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Detection and determination of poly aromatic hydrocarbons in vegetable and tree leaves, Baghdad city, Iraq

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***Abstract*---**Vegetables and tree leaves are an important part of healthy diet due to their nutritional value, so contamination of vegetables and tree leaves may be dangerous for the population. Polycyclic aromatic hydrocarbons (PAHs) are compounds that are widely distributed in the environment and many of them exhibit carcinogenic effects. PAHs can enter the food chain in a variety of ways; hence the examination of PAHs in food is important. The current study examines the amounts of PAHs for ten of these compounds which are acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), naphthalene (Np), anthracene (An), phenanthrene (Ph), benzo[α]anthracene (B[α]An), chrysene (Chry) , pyrene(Py), benzo[α]pyrene (B[α]Py) , In the following samples (celery , watercress , cress , Eucalpt , mandarin , orange). The current study showed the presence of naphthalene in all samples, and the highest concentration was recorded in the Eucalyptuse leaves sample from the Doura region (19.433ppm) and the lowest concentration recorded in the Taji watercress sample (0.0785ppm). It was noted that the presence of pyrene by 66.66% and benzo-a-pyrene by 77.77%, while chrysene did not appear in any of the samples.

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***Keywords*---**poly aromatic hydrocarbons, vegetables, healthy diet

Introduction

Hydrophobic chemical molecules with two or more aromatic rings are known as polycyclic aromatic hydrocarbons (PAHs). These compounds were formed during incomplete combustions. Due to a lack of ideal temperature and oxygen concentration, as well as a high wetness [1-2] Forest and brush fires, volcanoes, bacterial and algal synthesis, petroleum seeps, erosion of sedimentary strata containing petroleum hydrocarbons, and breakdown of vegetative liter fall are all examples of natural sources of PAHs creation. Anthropogenic sources of PAHs include the following: Incomplete combustion (such as incinerators) and various industrial processes are examples of large point sources.

Dispersed sources, for example, are smaller point sources (such as automotive emissions, smoke from wood-burning stoves, jet aircraft exhausts, cigarette and cigar smoke, and back- yard barbecues).Petroleum product spills, sewage sludge, and tarries or creosote waste materials are all anthropogenic sources of PAHs.It's worth noting that incomplete combustion, whether natural or anthropogenic ally induced, has been identified as the single biggest source of PAHs in the environment. [3].PAHs has high melting and boiling temperatures (as a result of which they are solid), low vapor pressure, and very low aqueous solubility ,The latter two characteristics tend to diminish as molecular weight increases, whereas resistance to oxidation and reduction increases[4].PAHs can move through all environmental compartments, including air, water, sediments, soil, and plants, contaminating aquatic and terrestrial species. As a result, large quantities of PAHs could be found in human meals. [5].Dietary consumption is a key source of PAHs exposure, accounting for more than 70% of total exposure in non-smokers. [6]. In addition, climate change will have an impact on the accumulation and uptake of polycyclic aromatic hydrocarbons in plants. [7].

Furthermore, heat may promote the excretion of root exudates and the desorption of remaining PAHs. [8].Contamination of agricultural soil and crops with persistent organic pollutants (POPs) has emerged as a global environmental issue, posing a risk to human health via food chain transfers. [9].

Since their discovery as a carcinogenic family of molecules in the late 1920s, PAHs have sparked a lot of research. [10]. According to recent epidemiological studies, dietary exposure to PAHs is linked to an elevated risk of some human malignancies. [11].The International Agency for Research on Cancer re-classified Benzo[a] Pyrene (BaP) from group 2A (probably carcinogenic to humans) to group 1 (carcinogenic to humans), Chrysene (Chry) was re-classified from group 3 (not classifiable as carcinogenic to humans) to group 2B (possibly carcinogenic to humans), and Benzo[a] Anthracene (BaA) was changed from group 2A to 2B [12-13-14].

Low molecular mass PAHs can be adsorbed by surface adsorption in plants, while high molecular mass PAHs can contaminate the surface as a result of atmospheric fallout. The direct link between soil and plant PAHs concentrations

revealed a plausible channel from polluted soil to plant roots. [15].Low molecular mass PAHs can be concentrated on the waxy surface of vegetables and fruits by surface adsorption, while particle-bound high molecular mass PAHs can contaminate the surface due to atmospheric fall-out. [16].
The highest PAH levels in plants grown in areas with air pollution are specially found in vegetables with a large surface area exposed, e.g., lettuce, kale and spinach. [17].

In the Czech Republic, PAH intake owing to apple consumption is predicted to be 0.45 mg day-1. [18]. Ethier in Catalonia, the daily intake of PAHs for fruits and vegetables are set at. 0.2 mg and 0.3 mg, respectively. [19].
HPLC, LC-MS, LC-MS-MS, GC-FPD, GC-MSD, GC-ECD, or GC-MS are common analytical methods used to detect PAHs after extraction and purification from vegetables[20].

Aim and objective
The aim of this study was to look at PAHs levels and distribution in (celery , watercress , cress , Radish leaves , Eucalyptus leaves , ziziphus spina Christi leaves). Because many PAHs are carcinogenic, mutagenic, and teratogenetic, this could endanger human health.
Literature review

Since vegetables constitute an essential and important part of the human diet, whether they are leafy or root vegetables, as well as the leaves of trees, which have an important role in the therapeutic aspect of many diseases, so it is necessary to know the pollutants that affect these vegetables and trees and the most important pollutants that pose a threat to human health Its life and cause many diseases, including cancerous diseases and teratogenic genetic mutations, are polycyclic aromatic compounds consisting of at least two or more united aromatic rings that enter the plant by absorbing them from the atmospheric air or the plant absorbs them from the soil by the roots. Therefore, many previous studies have been conducted on this the topic. And compared to the permissible limit of these compounds in vegetables and leaves, 16 aromatic hydrocarbons were identified in vegetable samples taken from the industrial zone in Shanghai, China, with concentrations ranging from 7.65 to 0.458 ng/g[21].

Also, in a study that was conducted on vegetable samples in the center of the city of Accra in Metropolitan, 16 compounds of PAH compounds were found with concentrations ranging from 0.037 to 16.2μg / kg. The presence of naphthalene was also diagnosed in all samples and this is similar to the results of the samples currently studied[22]. In another study conducted in Seoul, Korea, there were eight PAH compounds with concentrations ranging from 0.67 to 0.82 in vegetables and fruits, respectively[23].While in a study conducted on vegetable samples collected from different regions of the Kingdom of Saudi Arabia, in which eight PAHs compounds in vegetables were analyzed, anthracene showed a high concentration in all vegetables, which showed a relatively lower level of benzo -b-fluoranthene[24]. But when conducting a study of polycyclic aromatic hydrocarbons in urban and rural areas in Constanta, Romania,The results showed a mean sum of 15 PAHs of 4.887 g/kg, and the tolerable daily intake of PAHs through vegetables ranged from 0.12 g/kg/d to 3.60 g/kg/d.[25].

Method

Extraction PAHs of samples
After the samples were dried, 10 g of each sample was weighed and placed inside the thumble (cylindrical filter) and we put on top of the sample the glass hair to prevent the sample particles from coming out, then we put 200 ml of a mixture of normal hexane and acetone in equal volumes (1:1) on top of the sample in the extraction flask We regulate the temperature of the heater according to the boiling point of the solvent used in the extraction, then we pass cold water in the condenser for the purpose of condensing the solvent and distilling it on the sample and so we continue until all the substance to be extracted is withdrawn.

Degassing of the solvents
The dissolved oxygen in the organic solvents used in the mobile phase was removed before using them in the experiment by using an ultrasonic bath for a while, in addition to the degassing device originally included in the HPLC device used in the study (vacuum degasser), which is a type (8891 Cole-Parmer) for the purpose of obtaining a high stability of the device.

Analysis
The RP-HPLC aKorea-made (YL9100) high-performance reverse liquid chromatograph was used to separate and detect polycyclic aromatic compounds and the column used C18 (4.6*25)cm was used with a gradient elution system using a mobile phase consisting of a mixture of water/acetonitrile solvents. 100 µl was injected into the loop at a temperature of 30 °C and a wavelength of 254 nm, a flow rate of 1 ml/sec, the detector connected to the device is UV / VIS YL9120.

Quantification of PAHs
It was conducted out using the HPLC instrument described in the previous item. The method of external standard plot was employed. 100 µl PAHs standard injections in triplicate. Linear regression lines were built using solutions .Peak area ratios versus PAH concentrations. LOQ is the lowest concentration at which the analyze can be reliably detected while also meeting some predefined bias and imprecision goals. The LOQ could be equivalent to the LOD or much higher in concentration.

Study area
Samples of (celery, cress, watercress, Radish leaves , Eucalyptus leaves , ziziphus spina Christi (leaves) were collected from three different places of Baghdad Governorate, which is the capital of Iraq, considering that it is an ideal and typical representation. All samples for each type of tree leaves and vegetables were from roadside (including highways) and rural areas and areas containing oil refineries.

The chemicals used in this research
Standard PAHs of high purity not less than (99.92%) and equipped from the company D-86926 Greifenberg Ammersse, Flurstrasse, Germany.Acenaphthene (Ace), Acenaphthylene (Acy),Fluorene (Flu),Naphthalene (Np), Anthracene (An),Phenanthrene (Phe) ,Benzo[α]Anthracene (B[α]An), Chrysene (Chry) , Pyrene (Py), Benzo[α]pyrene (B[α]Py)

Preparation of standard solutions

Standard solution was prepared for each aromatic compound with a concentration (100ppm) by dissolved(1mg) from PAHs in(10mL) from the ethanol from the standard stock solution ,other solutions were prepared (0.5 ,1 ,2 ,4 ,8 ,10)µg/mL with the compositions required for dilutions with acetonitrile.

Optimization of separation of PAHs by RP-HPLC

Experinmental Test

1. A standard solution was prepared for each compound of the PAHs from the stock solution with a concentration of 100 ppm and a volume of 100 µL. Standard solutions are also prepared for the mixture of these aromatic compounds, as the mixture contains different Concentrations of this compound.
2. 100µL of the first standard solution in a concentration of 100 ppm was injected into a column Reprosil 100 C18.5 with dimensions (25 * 4.6)cm in a device HPLC according to the Gradient system and a wavelength fixed at 254nm.

Table[1]

The program for the gradient elution of the mobile phase in the device HPLC.

Time (min)	H_2O [%]	CH_3CN [%]	Flow [mL/min]
Initial	60.0	40.0	1.00
7.00	40.0	60.0	1.00
11.00	0.0	100.0	1.00
17.9	0.0	100.0	1.00
18	60.0	40.0	1.00

3. The previous steps were repeated on standard solutions of different concentrations of PAHs.
4. The retention time of each compound was found from its chromatogram
5. A mixture of the standard solutions for PAHs was injected by using a mobile phase of (acetonitrile- water) at a flow rate (1 mL/min), wavelength and the same column temperature (25C°), thus detecting and determining the peak of each aromatic compound and then calculating the retention time for each compound.

Sample preparation

1. The Leaves of vegetables and leaves of trees were dried by placing them under direct sunlight for seven days
2. A special mill was used to grind the samples to a very fine size.
3. 10 grams weighed by sensitive scale used KERN ACJ/ACS of each sample was taken and placed in the cellulose Thimble, then it was placed in the Soxhlet apparatuses.
4. 200 ml of the extraction solvent was acetone and normal hexane were taken in a ratio 1:1 to perform the extraction process at the boiling point of the solvent 50 °C.
5. Use a rotary evaporator to concentrate each sample to dryness and then complete the volume with acetonitrile to 1mL.

6. The sample is placed in a small amber vial and the name of the sample and the date of extraction is written on it, then it is measured with a device HPLC.

Results and Discussion

Linearity

Different concentrations ranging from 0.5 to 10 ppm were used to create the standard calibration curve. This study looked at three replicate injections of each concentration. From the graph of peak area and concentration, the Detection limit and quantitative limit were calculated.Through this equation, we extract the concentration of PAHs compounds present in the samples, as shown in Figure1 , Figure2, Figure3, Figure4, Figure5, Figure6, Figure7, Figure8, Figure9, Figure10.

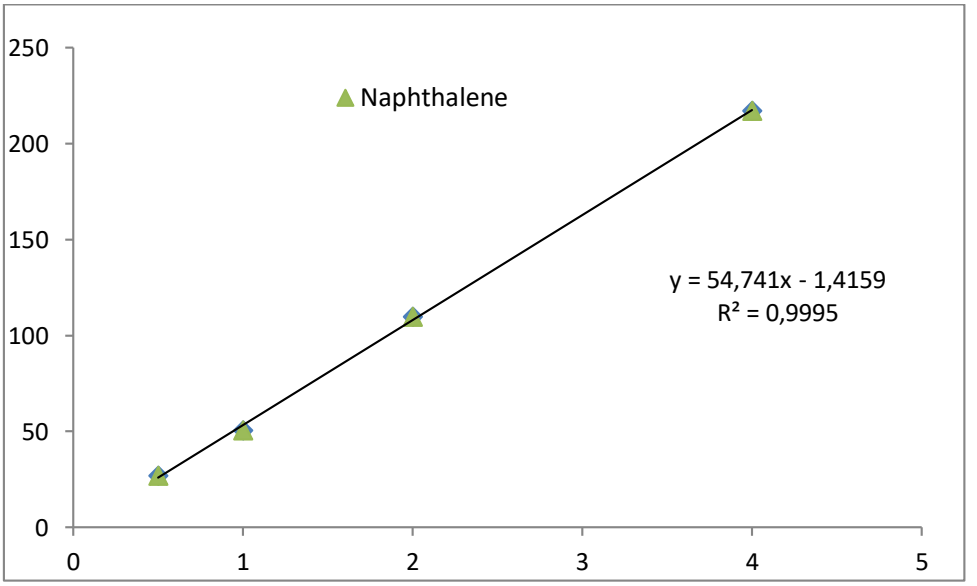
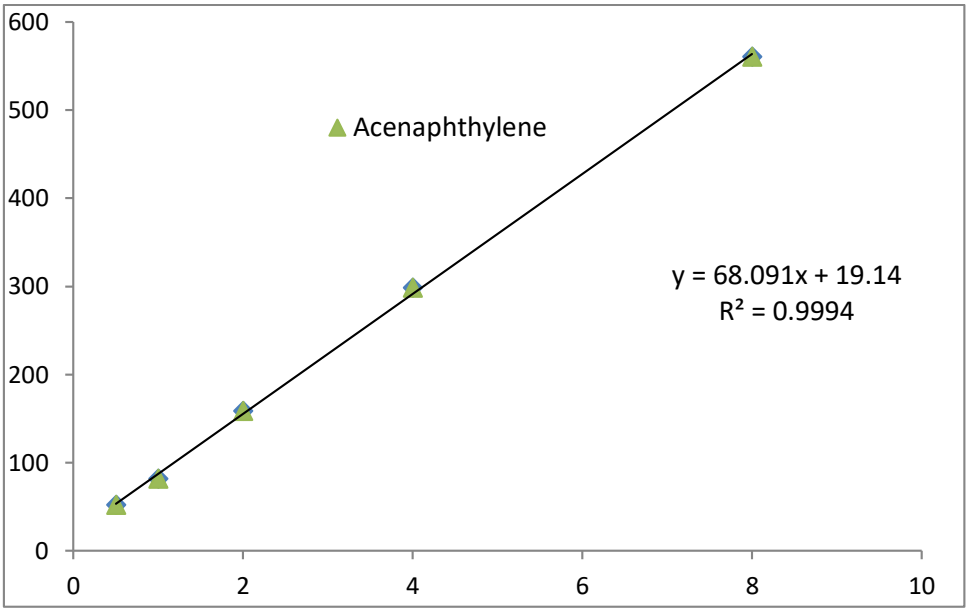


Figure 1 Calibration curve of Naphthalene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	27.138	27.558	0.915369	3.373015
2	Cal-std2	1	50.729	50.514	0.944044	1.860955
3	Cal-std3	2	109.89	110.08	0.841249	0.765537
4	Cal-std4	4	217.135	217.494	0.756354	0.348333



Figar 2 Calibration curve of Acenaphthylene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	52.032	52.015	0.071715	0.137828
2	Cal-std2	1	81.9	82.5	1.216553	1.485412
3	Cal-std3	2	158.496	158.374	0.4263	0.268966
4	Cal-std4	4	298.3207	298.531	0.684899	0.289923
5	Cal-std5	8	560.45	560.06	1.165032	0.207874

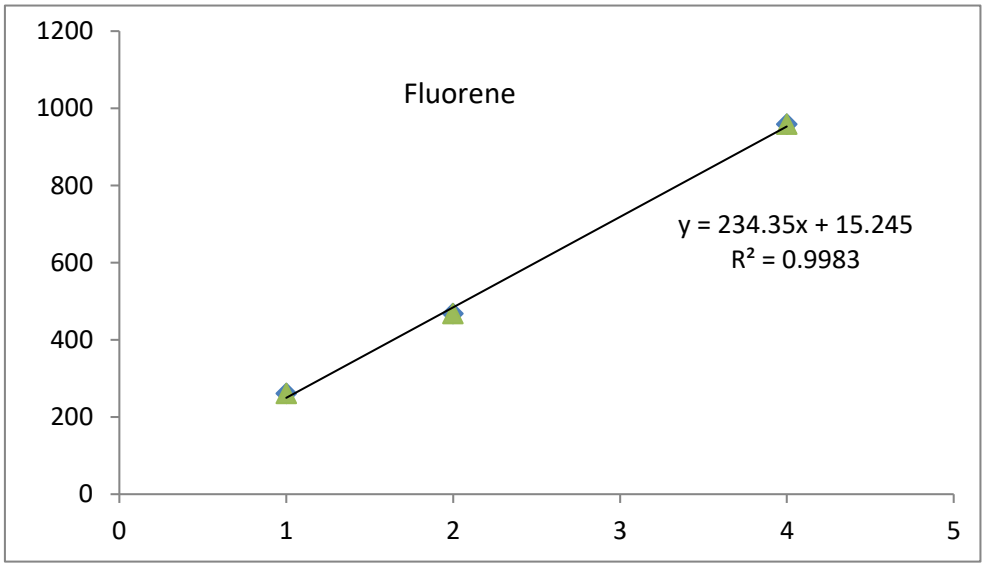


Figure 3 Calibration curve of Fluorene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	261.793	261.691	0.276491	0.105614
2	Cal-std2	1	482.158	482.311	0.616898	0.127945
3	Cal-std3	2	958.238	957.795	0.869694	0.09076

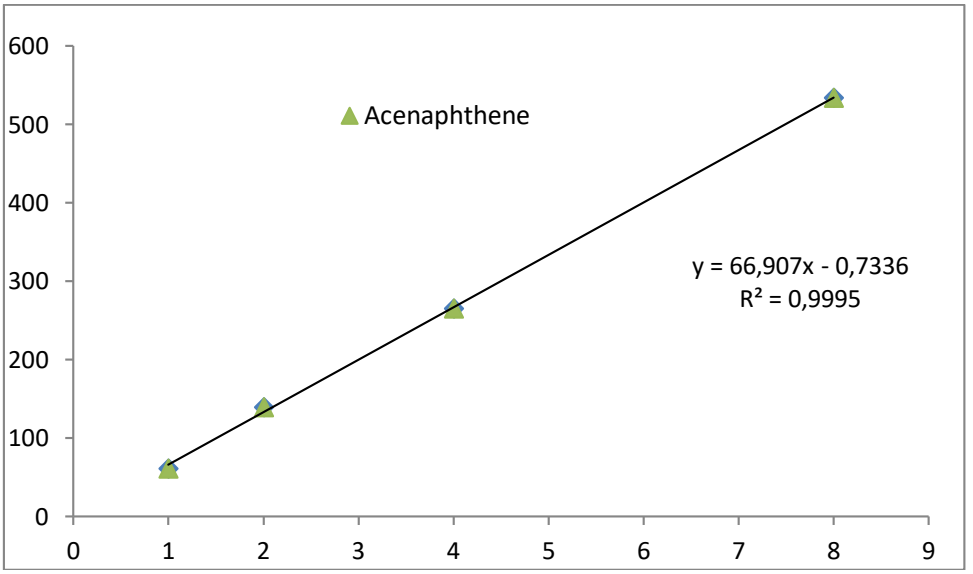


Figure 4 Calibration curve of Acenaphthene

No	Name	Conc.(µg/mL)	Area	median	S.D	R.S.D%
1	Cal-std1	0.5	61.531	61.418	0.765613	1.244273
2	Cal-std2	1	139.318	139.083	0.583165	0.418585
3	Cal-std3	2	265.666	265.108	0.970818	0.365428
4	Cal-std4	4	534.162	534.342	0.658711	0.123317

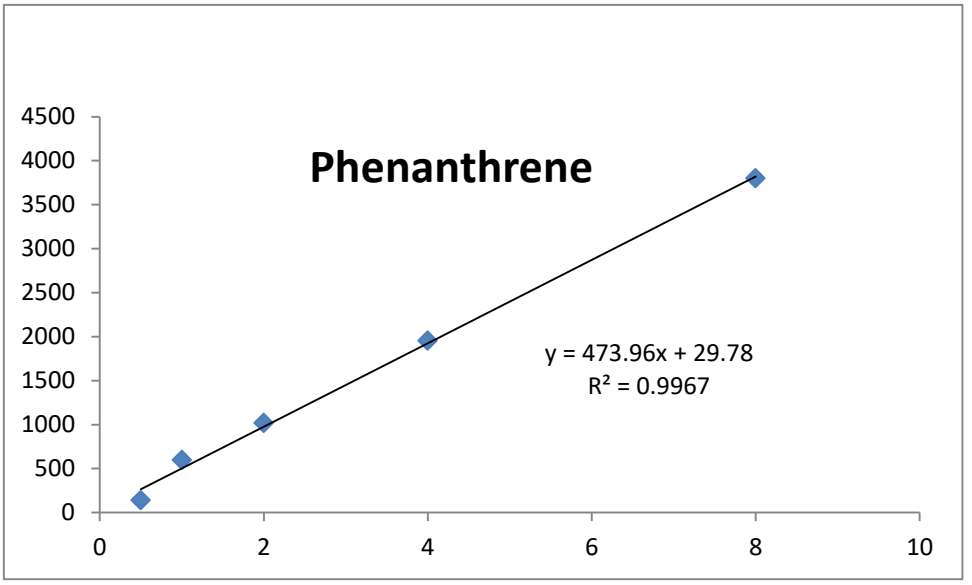


Figure 5 Calibration curve of Phenanthrene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	138.611	138.499	0.217791	0.157124
2	Cal-std2	1	594.865	594.880	0.034073	0.005728
3	Cal-std3	2	1015.281	1015.772	1.067816	0.105174
4	Cal-std4	4	1950.101	1950.093	0.019287	0.000989
5	Cal-std5	8	3796.49	3796.483	0.013892	0.000366

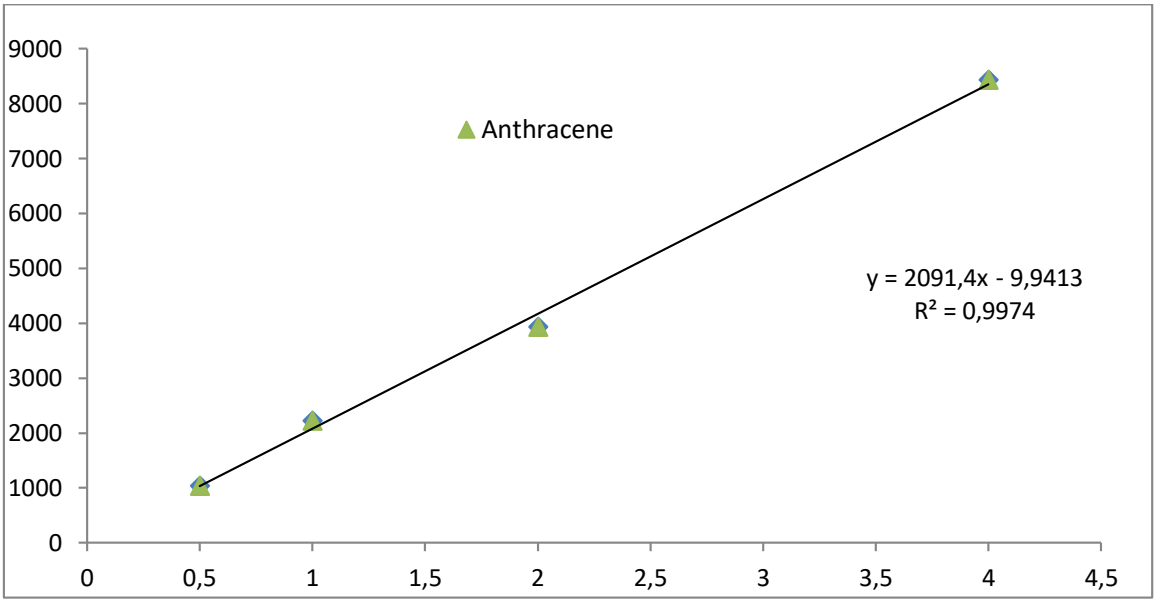


Figure 6 Calibration curve of Anthracene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	1043.502	1044.089	1.071736	0.102706
2	Cal-std2	1	2228.767	2229.333	1.229899	0.055183
3	Cal-std3	2	3938.52	3938.885	0.873687	0.022183
4	Cal-std4	4	8435.245	8435.776	1.071227	0.012699

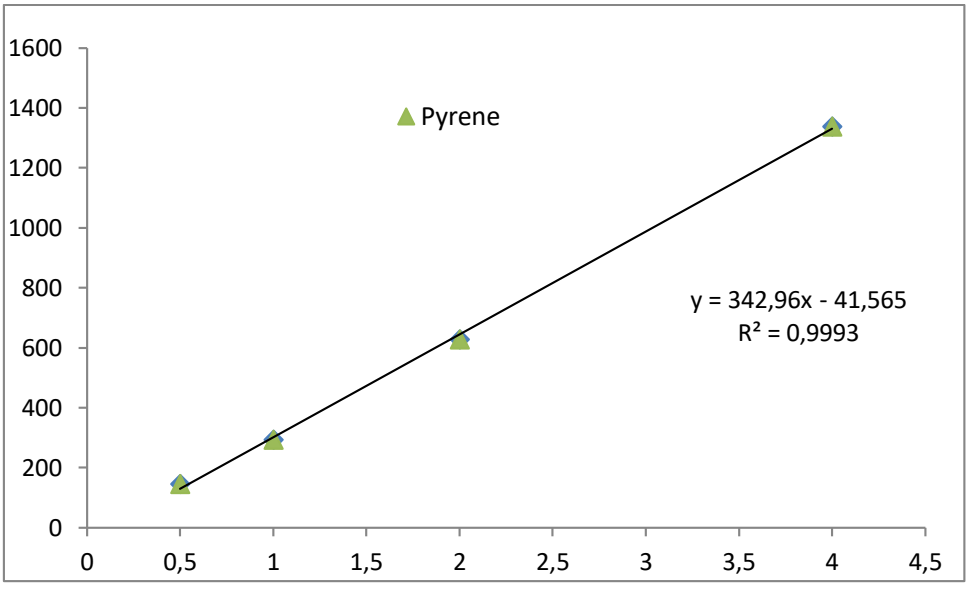


Figure 7 Calibration curve of Pyrene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	145.760	146.124	0.771355	0.529195
2	Cal-std2	1	293.598	294.144	0.990195	0.337262
3	Cal-std3	2	628.335	628.757	1.168616	0.185986
4	Cal-std4	4	1338.27	1338.019	0.585357	0.04374

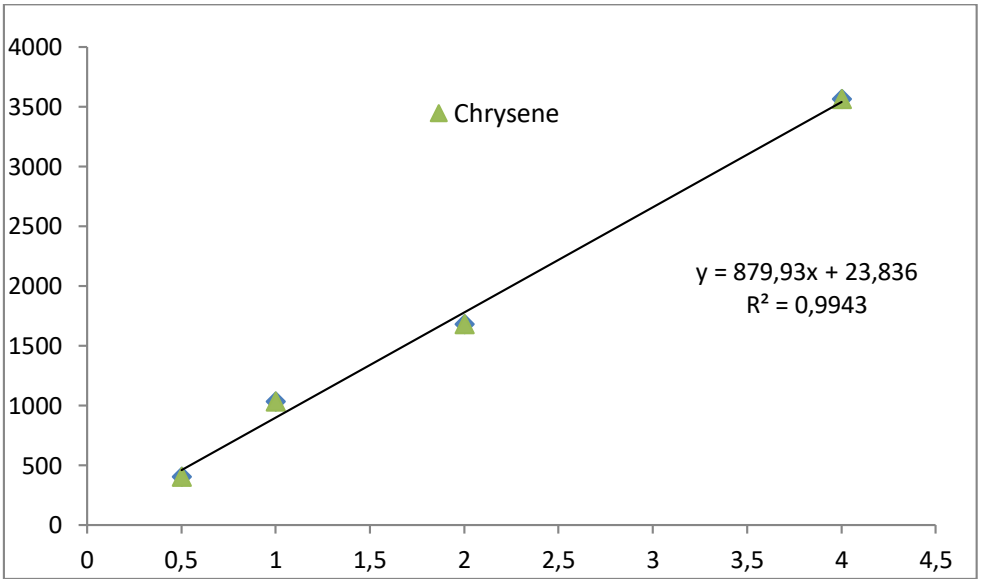


Figure 8 Calibration curve of Chrysene

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	406.125	406.470	0.72858	0.179398
2	Cal-std2	1	1037.704	1038.139	0.812157	0.078265
3	Cal-std3	2	1683.745	1683.304	0.825199	0.04901
4	Cal-std4	4	3567.283	3566.902	0.970323	0.027201

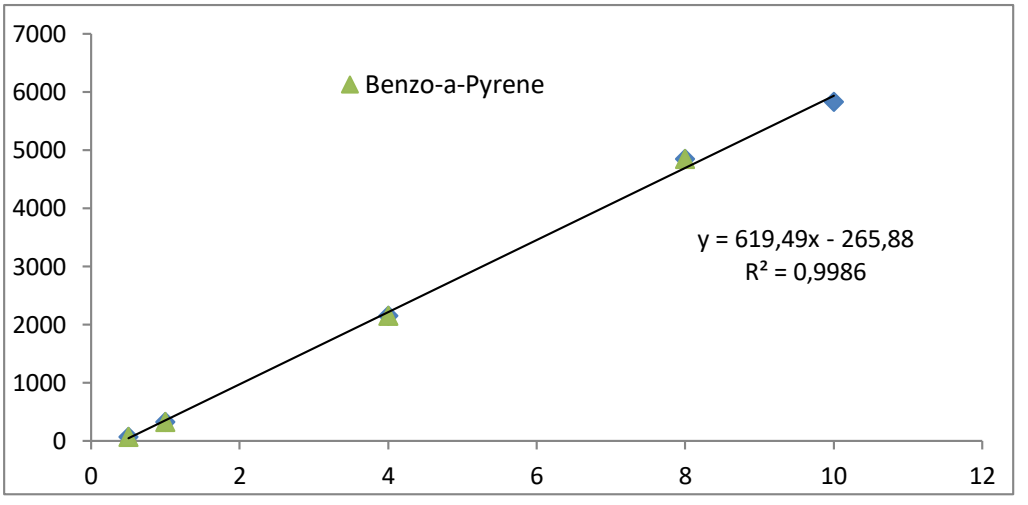


Figure 9 Calibration curve of Benzo(a)Pyrenees

No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	73.767	74.190	0.766681	1.039327
2	Cal-std2	1	328.070	327.680	0.965971	0.294441
3	Cal-std3	4	2151.181	2151.505	0.870464	0.040464
4	Cal-std4	8	4845.765	4845.498	0.935526	0.019306
5	Cal-std5	10	5829.922	5830.341	0.915484	0.015703

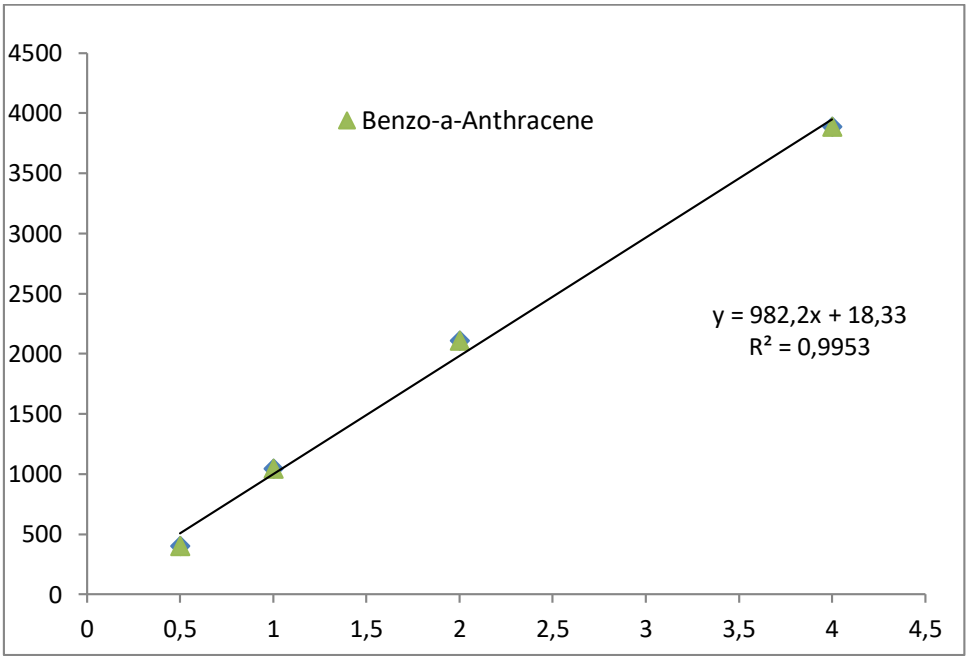


Figure 10 Calibration curve of Benzo(a) Anthracene

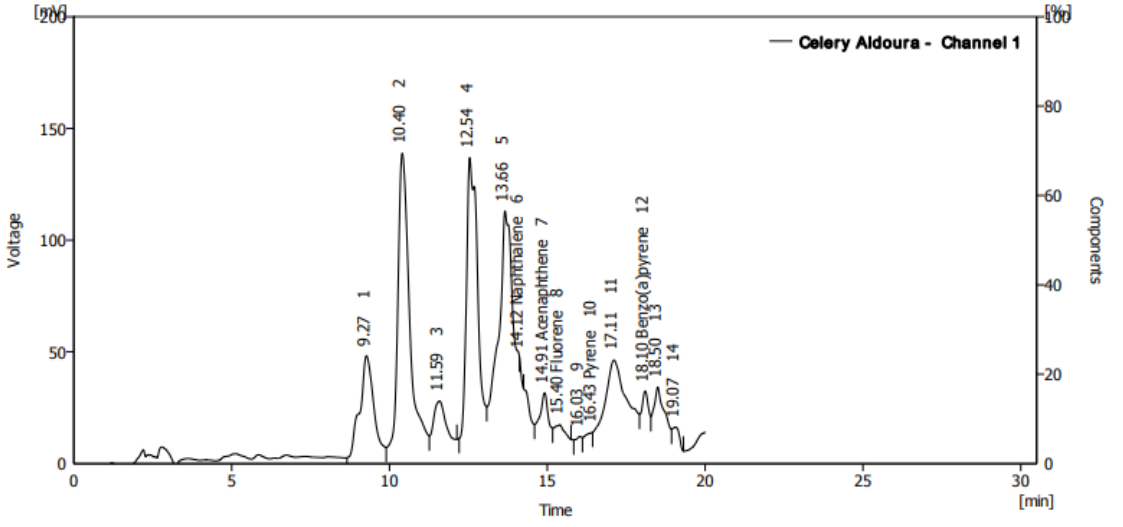
No	Name	Conc.(µg/mL)	Area	Median	S.D	R.S.D%
1	Cal-std1	0.5	402.2	400.860	0.75112	0.187218
2	Cal-std2	1	1042.999	1043.466	0.871965	0.083602
3	Cal-std3	2	2108.437	2108.074	0.712592	0.033797
4	Cal-std4	4	3887.198	3886.72	0.877747	0.02258

Table [2]
Polycyclic aromatic hydrocarbons (PAHs) that have been studied mainly monitored in tree leaves and vegetable.

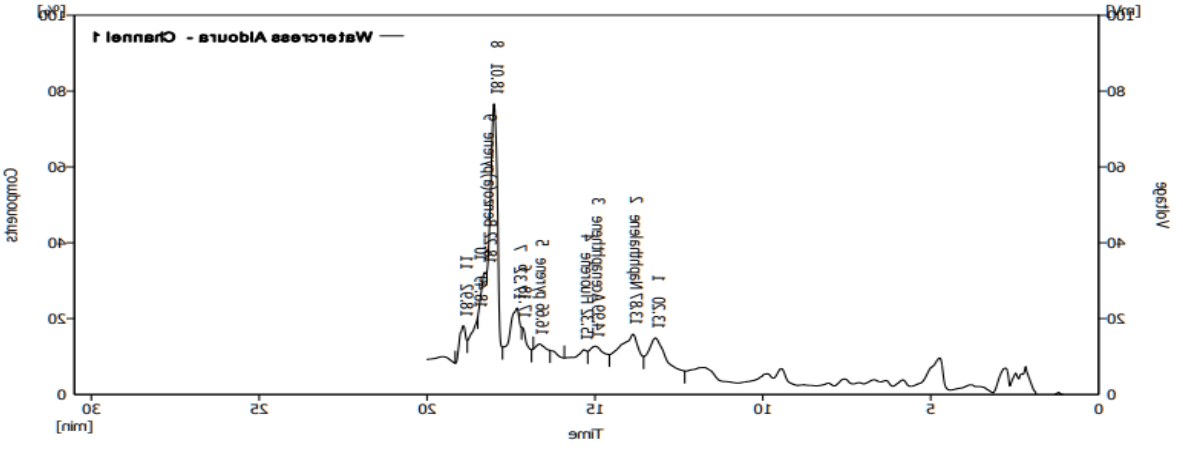
PAHs Abbreviation	MW($gmol^{-1}$)	Number of rings	t_R	Det.Limit Ppm	Quan.Limit Ppm
NPH	128	2	14.16	0.01559	0.168651
ACY	152	3	14.63	0.002067	0.006891
ACP	154	3	15.91	0.037328	0.124427
FLR	166	3	15.26	0.003168	0.010561
ANT	178	3	15.80	0.001541	0.005135
PHE	178	3	15.64	0.003821	0.012736
PYR	202	4	16.55	0.007938	0.026460
BaA	228	4	16.84	0.002808	0.009361
CHR	228	4	16.78	0.002691	0.00897
BaP	252	5	18.29	0.01559	0.051966

After finding the retention time of the standard aromatic compounds, these compounds were detected and identified as well as finding the detection limit and quantitative limit in the samples of fruits and vegetables injected into the RP-HPLC device, by using the optimal separation conditions for the samples mentioned in the method of work, the following results were obtained:

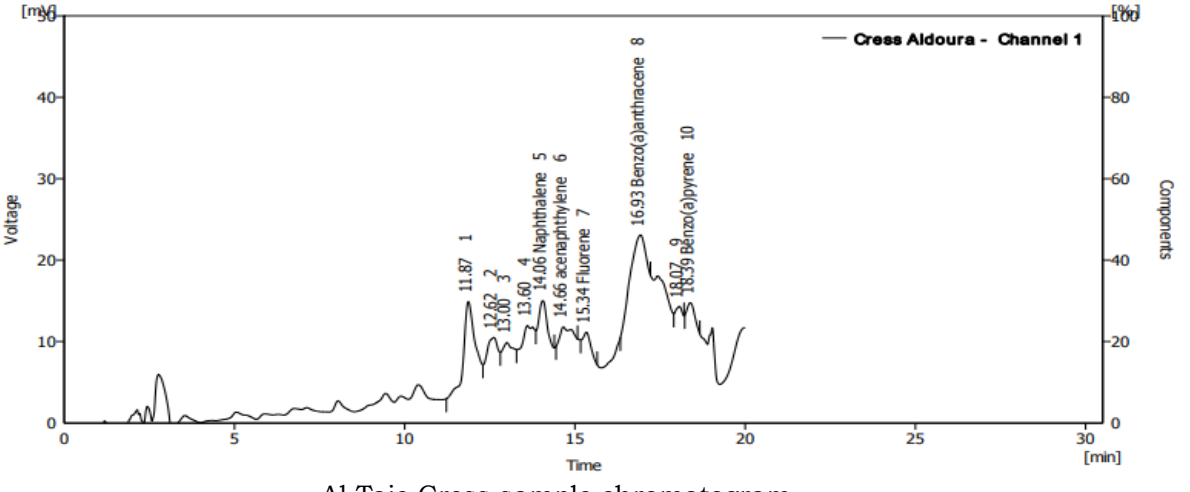
Alduora celery sample chromatogram



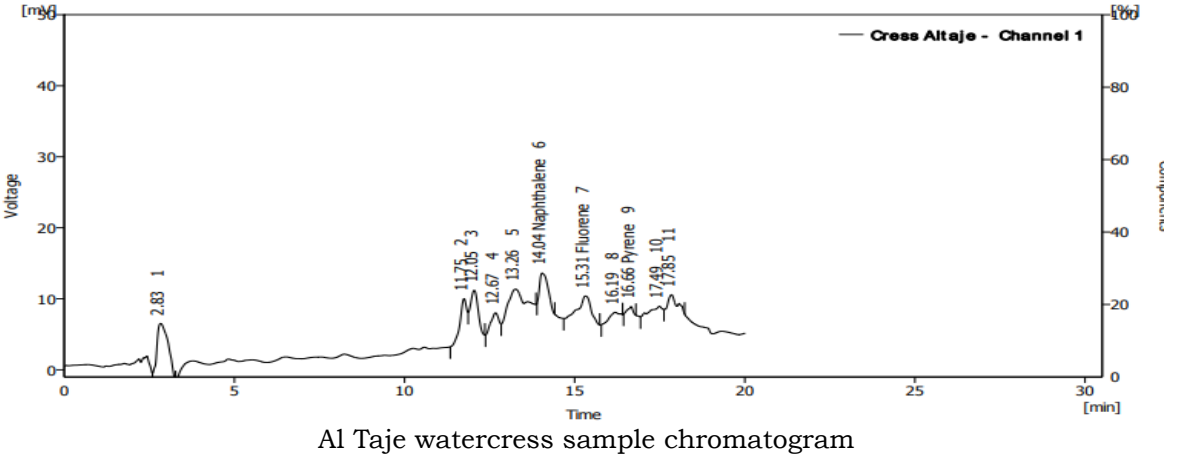
Al Doura Watercress sample chromatogram



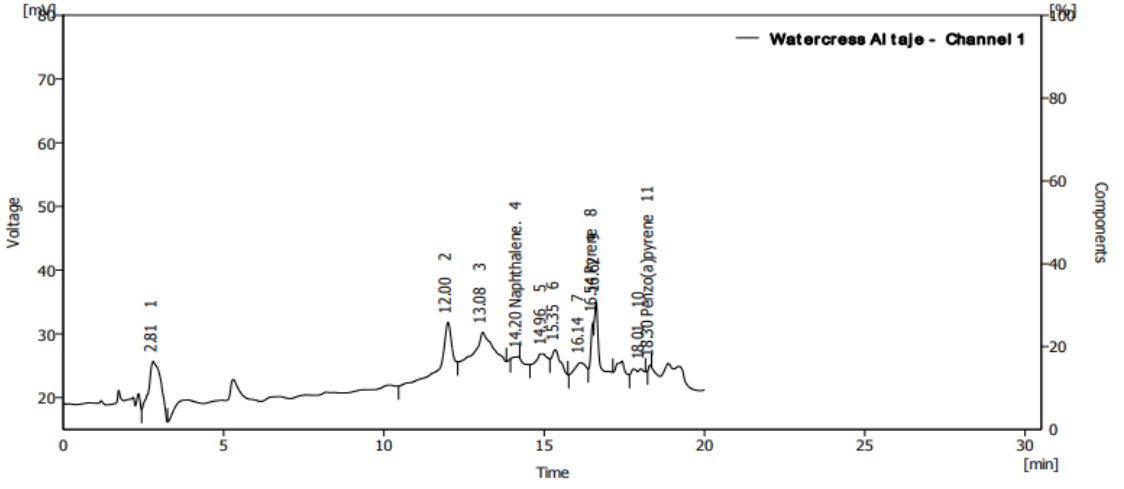
Al Doura Cress sample chromatogram



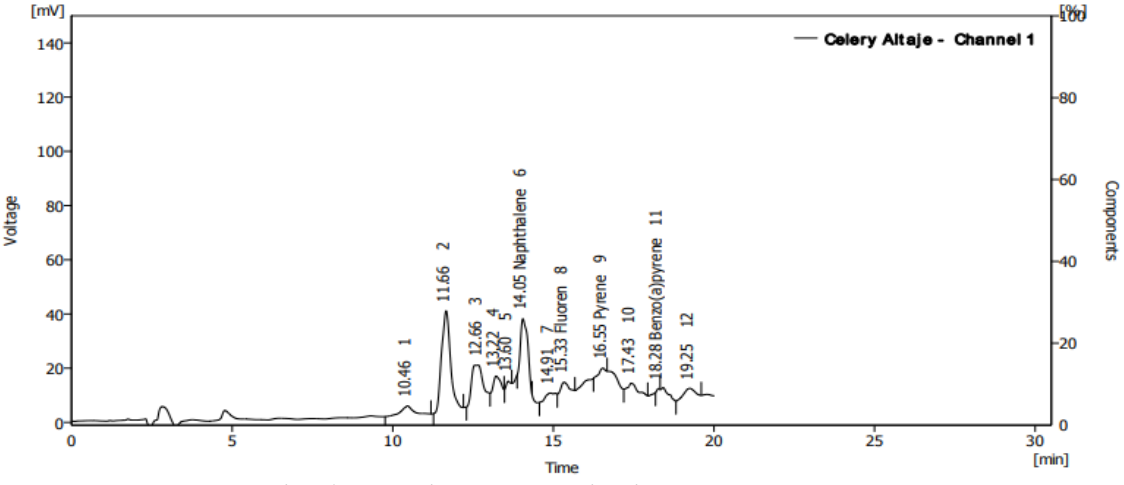
Al Taje Cress sample chromatogram



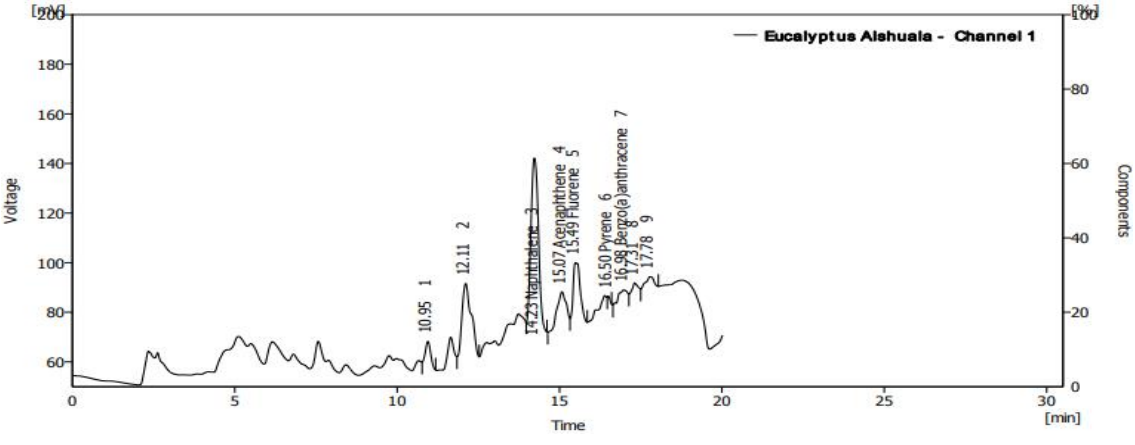
Al Taje watercress sample chromatogram



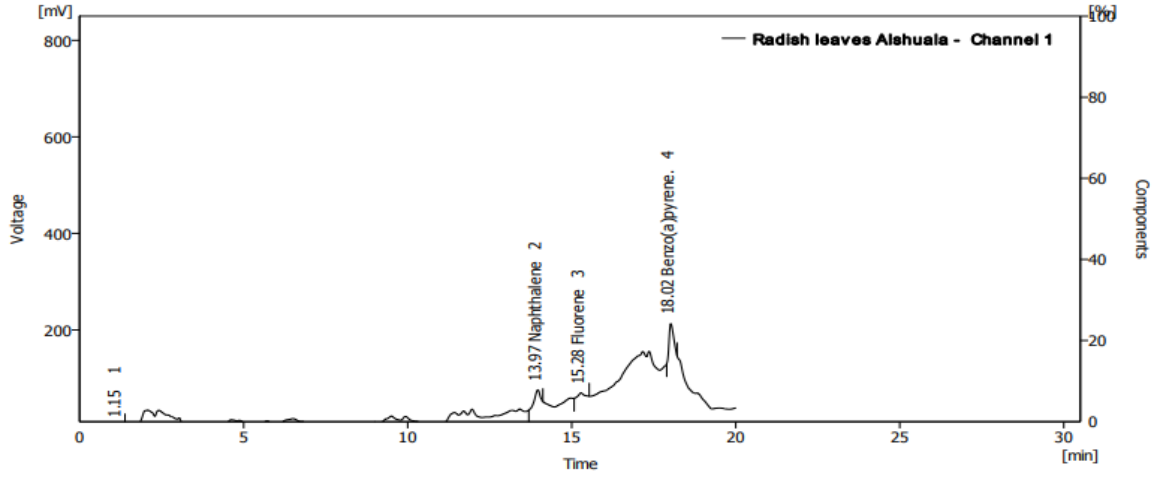
Al Taje Celery sample chromatogram



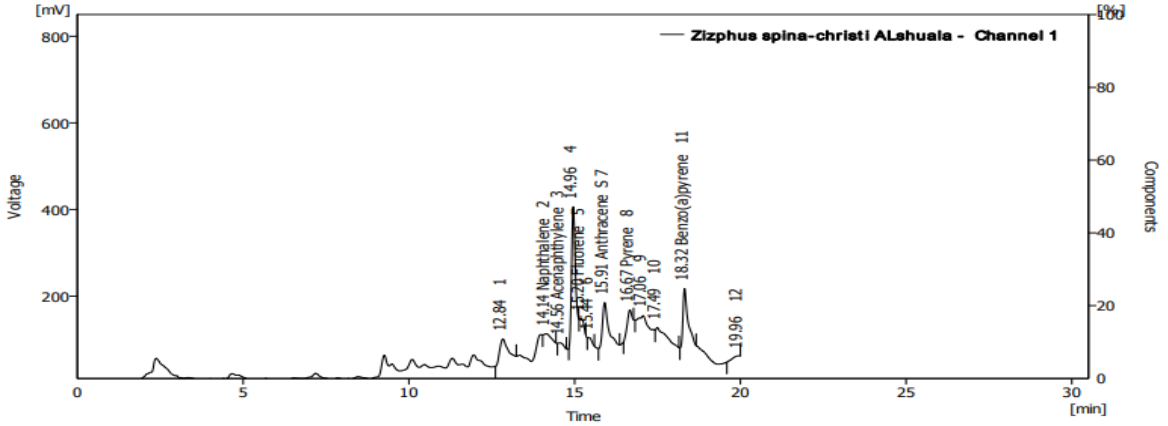
Al Taje Eucalyptus sample chromatogram



Al Shuala Radish leaves sample chromatogram



Al Shuala Zizphus spina christi leaves sample chromatogram



Below is a table [3],[4] showing the concentrations of PAHs compounds in the studied samples in units of ppm.

Table 3

No.	PAHs	Celery (Doura)	Cress (Doura)	Watercress (Doura)	Celery Taje	Cress Taje
1	Naphthalene	0.9907	2.1283	5.5358	6.8470	1.5211
2	Acenaphthylene		0.3610			
3	Acenaphthene	3.2859		1.7604		
4	Fluorene	0.29029	0.0516	0.1384	0.2343	0.3452
5	Phenanthrene					
6	Anthracene					
7	Pyrene			0.1833	0.1825	0.1570
8	Benzo[a]anthracene		0.2175			
9	Chrysene					
10	Benzo[a]pyrene	1.1018	0.4857	0.9216	0.4692	

Table 4

No.	PAHs	Watercress Taje	ziziphus spina christi Shuala	Eucalyptus Shuala	Radish leaves Shuala
1	Naphthalene	0.0785	1.9823	19.433	6.1481
2	Acenaphthylene		0.6041		
3	Acenaphthene		0.4558	4.3430	
4	Fluorene			1.6045	0.3510
5	Phenanthrene				
6	Anthracene				
7	Pyrene	0.2620	1.1392	1.3538	
8	Benzo[a]anthracene			0.0412	
9	Chrysene				
10	Benzo[a]pyrene	0.4326	2.7037		0.9463

Results

The results of the analysis of the above samples showed concentrations that exceeded the permissible limit of polycyclic aromatic compounds in vegetables and fixed leaves of trees, which was determined by the British Food Protection Agency, which is that the total of four polycyclic aromatic compounds in one sample does not exceed 0.14ppm. Accordingly, all samples from different regions contained concentrations higher than the permissible limit.

The Doura regions samples showed concentrations that ranged (0.0516 - 5.5358)ppm. The acenaphthene compound showed a very high concentration in the celery sample (3.2859ppm), while the naphthalene compound appeared in a high concentration in the cress sample (2.1283ppm) and a very high concentration in the watercress sample (5.5358ppm), and this could be attributed to the increase in the surface area of the watercress leaves and thus greater exposure to compounds PAHs present in the air or accumulated on sample leaves by soil particles.

As for the Tajik regions samples, they included concentrations ranging from 0.0785ppm to 6.8470ppm.

Naphthalene was found in a very high concentration in the celery sample and a high concentration in the cress sample. And in the Al-Shula regions, high concentrations of naphthalene and benzopyrene compounds were found in ziziphus spina Christi leaves and Eucalyptus leaves.

The concentration of PAH compounds in the ziziphus spina Christi leaves under study is considered low compared to the concentrations of these compounds in ziziphus spina Christi leaves samples in a previous study in the Muthanna Military Airport in Baghdad, Iraq, which showed very high concentrations of PAHS compounds[26].
The difference in the concentrations of samples of the same type taken from different regions is due to different environmental influences such as the concentration of these compounds in the air or soil or the difference in temperature or surface area of the leaf as well as precipitation containing PAH compounds or the absence of rain and other chemical or physical changes that occur in plants

It was also found in the Eucalyptus leaves at the concentrations of total PAHs compounds were very high (26.7755 µg/mL) compared to the concentrations in another study in the Qurna oil field in Basra, southern Iraq, where the highest concentrations of total pAHs compounds were found in Eucalyptus leaves (2.813 µg/mL)[27].
Conclusion

The results obtained from the current study using the HPLC technique with reversible phase, which showed that all samples contained PAHs compounds at a level higher than the permissible limit, and this is because of the places where these samples were planted, which contained industrial areas, oil fields or planted next to the road and thus They are exposed to pollution due to smoke from car exhaust

This contamination of plants leads to great dangers to living organisms, so the appropriate place and climate for cultivation must be taken into account, as well as the need to wash vegetables well before eating them. Also, the use of other separation techniques such as GC or GC-MS can lead to the detection of other PAH compounds present in the samples. We also note that all samples contained naphthalene because it is one of the lightest polycyclic aromatic compounds, so it is easily absorbed by plants, and pyrene, benzo[a]pyrene and Fluorene were found. As for chrysene, it was not seen in any of the studied samples.

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