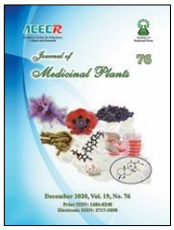
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Research Article

Screening of Apiaceae fruits discovered natural resources with considerable biological potential

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ABSTRACT

Background: Apiaceae fruits as common spices used for prevention of many chronic diseases including cancer. **Objective:** The present study compared the biological effects of different fruits from various Apiaceae tribes to compare and find the fraction source(s) with potential characteristics for further investigation including cancer prevention. **Methods:** Fruits of *Apium graveolens* L. (celery), *Bunium persicum* (Boiss.) B.Fedtsch. (black cumin), *Petroselinum crispum* (Mill.) Fuss (parsley), *Pimpinella anisum* L. (anise), *Trachyspermum ammi* (L.) Sprague (ajwain), *Coriandrum sativum* L. (coriander), *Foeniculum vulgare* Mill. (fennel), *Anethum graveolens* L. (dill), *Heracleum persicum* Desf. ex Fisch., C.A.Mey. & Avé-Lall. (Persian hogweed), *Ferula assa-foetida* L. (asafoetida), *Cuminum cyminum* L. (cumin) and *Daucus carota* L. (carrot) were extracted with 80 % methanol and fractionated by petroleum ether, chloroform, ethyl acetate and methanol, respectively. For different fractions and total extract of all 12 samples, cytotoxicity by brine shrimp test (BST) and MTT assay against cancer and normal cell (foreskin fibroblast cells), antioxidant effects by FRAP, and total phenols by Folin-Ciocalteu method were measured. **Results:** The general toxicity of ethyl acetate fractions (mean of data) was higher than others in the brine shrimp test ($P < 0.05$). The most cytotoxic fractions against colon carcinoma (HT-29), breast adenocarcinoma (MDA-MB-231) and alveolar basal epithelial adenocarcinoma (A549) cell lines were from Ammineae and Peucedaneae tribes while fruits fractions with high phenol contents and antioxidant powers were from Ammineae tribe. **Conclusion:** The Apiaceae fruits have significant biological effects, therefore the isolation of phytochemical compounds from active fractions with cytotoxicity is suggested in future studies.

Abbreviations: TE, Total methanol Extracts; PE, Petroleum Ether fraction; CL, Chloroform fraction; EA, Ethyl Acetate fraction; ME, Methanol residue fraction; BST, Brine Shrimp Test; FRAP, Ferric Reducing Antioxidant Power

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1. Introduction

Cancer is the second global cause of death after cardiovascular diseases. In 2015, 17.5 million cases were reported with cancer which 8.7 million of them have died. Despite the fact that the mortality from cancers was reduced in many countries, it is expected that its incidence will be increased. So, it is estimated by 2030; there will be 26 million new cancer cases and 17 million cancer deaths per year. Ranking of cancer demonstrated breast, lung and other parts of the respiratory system, and colorectal cancers have more incidences in both sexes [1, 2].

According to the prevalence and mortality rate of cancer, the importance of the continuing discovery of new anticancer agents is obvious. The potential of natural products and plant-derived compounds for cancer prevention and therapy was the reason for increasing attention to them over the previous few years [3]. On the other hand, over 60% of anti-cancer drugs have origins from natural sources or are related to them [4]. A significant portion of currently used antitumor drugs are synthetic or semi-synthetic derivatives of effective constituents elucidated from plants [5].

Apiaceae (Umbelliferae) family contains about 450 genera and 3700 species which can be found worldwide [6]. Iran is an important center of diversification of Apiaceae plants which is represented by 121 genera, 360 species and 122 endemic taxa. The fruits of this family have many culinary and medicinal properties [7, 8].

The purpose of present comprehensive study was toxicity investigation of common spices from Apiaceae fruits by brine shrimp lethally test (BST) and MTT assay. In addition, their antioxidant activities and total phenols were determined and compared between different tribes of Apiaceae family to find and introduce active fraction(s) for further studies.

2. Material and Methods

2.1. Plant material

The fruits of selected species of Apiaceae family including *Apium graveolens* (celery; PMP-687), *Bunium persicum* (black cumin; PMP-671), *Petroselinum crispum* (parsley; PMP-686), *Pimpinella anisum* (anise; PMP-684) and *Trachyspermum ammi* (ajwain; PMP-682) from Ammineae tribe, *Coriandrum sativum* (coriander; PMP-677) from Smyrneae tribe, *Foeniculum vulgare* (fennel; PMP-681) from Seselineae tribe, *Anethum graveolens* (dill; PMP-679), *Heracleum persicum* (Persian hogweed; PMP-659) and *Ferula assa-foetida* (asafoetida; PMP-685) from Peucedaneae tribe, *Cuminum cyminum* (cumin; PMP-670) and *Daucus carota* (carrot; PMP-676) from Caucalineae tribe were purchased in May 2016 from markets of Tehran, Iran. The plants were identified and deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The tribes were determined according to previous reference [9].

2.2. Extraction and fractionation

The fruits were powdered separately and extracted with 80% methanol via maceration at room temperature. Total methanol extracts (TE) were fractionated with petroleum ether (PE), chloroform (CL) and ethyl acetate (EA) and the residue was named methanol (ME) fraction. Total extracts and fractions were kept at the refrigerator prior to the test.

2.3. Brine shrimp lethality test (BST)

The toxicity of total extracts and different fractions of fruits of Apiaceae family were determined by brine shrimp test. The eggs of *Artemia salina* Leach (Shilat Center, Tehran, Iran) were hatched in aerated 35% saltwater under direct light and warmth (28-30 °C). The

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eggs transformed to nauplii 48 h later. Total extracts and various fractions of fruits were dissolved in normal saline (1 % (v/v) DMSO was used when necessary) to obtain different concentrations of 1-500 µg/ml in tubes containing 15 nauplii of brine shrimp. The control group contained the vehicle used for dilutions. Dead larvae were counted in each well after 24 h and mortality percentages (p) were determined according to Abbot's formula, p = pi-C/1- C; where pi was the observed mortality rate of each sample and C means the natural larvae mortality of negative control [10]. The lethal concentration (LC_{50}) value of each sample was calculated and reported.

2.4. Cell culture and MTT Assay

The colon carcinoma (HT-29), breast adenocarcinoma (MDA-MB-231) and alveolar basal epithelial adenocarcinoma (A549) cell lines were cultured in RPMI 1640 medium and foreskin fibroblast cells (primary culture) was cultured in DMEM medium containing 10 % Fetal bovine serum (FBS) and 1 % penicillin-streptomycin. Cell cultures were maintained at 37 °C in a humidified 5 % CO_2 and 95 % air incubator.

Cytotoxicity studies were performed by MTT assay [11]. Growing cells were incubated into 96-well plates at a density of 1×10^4 cells /well. After 24 h, samples with different concentrations (10-200 µg/ml) were added to wells. 48 h later, 20 µl of 5 mg/ml MTT reagent in phosphate-buffered serum (PBS) was added to each well. The plates were incubated at 37° C for 4 h. Then, the medium was replaced by 100 µl pure DMSO to dissolve formazan crystals which were quantified by reading the absorbance at 570 nm on microplate reader (Anthos, Austria). The cell survival was calculated according to the following equation:

% Cell viability = Mean absorbance of sample wells/Mean absorbance of control wells × 100

Three independent experiments were performed for each sample. The concentration of samples inducing 50 % growth inhibition (IC_{50}) was obtained from a dose response curve.

2.5. Antioxidant power assay

Antioxidant activities of different fractions of Apiaceae fruits were determined by ferric reducing antioxidant power (FRAP) assay [12]. In this method, ferric tripyridyltriazine (Fe (III)-TPTZ) complex was reduced to its blue colored form (Fe (II)-TPTZ) and the absorbance were measured by spectrophotometer. 50 µl of various fractions of fruits with a concentration of 100 µg/ml were added to 1.5 ml of FRAP reagent and incubated at 37 °C. After 10 min, the absorbance of samples was measured at a wavelength of 593 nm. FRAP reagent used as blank and contained 2.5 ml of TPTZ solution (10 mM) in HCl (40 mM), 2.5 ml of $FeCl_3$ (20 mM) and 25 ml of acetate buffer (0.3 M) with pH 3.6. $FeSO_4$. The standard curve was prepared by $FeSO_4.7\ H_2O$ aqueous solution (125-1000 µM) and antioxidant effects of samples were expressed as mM Fe^{2+}/100 g of fractions.

2.6. Total phenols determination

Total phenols amounts of different fractions of extract from Apiaceae fruits were determined by Folin-Ciocalteu method [13]. By the addition of molybdo tungstophosphoric heteropoly anion (Folin-Ciocalteu) reagent with yellow color to samples, phenol compounds were oxidized and molybdo tungstophophate with blue color was created. The maximum absorption of blue color was achieved in alkaline pH, usually by the addition of $NaHCO_3$ or Na_2CO_3. 2 ml of Folin-Ciocalteu reagent (1:10 diluted with distilled

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water) was mixed with 0.2 ml of methanol solutions of Apiaceae fruits fractions (100 µg/ml). After 5 min, 1.5 ml of sodium bicarbonate solution (60 g/L distilled water) was added to the mixture and incubated at room temperature for 90 min. Then the absorbance was measured at 725 nm by spectrophotometer and the experiment was carried out in triplicate. Different concentrations of Gallic acid methanol solution (10-100 mg/ml) were used for the preparation of standard curve and total phenols of samples were reported as gallic acid equivalents (GAE; mg of Gallic acid per g of samples).

2.7. Statistical Analysis

The data were average of three samples measurements and reported as Mean ± SD.

Statistical analysis was performed by Graph Pad Prism 5 via One-way ANOVA and post hoc of Tukey, and $P < 0.05$ were considered as a significant difference.

3. Results

3.1. Brine shrimp lethality test (BST)

Cytotoxic effects of different fractions from Apiaceae fruits were evaluated by BST and MTT assays. Brine shrimp lethality test is a simple, rapid and valid preliminary screening for definition of bioactive cytotoxic chemicals. Mortality ability of all extracts and fractions on *Artemia salina* were presented in Table 1.

Statistical analysis showed EA fraction of all plants (mean) demonstrated the best mortality rate and had a significant difference with other fractions ($P < 0.05$) and PE fraction was the second effective fraction (Fig. 1).

Among EA fractions of plants with 10 µg/ml concentration, *B. persicum* showed the lowest mortality rate with a significant difference with other EA fractions of plants ($P < 0.05$).

And among PE fractions with 10 µg/ml concentration, *D. carota* (carrot) is the best and showed significant difference with other PE fractions of plants ($P < 0.05$) except *A. graveolens* (dill).

Table 1. Cytotoxicity, antioxidant activity and total phenols of different fractions of Apiaceae fruits

Apiaceae Tribes	Plants	Fractions	Yield* (%)	LC_{50} of BST (µg/ml)	FRAP (mM Fe^{2+}/g)	Total Phenol (mg GAE/g)
Ammineae	*Apium graveolens*	PE	24.0	43.89 ± 0.9	342.10 ± 18.5	88.59 ± 0.7
		CL	17.5	246.00 ± 4.9	642.10 ± 33.2	218.42 ± 4.8
		EA	5.0	6.05 ± 0.4	1935.09 ± 58.5	123.68 ± 1.9
		ME	53.5	40.04 ± 1.8	515.78 ± 19.9	248.68 ± 9.7
		TE	20.7	66.22 ± 6.3	---	---
	Bunium persicum	PE	21.5	10.00 ± 4.2	403.50 ± 2.6	190.79 ± 2.0
		CL	7.6	110.34 ± 7.0	501.75 ± 9.0	295.60 ± 3.7
		EA	6.4	21.20 ± 5.4	1742.11 ± 9.7	278.95 ± 3.7
		ME	64.5	> 500	656.14 ± 3.8	435.96 ± 6.6
		TE	6.8	> 500	---	---
	Petroselinum crispum	PE	29.3	45.76 ± 1.2	1682.46 ± 37.0	260.96 ± 1.8
		CL	7.8	132.08 ± 5.0	538.59 ± 30.5	250.00 ± 2.6
		EA	4.9	6.22 ± 2.6	738.59 ± 8.9	410.96 ± 2.01
		ME	58.0	7.47 ± 4.9	457.87 ± 27.5	129.38 ± 6.7
		TE	18.1	10.19 ± 1.3	---	---
	Pimpinella anisum	PE	26.1	95.29 ± 0.9	464.91 ± 10.0	628.07 ± 7.5
		CL	9.1	327.54 ± 6.5	428.07 ± 19.9	279.38 ± 10.5
		EA	6.2	2.65 ± 4.6	963.15 ± 31.5	226.31 ± 6.5
		ME	58.6	141.5 ± 4.3	261.40 ± 17.4	832.90 ± 4.6

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Table 1. Cytotoxicity, antioxidant activity and total phenols of different fractions of Apiaceae fruits (Continued)

Apiaceae Tribes	Plants	Fractions	Yield* (%)	LC_{50} of BST (µg/ml)	FRAP (mM Fe2+/g)	Total Phenol (mg GAE/g)
Ammineae (Continued)	*Trachyspermum ammi*	TE	11.0	438.54 ± 2.0	---	---
		PE	31.6	44.46 ± 1.5	461.40 ± 1.7	137.72 ± 2.7
		CL	11.1	78.5 ± 2.6	631.57 ± 4.5	350.88 ± 1.7
		EA	7.2	2.77 ± 0.6	808.77 ± 3.9	776.75 ± 5.3
		ME	50.1	255.07 ± 4.6	917.54 ± 8.9	223.37 ± 7.9
		TE	12.3	49.84 ± 1.5	---	---
Smyrneae	*Coriandrum sativum*	PE	15.8	116.63 ± 5.7	782.45 ± 11.3	172.36 ± 4.6
		CL	6.6	275.37 ± 12.5	663.15 ± 6.9	112.28 ± 4.8
		EA	10.5	3.16 ± 4.3	701.75 ± 3.5	364.03 ± 2.9
		ME	67.1	27.12 ± 4.0	885.96 ± 5.8	453.07 ± 1.4
		TE	7.2	73.45 ± 3.2	---	---
Seselineae	*Foeniculum vulgare*	PE	15.1	41.27 ± 3.9	1470.18 ± 52.8	100.00 ± 2.7
		CL	10.2	19.78 ± 6.6	115.78 ± 1.4	190.13 ± 4.0
		EA	6.3	3.44 ± 0.3	654.38 ± 25.1	263.60 ± 2.3
		ME	68.4	> 500	1821.05 ± 56.5	284.65 ± 6.0
		TE	8.3	> 500	---	---
Peucedaneae	*Anethum graveolens*	PE	13.9	2.71 ± 0.6	126.27 ± 6.7	160.52 ± 6.0
		CL	6.3	22.57 ± 6.6	484.21 ± 13.8	156.14 ± 3.8
		EA	8.8	2.09 ± 0.3	859.64 ± 43.7	228.95 ± 4.7
		ME	71.0	190.88 ± 5.0	219.29 ± 10.3	71.93 ± 1.5
		TE	6.6	> 500	---	---
	Heracleum persicum	PE	14.4	17.65 ± 5.1	1471.05 ± 16.0	125.00 ± 2.8
		CL	9.9	40.94 ± 5.2	484.21 ± 2.9	76.88 ± 5.4
		EA	7.9	1.80 ± 0.4	1652.63 ± 23.9	128.95 ± 0.5
		ME	67.8	75.81 ± 5.4	702.58 ± 12.3	103.07 ± 2.0
		TE	11.3	23.11 ± 1.0	---	---
	Ferula assa-foetida	PE	13.0	7.77 ± 1.5	326.28 ± 5.2	264.91 ± 6.4
		CL	11.0	297.71 ± 5.4	560.53 ± 4.1	415.35 ± 3.9
		EA	6.6	9.21 ± 1.8	1084.21 ± 11.5	541.23 ± 2.1
		ME	69.4	> 500	1294.74 ± 12.6	109.65 ± 1.1
		TE	12.6	44.52 ± 3.0	---	---
Caucalineae	*Cuminum cyminum*	PE	22.4	33.68 ± 1.5	339.47 ± 6.3	39.91 ± 2.7
		CL	8.6	10.59 ± 4.2	436.84 ± 3.7	135.52 ± 1.0
		EA	8.0	1.62 ± 1.5	847.36 ± 2.9	243.42 ± 1.5
		ME	61.0	> 500	439.47 ± 2.6	117.98 ± 3.6
		TE	11.9	> 500	---	---
	Daucus carota	PE	21.5	5.11 ± 0.4	389.47 ± 9.6	148.24 ± 2.5
		CL	8.0	403.43 ± 3.9	663.15 ± 21.1	72.36 ± 3.2
		EA	6.3	6.16 ± 0.3	508.77 ± 27.5	161.40 ± 3.7
		ME	64.2	> 500	542.10 ± 34.6	52.63 ± 1.3
		TE	9.0	395.13 ± 0.7	---	---
Vitamin E		---	---	---	3130.7 ± 2.2	---
BHA		---	---	---	8800.3 ± 6.4	---

* Yield (%) calculated based on fraction (g)/total extract (g); PE: Petroleum ether fraction, CL: Chloroform fraction, EA: Ethyl acetate fraction, ME: Methanol fraction, TE: Total Extract

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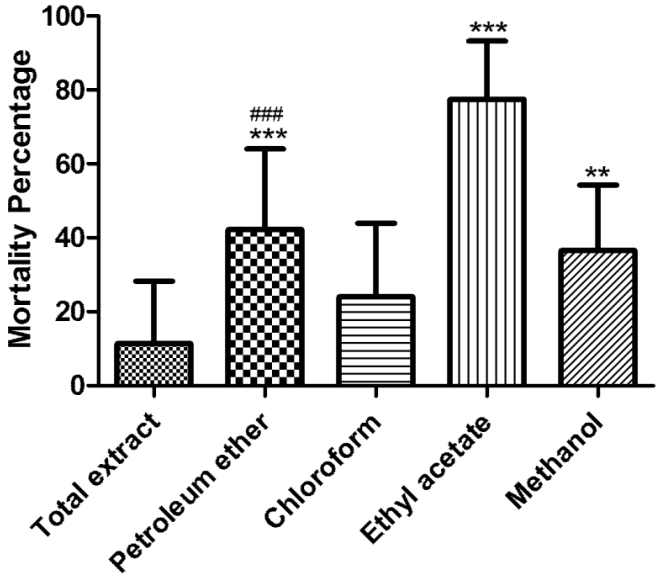


Fig. 1. BST mortality percentage of all fractions (10 µg/ml) of Apiaceae fruits, ** P ≤ 0.05, *** P ≤ 0.01 with total extract, ### P ≤ 0.05 with ethyl acetate fraction

3.2. MTT Assay

Cytotoxic fractions of Apiaceae fruits against cancer and normal cell lines were demonstrated in Table 2. Other samples showed IC_{50} upper than 200 µg/ml.

Most cytotoxic fractions were active against breast adenocarcinoma (MDA-MB-231) including PE of *P. anisum*, CL of *F. assa-foetida*, CL of *H. persicum*, PE of *A. graveolens* (celery), PE of *T. ammi* and PE of *D. carota* with IC_{50} equal to 147.76, 155.65, 177.61, 184.36, 195.83 and 197.00 µg/ml, respectively. Only CL and PE of *F. assa-foetida* demonstrated cytotoxicity on other cancer cell lines including CL and PE fractions with IC_{50} equal to 151.94 and 158.03 µg/ml on A549 alveolar and CL fraction with IC_{50} equal to 144.16 µg/ml against HT-29 colon cell lines.

Near all fractions of fruits demonstrated IC_{50} value of upper than 200 µg/ml against normal cell line except CL of *F. assa-foetida* and *A. graveolens* (celery) with IC_{50} equal to 140.66 and 193.71 µg/ml, respectively.

It was considerable greatest cytotoxic fractions against colon carcinoma (HT-29), breast adenocarcinoma (MDA-MB-231) and alveolar basal epithelial adenocarcinoma (A549) cell lines were from Ammineae and Peucedaneae tribes of Apiaceae family.

3.3. Antioxidant power assay

Total antioxidant activities of different fractions of Apiaceae fruits were measured based on $FeSO_4$ standard curve ($y = 0.001x + 0.049$, $r^2 = 0.932$) and reported in Table 1 in comparison to natural (vitamin E) and synthetic (BHA; butylated hydroxyanisole) antioxidants.

Antioxidant activities of some fractions of *A. graveolens*, *B. persicum* and *P. crispum* from Ammineae tribe, *F. vulgare* from Seselineae tribe, *H. persicum* and *F. assa-foetida* from Peucedaneae tribe were higher than others. All fractions of Apiaceae fruits have been shown no considerable antioxidant effects compare to vitamin E and BHA. On the other side, EA fraction of all fruits demonstrated greatest or significant reducing capacity among different fractions except *F. vulgare* and *D. carota*.

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Table 2. Cytotoxic fractions of Apiaceae fruits against cancer and normal cell lines.

Samples	IC_{50} of MTT assay			
	HT-29	**MDA-MB-231**	**A549**	**fibroblast**
PE[a] of *P. anisum*	> 200	147.76 ± 3.12	> 200	>200
PE of *T. ammi*	> 200	195.83 ± 2.44	> 200	>200
PE of *A. graveolens*	> 200	184.36 ± 2.93	> 200	>200
CL[b] of *A. graveolens*	> 200	> 200	> 200	193.71±2.64
CL of *H. persicum*	> 200	177.61 ± 1.24	> 200	>200
PE of *F. assa-foetida*	> 200	> 200	158.03 ± 1.63	>200
CL of *F. assa-foetida*	144.16 ± 1.25	> 200	> 200	>200
CL of *F. assa-foetida*	> 200	155.65 ± 2.86	> 200	>200
CL of *F. assa-foetida*	> 200	> 200	151.94 ± 1.74	>200
CL of *F. assa-foetida*	> 200	> 200	> 200	140.66±2.50
PE of *D. carota*	> 200	197.00 ± 3.47	> 200	>200

Results are expressed as IC_{50} value (µg/ml), mean of three determinations. [a] PE: petroleum ether fraction; [b] CL: chloroform fraction; *P. anisum*: *Pimpinella anisum*, *T. ammi*: *Trachyspermum ammi*; *A. graveolens*: *Apium graveolens*; *H. persicum*: *Heracleum persicum; F. assa-foetida*: *Ferula assa-foetida; D. carota*: *Daucus carota*

3.4. Total phenols determination

Total phenols contents of different fractions of Apiaceae fruits were determined according to the standard curve of Gallic acid ($y = 0.0076\ x$, $r^2 = 0.999$) and reported in Table 1. Some fractions of *P. anisum* and *T. ammi* from Ammineae tribe had the highest phenol contents among others. ME and EA were fractions of fruits with higher amounts of phenols except PE fraction of *D. carota* and *P. anisum* (after ME) which contained greatest content of phenols. Statistical analysis of total phenols showed no difference between fractions ($P < 0.05$) (Figure 2).

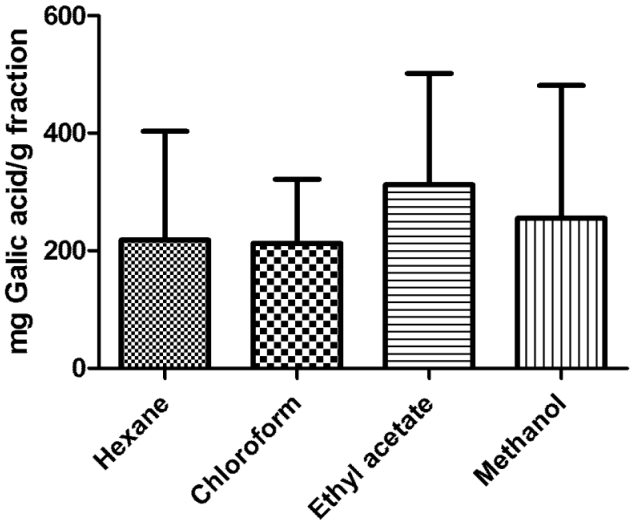


Fig. 2. Total phenols contents in all fractions of Apiaceae fruits

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4. Discussion

The fruits of Apiaceae family are widely used as spices around the world for culinary and medicinal properties [14, 15]. One of the important properties of consumption of plants as food is the prevention of chronic diseases including cancers. In the present study, cytotoxicity, antioxidant effects and total phenols of different fractions of twelve fruits from the Apiaceae family were measured and compared in various tribes of Apiaceae family.

The results were demonstrated cytotoxicity of almost all EA fractions and some other fractions of fruits measured by BST method were comparable with well-known cytotoxic alkaloid, berberine hydrochloride with LC_{50} of 26 µg/ml [16]. In fruits from Ammineae tribe, EA of *A. graveolens*, PE and EA of *B. persicum*, EA and ME of *P. crispum*, EA of *P. anisum* and EA of *T. ammi* had significant cytotoxic activities. A previous study about toxicity investigation on *A. salina* by traditional medicinal plants of Northern Peru showed LC_{50} of aqueous and ethanol crude extracts of *A. graveolens* aerial parts as 171 and 25 µg/ml, respectively [17]. There was an opposite report for none lethality activities of different extracts of *A. graveolens* of Pakistan on brine shrimp [18]. An investigation in Oman demonstrated ethyl acetate and hydro alcoholic extracts of the leaves of *P. crispum* killed the shrimp larvae with LC_{50} values equal to 51.95 and 88.15 µg/ml, respectively [19]. Assessment of brine shrimp cytotoxic activity of *T. ammi* seeds ethanol extract in Sundarbans mangrove forest region revealed LC_{50} and LC_{90} as 35.48 and 66.83 µg/ml, respectively [20]. The toxicity of crude extracts of present study was near to mentioned results.

EA of *C. sativum* from Smyrneae tribe and EA and CL of *F. vulgare* from Seselineae tribe were effective against *A. salina*. Aqueous and ethanol extracts of *C. sativum* aerial parts with LC_{50} of 22 and 0.015 µg/ml, and *F. vulgare* with LC_{50} of >10000 and 2.75 µg/ml exhibited their BST activity [17]. Another study on Sudanese plants demonstrated LC_{50} of 893.97 and 0.012 µg/ml for *F. vulgare* aqueous and ethanol extracts, respectively [21]. The data of present work about crude hydroalcoholic extracts of *C. sativum* and *F. vulgare* from Iran showed weaker toxicity than alcoholic extracts of Peru and Sudanese plants.

EA, PE and CL of *A. graveolens*, EA, PE and CL of *H. persicum*, and PE and EA of *F. assa-foetida* from Peucedaneae tribe showed potent effects as cytotoxic agents. Cytotoxicity of the aerial part of *A. graveolens* (dill) crude extract against brine shrimps showed it was toxic with LC_{50} value of 51.29 µg/ml [22]. A previous report revealed essential oil of *H. persicum* (LC_{50} equal to 0.0071 µl/ml) was known as active fraction in BST assay [23]. Our data about toxicity of crude extract of *H. persicum* was stronger than previous mentioned result.

EA and CL of *C. cyminum*, and PE and EA of *D. carota* from Caucalineae tribe were effective via brine shrimp bioassay. There were no previous reports about brine shrimp bioactivities of these plants.

Further cytotoxic investigations of different fractions of Apiaceae fruits were done by MTT assays against three cancer cell lines (HT-29, MDA-MB-231 and A549) and a normal foreskin fibroblast cell line. According to standard of National Cancer Institute (NCI) and Geran protocol, when a crude extract showed an IC_{50} less than 20 µg/ml, it was highly cytotoxic and active against cancer cell lines, when IC_{50} equal to 21 - 200 µg/ml, it is moderately cytotoxic, when IC_{50} equal to 201 - 500 µg/ml, it is weakly cytotoxic and when IC_{50} is upper than 501 µg/ml, it isn't cytotoxic [24].

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In details, all fractions showed IC_{50} higher than 200 µg/ml (weak or non-cytotoxic) except CL of *F. assa-foetida* on HT-29 with IC_{50} equal to 144.16 µg/ml; PE and CL of *F. assa-foetida* on A549 with IC_{50} equal to 158.03 and 151.94 µg/ml, respectively and CL of *A. graveolens* (celery) and *F. assa-foetida* on foreskin fibroblast cell line with IC_{50} equal to 193.71 and 140.66 µg/ml, respectively.

Based on this criterion, all fractions of fruits demonstrated no significant cytotoxic effects on cancer and normal cell lines. But there were maybe potent cytotoxic compounds in the most cytotoxic fractions of them including *P. anisum*, *F. assa-foetida*, *H. persicum*, *A. graveolens* (celery), *T. ammi* and *D. carota*, or they were maybe active against other cancer cell lines. It was interesting that most of cytotoxic fractions were from Ammineae and Peucedaneae tribes of Apiaceae. There were little reports about cytotoxic evaluation of extracts or fractions of mentioned plants. Antiproliferative effects of *A. graveolens* seeds extract has been confirmed on Dalton's lymphoma ascites (DLA) and mouse lung fibroblast (L929) cell lines by induction of apoptosis, DNA fragmentation and morphological changes [25]. Another study showed hexane extract of *A. graveolens* at concentrations of 100 and 200 µg/ml had the best cytotoxic activity on Rhabdomyosarcoma (RD) cell line [26]. In present research, more fractions of Apiaceae fruits were active against breast cancer (MDA-MB-231) cell line including PE of *P. anisum* and *T. ammi* from Ammineae tribe with IC_{50} equal to 147.76 and 195.83 µg/ml, respectively. Previous investigations showed treatment of liver HepG2 cell lines with anise seeds essential oil could exhibited a significant cytotoxicity [27]. In addition, ethanol extract of *P. anisum* revealed antiproliferative and apoptotic effects toward human prostate cancer cell line (PC-3) with IC_{50} value of 400 µg/ml [28]. Cytotoxicity of *T. ammi* essential oil on colon carcinoma cells was confirmed with an IC_{50} value of 9.6 µg/ml, too [29]. It was shown phytochemicals including thymol, γ-terpinene and *p*-cymene played an important role in anticancer activity of essential oil and hexane extracts of ajwain [30].

PE of *A. graveolens* (dill) and CL of *H. persicum* and *F. assa-foetida* from Peucedaneae tribe with IC_{50} equal to 184.36, 177.61 and 155.65 µg/ml, respectively and PE of *D. carota* from Caucalineae tribe with IC_{50} equal to 197.00 µg/ml showed cytotoxicity against breast cancer cell line, too. In before experiment, cytotoxicity evaluation of *H. persicum* aerial parts essential oil on three human cancer cell lines (HeLa, LS180 and Raji) demonstrated no effects with IC_{50} more than 2 mg/ml [31]. Cytotoxicity of carrot essential oil was reported by IC_{50} of 35.3 and 46.1 µg/ml on green monkey kidney (VERO) and human pharynx squamous cell carcinoma (FaDu) cell lines, respectively. Carotol, an important constituent of *D. carota* essential oil exhibited moderate cytotoxicity on both cell lines with no selectivity [32]. In another study, *D. carota* essential oil induced selective apoptosis in acute myeloid leukemia (AML) cell line via a MAPK-dependent mechanism [33]. It was interesting that most of cytotoxic plants fractions were from Ammineae and Peucedaneae tribes of Apiaceae.

Antioxidant activities of some fractions of *A. graveolens, B. persicum* and *P. crispum*, *F. vulgare, H. persicum* and *F. assa-foetida* were higher than others but it was not comparable with natural (vitamin E) and synthetic (BHA; Butylated hydroxyanisole) antioxidants. EA fraction of all fruits except *F. vulgare* and *D. carota* demonstrated greatest or significant reducing capacity among different fractions. The

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previous study showed methanol extract of *A. graveolens* seeds exhibited better antioxidant effects as Fe^{+2} chelating, reducing power activities, and the amounts of total phenols were higher in comparison to other extracts [34]. Another research demonstrated *P. anisum* extract showed the strongest radical scavenging activity among seven Apiaceae fruits from Iran. In addition, ethyl acetate fraction of *P. anisum* exhibited the highest antioxidant activity and flavonoid content [35]. *P. crispum* exhibited DPPH free radical-scavenging activity and cupric reducing antioxidant capacity [36]. There were no reports about antioxidant activities of different fractions of mentioned plants.

Total phenol contents of ME of *P. anisum*, EA of *T. ammi* and PE of *P. anisum* from Ammineae tribe were higher than other samples. According to previous results, anethole and thymol were the most abundant components of *P. anisum* and *T. ammi* essential oil which have phenol structures [37, 38]. It was interesting that all plants with potent antioxidant activities and high phenol contents were from Ammineae tribe of Apiaceae.

5. Conclusion

The brine shrimp lethally test is an ideal method in the initial biological screening of a broad range of phytochemical compounds including toxic components. Present investigation demonstrated despite potent larvicidal effects of EA fractions from Apiaceae fruits against *A. salina*, they didn't demonstrate considerable cytotoxicity against cancer and normal cell lines. Statistical analysis also confirmed that EA fraction is the best cytotoxic fraction and there was no significant difference between all fractions from the point of view of total phenols. Almost more cytotoxic fractions belong to fruits from Peucedaneae tribe while all plants with high phenol contents and antioxidant powers were from Ammineae tribe of Apiaceae. Secondary metabolites in active cytotoxic fractions have potential to act as toxic compounds on other cancer cell lines. In addition, there were not seen significant correlations between cytotoxicity, antioxidant and total phenols of different fractions from Apiaceae fruits.

Author contributions

Z.T., S.G. and E.M. conceived of the idea, planned and supervised the experiments. M.R., Sh.M., F.M., M.A., F.K., and B.Kh. carried out the experiments. M.P.H, S.T., M.J.T. S.J.N., and M.Sh. helped to perform experiments and analyzed the data. Z.T., M.P.H. and S.G. discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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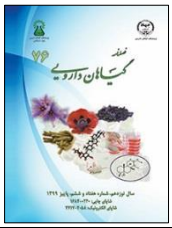
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مقاله تحقیقاتی

غربالگری میوه‌های خانواده چتریان، به عنوان منابع طبیعی بالقوه دارای خواص بیولوژیک

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اطلاعات مقاله

گل‌واژگان:
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میوه‌ها
سمیت سلولی
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محتوای فنولی

چکیده

مقدمه: میوه‌های چتریان به عنوان ادویه رایج برای پیشگیری و درمان بسیاری از بیماری های مزمن به کار می‌روند. **هدف:** مطالعه حاضر به بررسی خواص بیولوژیک میوه‌های مختلف از قبیله‌های گوناگون خانواده چتریان می‌پردازد و با مقایسه آنها سعی دارد فراکشن (های) دارای خواص بالقوه شامل پیشگیری از سرطان را برای مطالعات آینده بیابد. **روش بررسی:** میوه کرفس، زیره کرمانی، جعفری، انیسون، بادیان رومی (زنیان)، گشنیز، رازیانه، شوید، گلپر، آنقوزه، زیره سبز و هویج به وسیله متانول ۸۰ درصد عصاره‌گیری و سپس عصاره‌ها به ترتیب با پترولیوم اتر، کلروفرم و اتیل استات فراکشنه و باقیمانده فراکشن متانولی نامیده شد. میزان سمیت سلولی عصاره تام و فراکشن‌ها به وسیله تست BST و MTT در برابر سلول‌های سرطانی و نرمال بررسی گردید، میزان اثر آنتی‌اکسیدانی و فنول تام نمونه‌ها به ترتیب با تست FRAP و فولین سیوکالتو تعیین گردید. **نتایج:** در تست BST، سمیت عمومی فراکشن‌های اتیل استات (میانگین داده‌ها) بیش از سایر نمونه‌ها بود. سمی‌ترین فراکشن‌ها در برابر سلول‌های سرطانی HT-29، MDA-MB-231 و A549 از قبایل Ammineae و Peucedaneae بودند و فراکشن‌های حاوی مقادیر بالای ترکیبات فنولی و با قدرت آنتی‌اکسیدانی متعلق به قبیله Ammineae بودند. **نتیجه‌گیری:** میوه‌های خانواده چتریان دارای اثرات قابل توجه بیولوژیک می‌باشند، به همین دلیل جداسازی ترکیبات فیتوشیمیایی از فراکشن‌های فعال با اثر سمیت سلولی، در مطالعات آینده پیشنهاد می‌گردد.

مخففها: ET، عصاره تام متانولی؛ PE، فراکشن پترولیوم اتر؛ CL، فراکشن کلروفرمی؛ EA، فراکشن اتیل استاتی؛ME، فراکشن متانولی؛ BST، تست لارو میگوی آب‌شور؛ FRAP، قدرت آنتی‌اکسیدانی- احیاکنندگی آهن

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