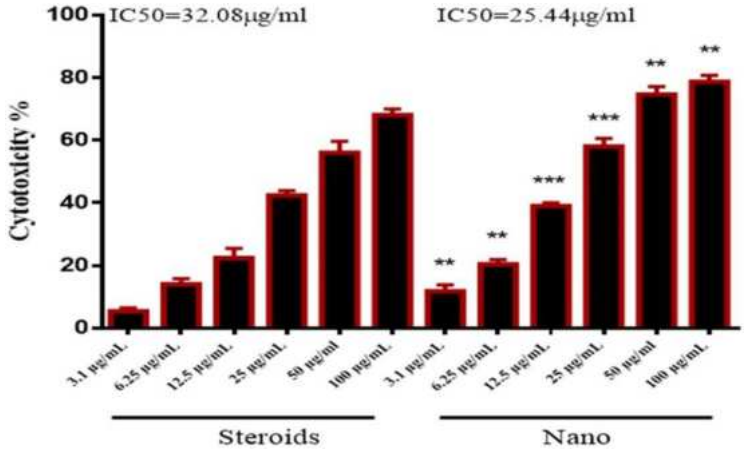

Fig. 9. Anticancer activity of Steroids and nanosteroids against PC3 cells.

Table 4. Anticancer activity of steroids. and nanosteroids. against PC3 cells.

	Cell line		
Concentration μg/ml	PC3 /steroid	PC3 /nanosteroid	LSD value
3.1	5.33 ±0.67 F b	11.67 ±1.20 F a	3.82 **
6.25	14.00 ±1.00 E b	20.33 ±0.88 E a	4.02 **
12.5	22.33 ±1.76 D b	39.00 ±0.57 D a	4.94 **
25	42.33 ±0.88 C b	58.00 ±1.52 C a	4.61 *8
50	56.00 ±2.08 B b	74.67 ±1.45 B a	4.39 **
100	68.00 ±1.15 A b	78.66 ±1.20 A a	4.52 **
LSD value	4.172 **	3.655 **	—

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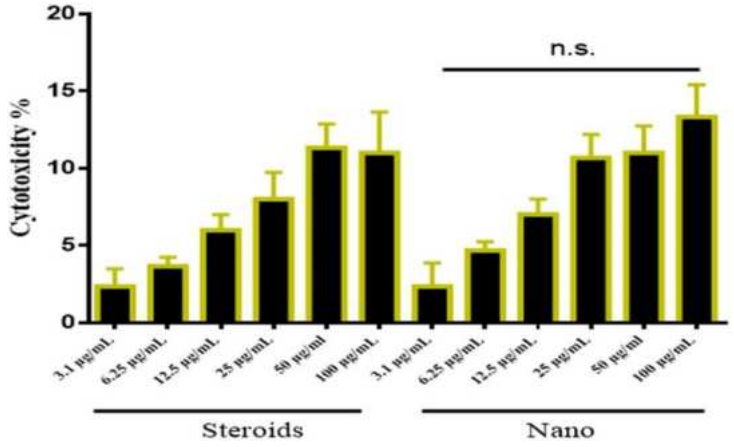

Fig. 10. Anticancer activity of Steroids and nanosteroids against MCF-10 cells.

Table 5. Anticancer activity of flavo. and nanoflavo. against MCF-10 cells.

	Cell line		
Concentration μg/ml	MCF10/steroid	MCF10/nanosteroid	LSD value
3.1	2.33 ±0.67 D a	2.33 ±0.88 D a	3.069 NS
6.25	3.67 ±0.33 CD a	4.67 ±0.33 CD a	1.05 NS
12.5	6.00 ±0.57 BC a	7.00 ±0.57 C a	1.16 NS
25	8.00 ±1.00 B a	10.67 ±0.88 B a	1.42 NS
50	11.33 ±0.88 A a	11.00 ±1.00 AB a	1.48 NS
100	11.00 ±1.52 A a	13.33 ±1.20 A a	2.37 NS
LSD value	2.812 **	2.652 **	—

Means with different big letters in the same column and small letters in the same row are significantly different. ** ($P \leq 0.01$).

that the lowest inhibitory dose of MCF-7 cells by steroid response was 6.25 μg/ml, 12.5 μg/ml, and 25 μg/ml (18.33%, 28.0 %, and 46.66%), respectively, and nanosteroids were 26.00%, 41.33%, and 62.00%. However, the maximum concentration of inhibitory steroids at 50,100 μg/ml (62.66% and 68.56%) and the maximum concentration of inhibitory nanosteroids (78.67% and 86.67%) were found, respectively, while the cytotoxicity of steroids against PC3 was (14.33%, 22.33%, and 42.33%), and that of nanosteroids (20.33 %, 39.00 %, and 58.00%), respectively. The greatest cytotoxicity of nanosteroids against PC3 was 74.67%, whereas the highest cytotoxicity of steroids was 56.00%, and 68.00%, respectively. A low cytotoxic effect in contradiction of normal cell lines (MCF-10 cells) has been detected in the results.

The inhibition percentages of MCF-7 and PC3 cells increased with growing concentrations of steroids and nanosteroids, and these differences were important in inhibition percentages as shown in Tables 3 to 5. However, there was no significant difference in inhibition percentages when treated with a normal cell line (MCF10 cells).

According to a previous study,[24] phytosteroids may be able to stop the growth of numerous human cancer cell lines at micromolar concentrations without impairing the growth of normal cells. Specifically, phytosteroids have been shown to inhibit the growth of prostate and breast cancer cells in a dose-dependent manner, stopping the cells' growth in the G1 phase of the cell cycle and inducing apoptosis. Strong cytotoxicity against breast and prostate cancer cell lines is confirmed by steroid-laden chitosan nanoparticles, which were produced and loaded with active anticancer steroidal chemicals. This is in line with our findings.[25]

Crystal violet staining method

Morphological changes were also used to examine the cytotoxicity effect on the tested cells, as shown in Figs. 11 to 13. The findings showed that whereas nano steroids exerted a greater harmful effect on MCF-7, and PC3 cells, their cytotoxicity was dependent on the quantity of exposure. However, normal cells (MCF-10) did not display any such changes. When comparing the growth rate of normal cells to that of cancer cells, the former showed fewer damaging effects. The affinity of the dye for the outer surface of the DNA double helix is the basis for the crystal violet test. One can estimate the number of live cells by measuring the amount of dye absorbed, which is proportionate to the culture's total DNA content.[26] The results additionally showed that nano steroids caused several additional alterations, such as adjustments to cell shape, treatment cell clumping with limited cellular extensions, and blockage of cell communication. The quantity of crystal violet staining in a culture is decreased when cells die because they lose their adherence and are

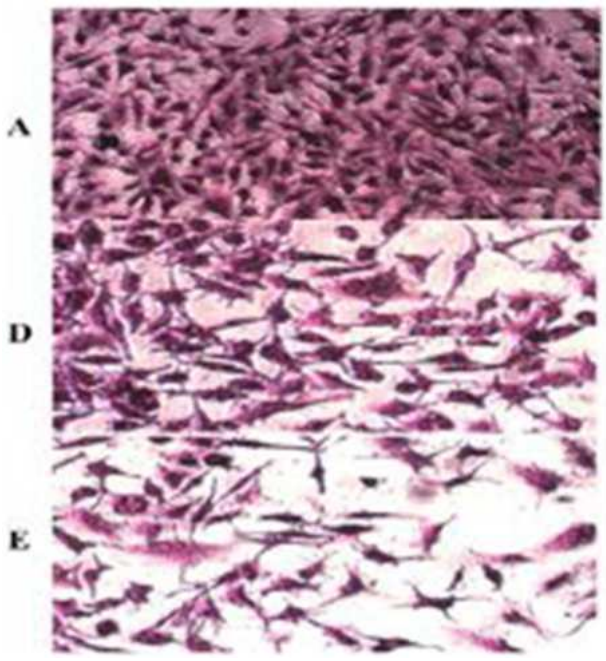

Fig. 11. MCF-7 cell morphological alterations as shown. A untreated control cells. D, cells were treated with steroids. E, cells were treated with nanosteroids.

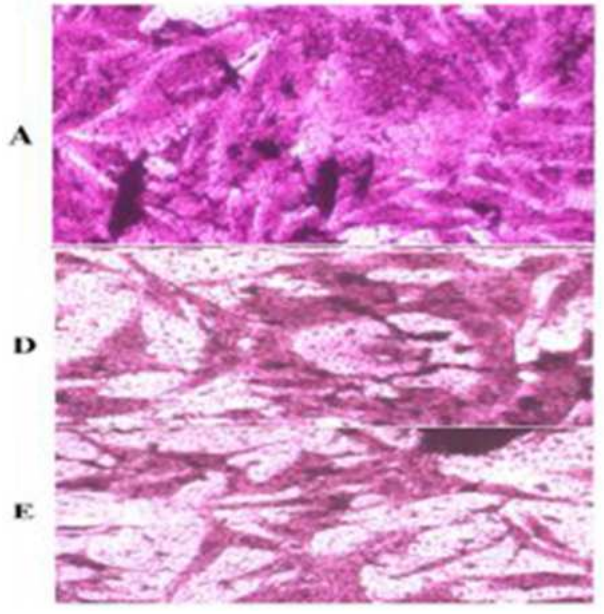

Fig. 12. The suggested morphological alterations in PC3 cells. A, untreated control cells.. D, cells were treated with steroids. E, cells were treated with nanosteroids.

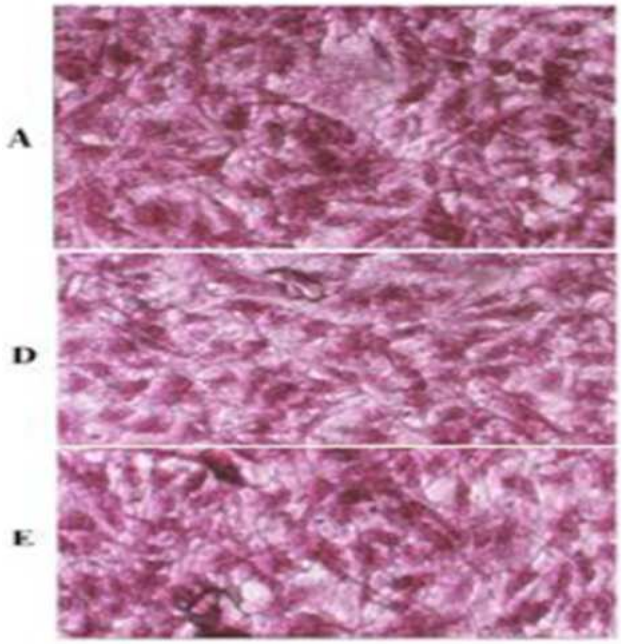

Fig. 13. MCF-10 cell morphological changes as shown. A, untreated control cells. D, cells were treated with steroids. E, cells were treated with nanosteroids.

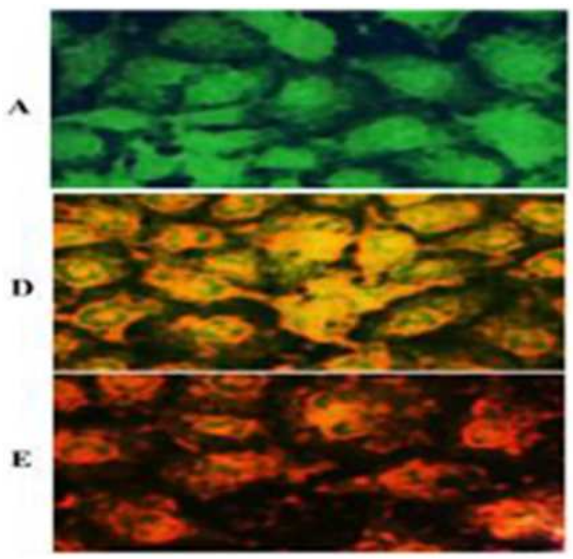

Fig. 14. Apoptosis markers in MCF-7 cells using AO/EtBr staining. A, untreated control cells. D, cells were treated with steroids. E, cells were treated with nanosteroids.

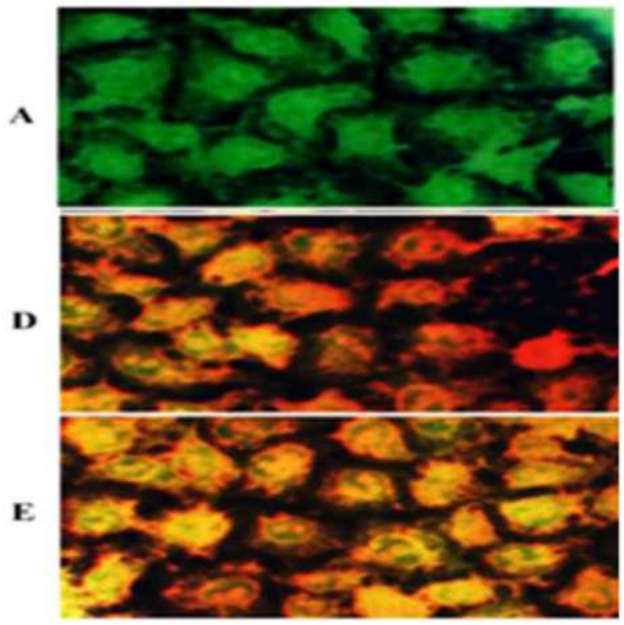

Fig. 15. Apoptosis markers in PC3 cells using AO/EtBr staining. A, untreated control cells.. D, cells were treated with steroids. E, cells were treated with nanosteroids.

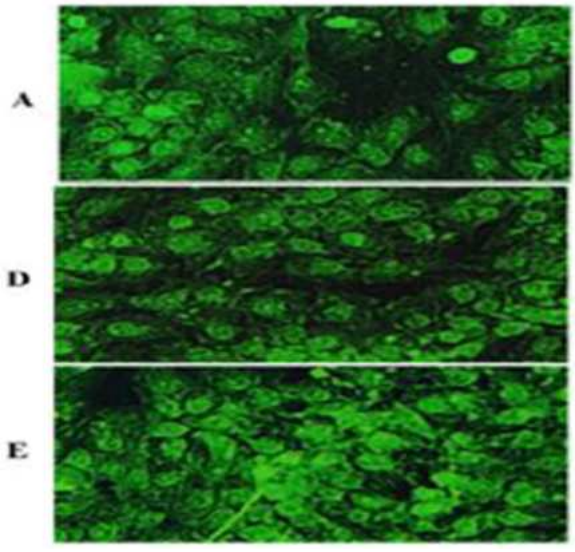

Fig. 16. AO/EtBr labeling of MCF-10 cells to identify apoptosis markers. A, untreated control cells D, cells were treated with steroids. E, cells were treated with nanosteroids.

subsequently eliminated from the cell population Fig. 12; E: Fig. 13; E. Conversely, less change was observed in the steroid-treated cells Fig. 12; D: Fig. 13; D, and no comparable effects were noted in the untreated control cells Fig. 14. Because of their small size, nanoparticles are easier to provide larger quantities of medication to get better results because they can enter cells more proficiently than microparticles. Significantly, encasing anticancer medications in polymeric nanoparticles may help avoid first-pass metabolism and P-glycoprotein-mediated drug efflux.[27] Numerous studies designate that the lethal effects of steroids and nano steroids on cancer cells may be related to activities that disrupt membranes, accumulate lactic acid, and reactive oxygen species (ROS), damage DNA, and induce apoptosis.[28,29]

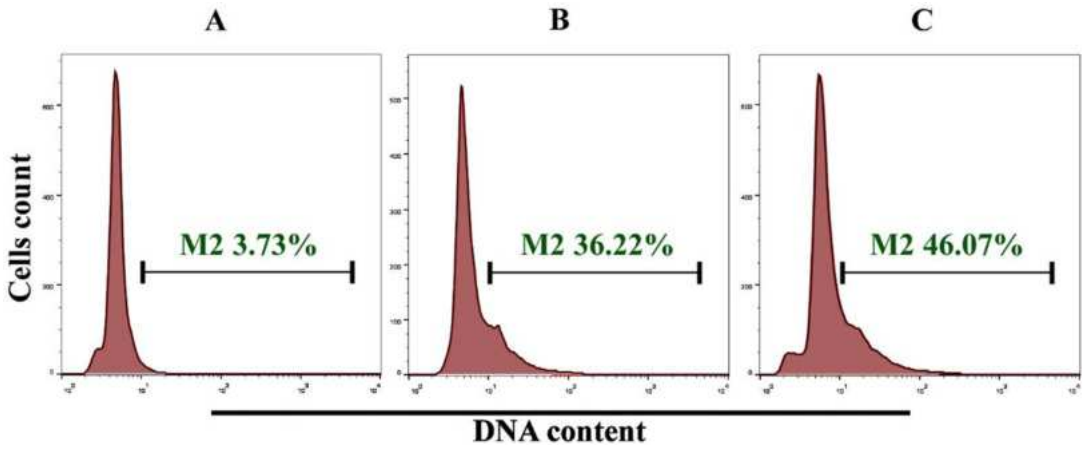

Fig. 17. DNA cycle in PC3 cells after treated as indicated. A, control untreated cells. B, cells were treated with steroids. C, cells were treated with Nanosteroids.

Results of the double acridine orange-ethidium bromide staining

The (AO/PI) dual stain is a fluorescent combination stain that produces distinctly colored fluorescence, making it possible to detect morphological changes in the nucleus. The permeability of fluorescent dye to the plasma membrane is enhanced in apoptotic cells.

Using the dual staining test with AO and EtBr dyes in MCF-7, PC3, and mcf10 cells, the potentials of the IC50 formulations of steroids and Nano steroids to induce cytotoxicity were examined Figs. 14 to 16. AO/EtBr dual staining fluorescence microscopy was used to evaluate and approve the changes in nuclear morphology in MCF-7, PC3, and MCF10 cells. Compared to control cells that were left untreated, these cells exhibited a more radical loss of membrane stability after being treated with steroids and nano steroids. A useful method for effectively identifying the strikingly induced changes, such as chromatin condensation and the appearance of red staining in the cytoplasm, which signify changes in RNA and lysosomes in drug-exposed cells, was to look at changes in fluorescence and morphology of treated cells. Early apoptotic cells have yellow nuclei, and their chromatin is either fragmented or condensation-defined. However, the chromatin of late apoptotic cells is either fragmented or condensed, and their nuclei are orange to red in hue.

Apoptotic cell death was discovered using AO/EtBr based analysis of the nuclear morphology of the cells inspected in this work. It is important to recall that AO can stain both living and dead cells. EtBr, however, will only dye cells that have had membrane deterioration. Previous research demonstrated that the treated cells were recorded as dead (red color), suggesting that the treatment had a cytotoxic effect, but the living cells (green color) had a uniform distribution and large nuclei.[30]

Additionally, phytosterols have confirmed characteristics that directly impede the formation of tumors, such as slowed cell cycle progression and the triggering of apoptosis through a reduction in blood cholesterol levels.[31] The outcomes are in line with[32] reports that designated numerous doses of phytosteroids had cytotoxic effects on (MCF-7,PC3) cells through the induction of apoptosis.

Effect of steroids and Nano steroids on DNA of cell lines

In the current work, we conducted an additional experiment to look into the potential inductive function of steroids and nanosteroids in the process of apoptosis. We used flow cytometry to measure the amount of DNA at the sub-G1 phase after treating MCF-7 and PC3 cells and staining their cellular DNA with propidium iodide (PI). The findings showed that, in PC3 cells treated with steroids, the percentage of cancer cells in the sub-G1 phase treated with steroids and nano steroids increased from 3.73% to 36.22% and 46.07%, respectively Fig. 17. The study's overall results showed that the produced steroids and nano steroids stimulated apoptotic cell death to have an anti-proliferative effect.[33] This is consistent with the findings of,[34] who discovered that steroids might cause cell cycle arrest in the G2/M phase and trigger dose-dependent cell death in PC-3 cells.

Conclusion

Phytosterols have been shown to inhibit the development of various cancers by inhibiting cancer cell growth and promoting apoptosis through the

activation of caspase enzymes. The enhanced activity of caspase enzymes may be explained by the alteration in membrane structure and function brought about by the incorporation of phytosterols into cell membranes. These changes also enhance the activities of proteins involved in extracellular and intracellular signal transduction pathways, which in turn activate caspase enzymes. Together, these data provide compelling evidence for phytosterols' anticarcinogenic properties. The outcomes demonstrated that steroid and nano steroid are effective chemotherapeutic agents that can be employed alone or in combination with other agents to treat various cancer cell types.

Author's declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.
- Authors sign on ethical consideration's approval.
- No animal studies are present in the manuscript.

Authors contribution

B.M., E.J. and Z.M. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Journal declaration

The Second author (B.M.J.Al.) serves as an editor for Baghdad Science Journal but was not involved in the peer review process of this manuscript beyond their role as an author. The authors declare no other conflict of interest.

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عزل وتنقية الفيتوستيرول من نبات شوكة المسيح المزروع في العراق بواسطة التحليل الكروماتوجرافي السائل عالي الأداء (PHPLC) وتقييم تاثيرها المضاد للسرطان

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الخلاصة

يشار إلى الستيرول والستانول النباتي مجتمعين على انها مواد مشابهة من الناحية الهيكلية للكوليسترول، ويطلق عليها فيتوستيرول. وان الفيتوستيرول يؤدي في النباتات نفس الأغراض التي يؤديها الكوليسترول في الحيوانات والبشر. وقد كان الهدف الأساسي لهذه الدراسة هو مقارنة نوعين مختلفين من خطوط الخلايا بالستيرويد المستخرج والستيرويد النانوي. وتم استخدام PHPLC لعزل جزيء الستيرويد الجديد من نبات *Euphorbia milii* في شكله النقي، وتم استخدام FTIR لتأكيد هويته. وكان زمن الاحتفاظ للمادة الكيميائية هو 13.87. وتم إنشاء الستيرويد النانوي عن طريق تفاعل مركب الكيتوسان وحامض الماليك . حللت مركبات الستيرويد النانوية بواسطة جهاز حيود الاشعة السينية(Philips PW1730 XRD). وتم التأكد من الخصائص المورفولوجية باستخدام المجهر الإلكتروني الماسح (SEM) والمجهر الإلكتروني النافذ (TEM) .وباستخدام اختبار MTT، درس النشاط المضاد للسرطان للستيرويد والستيرويد النانوي ضد خلايا سرطان البروستاتا (PC3)، وخلايا سرطان الثدي (MCF-7)، وخطوط الخلايا السليمة. تم استخدام تجزئة الحمض النووي وتقنية تلوين الخلايا بالصبغة المزدوجة AO/EtBr لفحص علامات موت الخلايا المبرمج. كما تم تحديد تشتت دورة الخلية باستخدام قياس التدفق الخلوي. وقد أكدت الأبحاث الحديثة أن التأثير السام للخلايا على خطوط خلايا سرطان البروستاتا والثدي قد تحسن بشكل ملحوظ من خلال كميات الستيرويدات والستيرويدات النانوية. وتشير هذه النتائج إلى أن الستيرويدات والستيرويدات النانوية قد يكون لها تأثير يعتمد على الجرعة في وقف تكاثر خلايا سرطان الثدي والبروستاتا حيث أنها تحفز موت الخلايا المبرمج وتوقف التطور في مرحلة G0 / G1 من دورة الخلية. وبناءً على البيانات الحالية، يمكن أن يستفيد علاج الخلايا السرطانية بأنواعها المختلفة من استخدام تركيبة الستيرويد والستيرويد النانوي كوسيلة علاجية.

الكلمات المفتاحية: موت الخلايا المبرمج، نشاط مضاد للسرطان، الستيرويد والستيرويد النانوي، الفايتوستيرول، التحليل الكروماتوجرافي السائل عالي الأداء.