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RESEARCH ARTICLE

EXTRACTION AND PURIFICATION FLAVONOID FROM CELERY PLANT AND APPLICATION ON HEPATIC AND BREAST CANCER CELL

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ABSTRACT

The hexanolic extracted and purification flavonoides from celery plant by using thin layer chromatography (TLC). Also studied the effect to block the development of cancer in human beinge, Is an extremely promising strategy for cancer prevention. Celery flavonoid have been assessed for chemopreventive activity. The anti proliferative effect of the hexanolic extract of celery was evaluated in vitro on two human cell lines (HepG2 hepatic cancer ,MCF-7 breast cancer), The HepG2 and MCF-7 cells were seeded 69-well culture plates of different concentration of celery flavonoid to determine their anticancer effects in using MTT (3-(4,5-Dimethyl thiazol-2-yle) 2,5-diphenyltetraloiumbromide) assay, Anticancer screening by 24 and 48 hrs cytotoxicity study showed that the extracts exhibited a dose dependent inhibition of growth .This study confirms that hexanolic flavonoid extract of celery possess potential candidate in the field of anticancer drug discovery .There are considerable effects to identify naturally occurring substances as new drugs in cancer therapy.

INTRODUCTION

Celery (Apium.graveolens L.) was introduced from Caucasia into China in about fifteenth century. Ancient medicinal herbs records and modern research have proved that Celery has a series of medicinal properties, such as reducing blood press, sedation, promoting digestion, diuresis and moistening lung. Celery is widely cultivated in the temperate zones as an important garden crop and the bleached leaf stalks are relished as a popular vegetable. A grave lens is one of ingredients in 8 of the 33 in dianpolyherbal formations with reputed life-protecting activity. Celery is also used as an effective remedy for various ailments such as bronchitis, liver and spleen disease, arthritic pain and this natural holistic approach to health is becoming more and more popular now days (Kolarovic *et al*., 2010; Jung *et al*., 2011). Flavonoids (or bioflavonoids), collectively known as Vitamin p and citrin , are a class of plant secondary metabolites which are ubiquitous in photosynthesizing cell and are commonly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, Wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human disease (Mink *et al.,* 2007., Hanneken *et al.,* 2006). The basic structural feature of flavonoid compounds is the 2-phenyl-benzopyrane or flavane nucleus, which consist of tow benzene rings (A and B) linked through a heterocyclic ring(C). Increasingly, this class of natural products is becoming the subjects of anti infective, and many groups have isolated and identified the structures of possessing antifungal, antiviral and antibacterial activity (Shohaib *et al*., 2011t; Punyasiri *et al*., 2004). The therapeutic effect of many traditional drugs are attributed to this group of compounds because of their inhibitory effects on certain enzymes and antioxidative activity. They have been shown to posses antibacterial, antifungal, antiviral and anti-inflammatory activities (Das *et al*., 2009), their antiallergic, antioxidative and antimutagen activities have been proven. Reduced risk of breast, prostate and colon cancers is related to flavonoid activity. Flavonoids have been studied in the prevention of menopausal symptoms and osteoporosis. It was shown that their biological activity depended on the location of the free hydroxyl groups on ring A more so than that on ring B (Dragan *et al*., 2007).
Celery flavonoids will play very important role in theoretical and practical significance for Celery. The distribution of flavonoids from different Celery resources in human was studied, and characteristics fingerprints of Apigenin, Apiin from Celery with chromatographic technique. Biological activity and pharmacological functions of Celery flavonoids compounds on resistance liver cancer, leukemia, lowering lipid, anti-inflammatory were studied (Amaowicza*et al*., 2004; Scott *et al.*, 2012). The aim of this study is to explore the flavonoid effecting on HepG2 hepatic cancer and MCF-7 breast cancer.

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MATERIALS AND METHODS

The Apium graveolens leaves were procured from local market in 2012 Baghdad Iraq. The leaves were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained, dried in a hot air oven at 50 temperature. The dried leaves were finely powdered. The dried powder (50) gm was extracted with 500 ml hexanol for 10 hrs in soxhlate. The obtain extracts were filtered through filter paper. The crude extracted concentrated in rotary in temperature 40 C^0, so green material that give, and suspended in 250 ml Ethanol for 8 h. Solvents were removed in Rotary and extracts were obtained, respectively.The residues were dissolved in 25 ml NaOH 5% in separation funnel, then added 25 ml chloroform for 15 min and show tow layer the upper layer (water layer), lower layer (chloroform layer). The upper layer had taken and megerment equal PH 7 by used HCL, Then added 25 ml Chloroform. Total flavonoid content of celery leaves were determined by using method of (Al

Zubaidy, 2007), By mixed 10 ml from extracted with 50 ml Ethyl alcohol 95% in 1:1 vol:vol. TLC sorbents and three mobile phase were used for the analysis of the flavonoid exudates.

Toluene: Diethyl ether: acetic acid 10% (50:50:50) V \ V \ V, was used for the development of the exudates on silica gel plates (20×20) cm (0.2) mm layer. Detection with U.V light at (280) nm. Flavonoid reaction products were identified by RF values and chromatography with authentic substances on TLC in different solvent systems. The Flavonoid extract was utilized as test material for *iv vitro* anticancer activity testing.

Cell Lines: Daltonn's HepG2 hepatic cancer cells and MCF-7 breast cancer cells were used for short term in Vitro cytotoxicity experiments. The cells were grown routinely as monolayer cultur in RPMI-1640 culture medium supplemented with 100 U/ml of penicillin, 100mg/ml of streptomycin and 10% heat-inactived FBS at 37 ^{0}c in 5% Co_2 incubator (Abdomohammadi *et al*., 2008).

In vitro cytotoxicity assay: Cells were used in cytotoxicity studies when 90% confluence was reached in cultur flask. Cell were harvested with trypsin/EDTA, washed with PBS and counted using trypan blue dye exclusion method. HepG2 and MCF-7 cells were seeded into 96-well plates at a density of 10^4cells /well and left to attach to the plates for 48 hrs. then cells were incubated for 24 and 48 hrs with various concentration of extract (2mg/ml,5mg/ml,10mg/ml,15mg/ml,20mg/ml). After the exposure time, the cells were incubated with 25 microliter of MTT (3-(4,5-Dimethyl thiazol-2-yle) 2,5-diphenyltetraloiumbromide) 20ml(5mg/ml)incubation for 2hrs. After dissolving the formazancrystalin DMSO, plates were read in microplate reader (hidexchamelon plate reader) set recorder absorbance at 570 nm (33). The percentage growth inhibition was calculated using the formula given below.

% Growth inhibition = 100-(OD of individual test group /OD of control group)× 100

Data and Statistical Analysis: All values obtained were expressed as means± standard error of the mean results were subjected to one- way analysis of variance (ANOVA) to assess treatment differences. Significant differences between means were determined at p<0.01. (Subhadradevi *et al*., 2011, Gao *et al*., 2011)

RESULTS AND DISCUSSION

The accumulation processing consider first important steps, Dring plante the second step to lowering moister of plant, to prevent enzymatic and microbial activity may be happen in plant tissue (jung *et al*., 2011).

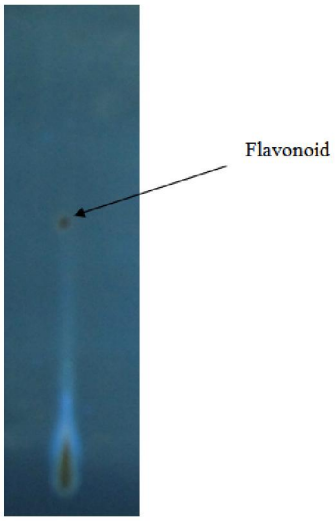

Figure (1): purification flavonoid on TLC.

The isolation and purification of flavonoids from the celery crud extracted was successfully established by using Hexanol to remove volitical oils and fatties were found in celery plant without effecting on flavonoid, so another step using ethyl alcohol as phase solvent, following separation process NaOH sodium hydroxide 5% due to it solve in water layer, and added equal volume from chloroform to remove large amount from chlorophyll material (kavaratskhella *et al*., 2004), (Das *et al.,* 2009). When show yellowish precipitate after added 10 ml extracted crude with 50 ml ethyle alcohol 95%. These lead to flavonoides found, these results successful with (Al Zubaidy, 2007). The isolation and purification of flavonoids from the celery crud extract was successfully established by using Toluene, diethyl either, acetic acid 10% (50:50:50) as the tow- phase solvent. Celery flavonoids glycosides monomer was tracked in 280 nm wavelength by TLC thin layer chromatography, can a good separation. Flavonoid reaction products were Identified by RF values (0.68) and cochromotography with authentic substances on TLC in different solvent systems (Nijveldt *et al*., 2001). These result show in Figure (1)

1- Cytototoxic effect on HepG2 hepatic cancer cells

Cytototoxic effect on HepG2 hepatic cancer cells was investigated by MTT assay. Cells were treated with celery flavonoid at concentration ranging from 2-20 µg/ml for 24 hrs and then the percentage of cell viability was analyzed. The plant flavonoid extract is significantly ($p<0.01$) inhibited the proliferation of HepG2 cells in a dose depended manner table (1: A, B). the best result in concentration 20µg/ml was 0.151µg/ml in OD 570, IR% (0.797µg/ml), While in table (2:A, B) indicates long term cytotoxicity of celery flavonoid extract against HepG2 cell lines, also the high result in concentration 20µg/ml was 0.166 µg/ml at 48 hrs in OD 570 nm and IR%(0.788µg/ml).

Table (1,A): IR% Inhibition growth rate of HepG2 cell line by celery flavonoid extract in different concentration for 24hrs by MTT assay, cell density 10^4 cells.

Concentrations Groups	IR % inhibition Rate				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
DMSO	0.271	0.271	0.271	0.271	0.271
MTX	0.330	0.437	0.479	0.580	0.728
Celery flavonoid	0.323	0.416	0.462	0.605	0.797

Table (1, B): Cytotoxicity of different concentration for 24 hrs of celery flavonoid against HepG2 cell line by MTT assay cell density 10^4 cells OD 570 nm wavelength with control OD 0.746 nm .the similar small leter mean no differences.

Concentrations Groups	OD 570nm wavelength Control OD = 0.746 nm				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
MTX	A,a 0.500 ±0.0127	A,b 0.420 ±0.0278	A,b 0.389 ± 0.0130	A ,c 0.313 ±0.0370	A , d 0.203 ±0.0096
Celery flavonoid	A , a 0.505 ±0.0200	A,b 0.435 ±0.0181	B , c 0.401 ± 0.0119	A , d 0.295 ±0.0155	B , e 0.151 ±0.0080

Table (2,A): IR% Inhibition growth rate of HpeG2 cell line by celery flavonoid extract in different concentration for 48hrs by MTT assay, cell density 10^4 cells

Concentrations Groups	IR % inhibition Rate				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
DMSO	0.254	0.254	0.254	0.254	0.254
MTX	0.374	0.483	0.539	0.597	0.725
Celery flavonoid	0.358	0.449	0.477	0.601	0.788

Table (2,B): Cytotoxicity of different concentration for 48hrs of celery flavonoid against HepG2 cell line by MTT assay cell density 10^4 cells in OD 570 nm wavelength with control OD 0.782 nm. the similar small leter mean no differences.

Concentrations Groups	OD 570nm wavelength Control OD = 0.782 nm				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
MTX	A , a	A , b	A , c	A ,d	A , e
	0.490	0.404	0.361	0.315	0.215
	± .0130	±0.0070	± 0.0266	±0.0075	±0.0167
Celery flavonoid	A , a	A,b	A ,d	A , c	A , d
	0.502	0.431	0.409	0.312	0.166
	± .0140	±0.0245	± 0.0091	±0.0115	±0.0070

2- Cytotoxic effect on MCF-7 breast cancer cells

The anti proliferatiive effects of plant flavonoid extract in comparison to 570 nM on MCF-7 cells was determined by MTT method. In vitro screening of the extracts of flavonoid on breast carcinonomaMCF-7 cell line produced a time and dose-dependent inhibition of the cell growth (Tan *et al.,* 2005; Ostad *et al*., 2006). Table (3: A,B), (4: A,B) show the active concentration as anticancer in 20 µg/ml for 24 hrs was 0.157µg/ml and IR% was 0.821µg/ml, Observed in 48 hrs the best result in concentration 20µg/ml was 0.146 µg/ml and IR %0.837µg/ml. In current study can observed, the effect of celery flavonoid on MCF-7 cell stronger than HepG2.

Table (3,A): IR% Inhibition growth rate of MCF-7 cell line by celery flavonoid extract in different concentration for 24hrs by MTT assay, cell density 10^4 cells

Concentrations Groups	IR % inhibition Rate				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
DMSO	0.349	0.349	0.349	0.349	0.349
MTX	0.632	0.638	0.662	0.690	0.715
Celery flavonoid	0.609	0.628	0.691	0.782	0.821

Table (3,B): Cytotoxicity of different concentration for 24hrs of celery flavonoid against MCF-7 cell line by MTT assay cell density 10^4 cells in OD 570 nm wavelength with control OD 0.874 nm . the similar small leter mean no differences

Concentrations Groups	OD 570nm wavelength Control OD = 0.874 nm				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
MTX	A,a	A,a	A, ab	A , b	A , b
	0.322	0.317	0.296	0.271	0.249
	± 0.0122	± 0.0180	± 0.007	±0.0213	±0.0188
Celery flavonoid	A , a	A,a	A , b	B , c	B , c
	0.342	0.325	0.270	0.191	0.157
	± 0.0260	± 0.0267	±0.0177	± .0201	±0.0126

Table (4,A): IR% Inhibition growth rate of MCF-7 cell line by celery flavonoid extract in different concentration for 48 hrs by MTT assay,cell density 10^4 cells

Concentrations Groups	IR % inhibition Rate				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
DMSO	0.358	0.358	0.358	0.358	0.358
MTX	0.651	0.653	0.668	0.695	0.730
Celery flavonoid	0.623	0.638	0.715	0.802	0.837

Table (4,B): Cytotoxicity of different concentration for 48hrs of celery flavonoid against MCF-7 cell line by MTT assay cell density 10^4 cells in OD 570 nm wave length with control OD 0.893nm. the similar small leter mean no differences.

Concentrations Groups	OD 570nm wavelength Control OD = 0.893 nm				
	2µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
MTX	A , a	A , a	A , ab	A ,b	A ,b
	0.312	0.310	0.296	0.273	0.241
	± 0.0098	±0.0075	±0.0204	±0.0270	±0.0090
Celery flavonoid	A , a	A, a	B , b	B , c	B , c
	0.337	0.323	0.255	0.177	0.146
	± 0.0096	±0.0180	±0.0526	±0.0145	± 0.0095

The majority of the cancer treatments are accompanied by a degree of herbal supplements. There are advantageous effects of medicinal plants on cancer (Pisani and Ferlay, 1999). Natural products discovered from medicinal plants have played avital role in the management of cancer. Natural products or natural product derivatives consistof 14 of top 35 drugs in 2000 based on worldwide sales (Verma *et al*., 2008). Plant based medication has definitely found a rol in cancer healing (chemotherapy) and mechanism of interaction between many phytochemicals and cancer cell has been studied extensively. The present study was undertaken to assess the cytotoxic activity of celery flavonoid. Chemoprevention, which consist of the use of synthetic or natural agents to block the development of cancer in human beings, is an extremely promising strategy for cancer prevention (Koppikar *et al*., 2010). *In vitro* confirmation of the extract's toxicity was done on HepG2 and MCF-7 cell lines. In our study, the hexanolic extracts of celery flavonoid in plant constituents may cause cell growth inhibition and induce apoptosis differentially in cancer cell, the result agreement with (Shrivastavate *et al*., 2010). In this study HepG2 hepatic and MCF-7 breast cancer cell were treated with hexanolic celery flavonoid for 24 and 48 hour with 5 concentration . The cell growth inhibitory effects of this plant extract support to exert their anti-cancer effects in vitro. We observed celery flavonoid inhibits growth of HepG2 cell in a dose dependent manner and maximum growth inhibitory effectson HpeG2 and MCF-7 at 20µg/ml.

The result of the experiment show that the HepG2 cells had under gone inhibition rat increasing manner after being incubated for 24 hrs with increasing flavonoid concentrations up to 20 µ/ml with which maximal inhibition rat response was attained , and it also clear that the percentage of inhibition cell dose differ significantly with increasing flavonoid concentration between 15 and 20 µg/ml. While after incubating the HepG2 for 48 hrs with increasing concentration of the flavonoid, the results reveal that most of the flavonoid concentration elicit an anticancer response which is directly in concentration 20 µg/ml these results observed in tables 1and 2. In this study, after 24, 48 hrs of exposure, extract treated cells showed a decreased viability of MCF-7 cells to MTX control by MTT assay. flavonid extract inhibited cell proliferation at various concentration with a time and dose dependent pattern. As a result, The IR % inhibition 0.821 in concentration 20µg/ml at 24 hrs, Wile in 48 hrsIR% 0.837 concentration 20µg/ml. Our present investigations have demonstrated that there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived from plants in recent years. Based on the evaluation done using the various in vitro assay models it may be concluded that celery line. Possess anticancer activity. Hexanolic extract of flavonoid of celery Line, Is moderately active for the treatment of cancer. Further pharmacological study using other cancer cells is necessary in order to establish whether these plants can be used as potential source for new anticancer medicine, agreement with (Jenifer *et al*., 2009).

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