

## ANALYSIS OF CHEMICAL PROFILES OF DIFFERENT *PISTACIA ATLANTICA* PARTS AT SULAYMANIYAH AND HALABJA REGION IN IRAQ

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**Abstract.** The current study estimated the chemical constituents of wild *Pistacia atlantica* parts. Leaves, fruits, and rachis in spring and autumn were chosen. In addition, the analysis covered the bark and gum of the plant. Between April and October 2020, samples were collected from Sulaymaniyah and Halabja governorates in Kurdistan (Northeastern Iraq). Notably, at various locations, spring leaves contained more nitrogen, phosphorus, potassium, and carbohydrates than autumn leaves. Moreover, Qaradagh autumn fruits contained the highest levels of fixed oil (32.08%). GC analysis of autumn fruit oil showed the concentrations of palmitic acid (11.02-11.69%), stearic acid (2.7-4.2%), oleic acid (44.25-45.39%), linoleic acid (13.11-15.36%) and linolenic acid (0.36-0.77%). Lastly, Qaradagh spring leaves had the highest total phenolic content (307.057 mg/g), while Ranya spring leaves had the highest total flavonoid concentration (101.483 mg/g). HPLC analysis of Ranya spring leaves revealed that the concentrations of quercetin, rutin, catechin, ferulic acid, and ellagic acid were 168.9 µg/g, 149.7 µg/g, 124.5 µg/g, 122.4 µg/g, and 97.4 µg/g, respectively. Phenolic compounds are abundant in Qaradagh spring leaves. As such, further studies are needed to investigate the potential therapeutic uses of bioactive compounds isolated from Qaradagh spring leaves of *P. atlantica*.

**Keywords:** plant parts, locations, fixed oil, phenolic composition, flavonoids

### Introduction

The *Pistacia* plant is a member of the Anacardiaceae family. The genus *Pistacia* consists of at least 11 species, some of which have edible nuts and are commercially important (Kafkas and Perl-Treves, 2001). Three *Pistacia* species are found in the Kurdistan region of Iraq; *P. atlantica* and *P. khinjuk* are wild species. *P. vera*, on the other hand, is a cultivated species (Shabaz, 2010). *P. atlantica* is known as Daraban or Qazwan in Kurdistan and is one of the most common *Pistacia* species worldwide (Dyary et al., 2017). In the Islamic Republic of Iran, *P. atlantica* is called Baneh and is the most economically important tree species in many rural areas (Saber-Tehrani et al., 2013). *P. atlantica* is indigenous to Kurdistan in northern Iraq, southern-east Turkey, northwestern Iran, Afghanistan, Syria, and Armenia (Shabaz, 2010). Leaves, fruits, and gum of *Pistacia* are valuable for their medicinal, cosmetic, and nutritional value; *Pistacia*'s gum is used to make natural Kurdish chewing gum (Paraschos et al., 2007). Parts of *Pistacia* plant contain phytochemical constituents such as phenolic compounds, terpenoids, fatty acids, and sterols (Labdelli et al., 2019; Hasheminya and Dehghannya, 2020; Najafiasl et al., 2022).

*Pistacia atlantica* fruits produce a considerable yield of oil. The fruits are widely consumed as a nutrient by the local population and are used in traditional medicine to treat a variety of diseases. Also, the oil can be used in food and cosmetics (Saber-Tehrani et al., 2013). Its oil contains both saturated and unsaturated fatty acids; the ratio of unsaturated fatty acids to saturated fatty acids indicates that the content of unsaturated fatty acids is approximately three times higher than that of saturated fatty acids (Labdelli et al., 2019). Also, the oil is rich in mono-unsaturated fatty acids, particularly oleic acid, which is inversely related to cholesterol levels (Ahmed et al., 2021).

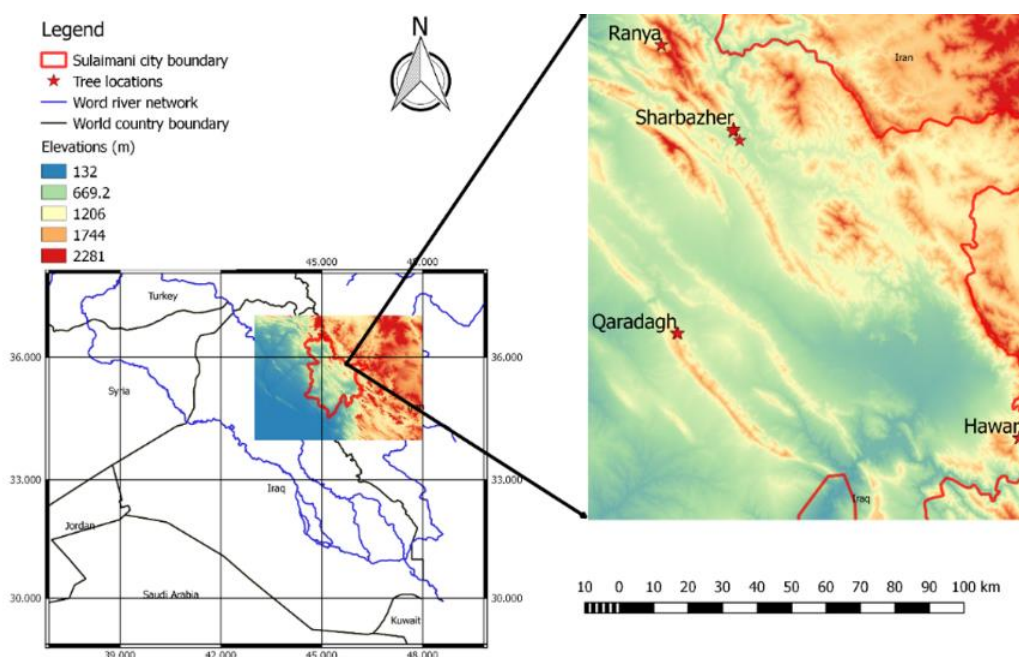
*P. atlantica* leaves are known to be an excellent source of phenolic compounds (Toul et al., 2017). Over 8000 phenolic compounds have been identified from various plant parts via different techniques, making them one of the most abundant bioactive molecules in the plant kingdom (Ramos, 2007). The majority of these compounds have the potential to be therapeutic agents (Tungmunnithum et al., 2018). Thus, the extraction and separation of polyphenols such as phenolic acids, flavonoids, lignans and stilbenes are vital for their potential uses in medicine (Baiano and Del Nobile, 2016). However, choosing and developing the right separation techniques can be challenging.

Despite the *Pistacia* tree's natural occurrence in Iraq's Kurdistan, there is a paucity of literature on the chemical constituents of various parts collected from local locations. As such, the goal of this study was to examine the bioactive components of plant materials and determine the best location containing plant parts with the highest concentrations of active compounds.

## Materials and methods

### Locations of the study

The plant parts were gathered from four locations; Qaradagh, Ranya, Sharbazher in Sulaymaniyah, and Hawar in Halabja (*Fig. 1*).



**Figure 1.** Map of the distribution of collection sites of the studied plant materials

Table 1 shows the highest and lowest temperatures, as well as rainfall, in the selected locations.

**Table 1.** Daily average of minimum and maximum temperatures and rainfall during January to October 2020 for the study locations

Month	Qaradagh			Ranya			Sharbazher			Halabja		
	Air temp. °c		Rain fall (mm)	Air temp. °c		Rain fall (mm)	Air temp. °c		Rain fall (mm)	Air temp. °c		Rain fall (mm)
	Min	Max		Min	Max		Min	Max		Min	Max	
January	2	9	171	0	7	277.4	-2.5	2.4	124	3	11.4	91.4
February	3	12	245.5	2	10	172.8	-5.2	4.1	242	4.7	13.8	67.9
March	9	19	241.3	6	15	171.8	2.5	11.3	270	9.7	20.8	124.5
April	13	21	101	13	20	71.4	6.3	12	94	12.9	25	83.6
May	19	29	16	16	25	29.5	11.6	21	31.5	18.6	35.3	10
June	20	35	0	22	33	0	16.8	30.8	0	23.6	42	0
July	26	42	0	25	41	0	15.9	32.2	0	29	45.9	0
August	24	40	0	24	39	0	17.1	29.7	0	26.4	43.5	0
September	22	37	0	20	37	0	19.8	25.9	0	24.4	42	0
October	16	30	0	17	27	0	13.1	20.1	0	17	34.3	0

\* The data were obtained from the meteorological station in Sulaimani

Regarding soil characteristics, Table 2 depicts some physical and chemical characteristics of the soil taken from the study locations.

**Table 2.** Physical and chemical characteristics of the soil of locations under study

Characteristics	Qaradagh	Ranya	Sharbazher	Halabja
Ec dS m <sup>-1</sup>	0.2	0.4	0.5	0.2
pH	6.95	7.12	7.08	7.05
Available N mg kg <sup>-1</sup>	45	32	31	39
Available P mg kg <sup>-1</sup>	16.65	16.42	15.52	18.23
Available K mg kg <sup>-1</sup>	172.63	160.98	140.53	180.23
O.M g kg <sup>-1</sup>	58	28.3	24.3	48.4
CaCo <sub>3</sub> g kg <sup>-1</sup>	331.2	114.8	103.7	100.0
Sand g kg <sup>-1</sup>	444	439	262	430
Silt g kg <sup>-1</sup>	367	316	480	380
Clay g kg <sup>-1</sup>	189	245	258	190
Texture	Loam	Loam	Loam	Loam

\*Soil analysis was carried out in a central laboratory for soil, water and plant analysis in the College of Agricultural Engineering Sciences / University of Baghdad

### Plant materials collection and preparation

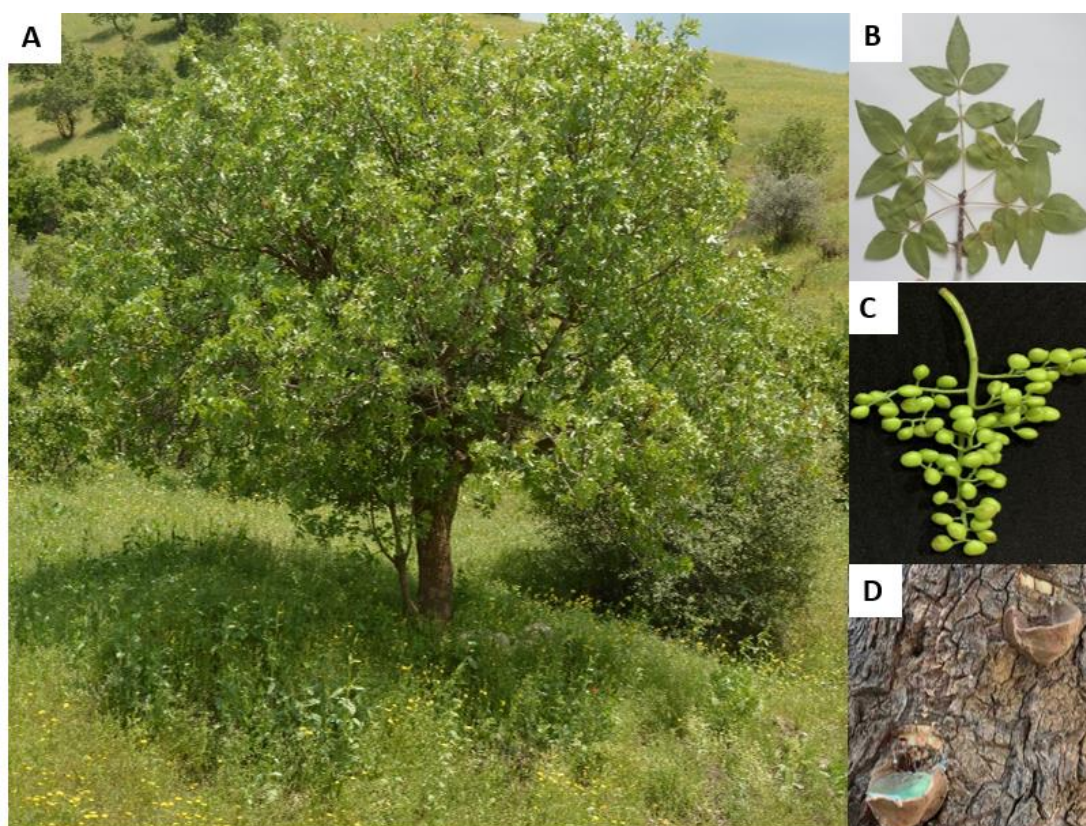
Between April and October 2020, spring leaf, spring fruit, spring rachis, bark, gum, autumn leaf, autumn fruit, and autumn rachis of *Pistacia atlantica* were collected from each location (Table 3). The samples were taken randomly with five replications. Figure 2 shows the tree, leaf, fruit, and gum of *Pistacia atlantica* with a gum collector.

Following collection, samples were prepared for analysis at the University of Sulaimani's Research Laboratory. All of the spring leaves, spring fruits, spring rachis, bark, autumn leaves, autumn fruits, and autumn rachis were air-dried at room temperature and ground to a fine powder with an electric blender, except the gum. All of the samples were then stored at 4 °C for future research.

**Table 3.** Number of samples, plant parts, and collection date at the different locations under study

No. of samples (trees)	Plant parts	Collection date
5	Spring leaf, bark spring fruit, spring rachis Gum Autumn leaf Autumn fruit, autumn rachis	April May July- August September October

\*Five trees for each location and eight parts for each tree



**Figure 2.** *P. atlantica* with different parts (A) Tree. (B) Leaf. (C) Fruit. (D) Handmade clay cup with collected gum (resin)

### Total nitrogen, phosphorus, and potassium %

After digesting half of each sample (0.5 g) with concentrated sulfuric acid and 30% hydrogen peroxide, total nitrogen in leaves was measured using a microkjeldahl apparatus (Labconco, Kansas). Phosphorus was determined using a colorimetric method by a spectrophotometer (Thermo Electron, UK) at 410 nm, and potassium was measured using a flame photometer (Janeway PFP 7, UK). The percentage of elements was calculated using dry weight (Estefan et al., 2013).

### **Carbohydrate %**

Carbohydrate was measured colorimetrically (Joslyn, 1970). Briefly, 0.2 g of dry samples were put into the test tubes separately, then 8 ml of HClO<sub>4</sub> (1N) was added. The mixture was placed in a water bath at 60 °C for 60 minutes. Thereafter, the samples were centrifuged at 3000 rpm for 15 minutes. All three mentioned steps are repeated three times. The supernatants collected the volume completed to 100 ml by adding distilled water. Then, 1 ml of the diluted solution was taken, and 1 ml of 5% phenol and 5 ml of H<sub>2</sub>SO<sub>4</sub> (97%) were added. Finally, total carbohydrates were determined using a spectrophotometer (Thermo Electron, UK) at 490 nm. Measurement of total carbohydrates in leaves was performed using the following equation:

$$\text{Carbohydrates \%} = \frac{\text{Concentration} \times \text{dilution}}{\text{Sample weight}} \times 100 \quad (\text{Eq.1})$$

### **Extraction and analysis the constituents of fixed oil**

Fixed oil was measured by Soxhlet (Simax, Czechia) method (Ferreira-Dias et al., 2003). 50 g dried-ground powder was placed in a Soxhlet thimble and extracted with 250 ml n-hexane solvent for 2 hours in Soxhlet extraction system. The remaining solvent was evaporated by a rotary evaporator (BUCHI, Germany). After drying, the dried extracts were weighed and the oil content calculated as follows:

$$\text{Total fixed oils \%} = \frac{\text{Oil weight (g)}}{\text{Sample weight (g)}} \times 100 \quad (\text{Eq.2})$$

The fixed oil compounds were analyzed using gas chromatography (Shimadzu, Japan), where the ionized flame detector (FID) and SE-30 capillary column (30 m length, 0.25 mm inner diameter) were used. Injection area, detector and separator column temperature were 280 °C, 310 °C and 120–290 °C (10 °C/min), respectively. The gas flow rate was 100 Kpa (Zhang et al., 2015).

### **Measurement of total phenolic and flavonoid contents**

Samples were prepared using the Tabart et al. (2007) and Michiels et al. (2012) methods. Briefly, 1 g of samples were placed in 15 ml tubes. The tubes were then filled with 10 ml of 80% methanol. The samples were shaken in a water bath for 3 hours at 38 °C before being centrifuged at 5000 rpm for 15 minutes at 4 °C. The upper layer was removed and transferred to a new, clean, labeled 15 ml tube, which was then stored in a refrigerator at 4 °C until use.

Total phenolic content was determined in the methanolic extract with a standard Folin-Ciocalteu reagent described by Rodrigues et al. (2019) with some modifications. 100 µl of each methanolic extract is mixed with 4 ml of 10% Folin-Ciocalteu reagent (Thomasbaker, India), and allowed to react for 5 minutes at room temperature. After that, 2 ml of 20% saturated Na<sub>2</sub>CO<sub>3</sub> solution was added then left for 60 minutes in the dark at 38 °C. Regarding blanks, same previous steps were repeated except 100 µl of water was used instead for the samples. The measurement was done at 765 nm using a spectrophotometer (Thermo Electron, UK) to calculate the phenolic content using the calibration curve made with gallic acid (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/ml) and expressed as milligrams of gallic acid equivalent (GAE) per gram dry weight.

Total flavonoid content was determined by the aluminum chloride (AlCl<sub>3</sub>) colorimetric method (Hassan et al., 2020) with minor modifications. In brief, 0.5 ml of each sample extract was mixed with 1.5 ml with 80% methanol, 0.1 ml of 10% (w/v) AlCl<sub>3</sub> solution, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. Then the mixture was incubated at room temperature for 45 minutes. 0.5 ml of water was mixed with the same amount of chemicals in the previous step as a blank. The absorbance of the reaction mixture was determined at 415 nm using a spectrophotometer (Thermo Electron, UK). The results of the total flavonoid content were calculated based on a standard curve prepared using quercetin at different concentrations (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 mg/ ml) and expressed as milligrams of quercetin per gram dry weight.

### ***Extraction and isolation of phenolic compounds for HPLC analysis***

Phenolic compounds were extracted from the homogenized plant sample (3 g) using ethanol/water (70/30) ratio. Extraction process was accomplished using an Ultrasonic Bath (Smith-Kline, USA) at room temperature for 60 sec. After filtration, the solvent was removed by the rotary evaporator (BUCHI, Germany) under vacuum, and dried at 40 °C to the constant mass. Dry extracts were stored in glass bottles at 4 °C to prevent oxidative damage until analysis. Reversed phase HPLC analysis was used to perform quantification of individual phenolic compounds, using a HPLC (SYKAMN, Germany) chromatographic system equipped with a UV detector, chemstation software, binary pump, online vacuum degasser, autosampler and Zorbax Eclipse Plus-C18-ODS column (4.6 x 250 mm). The gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water) was performed, as follows: 40 % B (0-4 min); 50 % B (4-10 min). The column temperature was 30 °C and flow-rate of 0.7 ml/min. The injected volume of samples and standards was 100 µl and was done automatically using an autosampler. The spectra were acquired in the 280 nm (Radovanović et al., 2015).

### ***Statistical analysis***

A statistical software package, XLSTAT (Version 2016.02.28451), was used to perform statistical analysis. Two way Completely Randomized Design (locations and plant parts) with five replicates (five trees) was conducted. The means were compared to determine critical values using the Duncan's Multiple Range Test at  $P \leq 0.05$ .

## **Results**

### ***Nitrogen, phosphorus, potassium, and carbohydrates content of *P. atlantica* leaves***

Table 4 shows the percentage of nitrogen, phosphorus, potassium, and carbohydrates in spring and autumn leaves of *P. atlantica* at different locations. The highest value (6.70%) of nitrogen content appeared in spring leaves collected from Qaradagh with significant differences compared to other treatments. Whereas, the lowest value was in autumn leaves (1.94%) collected from Ranya, which was not significantly different from spring leaves collected from the same location (2.30%).

The highest percentage of phosphorus occurred in the spring leaves collected from Halabja (2.03%) and the lowest value was in the autumn leaves (0.57%) collected from Ranya, which did not differ significantly with autumn leaves collected from Qaradagh (0.62%). Regarding the potassium percentage, it was noticed that the spring leaves collected from Halabja gave the highest value (2.25%), which did not significantly differ

from autumn leaves collected from the same place (2.14%); the lowest percentage occurred in the spring leaves collected from Ranya (0.48%).

Carbohydrate results showed the highest percentage (30.83%) was found in the spring leaves collected from Qaradagh. No significant difference was between the Ranya leaves collected from the same season (30.14%) and the lowest percentage was in the autumn leaves (20.31%) that were collected from Sharbazher.

**Table 4.** Nitrogen, phosphorus, potassium, and carbohydrates (%) in spring and autumn leaves

Locations	Nitrogen		Phosphorus		Potassium		Carbohydrate	
	Spring leaves	Autumn leaves	Spring leaves	Autumn leaves	Spring leaves	Autumn leaves	Spring leaves	Autumn leaves
Qaradagh	6.70 a	2.67 de	0.99 b	0.62 de	2.03 b	1.68 d	30.83 a	22.67 e
Ranya	2.30 ef	1.94 f	0.96 b	0.57 e	0.48 f	1.84 c	30.14 ab	23.57 de
Sharbazher	4.90 b	2.56 de	0.96 b	0.73 c	0.92 e	1.76 cd	29.26 bc	20.31 f
Halabja	3.70 c	2.74 d	2.03 a	0.68 cd	2.25 a	2.14 ab	28.89 c	24.21 d

\* Different letters have significant difference between them according to Duncan test at  $p < 0.05$

#### Total fixed oil and GC analysis for its constituents

Fixed oil content and GC analysis results in autumn fruit and bark of *P. atlantica* at different locations are illustrated in Table 5. Significant differences were found in the autumn fruit collected from various locations, while slight insignificant differences appeared among the barks collected from different locations. Significant differences were observed between the highest levels of total fixed oil found in autumn fruit collected from Qaradagh (32.08%) and the lowest level found in the bark collected from Halabja (3.10%).

Autumn fruit oil contained more saturated fatty acids (palmitic and stearic acids) and unsaturated fatty acids (oleic, linolic, and linolenic acids) than bark at various locations. The highest value of palmitic acid (11.69%), stearic acid (4.20%), oleic acid (45.39%), linolic acid (15.36%), and linolenic acid (0.77%) content appeared in autumn fruit oil collected from Ranya. While the lowest value of palmitic acid (2.55%), stearic acid (0.66%), oleic acid (5.89%), linolic acid (3.69%), and linolenic acid (0.14%) content was found in bark oil collected from Halabja.

**Table 5.** The concentration of total fixed oil, saturated and unsaturated fatty acids (%) in autumn fruits and bark oil

Locations	Total fixed oil		Palmitic acid		Stearic acid		Oleic acid		Linolic acid		Linolenic acid	
	Autumn fruits	Bark	Autumn fruits	Bark	Autumn fruits	Bark	Autumn fruits	Bark	Autumn fruits	Bark	Autumn fruits	Bark
Qaradagh	32.08 a	3.62 e	11.48 a	2.97 b	3.60 b	0.85 ef	45.02 ab	6.22 c	14.22 b	4.88 e	0.61 b	0.29 c
Ranya	31.16 b	3.48 e	11.69 a	3.05 b	4.20 a	0.91 e	45.39 a	6.39 c	15.36 a	5.00 e	0.77 a	0.20 c
Sharbazher	26.16 c	3.31 e	11.28 a	2.68 b	3.10 c	0.74 ef	44.79 ab	6.00 c	13.69 c	3.98 f	0.58 b	0.36 d
Halabja	18.04 d	3.10 e	11.02 a	2.55 b	2.70 d	0.66 f	44.25 b	5.89 c	13.11 d	3.69 f	0.36 c	0.14 d

\* Absence of this compound in other parts of the plant. \*Different letters have significant difference between them according to Duncan test at  $p < 0.05$

### Total phenolic content of *P. atlantica* parts (mg/g)

Table 6 shows the value of total phenolic content in all parts of *P. atlantica*. Spring and autumn leaves are richer in total phenolic content than other parts at all locations. On the other hand, at all locations, the gum contained a low amount of total phenol, with no significant differences observed between gum collected from different locations. There were significant differences between the maximum concentration in the Qaradagh spring leaves (307.057 mg/g) and the minimum concentration of the gum in the Sharbazher collection (1.409 mg/g).

**Table 6.** The concentration of total phenolic content (mg/g) in different parts

Locations	Spring leaves	Autumn leaves	Spring fruits	Autumn fruits	Spring rachis	Autumn rachis	Bark	Gum
Qaradagh	307.057 a	299.673 e	297.457 f	99.999 u	243.995 n	294.504 h	120.675 t	1.426x
Ranya	304.546 c	295.981 g	287.267 j	100.295 u	185.658 q	261.865 l	134.706 r	1.415x
Sharbazher	305.876 b	303.956 c	295.981 g	93.796 w	240.302 o	274.271 k	128.651 s	1.409x
Halabja	302.331 d	301.593 d	209.436 p	97.784 v	250.493 m	293.322 i	135.444 r	1.442x

\* Different letters have significant difference between them according to Duncan test at  $p < 0.05$

### Total flavonoid content of *P. atlantica* parts (mg/g)

Table 7 shows the concentration of total flavonoid content in eight parts of *P. atlantica*. Spring leaves at all locations contain a higher amount of total flavonoid than other parts and the significant differences were observed among locations. Bark and gum contain a low amount of total flavonoid and no significant value was observed between bark and gum collected from different locations. However, significant differences were observed between the highest concentration value in the spring leaves of Ranya (101.483 mg/g) and lowest concentration in the bark collected from Halabja (0.399 mg/g).

**Table 7.** The concentration of total flavonoids content (mg/g) in different parts

Locations	Spring leaves	Autumn leaves	Spring fruits	Autumn fruits	Spring rachis	Autumn rachis	Bark	Gum
Qaradagh	82.970 c	75.624 e	60.528 j	2.715 p	51.713 k	70.739 h	0.687 q	0.548 q
Ranya	101.483 a	63.136 i	49.619 l	2.494 p	40.510 n	45.285 m	0.433 q	0.426 q
Sharbazher	93.953 b	73.053 g	63.063 i	2.118 p	50.978 k	45.138 m	0.441 q	0.478 q
Halabja	81.979 d	74.596 f	26.552 o	2.521 p	51.603 k	60.087 j	0.399 q	0.609 q

\* Different letters have significant difference between them according to Duncan test at  $p < 0.0$

### HPLC analysis for phenolic compounds

The results of quantitative analysis of each identified phenolic compound by HPLC in *P. atlantica* parts are shown in Table 8. Spring leaves from all sites contain high amount of quercetin and have significant differences observed in comparison with other plant parts. The significant differences were observed between the highest levels of quercetin in Ranya spring leaves (168.9  $\mu\text{g/g}$ ) and the lowest level in Sharbazher spring rachis (61.4  $\mu\text{g/g}$ ). While quercetin in the Qaradagh spring leaves and Halabja autumn leaves



(160.7 µg/g and 158.9 µg/g, respectively) show a significant difference observed in other plant parts.

**Table 8.** HPLC analysis for phenolic compounds of *P. atlantica* (µg/g)

Phenolics compounds	Locations	Spring leaves	Autumn leaves	Spring fruits	Autumn fruits	Spring rachis	Autumn rachis	Bark	Gum
Quercetin	Qaradagh	160.7 b	138.9 f	101.4 j	71.5 p	68.7 qr	115.4 h	nd*	nd
	Ranya	168.9 a	144.2 e	106.9 i	77.5 n	70.4 pq	122.8 g	nd	nd
	Sharbazher	146.9 d	123.9 g	80.4 m	74.5 o	61.4 s	85.5 l	nd	nd
	Halabja	155.8 c	158.9 b	92.4 k	80.4 m	66.9 r	114.5 h	nd	nd
Rutin	Qaradagh	140.8 b	119.8 f	98.8 h	52.8 q	60.8 o	88.7 k	22.8 s	29.1 r
	Ranya	149.7 a	122.5 e	102.6 g	55.6 p	66.4 n	95.8 i	11.4 u	28.4 r
	Sharbazher	124.5 e	98.7 h	88.5 k	51.5 q	51.2 q	75.6 m	18.4 t	22.5 s
	Halabja	133.8 d	136.9 c	92.5 j	60.5 o	58.9 o	81.2 l	20.5 st	30.5 r
Cinnamic acid	Qaradagh	105.9 e	124.7 c	80.7 l	68.1 op	57.9 r	92.5 h	22.4 u	17.4 wx
	Ranya	120.6 d	133.5 b	84.9 k	73.9 m	60.8 q	104.8 e	12.8 y	15.8 x
	Sharbazher	88.5 j	94.5 g	66.8 p	62.5 q	50.1 t	80.1 l	18.9 vw	12.8 y
	Halabja	98.7 f	142.6 a	71.4 n	68.9 o	55.2 s	90.6 i	20.5 v	18.7 vw
Catechin	Qaradagh	120.9 b	nd	90.1 f	nd	78.7 h	nd	nd	nd
	Ranya	124.5 a	nd	98.7 e	nd	78.7 h	nd	nd	nd
	Sharbazher	112.5 d	nd	74.8 i	nd	66.2 j	nd	nd	nd
	Halabja	115.8 c	nd	84.5 g	nd	74.8 i	nd	nd	nd
Ferulic acid	Qaradagh	120.8 b	nd	78.9 f	nd	53.5 j	nd	18.9 q	26.8 n
	Ranya	122.4 a	nd	88.9 e	nd	55.4 i	nd	6.4 t	24.8 o
	Sharbazher	112.8 d	nd	66.5 h	nd	41.8 l	nd	13.6 s	22.5 p
	Halabja	118.9 c	nd	74.5 g	nd	50.1 k	nd	15.8 r	28.9 m
Ellagic acid	Qaradagh	91.4 b	62.8 i	60.1 j	42.7 q	38.7 s	66.2 g	25.8 v	nd
	Ranya	97.4 a	70.5 e	68.9 f	52.6 m	40.8 r	64.7 h	18.9 y	nd
	Sharbazher	64.5 h	66.9 g	44.5 p	41.5 r	32.5 u	50.9 n	20.1 x	nd
	Halabja	88.9 c	74.1 d	54.9 l	45.8 o	35.6 t	57.8 k	22.4 w	nd
Tannic acid	Qaradagh	nd	81.3 cd	nd	65.8 g	nd	80.4 de	28.9 j	21.7 l
	Ranya	nd	88.5 b	nd	79.8 e	nd	82.4 c	14.7 o	20.4 m
	Sharbazher	nd	79.4 e	nd	62.5 h	nd	51.7 i	22 l	18.9 n
	Halabja	nd	97.4 a	nd	74.5 f	nd	64.7 g	25.8 k	22.4 l
Kaempferol	Qaradagh	nd	81.4 f	nd	53.8 k	nd	86.9 d	nd	28.7 m
	Ranya	nd	91.4 b	nd	60.4 i	nd	84.6 e	nd	27.9 m
	Sharbazher	nd	88.9 c	nd	52.9 k	nd	63.5 h	nd	25.9 n
	Halabja	nd	98.7 a	nd	56.9 j	nd	71.5 g	nd	30.9 l
Stilbene	Qaradagh	nd	43.9 c	nd	26.9 i	nd	36.9 e	nd	nd
	Ranya	nd	50.9 b	nd	31.5 g	nd	41.9 d	nd	nd
	Sharbazher	nd	42.6 d	nd	24.8 j	nd	25.9 i	nd	nd
	Halabja	nd	55.2 a	nd	29.8 h	nd	33.6 f	nd	nd
Gallic acid	Qaradagh	nd	nd	nd	nd	nd	nd	nd	9.7 b
	Ranya	nd	nd	nd	nd	nd	nd	nd	9.2 c
	Sharbazher	nd	nd	nd	nd	nd	nd	nd	8.5 d
	Halabja	nd	nd	nd	nd	nd	nd	nd	10.5 a
Apigenin	Qaradagh	nd	nd	nd	nd	nd	nd	10.2 a	nd
	Ranya	nd	nd	nd	nd	nd	nd	6.2 d	nd
	Sharbazher	nd	nd	nd	nd	nd	nd	7.8 c	nd
	Halabja	nd	nd	nd	nd	nd	nd	8.9 b	nd

\* Not detected. \* Different letters have significant difference between them according to Duncan test at  $p < 0.05$

Rutin results showed that the highest value (149.7 µg/g) was found in spring leaves collected from Ranya, and the lowest value (11.4 µg/g) was obtained from the bark of the same location.

Autumn leaves from all sites except Sharbazher contain high amounts of cinnamic acid and have significant differences observed in comparison with other plant parts in other locations. The highest value (142.6 µg/g) of cinnamic acid content appeared in autumn leaves collected from Halabja with significant differences compared to other treatments. Whereas, the lowest concentration was present in bark collected from Ranya (12.8 µg/g) and gum collected from Sharbazher (12.8 µg/g).

The significant differences were observed between spring leaves collected from all locations and have high amounts of catechin in comparison with other plant parts. The highest value (124.5 µg/g) was recorded in spring leaves collected from Ranya followed by Qaradagh (120.9 µg/g) while the lowest value (66.2 µg/g) was recorded in spring rachis collected from Sharbazher.

The highest value (122.4 µg/g) of ferulic acid content was recorded for Ranya spring leaves while the lowest (6.4 µg/g) was for the bark of the same location.

Ranya spring leaves were significantly superior in ellagic acid content (97.4 µg/g) compared to (18.9 µg/g) in the Ranya bark.

The highest value (97.4 µg/g) of tannic acid appeared in autumn leaves collected from Halabja with significant differences compared to other plant parts. Whereas, the lowest value was in bark (14.7 µg/g) collected from Ranya.

Kaempferol results indicate that there were significant differences in the kaempferol concentration in the autumn parts and gum that collected from different locations. The highest value (98.7 µg/g) was found in autumn leaves from Halabja, followed by Ranya and Sharbazher (91.4 µg/g and 88.9 µg/g, respectively), and the lowest value was found in Sharbazher gum (25.9 µg/g).

Significant differences were observed between the highest level of stilbene in the autumn leaves at Halabja (55.2 µg/g) and the lowest concentration present in autumn fruits at Sharbazher (24.8 µg/g).

Regarding the gallic acid concentration in gum, the highest concentration of gallic acid was found in gum collected from Halabja (10.5 µg/g) with significant differences from Qaradagh and Ranya gum (9.7 µg/g and 9.2 µg/g, respectively), and the lowest concentration was found in Sharbazher gum (8.5 µg/g) with significant differences from other locations gum.

Apigenin results indicate that there were significant differences in the apigenin content of the bark collected from different locations. The highest value (10.2 µg/g) was found in bark from Qaradagh, followed by Halabja and Sharbazher (8.9 µg/g and 7.8 µg/g, respectively), and the lowest in Ranya (6.2 µg/g).

## Discussion

Analysis of chemical profiles of different parts of *Pistacia atlantica* at different locations in Kurdistan is reported for the first time. Concerning the spring and autumn leaves, it was discovered that nitrogen, phosphorus, potassium and carbohydrate levels were highest in the spring leaves. The environmental factors such as light, temperature, rainfall and soil fertility have significant effects on the efficiency of photosynthesis and chemical constituents (Muhammad et al., 2021). The leaves were fully expanded with more efficient photosynthesis during spring, so the production of carbohydrate

was higher. These findings corroborate those of Hassan (2016), who discovered that Qaradagh spring leaves contained the highest levels of carbohydrates. In addition, the average summer temperature was high, and the plant's respiration rate was increased. Autumn saw a gradual decrease in the concentrations of nitrogen, phosphorus, potassium, and carbohydrate, according to Crous et al. (2022). Also, in addition, the chemical constituents in autumn leaves decreased. The reason for this could be age, as photosynthetic capacity and efficiency decline with age (Bauerle et al., 2020).

It is noticed that the Qaradagh location gave the highest value for fixed oil in autumn fruit due to perhaps the difference of environmental conditions between the locations under study. Moreover, the Qaradagh soil contains a high proportion of organic matter which was considered to have a significant impact on the physicochemical properties of the soil. The main consequence was an improvement in the uptake of micro and macroelements (Khoshnaw and Esmail, 2020). Leskovar and Othman (2018) discovered that increasing the amount of organic matter increases porosity, which decreases soil-specific density and allows microorganisms to penetrate more easily into the soil environment to use organic compounds, providing plants with better access to nutrients.

GC analysis of autumn fruit oil showed the concentrations of unsaturated fatty acids (oleic, linoleic, and linolenic acids) were approximately four times higher than that of saturated fatty acids (palmitic and stearic acids). These results were similar with other researchers' data (Saber-Tehrani et al., 2013; Dorehgirae and Pourabdollah, 2015).

Hashemi et al. (2017) also found high levels of phenolic and flavonoid compounds in various locations in the leaves. Furthermore, the spring leaves in Qaradagh provided the highest value for the phenolic compound. The accumulation of secondary metabolites is strongly dependent on environmental factors such as light, temperature and soil fertility (Yang et al., 2018). Low temperature promotes the synthesis of phenolic compounds, whereas high temperature breaks the chemical bonds that exist between phenolic molecules because these bonds are temperature sensitive (Jan et al., 2021). When compared to other locations, Qaradagh soil contained the highest percentage of nitrogen; this is also thought to be a significant cause of high nitrogen contents in Qaradagh leaves harvested in the spring. The high nitrogen content increases phenylalanine synthesis as a precursor of phenolic compounds, resulting in a high phenol rate (Ibrahim et al., 2011). As a result, a positive relationship exists between the highest percentage of nitrogen and phenolic compounds. Moreover, Leskovar and Othman (2018) concluded that increasing organic matter in the soil increases plant biomass, which contributes to increased phenolic and antioxidant compound production.

Spring leaves collected from Ranya had the highest flavonoid content. Lowering temperatures, according to Wang et al. (2015), stimulate the enzymatic activity of some key enzymes involved in flavonoid biosynthesis, such as phenylalanine ammonia-lyase, which preside over the first step of general phenylpropanoid biosynthesis. This could explain why Ranya spring leaves had the highest total flavonoid content.

HPLC was used to identify eleven phenolic compounds from various parts, including quercetin, rutin, cinnamic acid, catechin, ferulic acid, ellagic acid, tannic acid, kaempferol, stilbene, gallic acid, and apigenin. These findings agreed with those of Karim (2014) and Hatamnia et al. (2014).

## Conclusion

*P. atlantica*'s various parts (leaves, fruits, and gum) are valuable for their medicinal, cosmetic, and nutritional value. The findings of this study confirm that the chemical profile of *P. atlantica* is affected by plant parts, and location, with autumn fruit in Qaradagh having higher fixed oil. Autumn fruit oil contained approximately four times more unsaturated fatty acids than saturated fatty acids. The Qaradagh spring leaves of *P. atlantica* were also found to be high in phenolic compounds. As a result, more research is needed to investigate the biological activity of phenolic compounds isolated from *P. atlantica* Qaradagh spring leaves.

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