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Phytochemical Analysis and Prostate Cancer Cytotoxicity of Iraqi *Apium graveolens*: A GC-MS Approach

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Abstract

Apium graveolens has been utilized for a multitude of purposes due to its diverse pharmacological characteristics. On the other hand, little is known about how the fatty acids (saturated and unsaturated) terpenes and steroids found in Iraqi *Apium graveolens* affect human cancer cells. The purpose of this study was to examine the effects of Iraqi *Apium graveolens* petroleum ether extract on the human prostate cancer cell line (PC3). Subsidiary extraction and phytochemical analysis by GC/MS were performed. The dry and fresh aerial parts (leaves and stem) of *Apium graveolens* were extracted using a Soxhlet device with 70 % ethanol, then fractionated with petroleum ether. Then, a Gas Chromatography System was used to identify the bioactive components, and The MTT assay was performed in human prostate cancer cell line (PC3) that was treated with different concentrations of the petroleum ether extract of both the fresh and dried parts (250, 500, and 1000µg/ml) and the results were compared to docetaxel as a control drug after 24,48, and 72 hrs of treatment, and cell viability was evaluated. The fresh part of the of Iraqi *Apium graveolens* was richer with important saturated fatty acids, steroids and terpenes more than the dry part. Furthermore, the fresh part showed a considerable cytotoxic activity in the human prostate cancer cell line (PC3), which is a concentration and time dependent effect; The half-maximum inhibitory concentration (IC50) values were reduced over time from 1549, 183.6, to 26.45µg/ml, and the cytotoxicity was increased with a remarkable reduction in the cancer cell viability. The fresh part of the petroleum ether fraction of Iraqi *Apium graveolens* has potential anticancer activity in prostate cancer more than the dried part. This result opens the minds to the fresh plant era and challenges the traditional belief that the dry plants produce better results than the fresh ones.

Keywords: Apium graveolens, Celery, Docetaxel, Dry part, Fresh part, PC3

Introduction

Prostate cancer (PCa) is the second most common type of non-skin solid cancer next to lung cancer and the fifth leading cause of cancer-related death in men. PCa mostly affects the population above 55 years old. Men over 65 years are predisposed by 65% to the disease, which is 25% in men older than 75 years [(1)]. Family history is a remarkable risk factor for PCa, as well as the wrong lifestyle, including a diet high in red or processed meat, sugar, and fat and low in healthy food, including fruits and vegetables. [(2,3)]. Despite significant progress with the available chemotherapy for prostate cancer, the toxicity towards healthy normal cells or non-specificity of these treatments remained a crucial constraint for treating PCa patients. Therefore, herbal medicines have gained very high popularity since early civilization because of their proven, wide pharmacological activity, including their anti-cancer effect [(4,5)]. One of these crucial plants that can be used as a treatment is celery, which revealed significant cytotoxicity effects against various tumor cell lines [(6-8)]. Celery, (*Apium graveolens* L.) is an indigenous plant of the family Apiaceae, or Umbelliferae, originating in the Mediterranean, and growing throughout Europe and the tropical and subtropical regions of Africa and Asia [(9-11)].

The generic name, *Apium* is derived from the Latin word "apis" meaning "bee", as its small white flowers have an attraction for the bees. The species name "*graveolens*" means "heavy-scented". Our English word "celery" is derived from the Latin word "celer" which means "swift" as celery is considered a fast-acting remedy [(12)]. Celery is an annual or biennial plant that grows to a height of 12 to 16 inches and is composed of leaf-topped stalks arranged in a conical shape joined at a common

base. Celery seed is very small, about 1.3 mm long, brown, ovoid, and ridged. The fruit is made up of two united carpels, each containing a seed [13]. It is widely distributed throughout the world and is known by different names in different regions of the world. In English, it is typically called "Celery", in Persian, it is called "Karafs" and it is known as "Apio" in Spanish.

It is commonly known as "Sellerie" in German. In Arabic, it is known as "Alkarafs". Celery is known as "Khuen chaai" in Thailand [14]. It is called "Tukhme karafs" in Urdu. *Apium graveolens* is commonly known as "Ajmod" in Hindi and the fruits are popularly known as "celery seeds"[15]. Celery has been used in traditional medication to manage stomach aches and spasms, as well as a laxative, diuretic, and sedative. This herb can also be employed as a heart tonic to lower blood pressure in traditional African medicine in Tobago and Trinidad [16]. In addition, a report stated that celery can be used to treat joint problems [17]. In traditional medicine, celery seeds can be used as a libido stimulant because of their role in strengthening sperm cells and as a protector against sodium valproate in the testes [18]. Celery seeds can also be used to increase breast milk secretion [19].

Celery has broad pharmacological activities such as antimicrobial activity, antifungal activity, antiparasite activity, anti-inflammatory activity, antiulcer activity, antioxidant activity, antidiabetic activity, anti-infertility activity, antiplatelet activity, antispasmodic effect, hepatoprotective activity, cardioprotective activity, neuroprotective activity, cytoprotective activity, hypolipidemic activity, analgesic activity, and anti-cancer activity [20]. Several phytochemical examinations for the constituents of *Apium graveolens* and different parts of the plant have been studied. Flavonoids, glycosides, coumarins, alkaloids, and carbohydrates are the most well researched chemical constituents have been detected [20,21].

While there are only limited phytochemical investigations that deal with terpenes and steroids in leaves, flowers, and stems (aerial parts) of *Apium graveolens* species. Therefore, the concept of the study concerned about terpenes and steroids in the Iraqi *Apium graveolens*, which contains a variety of pharmacological actions used in complementary medicine and has many potential uses in the future, embarking through extraction, phytochemical analysis by GC/MS, and cytotoxicity against the human prostate cancer cell line (PC3) via Iraqi *Apium graveolens* petroleum ether aerial parts extract.

Materials and Methods

Apparatus and Instrument

The following were used: rotatory evaporator type Buchi attached to a vacuum pump Buchi-Germany, an electrically sensitive balance (Sartorius, Germany), GC/MS type Agilent ١٩٠٩١S-433UI, cell culture plates (Santa Cruz Biotechnology, USA), CO_2 incubator (Cypress Diagnostics, Belgium), laminar flow hood (K and K Scientific Supplier, Korea), Micropipette (Cypress Diagnostics, Belgium), and microtiter reader (Gennex Laboratories, USA).

Chemicals and Reagents

Absolute ethanol and petroleum ether were purchased from Schar Lab S.L.-Spain. MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide was purchased from Promega (USA), while Docetaxel was supplied by Shanghai Roche Pharmaceuticals Ltd., China. Docetaxel and petroleum ether were dissolved in dimethyl sulfoxide (DMSO) (Zhengzhou Meyia Chemical Products Co., Ltd). To make the MTT stock solution, the MTT was diluted in phosphate buffer saline (PBS) at a rate of 5 mg/ml. To sterilize this solution, filtration with a 0.22µm filter unit was used. DMSO solvent was used to solubilize the formazan crystals at a concentration of 0.1% v/v in each well of the microtiter plate. Antibiotics (100 U/ mL penicillin and 100 µg/mL), RPMI-1640 medium, and fetal bovine serum (FBS) were purchased from Euroclone (Italy's Euroclone). All compounds were filter sterilized using a 0.22 µM Millipore syringe filter.

Plant material collection

The aerial parts of the *Apium graveolens* plant were collected from Baghdad city, at the College of Pharmacy, University of Baghdad farm in December 202٣. The plant was identified and authenticated by the Baghdad University Herbarium of the Department of Biology, College of Science at the University of Baghdad, registered at BUH No. ٨٩٦7, under the supervision of Prof. Dr. Sukaena Abass. Then the aerial parts of the plant were divided into two parts, one was dried under shade, and the other was used fresh [22].

Preparation of Apium graveolens extract

Extraction was performed according to the process with some modifications described by Al-Baaj A.S. [23]. The extraction was done for dry and fresh aerial parts (leaves and stem) of *Apium graveolens* L. by taking 100 gm of each dry plant and fresh parts, which were then extracted using a soxhlet device with 70 % ethanol. The extracted crudes were concentrated under reduced pressure at 40°C, then suspended in water and fractionated with 150 ml of petroleum ether three times. The yield of the vacuum-dried petroleum ether was then saved for later analysis [24].

Phytochemical analysis by GC/MS

Petroleum ether extract of both dry and fresh parts of the plant was analyzed to identify the bioactive components using Gas Chromatography System employing the following condition: Capillary

column: HP-5ms ultra inert column (30 m length and 250 µm diameter and 0.25 µm film thickness), Oven temperature: increasing from 80 to 265 °C, Aux Heater: 310°C, Carrier gas: high purity helium (99.99 %) was used with a flow rate of 1.53 ml/min, Inlets temp:250 °C, Split method:45.5 cm/sec, pressure at 11.933 psi, Injector volume: 1 µL.

Apium graveolens petroleum ether extract cytotoxicity testing

The methodology described here is to investigate the effects of the petroleum ether extract of *Apium graveolens* on the viability of the human prostate cancer cell line (PC3) compared with docetaxel (a positive control since it has anti-cancer effect) and its effect on the human prostate cancer cell line by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) colorimetric assay, which is used to assess cell metabolic activity. Under some conditions, NADPH-dependent cellular oxidoreductase enzymes may represent the number of live cells. These enzymes can convert the tetrazolium dye MTT to an insoluble formazan with a purple color.

Cell culture

The American Type Culture Collection ATCC (USA) provided the human prostate cell carcinoma cell line PC3. These cells were then collected and stored in the cell bank of the tissue culture research center of AL-Mustansiriyah University/College of Pharmacy. 10% fetal bovine serum (FBS), 2M of L-glutamine, and 1% penicillin/streptomycin were added to the standard RPMI 1640 medium to complement it. A humidified 5% CO2 atmosphere was used to sustain the cell culture at 37 °C. The cells were grown as a monolayer for no more than 15 passages. To subculture the PC3 cell line, after washing with Phosphate Buffer Saline (PBS), cells were incubated with 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (EDTA) for a few minutes. Finally, trypsin was inactivated by adding fresh serum-containing medium to detached cells. A logarithmic growth phase was maintained for the cells. Mycoplasma contamination in cells was routinely checked.

Cell viability assay

The MTT assay was used to assess the cytotoxic effects of the petroleum ether fraction of *Apium graveolens* on the human prostate carcinoma cell line PC3. Cells were seeded in 96-well plates at a density of $5x\ 10^{-3}$ cells per well and cultured in a CO2 incubator for 24 hr., 48 hr., and 72 hrs. Following overnight culture, cells were then treated with petroleum ether extract (the plant extracts were dissolved in DMSO, then diluted with cell culture medium to different serial dilutions while the concentration of vehicle control (DMSO) was kept below 0.1%) and docetaxel. The cells were exposed to each compound for 48 hours before 10 µL of MTT reagent (Sigma Aldrich) was added to each well. The medium was removed after 4 hrs. of incubation (37 ℃, 5% CO2) to generate formazan crystals, which were then dissolved in 100 µL of DMSO solution. The optical density of each well was measured at 53 nm using a Glomax microplate reader (Promega USA.). Each individual experiment was achieved in three replications. The following formula was used to calculate cell viability: Cell viability (%) = (absorption of treated cells) / (absorption of untreated cells) X 100. Graph Pad Prism 9.2 (Graph Pad Software Inc., USA) was used to calculate the IC50 (concentration required for 50% cell inhibition) [25,26].

Morphological changes study

The morphology of PC3 cells was studied under an inverted microscope with 40x magnification (Optika, Italy); to view and record the morphological alterations of apoptotic cells. Apoptotic characteristics were identified after 48 hours of incubation by the appearance of cell shrinkage and/or the presence of membrane-bound cellular bodies.

Statistical analysis

The MTT assay data were expressed using the mean and standard deviation (mean± STD). The IC50 values for each were calculated using Graph Pad Prism 9.2 software. (Graph Pad Software Inc., USA) [25]. The one-way analysis of variance (ANOVA) test was used to compare the mean percentage of inhibition between different concentrations. Regression analysis was used to calculate the IC50 value from the percentage of inhibition. $P<0.05$ was considered significant.

Results and Discussion

Extraction and fractionation of Iraqi Apium graveolens

The use of a hot extraction method is preferable, as heat will facilitate the penetration of plant material by the solvent by breaking the plant tissue fibers [27]. Because crude extract contains diverse classes of chemical constituents with varying polarities, fractionation of crude extract is recommended for full phytochemical profile screening for a given plant. This allows the main classes of plant constituents to be isolated from each other based on differences in polarity and solubility before chromatographic analysis is performed [28]. Petroleum ether was used to fractionate crude extracts of Iraqi *Apium graveolens* fresh and dried aerial parts of the plant then each fraction was subjected to gas- liquid chromatographic technique in order to further separate, purify, and identify major phytochemical constituents. The percentage yield (%w/w) of each fraction of plant extract was determined and the results are shown in “Table 1”

Table 1. Percentage of Different Fractions Obtained from *Apium graveolens*

Analyzed fraction	Percentage yield (w/w)
Fresh ethanolic *Apium graveolens*	11.6 %
Dry ethanolic *Apium graveolens*	9.7 %
Fresh petroleum ether *Apium graveolens*	5.7 %
Dry petroleum ether *Apium graveolens*	4.5 %

Gas chromatography/mass spectroscopy analysis for the petroleum ether fraction of both fresh and dry parts of the Apium graveolens plant

A GC/MS analysis was performed on the petroleum ether extract. In the GC-MS analysis, 21,13 bioactive photochemical compounds were identified in the petroleum ether extract of Iraqi *Apium graveolens* fresh and dried aerial parts, respectively. The identification of phytochemical compounds is based on the peak area, molecular weight, and molecular formula; some of the GC-MS peaks remained unidentified because of a lack of authentic samples and library data for the corresponding compounds. The major nine compounds have been identified in the fresh petroleum ether fraction, with only major five compounds identified in the dry part. The GC-MS spectrum for both parts is shown in "Figures. 1 and 2" respectively, and the retention time, peak regions, molecular weights, chemical formulas, and classes of the identified compounds are listed in "Tables. 2 and 3" respectively, according to their elusion from the HP-5ms ultra inert column. The major phytochemical compounds of the fresh part were methyl palmitate (peak area 19.76%), methyl linoleate (peak area 15.70 %), phytol (peak area 15.51%), ethyl palmitate (peak area 6.73%), and oxirane (peak area 6.56%), compared with the major compounds identified in the dry part were phytol acetate (peak area 39.78%), cis-13-octadecenoic acid methyl ester (peak area 11.07%), and phytol (peak area 6.50%).

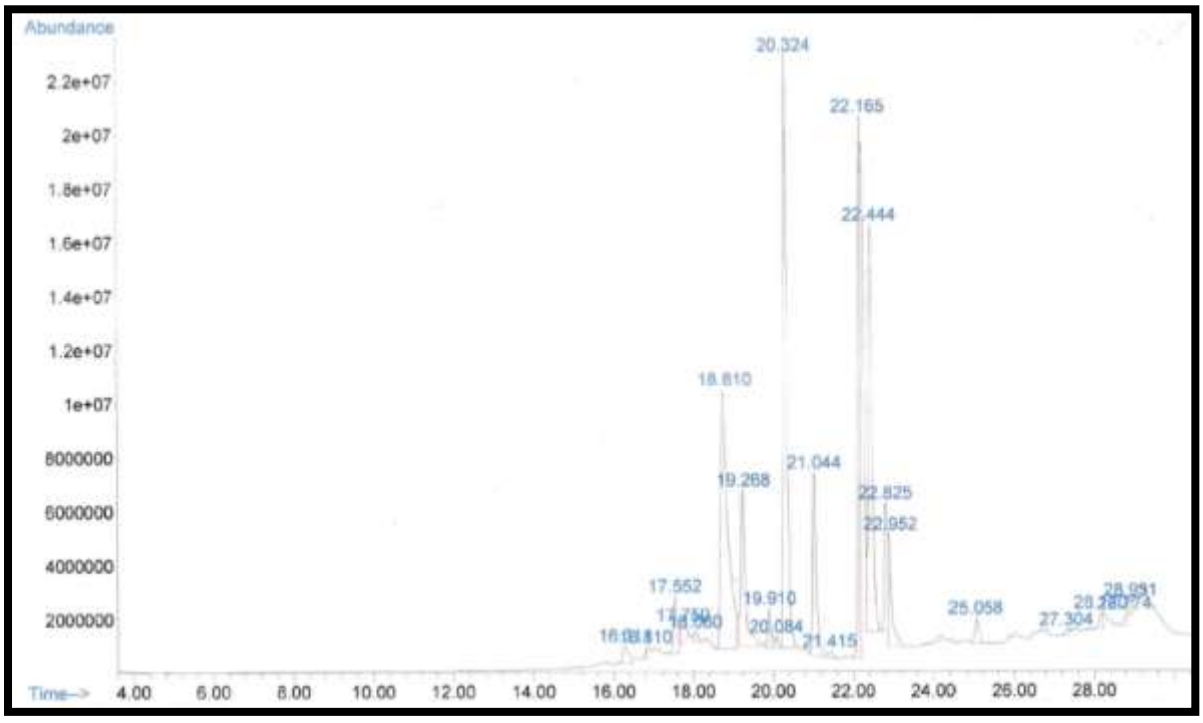

Figure 1. GC/MS chromatogram of the fresh petroleum ether fraction of Iraqi *Apium graveolens*.

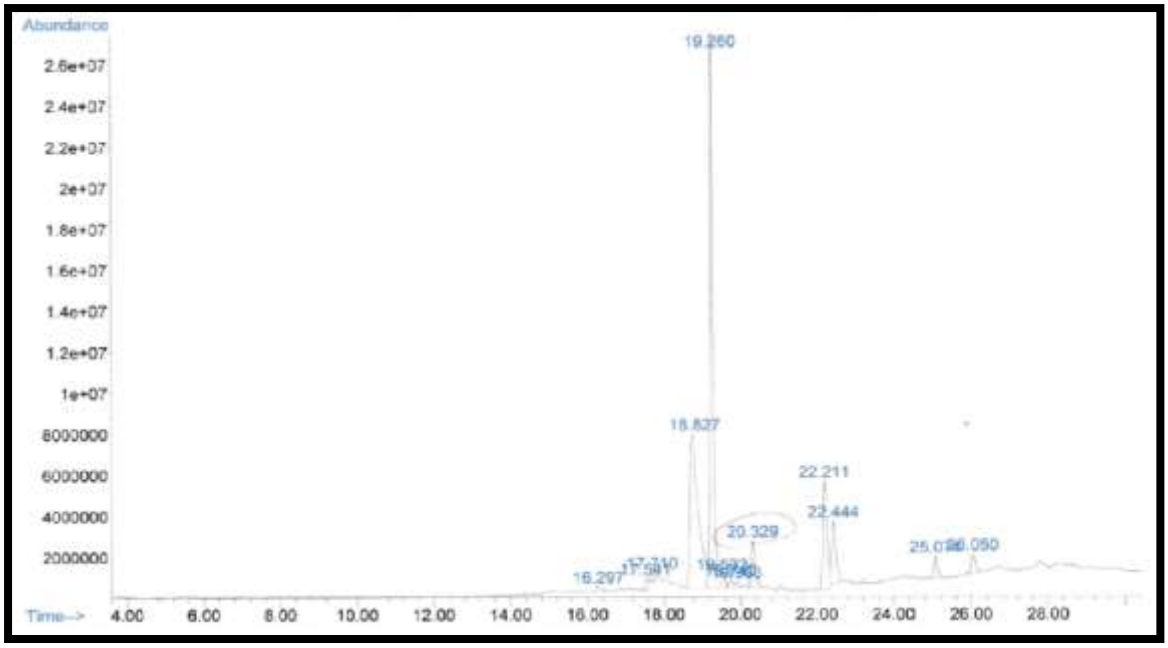

Figure 2. GC/MS chromatogram of the dried petroleum ether fraction of Iraqi *Apium graveolens*

Table 2. The result of GC/MS analysis was obtained from the petroleum ether fraction for the fresh part.

Peak No.	Retention time in Min.	Peak Area %	Chemical Formula	Molecular Weight	Compound Name	Similarity index	Class of Compounds
1	17.553	2.25	$C_{26}H_{54}$	366.707	Hexacosane	91%	Hydrocarbon lipid molecule
2	19.269	6.56	C_2H_4O	44.05	Oxirane	90%	Saturated organic heteromonocyclic
3	20.323	19.76	$C_{17}H_{34}O_2$	270.45	Methyl palmitate	99%	Fatty acid methyl ester of palmitic acid
4	21.045	6.73	$C_{18}H_{36}O_2$	284.484	Ethyl palmitate	98%	Fatty acid ethyl ester of palmitic acid
5	22.166	15.70	$C_{19}H_{34}O_2$	294.47	Methyl linoleate	99%	Fatty acid methyl ester of linoleic acid
6	22.447	15.51	$C_{20}H_{40}O$	296.53	Phytol	99%	Acyclic hydrogenated diterpene alcohol
7	22.956	3.32	$C_{20}H_{36}O_2$	308.5	Ethyl linoleate	99%	Fatty acid ethyl ester of linoleic acid
8	28.776	0.33	$C_{15}H_{24}O$	220.3505	Cis-Z-alpha-Bisabolene epoxide	90%	Sesquiterpenes
9	28.928	0.40	$C_{30}H_{50}O$	426.729	Lupeol	90%	Pentacyclic triterpenoid

Table 3. The result of GC/MS analysis was obtained from the petroleum ether fraction for the dry part.

Peak No.	Retention time in Min.	Peak Area %	Chemical Formula	Molecular Weight	Compound Name	Similarity index	Class of Compounds
1	19.261	39.78	$C_{22}H_{42}O_2$	338.6	Phytol, acetate	90%	Acyclic diterpene
2	19.532	1.32	$C_{10}H_{16}O$	152.2334	Alpha-Campholenal	90%	Monoterpene aldehyde
3	20.331	4.09	$C_{17}H_{34}O_2$	270.45	Methyl palmitate	98%	Fatty acid methyl ester of palmitic acid
4	22.208	11.07	$C_{19}H_{36}O$	296.4879	Cis-13-octadecenoic acid, methyl ester	99%	long-chain fatty acids
5	22.446	6.50	$C_{20}H_{40}O$	296.53	Phytol	93%	Acyclic hydrogenated diterpene alcohol

Petroleum ether fraction of both the fresh and the dried parts of the Iraqi celery plant was rich in methyl palmitate, which is a saturated fatty acid possesses a potent cardioprotective activity which exerted their effect through its significant antioxidant, anti-apoptotic, anti-inflammatory, and vasodilatation activities [29]. Phytol was another main compound in both parts of the plant. It is a valuable essential oil which was initially used as a fragrance, and also has a significant pharmacological importance. There are several publications suggesting that the cytotoxic activity of phytol is a concentration dependent action; it can stimulate both apoptosis and autophagy in human gastric adenocarcinoma AGS cells, and inhibit the growth of human T-cell leukaemia (Jurkat), counteract tumor development in a xenograft model of human lung adenocarcinoma in mice, and inhibit the invasiveness of the MDA-MB-231 cell line in breast cancer, and exert antitumor activity via apoptosis induction through activation of caspase-9/3 and inhibition of EMT in hepatocellular carcinoma cells. Furthermore, it has showen anticonvulsant antioxidant, anti-inflammatory, antimicrobial, sedative effects, and increased immunity [30]. Lupeol was detected by GC/MS in a trace amount in the fresh part only; however, it has a wide range of crucial pharmacological effects like anti-inflammatory, anti-microbial, anti-protozoal, cholesterol-lowering agent, cytotoxicity, anti-proliferative, anti-invasive, and anti-angiogenic agent [31]. Both fatty acid esters of linoleic acid (methyl linoleate, and ethyl linoleate) were contained in the fresh part with different uses, in which ethyl linoleate was known as a skin-whitening agent, anti-acne agent, and was used in many cosmetics for its antibacterial and anti-inflammatory properties [32]. On the other hand, the antifungal activity of methyl linoleate was exerted mainly against *Paracoccidioides* spp. with MIC values ranging from 15.6–250 µg/mL. as well as exhibiting antifungal activity with an MIC of 15.6 µg/mL against *Candida glabrata* and *C. krusei* and 31.2 µg/mL against *C. parapsilosis*. In addition, their potent candidate as an antioxidant agent [33].

Cytotoxic effect of Iraqi Apium graveolens against human prostate cancer cells

The cytotoxic test was performed to assess the toxicity of the fresh, and the dry parts of the petroleum ether fraction of *Apium graveolens* aerial parts using three different concentrations (250, 500, 1000 µg/ml) as compared with the same three concentrations of the docetaxel drug on the human prostate cancer cell line by the MTT test. The cytotoxicity results are illustrated in "Table 4", and "Figures. 3 and 4" show the IC50 for docetaxel when measured after 24hr., 48hr. ,and 72hr. was equal to (~181902, 97.31, 8.844 µg/dl) respectively, and these concentrations were lower compared to the plant extract, in which it found the IC50 for the fresh extract were (1549, 183.6, 26.45 µg/dl) respectively, while the dry part were (2529, 654.6, 12.19 µg/dl). According to the results for docetaxel and the plant extract, the fresh part is more potent than the dry part according to the amount of drug required to produce the cytotoxic effect.

Table 4. The IC50 for the fresh and dry parts and Docetaxel on the PC3 cell line after 24 hr., 48 hr., and 72 hr.

Sample	IC50 after 24 hr.	IC50 after 48 hr.	IC50 after 72 hr.
Docetaxel (control)	~181902	97.31	8.844
Fresh part	1549	183.6	26.45
Dry part	2529	654.6	32.19

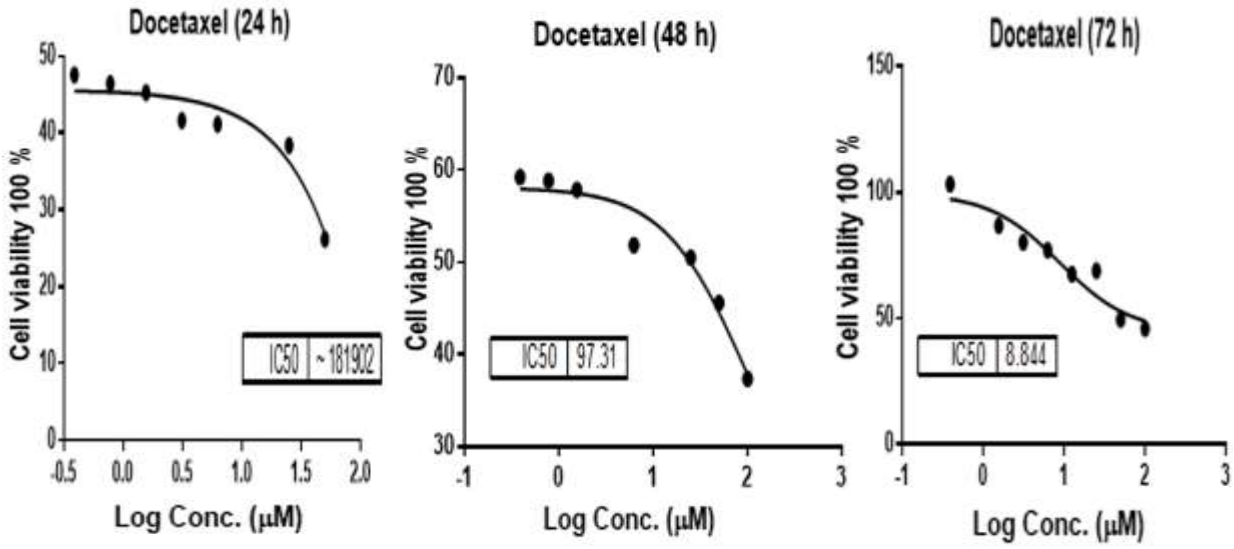

Figure 3. Dose-response curves of IC50 for assessment of the effect of Docetaxel against the PC3 cell line after 24, 48, and 72 hr.

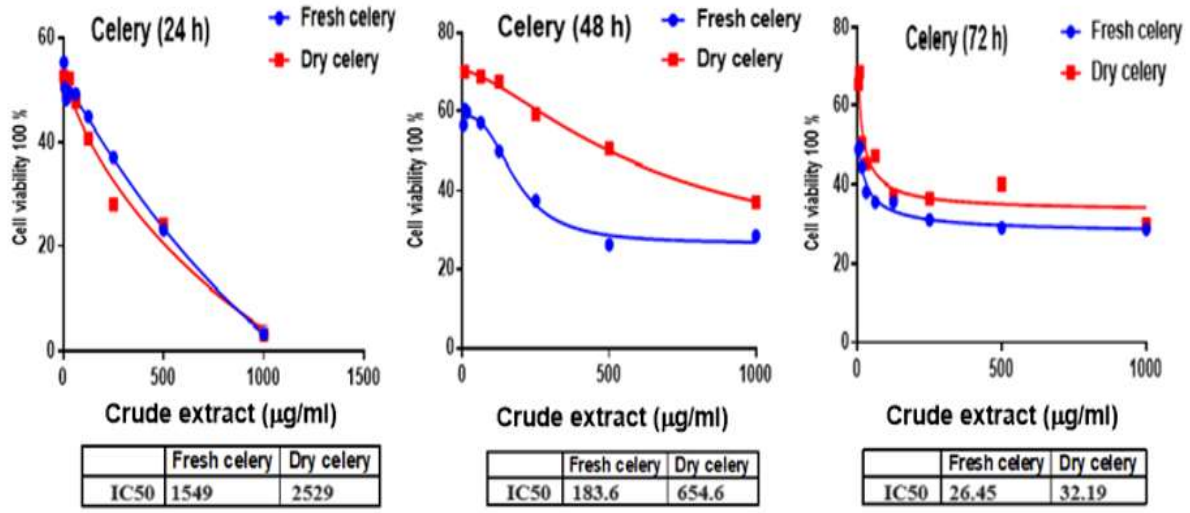

Figure 4. Dose-response curves of IC50 for assessment of the effect of the petroleum ether extract for the fresh and dry parts of *Apium graveolens* against the PC3 cell line after 24, 48, and 72 hr

In the MTT assay, as shown in "Figure. 5", the fresh extract showed significant toxicity ($p< 0.05$) towards the human prostate cancer cell line (PC3) at the three tested concentrations, with a greater effect at the higher concentration (1000 µg/ml), and as the time increased, the cytotoxicity increased. While the dry part exerted a remarkable effect only at a higher concentration after 72 hr. According to the IC50 and the cytotoxicity effect, the effect of the fresh part is more significant than that of the dried part, and the effect is concentration and time dependent as illustrated in "Figure and Table 5".

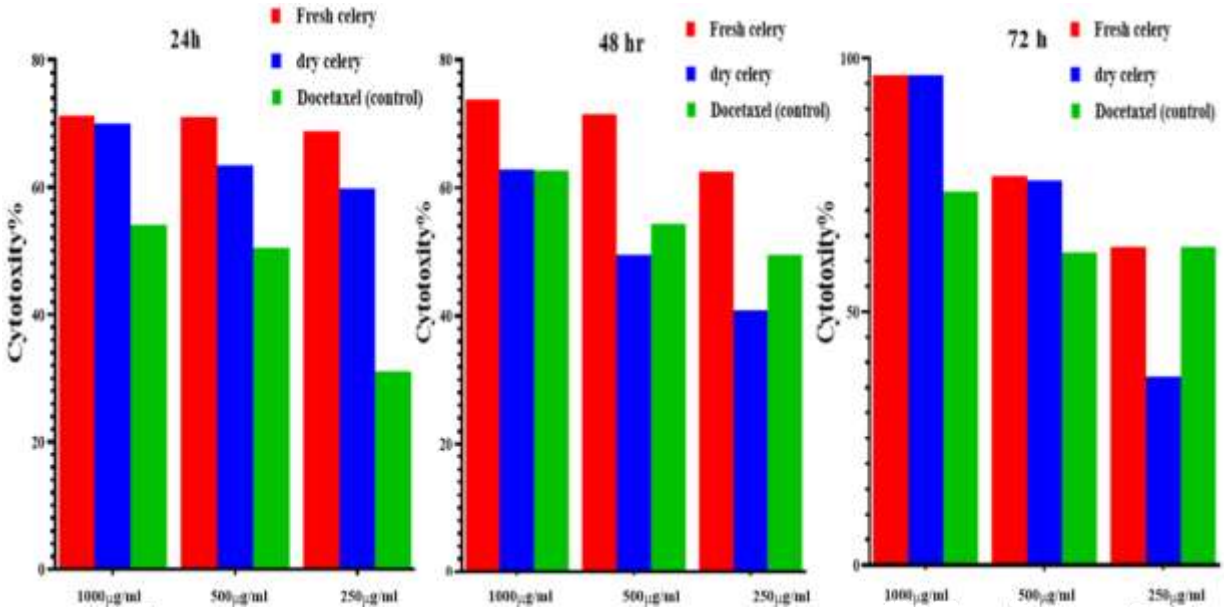

Figure 5. Cytotoxicity effects of the different concentrations (250, 500, and 1000 µg/ml) of the fresh and dry parts of *Apium graveolens* compared to docetaxel against the human prostate cancer cell line (PC3) after 24 hours, 48 hours, and 72 hours.

Table 5. % Cytotoxicity of the fresh and dry parts compared with Docetaxel at various concentrations after 24,48, and 72 hr.

Concentrations	Fresh part	Dry part	Docetaxel
After 24 hr.			
250 µg/ml	69%	59%	31%
500 µg/ml	70.5%	63%	51%
1000 µg/ml	71%	70%	54%
After 48 hr.			
250 µg/ml	62%	40%	49%
500 µg/ml	71%	49%	54%
1000 µg/ml	73.5%	62.5%	62%
After 72 hr.			
250 µg/ml	63%	37%	62%
500 µg/ml	77%	76%	61%
1000 µg/ml	97%	97%	74%

Morphological comparison of prostate cancer cell line (PC3) upon exposure to docetaxel and apium graveolens extract

As shown in "Figure. 6", there is a significant decrease in the number of viable cancer cells, as well as morphological alterations, including cell de-attachment and cell separation. Morphological comparison of the prostate cancer cell line (PC3) upon exposure to docetaxel, fresh, and dry parts of the plant show that both the fresh and dry parts were better than docetaxel in reducing the number of cancer cells, with remarkable cytotoxicity of the fresh part towards the cancer cell.

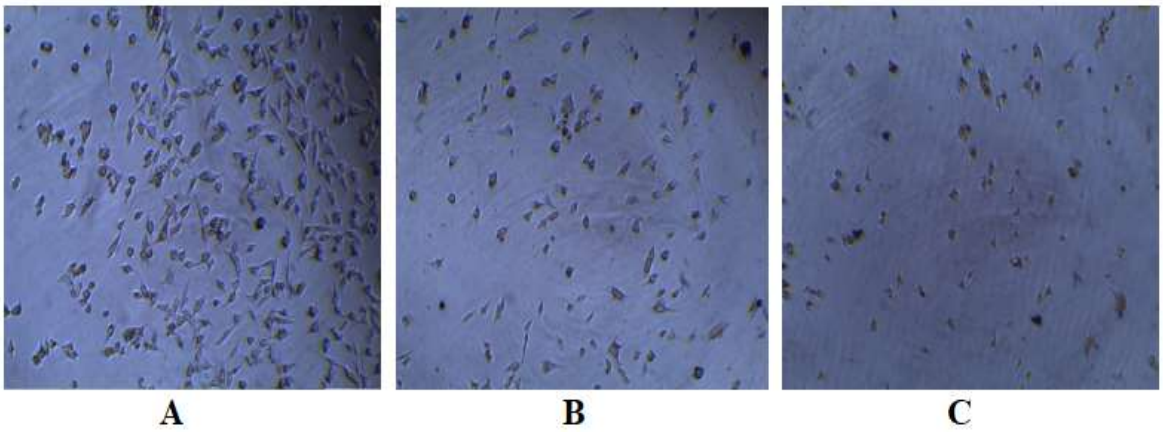

Figure 6. Effect of the Iraqi *Apium graveolens* petroleum ether extract and Docetaxel on the morphology of the Human Prostate Cancer Cell Line (PC3). A: After treatment with Docetaxel, B: After treatment with the dried part of the *Apium graveolens* petroleum ether extract, C: After treatment with the fresh part of the *Apium graveolens* petroleum ether extract.

The present study resulted in the identification of fatty acids (saturated), steroids, and terpenes (sesqui, di, and tri terpenes) in *Apium graveolens* petroleum ether extract, and these compounds have been identified in previous studies as well as known for their efficacy against various cancer cells [34-36]. Therefore, the reported cytotoxic effect of the Iraqi *Apium graveolens* can be attributed to the active component present in the extract; research by Song and coworkers demonstrated the efficacy of the phytol (acyclic diterpene) against the human gastric adenocarcinoma AGS cells through induction of both apoptosis and autophagy; research by Kim and coworkers revealed the antitumor activity through apoptosis induction and activation of caspase-9/3 and inhibition of EMT in hepatocellular carcinoma Huh7 and HepG2 cells; and research by Chikati tested the inhibition of the MDA-MB-231 cell line invasiveness in breast cancer MDA-MB-231 cell line [37-39]. In addition to the anticancer effect of lupeol (pentacyclic triterpenoid) on the lung cancer A427 cell line, the human cervical carcinoma HeLa cell line and the colorectal cancer HCT116 and SW620 cell lines [40-42]. Furthermore, the hepatocellular carcinoma cell Hep-G2 cell line was tested by MTT using methyl palmitate (saturated fatty acid) in combination with Sorafenib, which resulted in increasing the cytotoxicity via apoptosis and decreasing the metastatic migration [43]. however, further research is needed to establish direct links between these compounds and their effects on prostate cancer cells, and to confirm the findings in vivo study.

Conclusion

According to the findings of this study, the phytochemical analysis by the GC/MS for the fresh and dried petroleum ether extract present in the aerial parts of the Iraqi *Apium graveolens* showed that the plant is a good source of essential fatty acids, which possess a wide pharmacological importance. Phytol, lupeol, methyl palmitate, methyl linoleate, ethyl palmitate, and ethyl linoleate were the major terpenes and fatty acids detected in the Iraqi plant. Each active component was used for its cardioprotective, anti-inflammatory, anti-microbial, antioxidant, and cytotoxicity effects through several mechanisms on different tested cell lines. Additionally, the high cytotoxic potential of the petroleum ether fresh aerial extract of this plant, in which the cytotoxicity was concentration and time-dependent resulted in a remarkable reduction in the growth of the prostate cancer cell line PC3.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of the manuscript.

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Ethics Statements

The manuscript did not include human and/or animal studies, so ethical approval is not needed for this research.

Author Contribution

The authors planned the experiments, accomplished the planned procedures, including sample preparation, extraction process, phytochemical analysis by GC/MS, evaluation of cytotoxic activity, statistical analysis, and wrote the manuscript.

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التحليل الكيميائي النباتي والسمية الخلوية لسرطان البروستات لنبات الكرفس العراقي: طريقة كروماتوغرافيا الغاز-والطيف الكتلي

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الخلاصة

تم استخدام نبات الكرفس العراقي في العديد من الأغراض نظرًا لخصائصها الدوائية المتنوعة. من ناحية أخرى، لا يُعرف سوى القليل عن كيفية تأثير الأحماض الدهنية (المشبعة وغير المشبعة) والستيرويدات الموجودة في نبات الكرفس العراقي على الخلايا السرطانية البشرية.كان الغرض من هذه الدراسة هو دراسة تأثير مستخلص البترويليوم ايثر لنبات الكرفس العراقي على خط خلايا سرطان البروستات البشرية وتم إجراء التحليل الكيميائي النباتي بواسطة كروماتوغرافيا الغاز والطيف الكتلي. تم استخلاص الأجزاء الهوائية الجافة والطازجة (الأوراق والسيقان) من نبات الكرفس العراقي باستخدام السوكسيلت مع ٧٠٪ ايثانول كمذيب، ثم تجزئتها مع بترويليوم ايثر. ثم استخدام نظام كروماتوغرافيا الغاز لتحديد المكونات الفعالة،وتم إجراء اختبارMTTفي خط خلايا سرطان بروستات الانسان التي تم علاجها بتراكيز مختلفة من مستخلص البترويليوم ايثر لكل من الأجزاء الطازجة والمجففة (٢٥٠، ٥٠٠، و ١٠٠٠ ميكروغرام/مل) وتمت مقارنة النتائج مع الدوسيتاكسيل كدواء فعال ضد الخلايا السرطانيه بعد ٢٤،٤٨، و٧٢ ساعة من العلاج، وتم تقييم بقاء الخلية السرطانية. كان الجزء الطازج من نبات الكرفس العراقي غنيا بالأحماض الدهنية المشبعة المهمة والستيرويدات والتربينات أكثر من الجزء الجاف. علاوة على ذلك، أظهر الجزء الطازج نشاطًا كبيرًا سامًا للخلايا في خط خلايا سرطان بروستات الانسان ، وهو تأثير يعتمد على التركيز والوقت؛ تم تخفيض قيم التركيز التثبيطي الاقصى حتى النصف بمرور الوقت من ١٥٤٩، ١٨٣,٦، إلى ٢٦,٤٥ ميكروغرام/مل، وتمت زيادة السمية الخلوية مع انخفاض ملحوظ في قابلية الخلايا السرطانية للبقاء. الجزء الطازج من جزء بترويليوم ايثر من نبات الكرفس العراقي له نشاط محتمل كمضاد لسرطان البروستات أكثر من الجزء المجفف. تغير هذه النتيجة االتفكير بمدى فعالية النباتات الطازجة وتتحدى الاعتقاد التقليدي بأن النباتات الجافة تنتج نتائج أفضل من النباتات الطازجه.

الكلمات المفتاحية: نبات الكرفس العراقي، سليري، دوسيتاكسيل، الجزء الجاف، الجزء الطازج، خلايا سرطان البروستات البشرية.