

Evaluation of Anticancer Activities of Crude Extracts of *Apium graveolens L.* Seeds in Two Cell Lines, RD and L20B *in vitro*

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Abstract:

Present study was designed to evaluate anticancer effect of *Apium graveolens* seed extracts (aqueous, ethanolic and hexane extracts) *in vitro* on two cell lines (RD and L20B) using different concentrations of *A. graveolens* seed extracts (6.25, 12.5, 25, 50, 100 and 200 µg/ml) for an incubation period of 48 hours. The results revealed a clear cytotoxic activity of those extracts on growth of RD cancer cell line, and the effect was concentration-dependent. The results also, suggested that the hexane extract of *A. graveolens* showed the best cytotoxic activity on RD cell line, especially at the concentrations 100 and 200 µg/ml. In contrast, there was no significant cytotoxic effect of the extracts on the L20B transformed cells, with the exception of hexane extract at the concentration 200 µg/ml, in which a significant growth inhibitory effects were observed.

Keyword: Anticancer, *Apium graveolens* Sytotoxicity, invitro

Introduction:

Throughout medical history, plant products have been shown to be the main sources of drugs for the world's population .

Today , about 80% of the world population residing in third world countries rely almost entirely on plant products for their primary health care. The remaining 20% of individuals living in the first world , in more than 25% of cases, use pharmaceuticals, which have been directly derived from plant products (1,2). In the United States of America, it has been reported that one of three people is using one type of alternative therapy(3).

Scientific studies confirmed that the promising phytochemicals can be developed from medicinal plants for many health problems(4). One of these plants is *Apium graveolens* (Apiaceae family), which is also known as celery or celeriae (5). Many pharmaceutical properties of *A. graveolens* have been described, some of them are documented by folkloric medicine, while others have been revealed by some scientific publications. During ancient times, Ayurvedic physicians used celery seeds to treat colds, flu, water retention, anemia, poor digestion, various types of arthritis, and certain ailments of the liver and spleen (6,7). Laboratory studies have suggested that a combination of the plant with other plants may have some medicinal effects; for instance, antimicrobial(8),

anti-inflammatory(9), antioxidant (10), diuretic properties and can help lower cholesterol levels (11).

Cancer is one of the major causes of death worldwide. It is estimated that 12.8% of the world population die due to cancer(12) . It is well known that chemotherapy and radiotherapy are toxic not only to cancer cells, but also to healthy cells. However, the use of nature sources, particularly plant-derived products in cancer treatment, many adverse side effects of conventional therapy are reduced (13). Moreover , the herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness(14). Celery contains several substances with suspected or demonstrated cancer fighting properties, including apigenin, apiuman, luteoline, chrysoeriol, coumarin, and several polycetylenes and polyphones (15). Celery seeds also contain perillyl alcohol, which has been found to have achemopreventive activity(16). Therefore the present study was conducted to evaluate anticancer activity of *A. graveolens* seed extracts on the growth of cancer cell lines *in vitro*.

Materials and Methods:

Plant collection

The dried seeds of *A. graveolens* were purchased a from traditional and folk medicine store in Baghdad. The seeds were identified by a plant taxonomist at th Biology Department, College of Science, University of Baghdad. Preparation of *A. graveolens* extraction

According to (17), three types of extracts of the plant were prepared as follows:

1-Aqueous extract: The seeds were powdered using an electric blander, and 50 grams of the powder was extracted with 100 ml of distilled water by the soxhlet

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apparatus for three hours at 45°C. A further separation was done by centrifugation at 300 rpm for 10 min to obtain clear solution of the extract. Then, it was dried at 45°C by using hot air oven with circulatory fan, and kept at 4°C until use.

2-Ethanol extract: Fifty grams of powdered seeds were extracted with 250 ml of absolute ethanol using soxhelt apparatus for four hours at 45°C. The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure at 45°C and stored at 4°C until use.

3-Hexane extract: Fifty grams of powdered seeds were infused in hexane overnight. Plant materials were then extracted by soxhelt apparatus using hexane for 20 hours. Solvent was then distilled off under reduced pressure below 4°C using rotary evaporated. The extract was kept in the refrigerator (4°C) until use.

Cell culture and Cytotoxicity

This study was conducted between 2009-2010 at Tissue Culture Unit of the Biotechnology Research Center/ Al-Nahrin University. Two types of cell lines; rhabdomyosarcom (RD) and murine L20B cells, were used. Cells were grown in RPMI-1640 medium containing 10% fetal calf serum (FCS and 1% penicillin-streptomycin antibiotic. The cytotoxicity of the different extracts (aqueous, ethanolic and hexane) was tested using the method of (18). In brief, the extracts was dissolved in dimethyl sulfoxide (DMSO) and diluted with complete RPMI-1640 medium to give concentrations ranging from 6.25-200 µg/ml. The cells were grown in tissue culture flasks containing growth medium at 37°C in an atmosphere of 5% CO and 95% relative humidity in a CO incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin-versine solution (20 ml trypsin in 370 ml PBS containing 10 ml versine) and suspended in the medium. Cells with more than 97% viability (trypan blue exclusion) were used for

determination of cytotoxicity. Cells were plated in 96-multiwell plate for 24 hours in a CO incubator at 37°C. Different concentration of the tested substance (6.25, 12.5, 25, 50, 100 and 200 µg/ml) were added to the cells (four replicate wells were prepared for each individual concentration) and reincubated for further 48 hours. Control cultures containing RPMI-1640 alone were tested for a back ground cytotoxicity. After that, 50µl of crystal violet stain were added to the wells, and the plates were incubated in a CO incubator for 30 minutes at 37°C. The stain was washed gently with tap water for three times and air-dried. The optical density was recorded on ELISA reader at 492 nm. The inhibitory rate of cell growth was calculated as following formula (19):

$$\text{Inhibition(\%)} = \frac{[(\text{optical density of control wells} - \text{optical density of test wells}) / \text{optical density of control wells}] * 100}{* 100}$$

Statistical analysis

the experiments data were analyzed using statistical software SPSS (SPSS 16.0 for windows ,SPSS Ins. III, USA). Significant difference between control and sample means was assessed using student's t-test and p values < 0.05 were considered significant.

Results:

Four concentrations (25, 50, 100 and 200 µg/ml) of the three extracts (aqueous, ethanolic and hexane) were Significant (p<0.05) effective in reducing the optical density of RD cultural cells as compared to control (Figure1). In contrast, no such effects were observed when the L20B cell line was used, with the exception of hexane extract at the concentration 200 µg/ml, in which the reduction in optical density of cultural cells was significant (p<0.05) as compared to control cultural cells (Figure2).

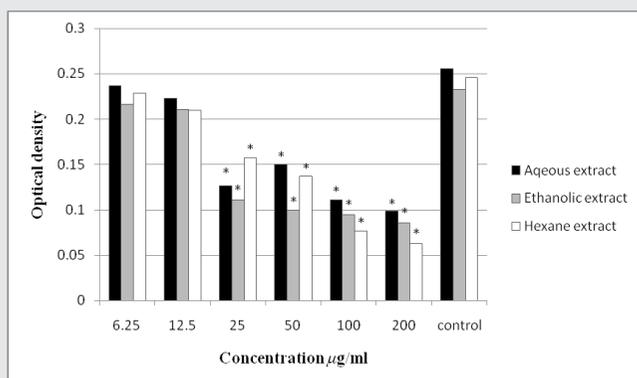


Figure 1: Effect of *A. graveolens* seed extracts at different concentrations on optical density of RD cell line during 48 hours of exposure.

*Significant at $p < 0.05$.

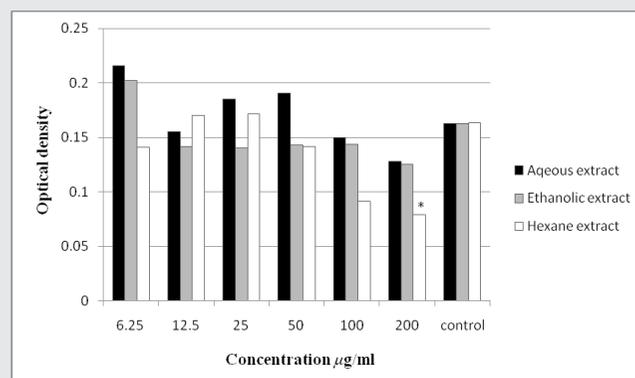


Figure 2: Effect of *A. graveolens* seed extracts at different concentrations on optical density of L20B cell line during 48 hours of exposure.

*Significant at $p < 0.05$.

Based on The results of optical density the growth inhibiting (GI) effect was calculated for each concentration of the extracts. The results In table1 revealed that the hexane extract of *A. graveolens* had the greatest GI effect on RD cell line than the ethanolic and aqueous extracts. The GI percentage for hexane extract at 100 and 200 µg/ml concentrations was 68.70% and 81.71% respectively. Whereas the GI percentage for ethanolic and aqueous extracts by the same concentrations was 59.23%, 63.09%

and 56.64%, 61.33%, respectively. The results also showed that the GI for RD cell line was decreased at the lower concentrations (6.25 and 12.5 µg/ml) of the three extracts. In the other hand, the study of the effect of the three types of *A. graveolens* extracts on L20B cell line showed that there was a slight increase in the percentage inhibition in all concentrations during 48h of exposure, expect hexane extract at concentration 200 µg/ml that caused an increase in the cell GI.

Table (1): Percentages of inhibition of RD and L20B cells by the crude extracts of *A. graveolens* during 48 hours of exposure.

Type of Cell Line	Concentrations (µ/ml)	Growth Inhibition (%)		
		Aqueous Extract	Ethanolic Extract	Hexane Extract
RD	6.25	7.42	6.87	6.91
	12.5	12.89	9.44	14.63
	25	50.39	52.36	35.58
	50	41.41	57.08	44.31
	100	56.64	59.23	68.70
	200	61.33	63.09	81.71
L20B	6.25	-	-	13.50
	12.5	4.91	12.88	-
	25	-	14.11	-
	50	-	12.29	12.88
	100	7.36	11.66	31.29
	200	21.47	23.31	51.53

- : No inhibition

Discussion:

According to (20), cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties. However, the present study observed that the crude extracts of *A. graveolens* seeds inhibited significantly the proliferation of human cancer cell line RD and its activity was in a concentration-dependent manner. These results are in agreement with the results of (21), which reported that the *A. graveolens* seeds extract act as an inhibitory agent against tumor formation. Similar results indicated that the celery seed extracts have antiproliferative activities and growth inhibition on various cancer cell lines including acute lymphoblastic leukemia cell line CEM-C7H2 and human neuroblastoma SH-SY5Y cells (22,23).

These effects can be justified in the ground of *A. graveolens* nature and its chemical constituents. According

to (10,24), celery seeds are particularly known for their anti-cancer and antioxidant effects. These effects may be due in part to the phthalide constituents (d-limonene, selinene and related phthalides) found in celery seeds. Phthalides are a bioactive compound that give celery its characteristic odor and also have been shown to Induce the detoxifying enzyme glutathions S-transferase (GST). GST aids the defense mechanisms in the body to detoxify carcinogens and xenobiotics, as well as reduce oxidative stress (25,26).

A further chemical constituent is apigenin, which is an antioxidant that was documented as one of the major celery's active principals in *A. graveolens* (27). Apigenin inhibited the growth of many human cancer cell lines, including cervical carcinoma cells, breast cancer cells and leukemia through the activity of apoptosis (28,29). The apigenin anticancer effect has been suggested to

be mediated through induction of p53 expression, which causes cell cycle arrest and apoptosis (30). As it was documented by (28), apigenin inhibits directly the phosphatidylinositol-3 kinase (PI3K) activity which is responsible for proliferation leading to inhibition in cell growth. So, the observed inhibiting effects might be related to their mechanisms.

Three phenolic acids, p-coumaric acid, ferulic acid and caffeic acid are also found in *A. graveolens* (15); they have antimutagenic activity by blocking the metabolic activation of the mutagens and scavenging the free radicals produced from mutagen metabolism. Phenolic compounds can also reduce the DNA-adduct formation by binding to the target sites in the DNA to prevent the binding of the mutagen (31). Tannins is another compound, which can be extracted from *A. graveolens* seeds and they are considered to have cancer preventive properties (32).

The coumarins are generally considered to be the most important element of *A. graveolens*, and it has been demonstrated that coumarins have antioxidant properties and exerted a reduced proliferative activity of cancer cell lines (11,22). Certain bioactive compounds derived from *A. graveolens* seeds have been described and identified; including Luteolin, linolenic acid, psoralen and oleic acid, all of them have antioxidant properties and growth inhibition on various cancer cell lines through inhibition of tumor cell proliferation by inducing cell cycle arrest and by inducing of apoptosis via intrinsic and extrinsic signaling pathway including glioma cell line C6 and breast

cancer MCF-7, Keratinocytes HaCaT (33,34). It has also been reported that *A. graveolens* contain high levels of vitamins C, A and B. These vitamins are antioxidants that are useful in reducing the oxidative stress caused by toxic agents (35).

L-3-n-butylphthalida (NBP), the active component of essential oil extracted from seeds of *A. graveolens*, has been demonstrated to have multiple neuroprotective effects in vivo and in vitro by inhibiting oxidative stress, improving mitochondria function, increasing the cellular GSH content, blocking inflammatory reaction and reducing neuronal apoptosis and protect neurons against amyloid-beta 25-35 induced neurotoxicity via inhibiting protein hyperphosphorylation (36,37).

In the current study, it was also observed that hexane extract was more active than ethanolic and aqueous extracts against the RD cell line. These results are in agreement with the results of (38), which indicated that the hexane extract of *Cyperus rodundus* had the best cytotoxic activity on RD cell line. Through the results, it was also observed that the cytotoxicity of extracts against RD cancer cell line was higher than L20B cell line, while no significant effect on transformed cell was observed. These results were agreement with the results of (21,39).

In conclusion, the extracts showed selective (cell line-dependent) inhibitory effects on cancer cell lines and the plant may be a promising anticancer drug. Further studies will be needed to determine the effects of compounds isolated from *A. graveolens* and other more advanced anticancer assay must be applied.

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تقييم التأثيرات المضادة للسرطن لمستخلصات الخام لبذور نبات الكرفس *Apium graveolens* L في اثنين من الخطوط الخلوية RD و L20B في الزجاج

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الخلاصة:

صممت هذه الدراسة لتقييم التأثيرات المضادة للسرطن لمستخلصات بذور نبات الكرفس (*Apium graveolens* L) في اثنين من الخطوط الخلوية RD و L20B المنماة في الزجاج وباستخدام تراكيز مختلفة (6.25، 12.5، 25، 50، 100 و 200 مايكروغرام/ملليتر) وخلال مدة تعريض 48 ساعة. أظهرت النتائج وجود تأثير سمي واضح وبمعنوية عالية لتلك المستخلصات الخام في نمو الخط الخلوي السرطاني RD، علما ان نسبة السمية زادت بزيادة التركيز، وكان المستخلص الهكساني أكفأ المستخلصات سمية في الخط الخلوي السرطاني RD بتركيز 100 و 200 مايكروغرام/ملليتر، في حين لم يكن هناك تأثير واضح لتلك المستخلصات الخام في نمو الخلايا المتحولة L20B.