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Isolation and Identification of Flavonoid Compounds from *Euphorbia Milii* Plant Cultivated in Iraq and Evaluation of its Genetic Effects on Two Types of Cancer Cell Line

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Abstract

The "crown of thorns" or *Euphorbia milii*, a decorative and therapeutic plant species, is regarded as belonging to the genus Euphorbia. *E. milii* is abundant in phenolic compounds, terpenoids, steroids and alkaloids. The main objective of this study was to investigate the extracted flavonoid and nano-flavonoids against two types of cell lines. Nano-flavonoids were synthesized via chitosan-maleic complex reaction. The structure of nano-flavonoids was analyzed by UV-sp -8001 spectrophotometer with a 200–1000 nm scanning wavelength and the produced compounds were examined. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used to determine the morphological properties. A strong and cutting-edge anti-proliferative drug against breast cancer cells (MCF-7) and prostate cancer cells (PC3) is the flavonoid and nano-flavonoid. The anti-cancer efficacy of flavonoids and nano-flavonoids against two different cancer cell lines as well as healthy cell lines was examined using the MTT assay. DNA fragmentation and the AO/ EtBr double staining technique were used to examine apoptotic markers. To determine the dispersion of the cell cycle, flow cytometry was used. The recent findings showed that the quantities of flavonoid and nano-flavonoid considerably improved the cytotoxic effect against breast and prostate cancer cell lines. Additionally, the cell cycle arrest in the G0/G1 phase was linked to the apoptosis of the cell lines induced by flavonoids and nano-flavonoids. According to these results, flavonoids and nano-flavonoids prevent cancer cell lines from proliferating, which causes the cell cycle to be arrested and apoptosis to be induced. The available findings suggested that the flavonoid and nano-flavonoid complex would represent a promising therapeutic approach for treating cancer cells of other types.

Keywords: Anticancer activity, Apoptosis, DNA fragmentation, Euphorbia milii, The flavonoid and nano-flavonoid.

Introduction

The worldwide search for new medications and drugs is a continuous exploration. Improvements, modifications, and new drug detection are constant processes worldwide that seek to find a remedy for numerous diseases, especially cancer. Among many rich sources for curing cancer are medicinal plants that evermore represent a fruitful exporter of modern, unique drug detection and historical folk medicine[1-3]. *Euphorbia milii* is a frilly and medical herbal species universally recognized as the “crown of

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thorns"in the medicinal genus *Euphorbia*[4]. 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[5]. Modern research states numerous pharmacological properties of *E. milii* such as analgesic, anti-inflammatory, antimicrobial, antitumor as well as many others[6]. Different phytochemical studies exposed that *E. milii* is rich in many phytochemical compounds namely phenolic compounds and terpenoids; nevertheless, other studies noticed cardiac glycosides, alkaloids, steroids, and anthocyanins[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. 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 representing marks of skin variations. A persistent cough and respiratory issues are some potential symptoms [9]. The most common malignancies all over the world are women's breast cancer and men's prostate cancer, respectively[10]. Moreover, gastric cancer is considered to be one of the most universal cancers around the world as well as being the fourth leading source of cancer-related deaths all over the world[11]. With roughly 9000 different compounds, flavonoids are one of the classes of bioactive particles with the greatest diversity[12]. The most diverse group of polyphenols are flavonoids, which have a wide variety of structural and functional properties[13]. The name "flavonoid" refers to phenyl-substituted propylbenzene compounds and flavonolignans, which are formed by combining these compounds with C6-C3 flavonoid substrates with a C15 backbone[14]. Flavonoids are divided into 10 different species based on their chemical makeup, with flavones, flavanones, anthocyanidins, flavonols, isoflavones, and catechins being the most prevalent in foods consumed by humans. In both in vitro and in vivo experiments, it has been demonstrated that several of these flavonoids have anti-tumor effects[15]. The aim of this study was to isolate flavonoids from *E. milii*, then loaded them on chitosan nanoparticles to develop their anticancer activity against breast and prostate cancer cell lines. The results proved that the load nanoparticles have cytotoxic influence against cancer cell lines higher than the flavonoids alone.

Materials and Methods

Fractionation and extraction of several active compounds

The different plant components were separated according to the scheme shown in Fig. 1,[16-18].

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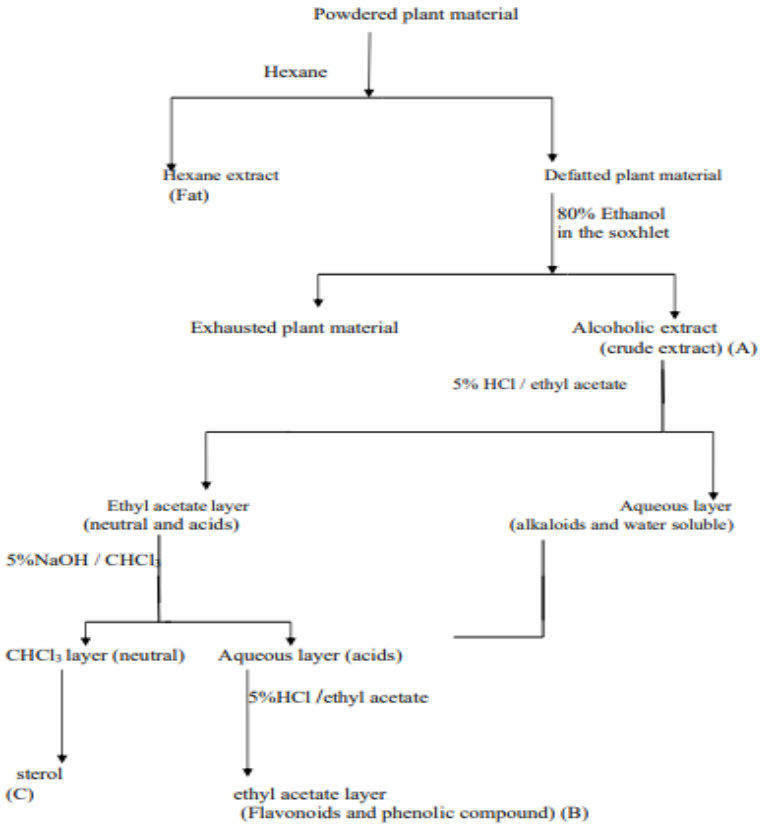

Figure 1. General strategy for separating various plant components.

Qualitative analysis of active compounds in *Euphorbia milii*

The medicinal plants under research were subjected to phytochemical analysis using the standard techniques outlined by Harborne[19, 20].

Analyze for phenolic compounds

Extracts (10 mg) were diluted in 10 ml of distilled water, filtered, and the filtrate was then combined with 3 ml of a 5% w/v FeCl3 solution. It was thought that the production of a blue-black or dark-green precipitate.

Analyze for Flavonoids compounds

A few milligrams of extracts were dissolved in ethanol, followed by the addition of a few drops of 5% ethanolic KOH and a few drops of 5% HCl. The variations in color were noted.

Quantitative analysis of flavonoids in *Euphorbia milii* [21]

Used HPLC equipment to measure the presence of flavonoids.

Chitosan-Maleic-Flavonoid 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-Flavonoid 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 □m filter[22, 23].

Characterization of chitosan- flavonoid nanocompound

Utilizing a UV-sp -8001 spectrophotometer with a 200–1000 nm scanning wavelength, the produced

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compounds were examined. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to determine the morphological properties.

Maintenance of Cell Line Cultures

The effects of flavonoids and nano-flavonoids were examed on MCF-7 and PC3 cancer cell lines as well as MCF-10 normal cells used[24].

Anticancer activity of compounds

The cytotoxic activity was conducted using established procedures [25, 26], for Flavonoids and nanoflavonoids on MCF-7 ,PC3 and MCF-10.

Acridine Orange-Ethidium Bromide Staining

AO/ EtBr was used to test the flavonoids and nano-flavonoids capacity to cause apoptosis. Briefly stated, cells were exposed to the IC50 preparations of the flavonoids, Nano flavonoids, 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[27].

Flavonoids, Nano flavonoids and their impact on DNA content

In order to study the cell cycle in cancer cell lines that had been exposed to flavonoids and nano flavonoids, flow cytometry was used. Briefly stated, cells (5104 cells mL^{-1}) were displayed for 24 hours to the nano flavonoids' 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[28].

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[29].

Results and Discussion

Qualitative analysis of active compounds in *Euphorbia milii*:

The results of the phytochemical screening are shown in Table 1.

Table 1. Screening of crude extract and various fractions for phytochemical

Sample	Alkaloids	Flavonoids and phenols	Steroids	Terpenoids
Fraction A	+	+	+	+
Fraction B	-	+	-	-
Fraction C	-	-	+	+

+, - represent presence and absence of phytoconstituents respectively.

Quantitative analysis of flavonoids in *Euphorbia milii*

By using a hyper clone ODCC C18 V-25cm column and a mixture of ethyl acetate: water (7:30 ratio) as the mobile phase with a flow rate of 0.5 ml/min, the active constituents were estimated qualitatively and quantitatively using HPLC. Identifications were made by based on retention times obtained at equal chromatographic circumstances of analyzed samples and authentic criteria, and detected at 280 nm. Table 2 Fig. 2.

Table 2. Retention time (Rt) in minutes for the isolated flavonoid compounds and their corresponding standards.

Isolated compound	Standard compound	Rt standard	Rt sample
1	Quercitrin	5.61	5.80
2	Coumaric acid	8.85	8.72
3	Caffeic acid	10.5	10.21

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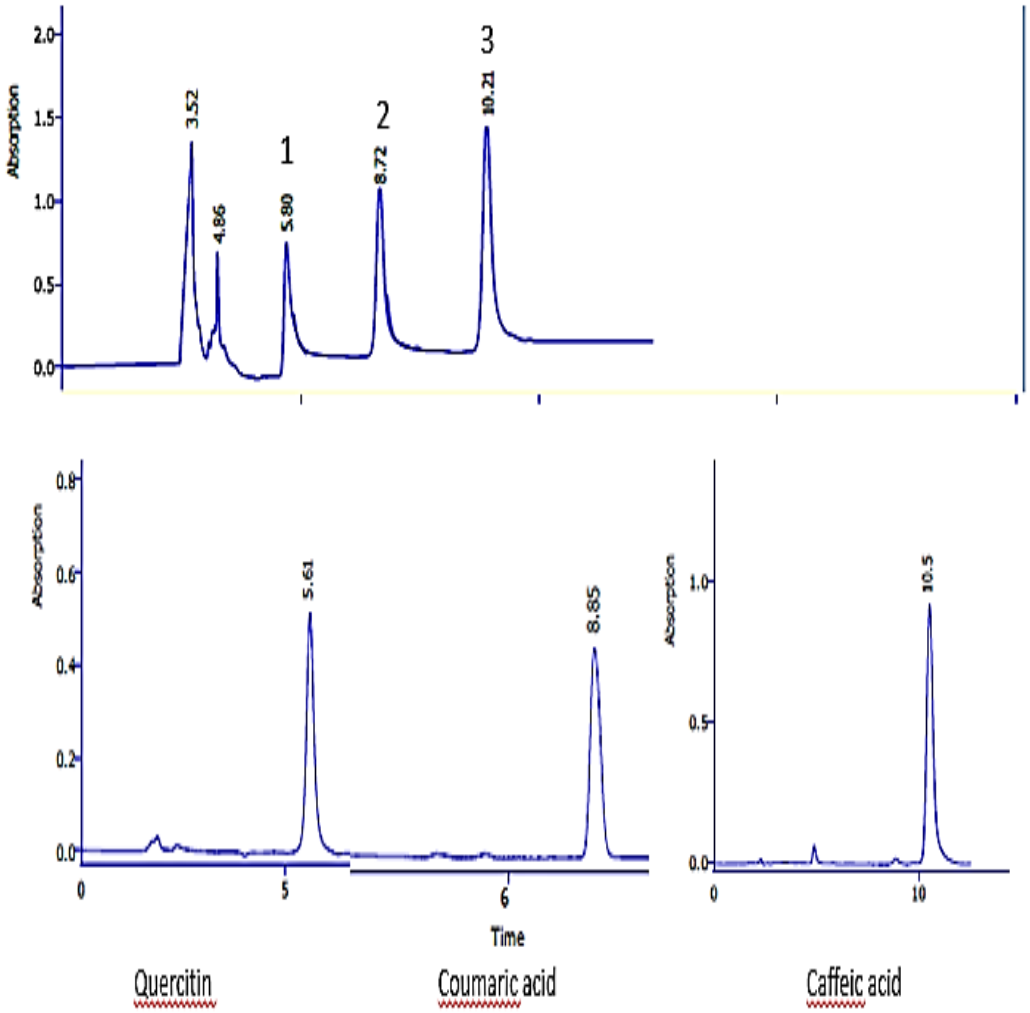

Figure 2. HPLC chromatogram of isolated compound and standard compound.

Characterization of chitosan- flavonoid nanocompound

1. UV-Vis spectrum

This compound's UV-visible spectrum revealed the existence of a single high-pitched peak with an absorbance value of 1.028 at 206 nm, which is attributable to π-π* electronic transitions [30]. This peak's location, which differs from the primary peak of nano-chitosan, which typically appears at about 250 nm [31], is proof of the success of the reactivity between flavonoids and nano-chitosan, as shown in Fig. 3.

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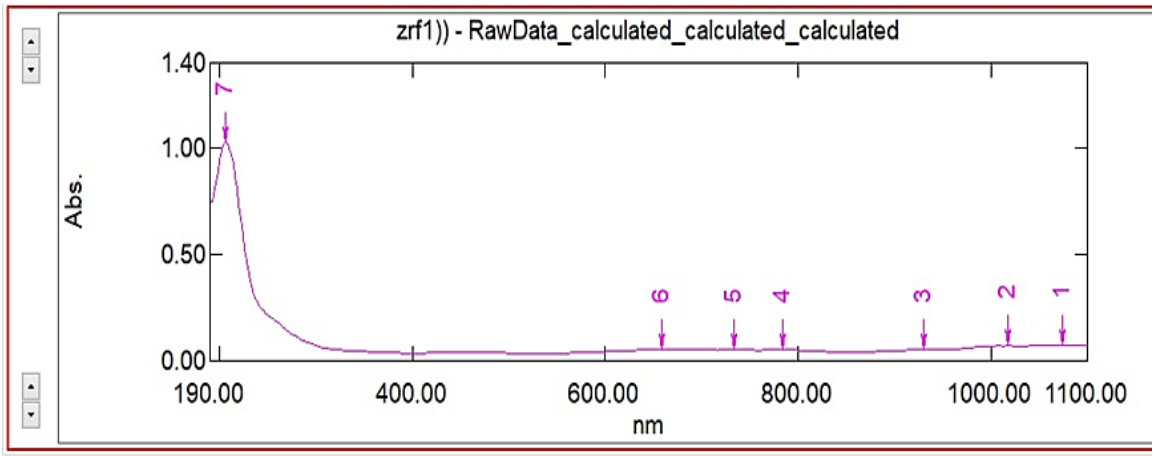

Peak | Peak Area | Point Pick | Calculation | Parameter | History | Summary

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	1074.0	0.075	
2	↑	1018.0	0.070	
3	↑	930.0	0.054	
4	↑	784.0	0.053	
5	↑	734.0	0.051	
6	↑	658.0	0.053	
7	↑	206.0	1.028	

Figure 3. UV-Vis spectrum of Chitosan- flavonoid nano-compound.

2. SEM

The produced chitosan-alkaloid nanoparticles' size and shape were determined using this measurement. The measurement revealed that regular sphere-like particles, as shown in Fig. 4, were present. Additionally, as shown in Fig. 5, the measurement demonstrated that the prepared particles' size was within the nanoscale range, which spans 1 to 100 nanometers. The prepared particles' size ranged from 22 to 97 nanometers. It was noted that the average particle size as obtained by SEM and the particle size as determined by Scherer's equation are quite close.

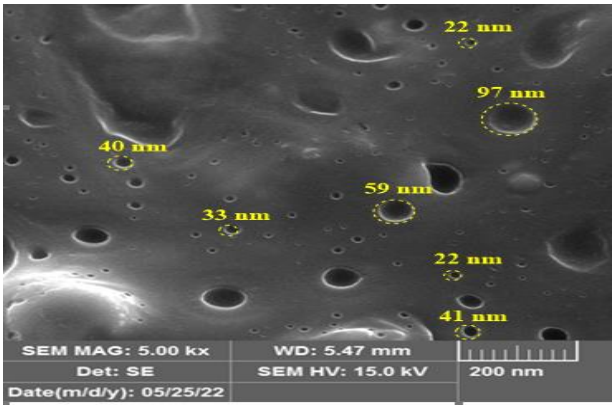

Figure 4. SEM of Chitosan- flavonoid nano-compound.

3-TEM

The characterization of the flavonoids-coated chitosan nanoparticles was studied using TEM. In accordance with the findings displayed in Fig. 5(A,B), chitosan nanoparticles (NPs) are exhibited as semi- or spherical nanoparticles with an average size of nearly 25–65 nm. FLavonoides, on the other hand, resemble erratic forms. The combination of Chitosan NPs and flavonoids is demonstrated by coated nanoclusters with spherical forms as shown in Fig. (5(D), [32].

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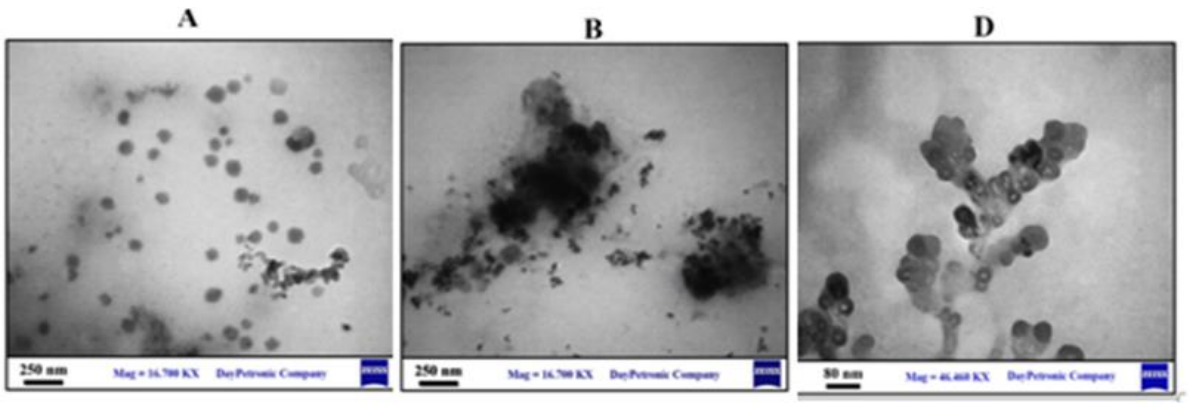

Figure 5. TEM images of A, Chitosan nanoparticles. B, Flavonoid., D, flavonoid coated Chitosan NPs.

Anticancer activity of compounds

When doing colorimetric experiments to determine cytotoxicity or cell viability, it is clear that MTT (3-(4,dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) is one of the chemicals that are most frequently used[33]. This assay identified the cell viability of the examined cell lines in response to treatment with flavonoids, Nano flavonoids. (Table 3,4,5 /Fig. 6,7,8). When MTT enters metabolically active cells, it is transformed into insoluble purple MTT-formazan crystals, which are then dissolved in a solvent and spectrophotometrically assessed[34]. Flavonoids used in the current study compared to MCF10, nano flavonoids showed more effectiveness against MCF-7 and PC3. Fig. 1-6 illustrates that when cells were exposed to nano flavonoids, there was dose-dependent cellular damage. The cytotoxicity of flavonoids against MCF-7 was the highest (71.66%) and the highest for nano-flavonoids was (90.67%). Flavonoids and nano-flavonoids had the maximum cytotoxicity against PC3 at 67% and 84.33%, respectively. The results against the typical cell line (MCF-10 cells) revealed a negligible cytotoxic effect. This effect of flavonoids and nano-flavonoids on NCF-7 and PC-3 cells may be indicative of a mechanism that inhibits proliferation by triggering apoptosis[35]. In contrast, MCF-10 showed no signs of such action. When compared to cancer cells, normal cells rate of proliferation showed less evidence of harmful effects. this study is consistent with [36-38] Flavonoids have a wide range of anticancer properties, including the ability to regulate ROS-scavenging enzyme activity, participate in cell cycle arrest, reduce the proliferation or invasiveness of cancer cells, and trigger apoptosis[39]**.**

Table 3. Anticancer activity of flavo. and nanoflavo. against MCF-7 cells.

Concentration µg/ml	Cell line		LSD value
	MCF7/ flavonoid	MCF7/ nanoflavonoid	
3.1	9.00 ±0.58 F b	16.67 ±1.45 F a	**4.340****
6.25	19.66 ±1.45 E b	28.33 ±2.33 E a	**5.03 ****
12.5	30.33 ±0.88 D b	45.00 ±2.31 D a	**5.92 ****
25	48.67 ±2.40 C b	66.67 ±1.20 C a	**6.33 ****
50	64.33 ±1.45 B b	81.66 ±2.33 B a	**5.42 ****
100	71.66 ±1.45 A b	90.67 ±1.45 A a	**5.39 ****
LSD value	4.574 **	5.885 **	---

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

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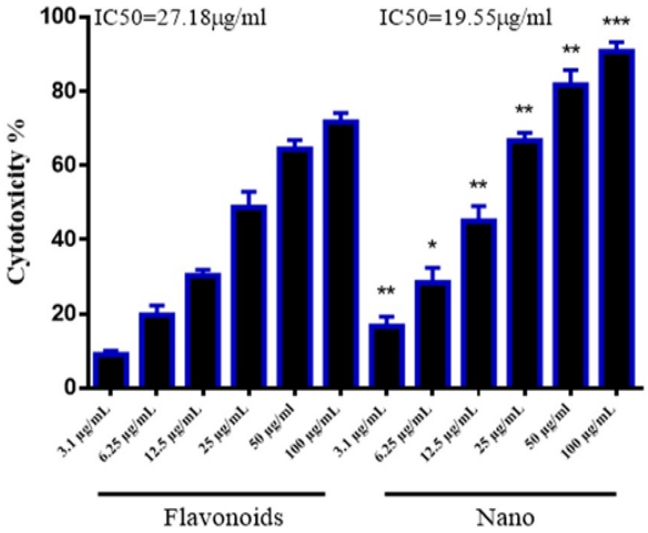

Figure 6. Anticancer activity of flavo. and nanoflavo. against MCF-7 cells.

Table 4. Anticancer activity of flavo. and nanoflavo. against PC3 cells

Concentration µg/ml	Cell line		LSD value
	PC3/flavonoid	PC3/nanoflavonoid	
3.1	6.33 ±0.67 F b	13.33 ±1.20 F a	**3.46 ****
6.25	15.67 ±0.88 E b	22.67 ±1.20 E a	**4.73 ****
12.5	26.00 ±2.08 D b	39.67 ±2.40 D a	**4.87 ****
25	44.33 ±1.76 C b	61.66 ±1.45 C a	**6.02 ****
50	60.33 ±1.20 B b	78.33 ±1.20 B a	**5.19 ****
100	67.00 ±1.52 A b	84.33 ±1.76 A a	**5.87 ****
LSD value	4.377 **	4.925 **	---

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

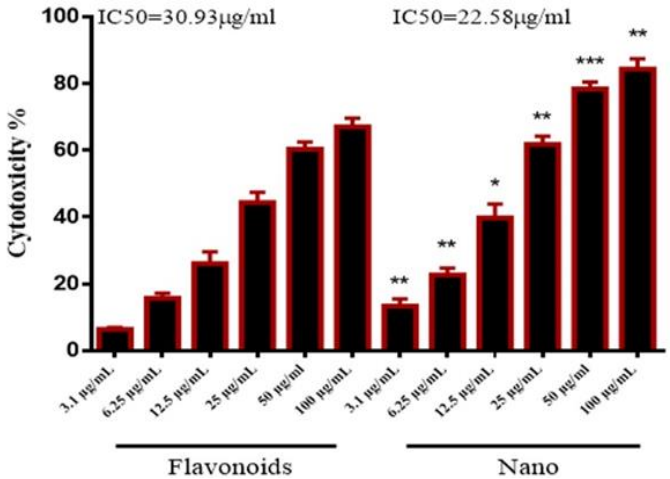

Figure 7. Anticancer activity of flavo. and nanoflavo. against PC3 cells.Table 5. Anticancer activity of flavo. and nanoflavo. against MCF-10 cells.

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

Concentration µg/ml	Cell line		LSD value
	MCF10/ flavonoid	MCF10/ nanoflavonoid	
3.1	2.00 ±0.57 D a	2.67 ±0.33 D a	**1.851NS**
6.25	3.33 ±0.88 CD a	4.00 ±0.57 D a	**1.37 NS**
12.5	5.66 ±0.88 BC a	6.33 ±0.67 C a	**1.62 NS**
25	8.33 ±0.88 AB a	10.00 ±0.58 B a	**1.76 NS**
50	10.66 ±0.88 A a	11.00 ±0.57 AB a	**1.81 NS**
100	11.00±1.52 A a	12.33 ±1.20 A a	**1.59 NS**
LSD value	3.024 **	2.178 **	---

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

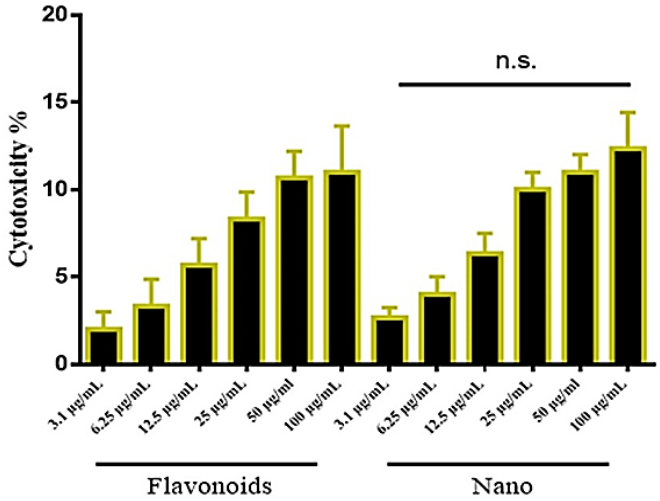

Figure 8. Anticancer activity of flavo. and nanoflavo. against MCF-10 cells.

Morphological changes in cancer cells

Morphological changes were used to investigate the cytotoxicity effect of the tested cells, as shown (Figure 9,10,11). The findings showed that nano-flavonoids and flavonoids have less cytotoxicity against MCF-7 and PC3 cells. Normal cells (MCF-10) did not exhibit any such modifications. When compared to cancer cells, normal cells proliferated at a lower rate and showed fewer cytotoxic effects. The basis of the crystal violet test is the dye's affinity for the outside of the DNA double helix. It is feasible to determine the estimated number of viable cells by correlating the dye absorption and the culture's overall DNA content[40]. Other alterations caused by flavonoids and nano flavonoids, according to the research, were modifications to cell structure, clumping of treated cells with few cellular extensions, and cell communication inhibition. Cells going through cell death create less crystal violet staining in culture because they lose their adherence and are subsequently eliminated from the population of cells. Cells were treated with nano flavonoids, although there was less change than what was shown in the control cells that weren't treated. Numerous studies suggest that the fatal effects of flavonoids and nano-flavonoids on cancer cells may be caused by the membrane-disrupting, accumulation of lactic acid, reactive oxygen species (ROS), DNA damage, and apoptosis-inducing activities of these substances[41]. our results in consistent with [42]When treated with flavonoids and nano-flavonoids, the noticeable morphological alterations in the cancer

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cells section were indicated by a decrease in the number of darkly pigmented cells, cell disintegration, irregular form, cell lysis, and necrotic cells because of the onset of programmed cell death.

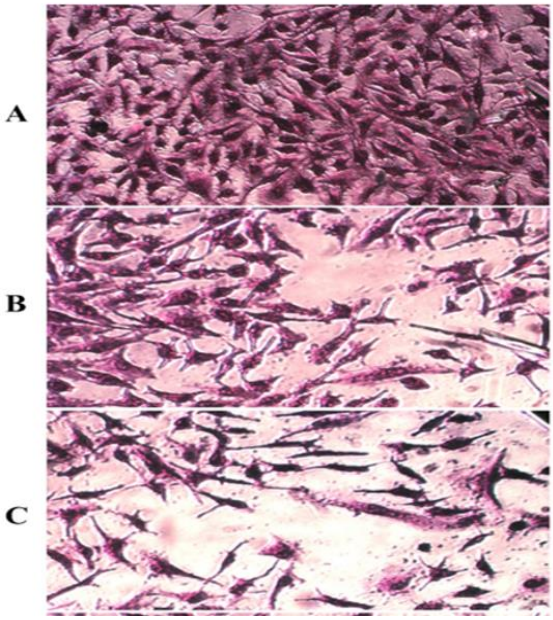

Figure 9. MCF-7 cell morphological alterations as shown. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells

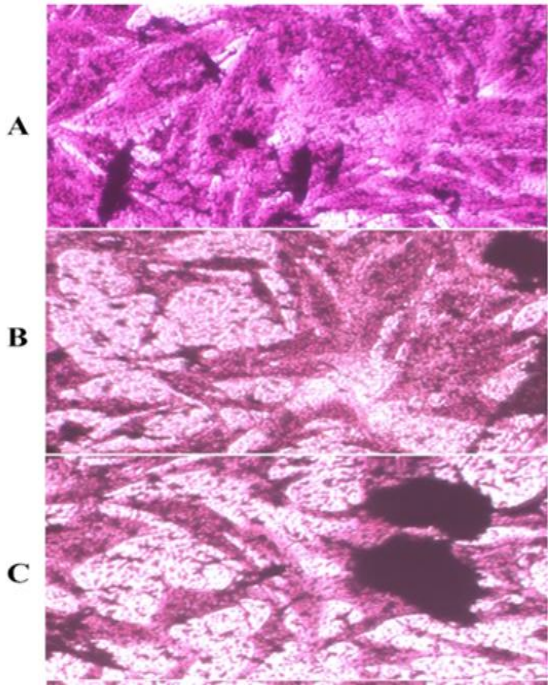

Figure 10. The suggested morphological alterations in PC3 cells. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells

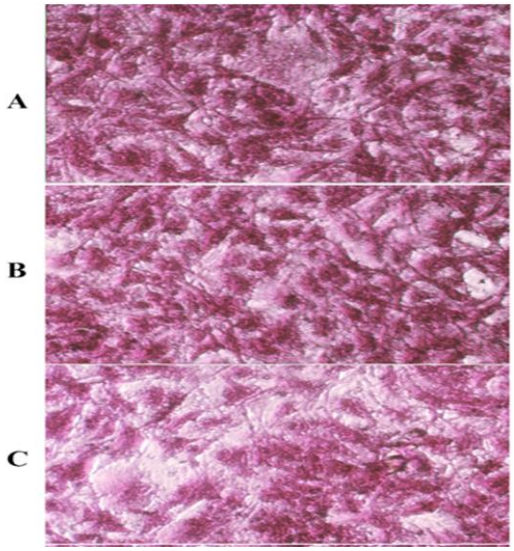

Figure 11. MCF-10 cell morphological changes as shown. A, untreated control cells. B, cells received a flav treatment. C, Nanofalvo was used to treat the cells.

Results of the double acridine orange-ethidium bromide staining

Utilizing the dual staining test in MCF-7, PC3, and MCF-10 cells with AO and EtBr dyes, it was possible to determine the ability of IC50 formulations of flavonoids and nano-flavonoids to produce cytotoxicity (Fig. 12,13,14). AO/EtBr dual staining fluorescence microscopy was used to evaluate and further confirm the changes in nuclear morphology in MCF-7, PC3, and MCF-10 cells. In comparison to untreated control cells, these cells displayed a more pronounced breakdown of membrane stability after being exposed to flavonoids and nano-flavonoids. Examining changes in fluorescence and morphology of treated cells was an efficient way to spot the impressively induced changes, such as chromatin condensation and the appearance of red staining in the cytoplasm, which reflect changes in RNA and lysosomes in cells exposed to drugs. Early apoptotic cells have a yellow tint in their nuclei, and their chromatin is either condensed or fragmented. However, the chromatin of late apoptotic cells is either fragmented or condensed, and the nuclei of these cells are orange to red in hue. Apoptotic cell death was discovered by AO/EtBr based on investigation of the nuclear morphology of the tested cells. The fact that AO can stain both live and dead cells should not be overlooked. Contrarily, EtBr is only employed to stain the DNA of cells whose membrane integrity has been disrupted. Previously,

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it was shown that the treated cells were recorded as dead (red color), indicating that it had a cytotoxic impact, whereas the live cells (green color) had a uniform distribution and huge nuclei[43]. By causing cell cycle arrest or apoptosis in oral squamous cancer cells, human esophagus, lung, liver, colon, and prostate cancer cells, flavonoid, nano-flavinoid can have anti-cancer effects. By simultaneously activating anti-apoptotic proteins and decreasing pro-apoptotic proteins and caspases, flavonoids can trigger cell death signaling pathways in cancer cells[44, 45]. this study is consistent with[46] After the treatment cellS (MCF-7) with flavonoids, we noticed fragmented and pyknotic nuclei with condensed chromatin, which became more noticeable after a longer incubation period.

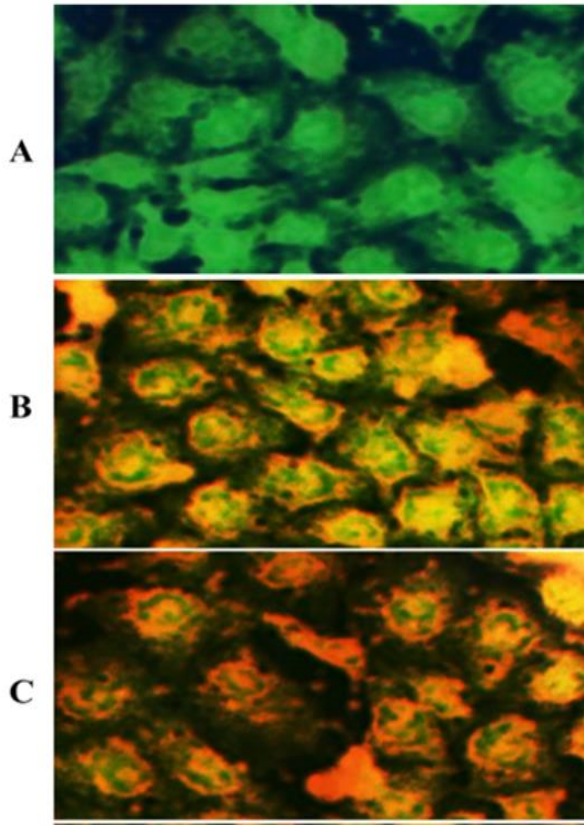

Figure 12. Apoptosis markers in MCF-7 cells using AO/EtBr staining. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells.

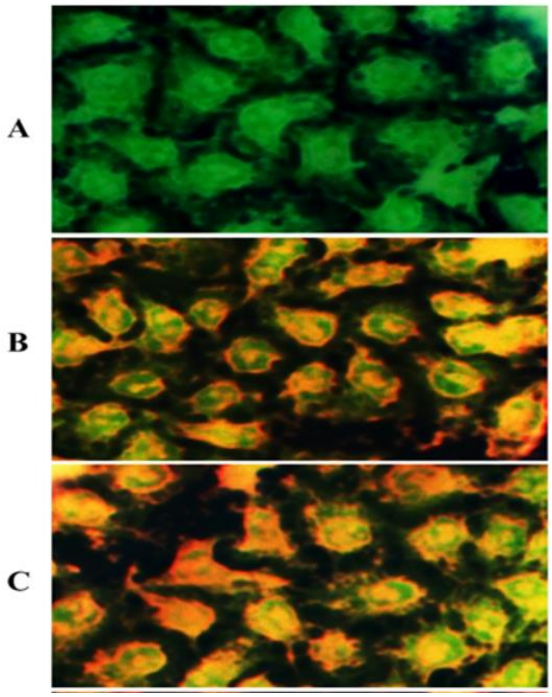

Figure 13. Apoptosis markers in PC3 cells using AO/EtBr staining. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells.

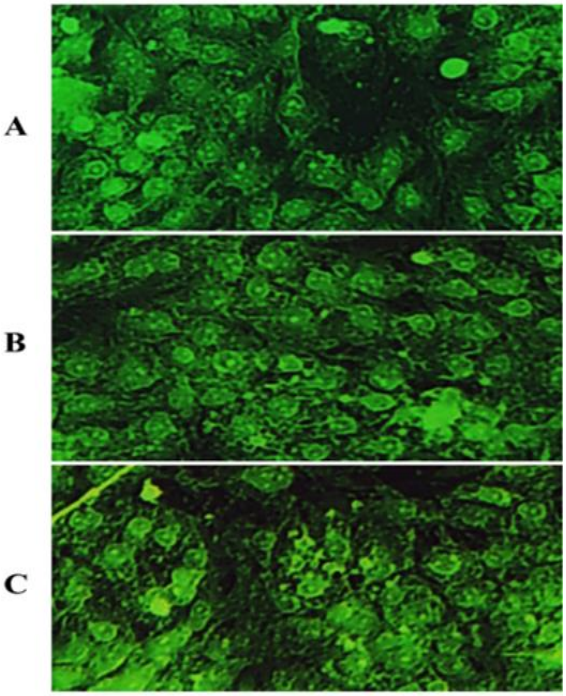

Figure 14 . AO/EtBr labeling of MCF-10 cells to identify apoptosis markers. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells.

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Flavonoids, Nano flavonoids and their impact on DNA content

In the current investigation, an additional experiment was conducted to look into the potential inductive function of flavonoids, particularly nano-flavonoids, in apoptosis. After labeling the cellular DNA of treated MCF-7 and PC3 cells with PI, flow cytometry was used to determine the DNA content in the sub-G1 phase. The outcomes showed that treatment with flavonoids, nano flavonoids, or both raised the proportion of cancer cells in the sub-G1 phase in MCF-7 cells from 2.11% to 34.74% (Fig. 15). Generally, the findings of this study showed that manufactured flavonoids, or nano flavonoids, had an anti-proliferative effect via inducing apoptotic cell death[47, 48].

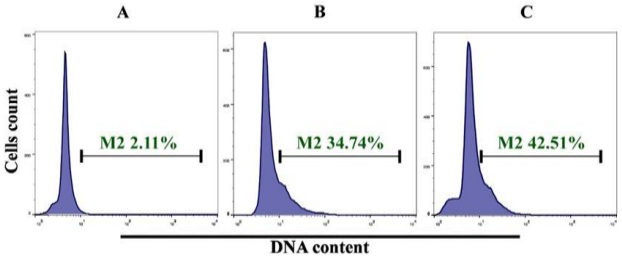

Figure 15. The DNA cycle in MCF-7 cells follow the recommended treatment. A, untreated control cells. B, Nanofalvo was used to treat cells; C, Flav was used to treat cells.

Conclusion

In the past two decades, there has been a lot of research on the potential of using natural components as chemo preventive agents against cancer. According to studies, using anti-cancer phytochemicals with various mechanisms or modes of action may be more efficient in treating diseases and reducing side effects. Flavonoids could have an important role in the prevention and treatment of diseases like cancer, as well as nutritional supplements. The outcomes demonstrated that flavonoids and nano-flavonoid are effective chemotherapeutic agents that can be employed alone or in combination with other agents to treat various cancer cell types.

Authors' 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 re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

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:

Dr. Bushra M.J. Alwash is an Editor for the journal but did not participate in the peer review process other than as an author. The authors declare no other conflict of interest.

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عزل وتشخيص المركبات الفينولية من نبات شوكة المسيح المزروع في العراق وتقييم تاثيرها الوراثي على نوعين من الخطوط السرطانية

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الخلاصة

يعتبر "تاج الأشواك" أو نبات شوكة المسيح، وهو من نباتات الزينة الطبية ، ينتمي إلى جنس يوفوربيا. E. milii يحتوي كميات وفيرة من المركبات الفينولية ، التربينات، الستيرويدات والقلويدات. كانت الأهداف الرئيسية لهذه الدراسة هي فحص مستخلصات الفلافونويد والنانو فلافونويد ضد نوعين من خطوط الخلايا السرطانية. تم تصنيع مركبات الفلافونويد النانوية عن طريق تفاعل مركب الكيتوسان والماليك اسد. تم تحليل مركبات الفلافونويد النانوية بواسطة مقياس الطيف الضوئي UV-sp -8001 بطول موجي 200-1000 نانومتر ، تم استخدام المجهر الإلكتروني (TEM) والمجهري الإلكتروني الماسح(SEM) لتحديد الخصائص المورفولوجية لمركبات الفلافونويد النانوية. يعتبر الفلافونويد والنانوفلافونويدعقار قوي ومتطور ضد خلايا سرطان الثدي (MCF-7) وخلايا سرطان البروستات(PC3) . تم فحص الفعالية المضادة للسرطان من مركبات الفلافونويد والنانوفلافونويد على خطين مختلفين من الخلايا السرطانية وكذلك خطوط الخلايا السليمة باستخدام اختبار MTT. تم استخدام تجزئة الحمض النووي وتقنية تلوين الخلايا بالصبغة المزدوجة AO / EtBr لفحص علامات موت الخلايا المبرمج. لتحديد تشتت دورة الخلية ، تم استخدام قياس التدفق الخلوي ، وأظهرت النتائج أن كميات الفلافونويد والنانوفلافونويد لها نشاطا ساما وفعالا ضد خطوط خلايا سرطان الثدي والبروستات. بالإضافة إلى ذلك ، تم ربط إيقاف دورة الخلية في مرحلة G0 / G1 بموت الخلايا المبرمج لخطوط الخلايا التي يسببها الفلافونويد والنانوفلافونويد. وفقًا لهذه النتائج ، تمنع مركبات الفلافونويد والنانوفلافونويد من تكاثر خطوط الخلايا السرطانية ، مما يؤدي إلى توقف دورة الخلية وتحفيز موت الخلايا المبرمج. تشير النتائج المتاحة إلى أن مركب الفلافونويد والنانوفلافونويد سيمثل نهجًا علاجيًا واعدًا لعلاج الخلايا السرطانية من الأنواع الأخرى.

الكلمات المفتاحية: نشاط مضاد للسرطان ،موت الخلايا المبرمج، تفتيت الحمض النووي ، الفلافونويد والنانوفلافونويد.