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## Analysis of Active Compounds in Hawthorn Species Widespread in Northwestern Syria

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### Abstract

The research was conducted during the year 2022 to estimate the most important active compounds in the different plant parts of some types of hawthorns (*Crataegus* L.) widespread in northwestern Syria (Aronia, Yellow Azarolus, Red Azarolus, and Monogyna). The plant material was collected during the growth period. Flavonoids were estimated in the dry matter of the leaves, flowers, and fruits of the studied hawthorn types, vitamin C in the dry flowers and fresh fruits, carotenoids in the dry fruits, total acidity (TA%), and total soluble solids (TSS%) in the fresh fruits. The results showed that the value of flavonoids in the leaves reached 29.66, 15.76, 14.42, and 14.66 mg/g DM in the studied hawthorn types Monogyna, Aronia, Red Azarolus, and Yellow Azarolus, respectively. The leaves outperformed the flowers and fruits in their flavonoid content in most types, as the average flavonoid content of the leaves, flowers, and fruits in the different types was 18.60, 17.71, and 10.90 mg/g DM, respectively. The highest value of flavonoids (29.66 mg/g) was in the leaves of the Monogyna type, and the lowest value (6.23 mg/g) was in the fruits of the Red Azarolus type. As for vitamin C in the dried flowers of the different types, its value reached 1018.6, 917.5, 814.9, and 799.1 mg/100g, while the value of vitamin C in fresh fruits was 11, 8.14, 7.04, and 6.53 mg/g, in Monogyna, Aronia, Yellow Azarolus and Red Azarolus, respectively (in dried flowers and fresh fruits). The Monogyna type outperformed the other types of vitamin C content, whether in dried flowers or fresh fruits. As for carotenoids, the values were 0.202, 0.077, 0.093, and 0.156 mg/cm<sup>3</sup>, respectively, for the types Monogyna, Red Azarolus, Yellow Azarolus, and Aronia. The Monogyna type outperformed the other types in the carotenoid content of its fruits. As for the total acidity, the Red Azarolus type outperformed the other types. The total acidity values were 2.54, 1.94, 1.8, and 0.46% for Red Azarolus, Yellow Azarolus, Aronia, and Monogyna, respectively. Finally, there were no significant differences between the total soluble solids content of the fruits of the different types, as the TSS values reached 24.60, 23.33, 21.27, and 20.97%, in the Red Azarolus, Yellow Azarolus, Monogyna, and Aronia types. Respectively.

**Key words:** Hawthorn, Flavonoids, Carotenoids, Vitamin C, Total Acidity, Total Dissolved Solids.

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## Introduction

Plants have gained increasing importance as a source of active medicinal ingredients. It is known that the use of medicinal plants is an important factor that improves the general health of people, and interest in plant extracts, herbs, spices, and other plant compounds rich in phenols is increasing in the food industry due to their protective effect against oxidative decomposition of fats and improving the quality and nutritional value of foods (Wojdyło *et al.*, 2005). The race to extract and purify many of these active compounds with biological effects continues to occupy pharmaceutical, chemical, and life science scientists (Salmanian *et al.*, 2014).

The hawthorn plant is one of the important medicinal plants. It is a medium-sized shrub with deciduous leaves, not more than 8 m high. Its branches end with thorns. Its flowers are white, red, or pink with a strong smell. It blooms between April and June in the form of large bouquets, then the buds grow. Veganism. The fruits are spherical in shape, small, and are red or yellow when ripe. They contain 2-3 seeds. The leaves are shiny, and silvery green, grow in many shapes and sizes, and are winged and tough-skinned. Hawthorn belongs to the genus *Crataegus*, which in turn belongs to the subfamily Pomoideae and the rose family Rosaceae. The genus *Crataegus*, commonly known as the hawthorn, includes about 200 species, distributed in the temperate and subtropical regions of the Northern Hemisphere (Dönmez, 2004; Makhoul and Mahfoud, 2007). Hawthorn bushes are usually thorny shrubs or low trees about 5-15 meters high. The lifespan of hawthorn can reach more than 200 years, and today more than 20 species of hawthorn are used in folk medicine, some of which are found in pharmacies in many countries (Chang *et al.*, 2002).

The importance of hawthorn comes from the fact that it is a plant that is ranked fourth in the world in terms of its benefits, according to the American Food and Drug Administration (FDA), the German Authority for Medicinal Herbs, and the European Food and Drug Safety Authority, because of its medical benefits at the heart level and for treating heart attacks by expanding the diameter of the coronary blood vessels (Critu *et al.*, 2011). Hawthorn is among the most valuable and effective plants for protecting the heart. It has been observed that extracts of hawthorn fruits, leaves, and flowers have a beneficial effect on coronary artery flow and myocardial contraction and are used as effective agents to strengthen the heart, regulate blood pressure, and as a stimulant for the digestive and circulatory systems. Modern pharmaceutical extracts are taken from flowers, leaves, and fruits (Balick, 2013; Alirezalu *et al.*, 2018; Long *et al.*, 2006; Degenring *et al.*, 2003). In some countries, certain species of the genus *Crataegus* are used to treat gastrointestinal problems, shortness of breath, and kidney stones (Rigelsky and Sweet, 2002). Hawthorn extracts also contain a wide range of antioxidant, antimicrobial, antifungal, anti-inflammatory, and antiviral activities (Orhan *et al.*, 2007; Benli *et al.*, 2008; Tadic' *et al.*, 2008; Garcí'a-Mateos *et al.*, 2013). Phenols are among the most common compounds, due to their pronounced antioxidant activity and the ability to directly bind (Rice-Evans *et al.*, 1996; Kirakosyan *et al.*, 2003; Kancheva, 2009). Scientific evidence has proven that hawthorn fruits possess powerful antioxidants and free radical scavenging activities due to the presence of bioactive compounds such as epicatechin and chlorogenic acid (Sallabanks, 1992; Özcan *et al.*, 2005; Barros *et al.*, 2011). It has been found that these compounds have many pharmacological effects, including protecting nerve cells, the liver, and the heart, and preventing kidney disease, among others (Nabavi *et al.*, 2015). Hawthorn is known for its ability to treat cardiovascular diseases such as congestive heart failure, myocarditis, and chest pain. It is also used to treat atherosclerosis, cases of angina, and high cholesterol, and regulates blood pressure. In addition, hawthorn extract is useful in treating heart muscle disorders and heart valve diseases (Tassel *et al.*, 2010; Kostic *et al.*, 2012; Veveris *et al.*, 2004). Hawthorn is of great importance because of its astringent properties. It has been used to treat digestive problems such as indigestion, diarrhea, and stomach pain, and to get rid of tapeworms and other intestinal infections. It is also used as a sedative, relieves anxiety and insomnia, increases diuresis, and treats menstrual problems and heavy menstrual bleeding in women. It has also been used to treat boils and ulcers on the skin and in first aid to remove splinters over the past century. Hawthorn preparations are used as a lotion for sores, itching, and infections caused by frost (Castleman, 2000; Ruwaiha, 2001).

Reference studies show that the content of hawthorn leaves and flowers of flavonoids ranges between 0.1-2%, including rutin, hyperoside, vitexin, and quercetin (Taherovic, 2014). One study conducted on hawthorn leaves and flowers confirmed that the flavonoid contents in dried samples ranged between 0.16-1%, while their amount in fresh samples was between 0.03-0.18%,

and that the flavonoid contents in hawthorn flower samples, whether dried or fresh, is greater than in leaf samples. (Chouman and Aldeen, 2017). In one study of the hawthorn plant, the content of flavonoids was different in the fruits and leaves of the hawthorn and ranged between 21.11 and 122.98 mg/g dry matter, where the highest content of flavonoids was found in the leaf extract, 122.98 mg/g dry matter (Vidoslva *et al.*, 2020). The total amount of flavonoids varied depending on the type of plant parts and ranged between 2.27 and 17.40 mg/g dry matter, and phenolic compounds were the most abundant in hawthorn flower extracts in most genotypes (Alirezalu *et al.*, 2018). Phenolic compounds are the ones that contribute most to the antioxidant activity of fresh foods that are consumed, which enables us to conclude that some of the antioxidant activity in hawthorn fruits is due to a high percentage of phenols, and in other cases perhaps to a joint effect of vitamin C and flavonoids (Scalbert and Williamson, 2000).

Ascorbic acid is an antioxidant found in animal and plant cells (Davey *et al.*, 2000), and plays a major role in the detoxification of activated oxygen (AO) which acts as an antioxidant (Foyer *et al.*, 1991). Studies indicate that the ascorbic acid content in hawthorn fruits ranges approximately between 102 and 231 mg/100 g (Tahirovic *et al.*, 2012). Hawthorn also contains vitamins B1, B2, B6 and others. In addition, 17 amino acids were identified in hawthorn (Guo *et al.*, 1995). In one study to detect total carotenoids in the fruits of 8 hawthorn species, it was found that their quantity varied from one species to another and ranged from 86-405 micrograms/g.

From the above, previous studies indicate that there are large differences in the amount of active chemical compounds (especially antioxidants and phenolic compounds) between hawthorn species. Therefore, evaluating the genetic resources of hawthorn and identifying models with active contents of these effective medicinal compounds, contributes significantly to the production of antioxidants. Natural antioxidants and other phytochemical compounds with nutritional and pharmaceutical value. Hence the importance of this research in evaluating the hawthorn types widespread in northwestern Syria and determining their content of active chemical compounds such as flavonoids, vitamin C, carotenoids, and others.

## Materials and Methods

### Research Location:

Plant samples were collected from hawthorn distribution sites in Idlib Governorate during 2021 and 2022. Chemical analyses were conducted on the samples collected in the laboratories of the Agricultural Engineering Faculty at the University of Idlib.

### Plant Material:

The plant material for this research included four types of hawthorns that were identified and described (Aronia, Yellow Azarolus, Red Azarolus, and Monogyna), (Fig. 1). The various plant parts (leaves, flowers, fruits) were collected to conduct the required chemical analyses. Readings were taken on several trees from every type and every site.



Fig. (1): Leaves (A), flowers (B), and fruits (C) of studied hawthorn types

**(1-Aronia; 2-Yellow Azarolus; 3-Red Azarolus; 4-Monogyna)****Sample Preparation:**

The leaves and flowers were collected during their growth period, then dried away from sunlight, then ground with an electric blender, and kept in a dry place away from heat, humidity, and light until analyses were conducted to extract the active compounds in them. Fresh fruits were collected at the ripening time for each type from the hawthorn distribution areas in northwestern Syria and then immediately stored in the refrigerator until the experiment was conducted. As for the dry fruits, they were collected and dried in a dryer for 24 hours, then ground and preserved until analysis.

**Chemical analyses:**

After collecting samples from the different parts (leaves, flowers, and fruits) of the hawthorn types (Aronia, Yellow Azarolus, Red Azarolus, and Monogyna), the following chemical analyses were performed:

- a) Flavonoids (mg/g of dry matter DM):** Flavonoids were determined from leaves, flowers, and fruits, where extraction was first done using an alcohol method, while detection and estimation were done with aluminium chlorine and a spectrophotometer, according to the method described by Garcí'a-Mateos *et al.* (2017).
- b) Vitamin C (mg/100g):** The vitamin C content of fruits was estimated by titrating the vitamin with an iodine solution in the presence of a starch indicator, according to the method used by Outreach (2017). As for the flowers, extraction was done with glacial acetic acid and then titrated with iodine solution in the presence of starch indicator according to the method described by Azra *et al.* (2012).
- c) Carotenoids (mg/100g DM):** Carotenoids were determined using a spectrophotometer according to the method described by Goodwin (1984).
- d) Total Acidity (TA%):** Total acidity was determined by titration by adding sodium water and in the presence of the phenolphthalein reagent, according to the method described in Nielsen (2017).
- e) Total Soluble Solids (TSS%):** These include sugars, organic acids, vitamins, and salts present in the food substance, which were measured in the fruit juice using a refractometer according to the method described in Howrtiz (1975).

**Statistical analysis:**

The results were tabulated into tables, and the averages were compared by calculating the least significant difference (LSD) at the 1% significance level for laboratory readings using the GenStat statistical analysis program. Cluster analysis, correlation analysis, and PCA analysis were performed.

**Results and Discussion****Flavonoids analysis:**

Flavonoids were estimated in dried samples of leaves, flowers, and fruits. The extraction was done first using the alcohol method, then detection and estimation using the aluminum chlorine method and a spectrophotometer. Then flavonoid values were calculated, and the results shown in Table (1) were given.

**Table (1): Flavonoid contents (mg/g DM) in hawthorn leaves, flowers, and fruits**

Type	Leaves	Flowers	Fruits
<b>Aronia</b>	15.67 b	16.43 c	18.05 a
<b>Yellow Azarolus</b>	14.66 b	17.40 b	10.22 b
<b>Red Azarolus</b>	14.42 b	17.50 b	6.23 d
<b>Monogyna</b>	29.66 a	19.50 a	9.11 c

<b>Mean</b>	18.60	17.71	10.90
<b>L.S.D. (1%)</b>	8.93	0.66	0.22
<b>C.V.%</b>	15.9	1.2	0.7

The results of the statistical analysis (Table 1) showed that there were significant differences in the flavonoid content of the leaves of the studied hawthorn types. The Monogyna type outperformed the other types with a value of 29.66 mg/g DM, with no significant differences between the other types, as the values reached 15.76, 14.66, and 14.42 mg/g DM in the Aronia, Yellow Azarolus, and Red Azarolus types, respectively. These results are consistent with the results of Alirezalu *et al.* (2018), where the total amount of flavonoids in the leaves of the studied hawthorn types varied and ranged from 3.34 to 6.86 mg/g DM for Monogyna and 5.17-7.09 mg/g DM for Aronia.

The results in Table (1) displayed that there were significant differences in the quantity of flavonoids between the flowers of the studied hawthorn types. Where the Monogyna type outperformed the other types with a value of 19.50 mg/g DM, followed by the Red Azarolus and Yellow Azarolus types, which outperformed the Aronia type, with values reaching 17.50, 17.40, 16.43 mg/g DM, respectively, with no significant differences between the Red Azarolus and Yellow Azarolus types. These results are consistent with those of Alirezalu *et al.* (2018), where the total amount of flavonoids in the flowers of the studied hawthorn types varied between 3.43 mg/g DM for Monogyna and 8.96 mg/g DM for Aronia.

Table (1) also showed that there were significant differences in the flavonoid content of the fruits of the studied hawthorn types. Aronia outperformed the other types with a value of 18.05 mg/g DM. Yellow Azarolus also outperformed the other two types with a value of 10.22 mg/g DM, while Monogyna outperformed Red Azarolus, with values of 9.11 and 6.23 mg/g DM, respectively. These results are consistent with the results of Alirezalu *et al.* (2020), where the total amount of flavonoids in the fruits of the studied hawthorn species varied and reached 5.75 mg/g DM for Monogyna and 2.89 mg/g DM for Aronia.

As for the content of the plant parts of the Aronia types, the fruits were superior in their flavonoid content to the flowers, which in turn were superior to the leaves. The values reached 18.05, 16.43, and 15.67 mg/g DM, respectively. As for the Yellow Azarolus type, the flowers had a higher flavonoid content than the fruits, and the values were 17.40, 14.66, and 10.22, respectively. The same applies to Red Azarolus, where the flowers outperformed the leaves in their flavonoid content, which in turn outperformed the fruits. The values were 17.50, 14.42, and 6.23 mg/g DM. While the Monogyna type differed from the other types, the leaves were superior in their flavonoid content to the flowers, which in turn were superior to the fruits. The flavonoid content was 29.66, 19.50, and 9.11 mg/g DM, respectively.

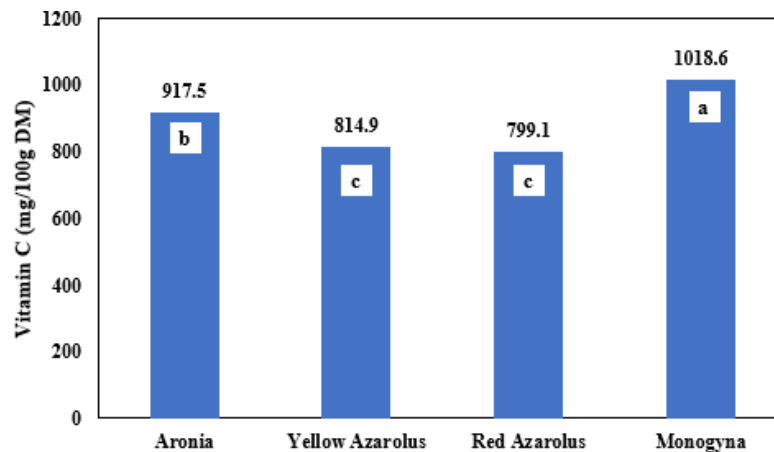
In general, the content of the leaves of the Monogyna type exceeded the other plant parts (leaves, flowers, and fruits) of all other types (Red Azarolus, Yellow Azarolus, and Aronia). Our results are consistent with the results of previous studies in terms of the difference in the content of hawthorn's plant parts (leaves, flowers, and fruits) and the differences in the flavonoid content of the studied types. In a study on the flavonoid content of hawthorn fruits and leaves, the results showed that the flavonoid content of the fruits was between 21.11 and 58.81 mg/g DM, while the leaves content ranged between 36.01 and 122.98 mg/g DM (Vidosla *et al.*, 2020). The total content of flavonoids also varied depending on the species and plant organs (leaves and flowers). It ranged between 2.27 and 17.40 mg/g DM, and the flowers were superior in some types and the leaves in others (Alirezalu *et al.*, 2018).

#### 4-1- Vitamin C analysis:

##### 4-2-1- Flowers (Dry Matter):

The vitamin C content of dried flowers was determined by extraction with glacial acetic acid and then titration with iodine solution in the presence of starch indicator by the method described by Azra *et al.* (2012). Table (2) shows the results of estimating the vitamin C content of dried flowers in the studied hawthorn types.





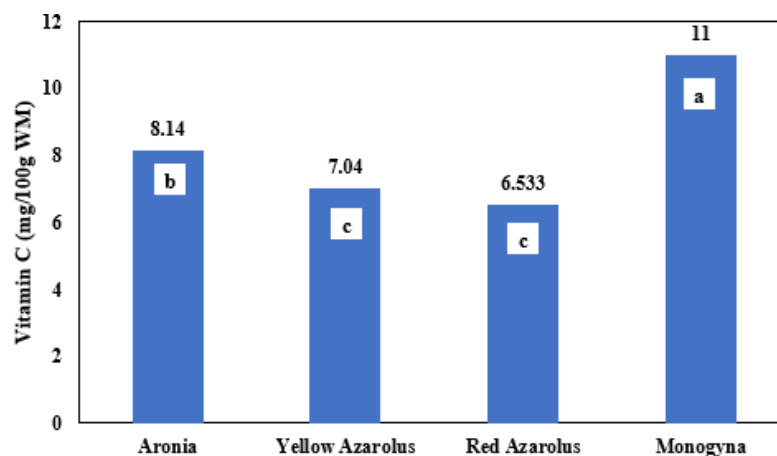
**Fig. (2): Vitamin C content of dried flowers in the studied hawthorn types**

**(The columns carrying the same letter are not significantly different, L.S.D. (1%) =24.89)**

The results of the statistical analysis of vitamin C in the dried flowers of the hawthorn types (Fig. 2) showed that there were significant differences between the types, as the Monogyna type outperformed the other types with a value amounting to 1018.6 mg/100g DM, while the Aronia type outperformed the Yellow Azarolus and Red Azarolus types with values amounting to 917.5, 814.9, and 799.1 mg/100g DM respectively. However, there are significant differences between the Yellow Azarolus and Red Azarolus types. These values are consistent with the results of previous studies, in which the values of vitamin C in Monogyna were estimated at 1108 mg/100 g DM (Tahirovic' *et al.*, 2012).

#### 4-2-2- Fruits (Wet Matter WM):

The vitamin C content of fruits was estimated by titrating the vitamin with an iodine solution in the presence of a starch indicator, according to the method used by Outreach (2017). Table (3) shows the results of estimating the vitamin C content of fresh fruits in the studied hawthorn types.



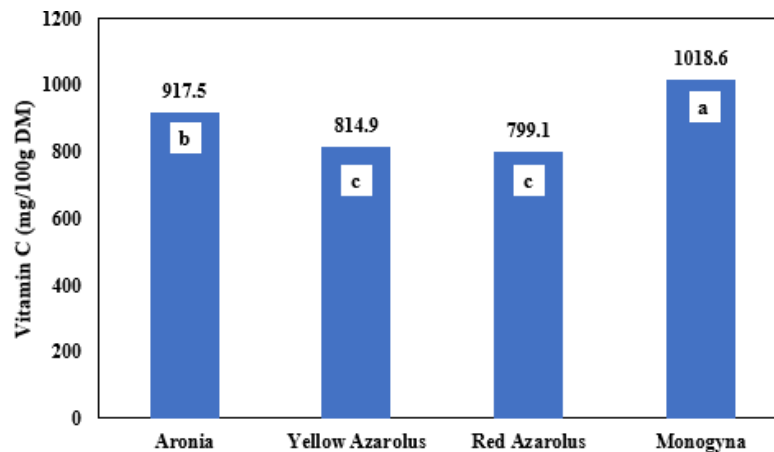
**Fig. (3): Vitamin C content of fresh fruits in the studied hawthorn types**

**(The columns carrying the same letter are not significantly different, L.S.D. (1%) =0.56)**

The results of the statistical analysis of the vitamin C content of fruits (Fig. 3) showed that there were significant differences between the studied hawthorn types, as the Monogyna type outperformed the other types, while the Aronia type outperformed the Yellow Azarolus and Red Azarolus types, with values reaching 8.14, 7.04, and 6.53 mg/100 g WM, respectively, while there were no significant differences between the Yellow Azarolus and Red Azarolus types. These results are consistent with a study conducted on 11 varieties of hawthorn, where vitamin C values in the studied varieties, belonging to the species *Crataegus pontica* Koch., ranged between 1.555-9.418 mg/100 g WM (Gundogdu *et al.*, 2014).

#### 4-2- Carotenoids (Dry Matter):

The fruits were dried and ground, then soaked with 80% acetone, then the percentage of carotenoids in the extract was measured with a spectrophotometer. Table (4) shows the results of estimating the carotenoid content of dried fruits in the studied hawthorn types.



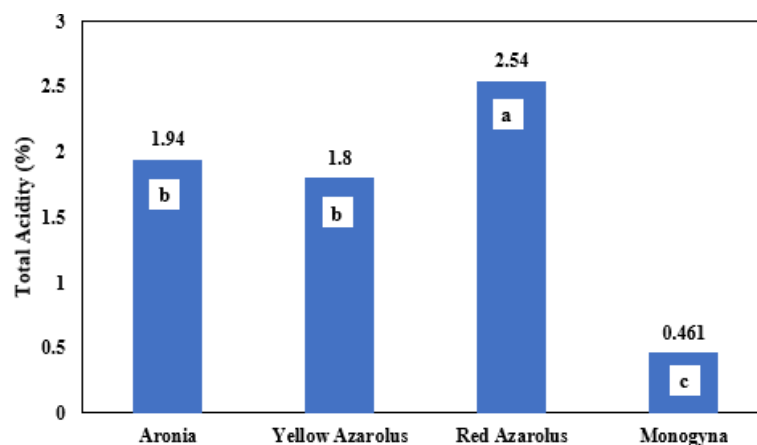
**Fig. (4): Carotenoid content of fresh fruits in the studied hawthorn types**

**(The columns carrying the same letter are not significantly different, L.S.D. (1%) =0.068)**

The results of the statistical analysis of the carotenoid content of dry fruits (Fig. 4) showed significant differences between the studied hawthorn types. The Monogyna type was significantly superior to the Yellow Azarolus and Aronia types, with values reaching 0.2020, 0.930, and 0.077 mg/cm<sup>3</sup>, respectively. There were no significant differences between the Monogyna and the Red Azarolus types, in which their dry fruit had a content of carotenoids of 0.1563 mg/cm<sup>3</sup> and was significantly superior to the Aronia type. On the other hand, there were no significant differences between the Red Azarolus and Yellow Azarolus types, and between the Yellow Azarolus and Aronia types. According to previous results, the carotenoid content of dry fruits in the studied types ranged between 0.0776 mg/cm<sup>3</sup> in the Aronia type and 0.2020 mg/cm<sup>3</sup> in the Monogyna type, which are values close to the results of previous studies.

#### 4-3- Total acidity (TA%):

The total acidity (TA%) in fresh hawthorn fruits was determined by titration by adding sodium water and in the presence of the phenolphthalein reagent, according to the method described by Nielsen (2017). Table (5) shows the results of estimating the total acidity content of hawthorn fruits in the studied hawthorn types.



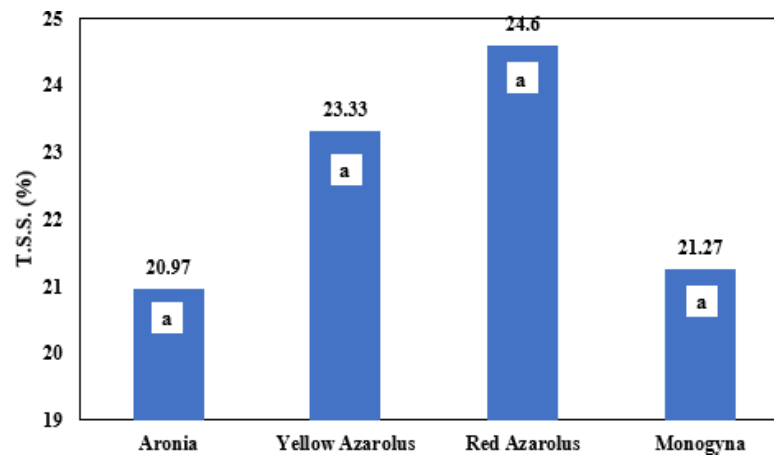
**Fig. (5): Total acidity (%) in the fresh fruits of the studied hawthorn types**

**(The columns carrying the same letter are not significantly different, L.S.D. (1%) =0.186)**

The results of total acidity in fresh hawthorn fruits (Fig. 5) showed significant differences between the studied types. The Red Azarolus outperformed the other types by a value of 2.54%, while the Yellow Azarolus and Aronia types outperformed the Monogyna type, with values of 1.94, 1.8, and 0.46%, respectively, with no significant differences between the Yellow Azarolus and Aronia types. Through this study, the total acidity value in the fruits of the studied hawthorn types ranged between 0.461% in the Monogyna type and 2.540% in the Red Azarolus type. These results are like some studies where some chemical compounds in hawthorn (*Crataegus*) fruits were analyzed, including acidity, which reached 1.98% (Ö zcan *et al.*, 2005).

#### 4-4- Total Soluble Solids (T.S.S.%):

Total Soluble solids include sugars, organic acids, vitamins, and salts present in the food substance, and were measured in the fruit juice using a refractometer according to the method described by Howrtiz (1975). Table (6) shows the results of estimating the total soluble solids content of fresh fruits in the studied hawthorn types.



**Fig. (6): Total soluble solids (%) in the fruits of the studied hawthorn types**

(The columns carrying the same letter are not significantly different, L.S.D. (1%) = 3.91)

The results of the statistical analysis (Fig. 6) show that there are no significant differences between the total soluble solids content of fruits (%) in the studied hawthorn types. The value of total soluble solids (%) ranged between 20.97% in the Aronia type and 24.60% in the Red Azarolus type. The overall average of total soluble solids was 22.17%.

#### 4-5- Correlation analysis:

The correlation analysis was conducted between the different compounds of the studied hawthorn types. Table (2) shows the results of the correlation analysis.

**Table (2): Correlation analysis between the studied active compounds**

	Correlations							
	Leaves flavonoids	Flower flavonoids	Fruits flavonoids	Fruit vitamin C	Flower vitamin C	Carotenoids	TA	T.S.S.
Leaves flavonoids	1							
Flower flavonoids	0.897	1						
Fruits flavonoids	-0.166	-0.571	1					
Fruit vitamin C	.964*	0.751	0.104	1				
Flower vitamin C	0.916	0.645	0.236	.987*	1			
Carotenoids	-0.942	-0.702	-0.175	-.997**	-.994**	1		
TA	-0.942	-0.806	-0.004	-.952*	-0.902	0.944	1	
T.S.S.	-0.553	-0.141	-0.730	-0.755	-0.832	0.800	0.662	1

\* Correlation is significant at the 0.05 level; \*\* Correlation is significant at the 0.01 level

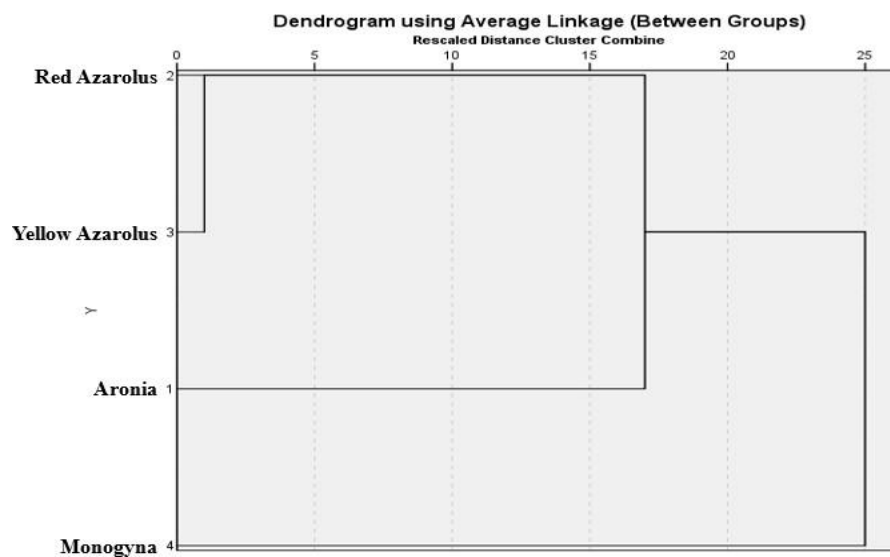
Table (2) shows that there is a positive correlation between vitamin C in fruits and flavonoids in leaves, as well as a positive correlation between vitamin C in flowers and fruits, and that vitamin C in fruits has a high negative correlation between it and carotenoids, and a negative correlation



between it and the total acidity (TA) of fruits. Vitamin C in flowers has a high negative correlation with total carotenoids.

#### 4-6- Cluster analysis:

Hierarchical cluster analysis gave the results shown in Fig. (7).



**Fig. (7): Hierarchical cluster analysis of the studied hawthorn types**

We notice from Fig. (7) that the hawthorn types were divided into two main groups: Group (A) included three types, divided into two subgroups: the first group included the Yellow Azarolus and the Red Azarolus (we notice a very strong similarity between the two types), and the second subgroup included the Aronia type. Group B included one type, the Monogyna. The importance of cluster analysis in determining the degree of relatedness between the studied types is demonstrated by its importance in breeding and hybridization programs between species and varieties, by reducing the number of inputs used in the hybridization and pollination processes and relying on genetically divergent parents, which ensure obtaining a broad genetic base (Thanh *et al.*, 2006). Cluster analysis has been used to determine the degree of morphological relatedness between hawthorn species (Albarouki and Peterson, 2007; Erfani-Moghadam, 2016; Ma and Lu, 2016; Yildiz, 2022).

#### 4-7- Principal Component Analysis (PCA):

Tables (3 and 4) and Fig. (8) show the results of the principal components analysis (PCA) responsible for the variation between the studied hawthorn types.

**Table (3): The principal components responsible for the total variance**

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	5.963	74.539	74.539
2	1.952	24.405	98.944
3	0.084	1.056	100.000
4	4.047E-16	5.059E-15	100.000
5	6.057E-17	7.571E-16	100.000
6	-1.205E-16	-1.506E-15	100.000
7	-3.312E-16	-4.140E-15	100.000
8	-4.664E-16	-5.830E-15	100.000



**Fig. (8): Factors constituting variance**

**Table (4): Principal components of the factors responsible for the variance**

Component Matrix <sup>a</sup>		
	Component	
	1	2
Leaves flavonoids	0.992	0.113
Fruit vitamin C	0.987	-0.156
Caroten	-0.973	0.227
TA	-0.970	0.043
Flower vitamin C	0.948	-0.291
Flower flavonoids	0.847	0.531
Fruits flavonoids	-0.051	-0.998
T.S.S.	-0.645	0.764

*Extraction Method: Principal Component Analysis*

Principal components analysis (PCA), Fig. (8), Table (3), and Table (4) show that there are two main factors responsible for approximately 99% of the variances between the types. The PC1 factor was responsible for 74.54% of the variances, which were positively identified by leaf flavonoids (0.992), vitamin C for fruits (0.987), vitamin C for flowers (0.948), and flavonoids for flowers (0.847). As for the variables that were combined by PC2, which is responsible for 24.40% of the variances, they were positively identified by leaf flavonoids (0.113), fruit carotenoids (0.227), total acidity TA (0.043), flower flavonoids (0.531), and total soluble solids TSS (0.764).

### Conclusion

The research showed that the Monogyna type was superior to the other types in the flavonoid content of leaves and flowers, while the Aronia type was superior in the flavonoid content in the fruits. In general, the percentage of flavonoids in flowers was higher than in leaves in almost all types, except for Monogyna, where leaves outperformed flowers. Regarding vitamin C, the fruits of the Monogyna type outperformed the other types, whether in flowers or fruits. As for the carotenoid content in the fruits, the Monogyna type outperformed the other types. While the Red Azarolus type outperformed the other types in total acidity. Finally, there were no significant differences in total soluble solids values between the studied types.

### References

- Albarouki, E., and Peterson, A. (2007). Molecular And Morphological Characterization of *Crataegus L.* Species (Rosaceae) In Southern Syria. Botanical Journal of The Linnean Society, 153(3): 255-263.
- Alirezalu, A., Ahmadi, N., Salehi, P., Sonboli, A., Alirezalu, K., Mousavi Khaneghah, A., and Lorenzo, J. M. (2020). Physicochemical characterization, antioxidant activity, and phenolic compounds of hawthorn (*Crataegus spp.*) fruits species for potential use in food applications. Foods, 9(4): 436.
- Alirezalu, A., Salehi, P., Ahmadi, N., Sonboli, A., Aceto, S., Maleki, H. H., and Ayyari, M. (2018). Flavonoids profile and antioxidant activity in flowers and leaves of hawthorn species (*Crataegus spp.*) from different regions of Iran. International Journal of Food Properties, 21(1): 452-470.
- Azra, T., Amira, C., Nedz'ad, B., Lela, K., and Mirel, S. (2012). Determination of vitamin C in flowers of some Bosnian *Crataegus L.* species. Radovi, 2, 1-12.
- Balick, M., 2013. Herbal gram. The journal of the American Botanical Council, 96.
- Barros, L.; Carvalho, A. M.; and Ferreira, I. C. (2011). Comparing the Composition and Bioactivity of *Crataegus monogyna* Flowers and Fruits Used in Folk Medicine. Phytochemical Analysis, 22, 181–188.
- Benli M., Yig'it, N., Geven, F., Güney, K., and Bingöl, Ü . (2008), Antimicrobial activity of endemic *Crataegus tanacetifolia* (Lam.) Pers and observation of the inhibition effect on bacterial cells. Cell Biochemistry and Function, 26(8): 844-851.
- Castleman, M. (2000). Dictionary of Healing Plants, Al-Shafaq Printing and Publishing, Beirut. Lebanon, 582 p.
- Chang, C., Yang, M., Wen, H., and J. Chern. (2002). Estimation of total flavonoids content in propolis by two complementary colorimetric methods. Journal Food and Drug Analysis. 10:176-182.
- Chouman, F., and Aldeen, S. H. (2017). Determination of total content of flavonoids in dried and fresh hawthorn leaves and flowers. Tishreen University Journal-Basic Sciences Series, 39(6).
- Critu, R; Mihailescu, R; Mitroy, G; Iacob, E., and Verdis, R. (2011). Phytochemical investigation of crataegi folium cum flos (Hawthorn leaves and flowers) and hyperici herba (ST john's wort aerial parts) hydroalcoholic extracts.
- Davey, M. W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J. J., Strain, J. J., Favell, D., and Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture. 80: 825-860.
- Degenring, F. H., Suter, A., Weber, M., and Saller, R. (2003). A randomised double-blind placebo controlled clinical trial of a standardised extract of fresh *Crataegus* berries (Crataegisan®) in the treatment of patients with congestive heart failure NYHA II. Phytomedicine, 10(5): 363-369.
- Dönmez, A. (2004). The Genus *Crataegus L.* (Rosaceae) with Special Reference to Hybridisation and Biodiversity in Turkey. Turkish Journal of Botany. 28(1): 29-37.
- Erfani-Moghadam, J., Mozafari, M., and Fazeli, A. (2016). Genetic Variation of Some Hawthorn Species Based on Phenotypic Characteristics and RAPD Marker. Biotechnology and Biotechnological Equipment, 30(2): 247-253.
- Foyer, C.H., Lelandias, M., Edwards, E.A., and Mullineaux, P. M. (1991). Current topics in plant physiology, 6, ASPP, Rockville, 131.
- García-Mateos M.R., Ibarra-Estrada E., and Nieto-Angel R. (2013). Antioxidant compounds in hawthorn fruits (*Crataegus spp.*) of Mexico Compuestos antioxidantes en frutos de tejocote (*Crataegus spp.*) de México, Revista Mexicana de Biodiversidad. 84(4):1298-1304.
- Goodwin, T.W., and Mercer, E.I., (1984). Introduction to plant biochemistry. Second ed. Pergaman Press, Oxford, 677.
- Gundogdu, M., Ozrenk, K., Ercisli, S., Kan, T., Kodad, O., and Hegedus, A. (2014). Organic acids, sugars, vitamin C content and some pomological characteristics of eleven hawthorn species (*Crataegus spp.*) from Turkey. Biological Research, 47(1): 1-5.
- Guo, T., and Jiao, P. (1995). Hawthorn (*Crataegus*) resources in China. HortScience, 30(6): 1132-1134.

- Horwitz, W. (1975). Official methods of analysis (Vol. 222). Washington, DC: Association of Official Analytical Chemists.
- Howrtiz, W. (1975). Official methods of analysis. Association of Official Analytical Chemists, Washington, D.C., U.S.A. 86p.
- Imanishi, S., Sugiyama, K., Nagata, M., and Noguchi, Y. (2006, August). A simple method for the estimation of alpha-and beta-carotene in carrots. In XXVII International Horticultural Congress-IHC2006: International Symposium on The Role of Postharvest Technology in the 768 (pp. 565-569).
- Kancheva, V. D. (2009). Phenolic antioxidants–radical-scavenging and chain-breaking activity: A comparative study. *European Journal of Lipid Science and Technology*, 111(11): 1072-1089.
- Kirakosyan, A., Seymour, E., Kaufman, P. B., Warber, S., Bolling, S., and Chang, S. C. (2003). Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (Hawthorn) subjected to drought and cold stress. *Journal of agricultural and food chemistry*, 51(14): 3973-3976.
- Kostic', D. A., Velickovic', J. M., Mitic', S. S., Mitic', M. N., and Randelovic', S. S. (2012). Phenolic content, and antioxidant and antimicrobial activities of *Crataegus oxyacantha* L (Rosaceae) fruit extract from Southeast Serbia. *Tropical Journal of Pharmaceutical Research*, 11(1): 117-124.
- Long, S. R., Carey, R. A., Crofoot, K. M., Proteau, P. J., and Filtz, T. M. (2006). Effect of hawthorn (*Crataegus oxyacantha*) crude extract and chromatographic fractions on multiple activities in a cultured cardiomyocyte assay. *Phytomedicine*, 13(9-10): 643-650.
- Ma, S. L. Y., and Lu, Y. M. (2016). Classification And Phylogenetic Analysis of Chinese Hawthorn Assessed By Plant And Pollen Morphology. *Genet. Mol. Res. Gmr*, 15, Gmr-15038739.
- Makhoul, G., and Mahfoud, M. (2007). The Medicinal and Economic Importance of Hawthorn. Symposium on Neglected Trees in Syria, Tishreen University, Faculty of Agriculture, 13p.
- Nabavi, S. F., Habtemariam, S., Ahmed, T., Sureda, A., Daglia, M., Sobarzo-Sa'nchez, E., and Nabavi, S. M. (2015). Polyphenolic composition of *Crataegus monogyna* Jacq. from chemistry to medical applications. *Nutrients*, 7(9): 7708-7728.
- Nielsen, S. S. (2017). Correction to: Food Analysis Fifth Edition. *Food Analysis*, C1-C3.
- Orhan, I., Ö zçelik, B., Kartal, M., Ö zdeveci, B., and Duman, H. (2007). HPLC quantification of vitexine-2 "-O-rhamnoside and hyperoside in three *Crataegus* species and their antimicrobial and antiviral activities. *Chromatographia*, 66: 153-157.
- Outreach C., 2017, Vitamin C determination by iodine titration. *Canterbury Ac. Nz.*, 17-20.
- Ö zcan, M., Hacıseferođ ullaří, H., Marakođ lu, T., and Arslan, D. (2005). Hawthorn (*Crataegus* spp.) fruit: some physical and chemical properties. *Journal of Food Engineering*, 69(4): 409-413.
- Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, 20(7): 933-956.
- Rigelsky, J. M., and Sweet, B. V. (2002). Hawthorn: pharmacology and therapeutic uses. *American Journal of Health-System Pharmacy*, 59(5): 417-422.
- Ruwaiha, A. (2001). Herbal medicine. *Dar Al-Qalam*, Beirut, 390p.
- Sallabanks, R. (1992). Fruit fate, frugivory, and fruit characteristics: a study of the hawthorn, *Crataegus monogyna* (Rosaceae). *Oecologia*, 91: 296-304.
- Salmanian, S. S. M. A., Sadeghi Mahoonak, A. R., Alami, M., and Ghorbani, M. (2014). Phenolic content, antiradical, antioxidant, and antibacterial properties of hawthorn (*Crataegus elbursensis*) seed and pulp extract. *Journal of Agricultural Science and Technology*, 16(2): 343-354.
- Scalbert, A., and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition* 130(S): 2073-2085.
- Tadic', V. M., Dobric', S., Markovic', G. M., Đorđevic', S. M., Arsic', I. A., Menkovic', N. R., and Stevic', T. (2008). Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. *Journal of agricultural and food chemistry*, 56(17): 7700-7709.
- Tahirovic', A., and Bas'ic', N. (2014). Phenolic content and antioxidant activity of *Crataegus monogyna* L. fruit extracts. *Radovi S'umarskog fakulteta Univerziteta u Sarajevu*, 44(2): 29-40.

- Tahirović, A., Čopra–Jančićević, A., Bašić, N., Vidić, D., Klepo, L., and Delić, D. (2012). Determination of vitamin C in some Bosnian *Crataegus* L. species by spectrophotometric method. *Radovi Šumarskog fakulteta Univerziteta u Sarajevu*, 42(1): 43-55.
- Tassell, M. C., Kingston, R., Gilroy, D., Lehane, M., and Furey, A. (2010). Hawthorn (*Crataegus* spp.) in the treatment of cardiovascular disease. *Pharmacognosy reviews*, 4(7): 32.
- Thanh, V.C., Phuong, P.V., Uyen, P.H.H., and Hien, P.P. (2006). "Application Of Protein Electrophoresis SDS-PAGE To Evaluate Genetic Purity and Diversity of Several Varieties". *Proceedings Of International Workshop on Biotechnology In Agriculture*. Nong Lam University Ho Chi Minh City, October 20-21, p192-194.
- Veveris, M., Koch, E., and Chatterjee, S. S. (2004). *Crataegus* special extract WS® 1442 improves cardiac function and reduces infarct size in a rat model of prolonged coronary ischemia and reperfusion. *Life Sciences*, 74(15): 1945-1955.
- Vidoslva, D., Novica, R., Biljana, D., Milenko, R. (2020). Phenolic And Flavonoid Content and Antioxidant Evaluation of Hawthorn (*Crataegus monogyna* Jacq.) Fruits and Leaves Extrats. *Publication in Natural Sciences*, 10(1): 20-25.
- Wojdyło, A., Oszmianowski, J., and Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food chemistry*, 105(3): 940-949.
- Yildiz, E., Erkek, B., Yilmaz, K. U., and Yaman, M. (2022). Determination Of the Effect of Distance to Highway on The Accumulation of Some Heavy Metals in Apple Orchards. *Current Trends in Natural Sciences*, 11(21): 145-152.
- Yildiz, E., Sümbül, A., Yaman, M., Nadeem, M. A., Say, A., Baloch, F. S., and Popescu, G. C. (2023). Assessing the genetic diversity in hawthorn (*Crataegus* spp.) genotypes using morphological, phytochemical, and molecular markers. *Genetic Resources and Crop Evolution*, 70(1): 135-146.